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***BRCA1* and *BRCA2* Predictive Genetic Testing for Breast and Ovarian Cancers: A Systematic Review of Clinical Evidence**

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CCOHTA
600-865 Carling Avenue
Ottawa ON Canada K1S 5S8
Tel. (613) 226-2553
Fax. (613) 226-5392
Email: pubs@ccohta.ca

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Canadian Coordinating Office for Health Technology Assessment

BRCA1 and *BRCA2* Predictive Genetic Testing for Breast and Ovarian Cancers: A Systematic Review of Clinical Evidence

Lynda McGahan MSc[1]
Ritsuko Kakuma MSc[2]
Chuong Ho MD MSc[1]
Ken Bassett MD PhD[1]
Hussein Z. Noorani MSc[1]
Janet Joyce MLS[1]
Judith Allanson MD FRCPC FCCMG DABMG[3]
Sherry Taylor PhD FCCMG[4]

March 2006

[1] Canadian Coordinating Office for Health Technology Assessment, Ottawa ON Canada
[2] Agence d'évaluation des technologies et des modes d'intervention en santé, Montréal QC Canada
[3] Children's Hospital of Eastern Ontario, Ottawa ON Canada
[4] Queen's University, Kingston ON Canada

Reviewers

This report results from a collaboration between the Agence d'évaluation des technologies et des modes d'intervention en santé (AÉTMIS) and the Canadian Coordinating Office for Health Technology Assessment (CCOHTA). The Board and the Genetic Advisory Board of AÉTMIS (comprised of experts in medical genetics, bioethics, law, biochemistry, and public health) reviewed an initial draft. A subsequent draft underwent internal review by CCOHTA before external review.

These individuals kindly provided comments on this report.

External Reviewers

Peter J Bridge, PhD FCCMG FACMG
Associate Professor,
Departments of Medical Genetics, and
Pathology & Laboratory Medicine,
Faculty of Medicine
Director, Molecular Diagnostic Laboratory,
Alberta Children's Hospital
Edmonton AB

Bartha Maria Knoppers, PhD O.C.
Professor, Canada Research Chair in Law and Medicine
Université de Montréal, C.R.D.P.
Montréal QC

Jacques Simard, PhD Physiology
(Molecular Endocrinology)
Canada Research Chair in Oncogenetics
Professor, Department of Anatomy and Physiology,
Laval University
Sainte-Foy QC

CCOHTA Scientific Advisory Panel Reviewers

Ruth Collins-Nakai, MD MBA FRCPC FACC
Cardiologist, University of Alberta
Edmonton AB

Kenneth Marshall, BA MSc MD FRCPC FCFP
Professor of Family Medicine (Retired)
University of Western Ontario
London ON

Agence d'évaluation des technologies et des modes d'intervention en santé (AÉTMIS) and the Canadian Coordinating Office for Health Technology Assessment (CCOHTA) collaborated to systematically examine the available evidence regarding the analytical and clinical validity of available molecular technologies, and review inherent issues associated with testing. The results pertaining to molecular methods, analytical validity, psychosocial impact, ethical implications, and clinical management are presented in this report. Results related to prevalence, penetrance, risk assessment, clinical validity, and genetic counselling will be presented separately in forthcoming AÉTMIS monographs.

This report is a review of existing public literature, studies, materials and other information and documentation (collectively the "source documentation") which are available to CCOHTA. The accuracy of the contents of the source documentation on which this report is based is not

warranted, assured or represented in any way by CCOHTA and CCOHTA does not assume responsibility for the quality, propriety, inaccuracies or reasonableness of any statements, information or conclusions contained in the source documentation.

CCOHTA takes sole responsibility for the final form and content of this report. The statements and conclusions in this report are those of CCOHTA and not of its Panel members or reviewers.

Authorship

All authors participated in planning the project.

Lynda McGahan is a research officer at CCOHTA. She developed the protocol; contributed to designing the literature search strategy; reviewed abstracts and selected articles for inclusion for molecular methods, analytical validity, ethical and psychosocial issues; assessed study quality; abstracted and analyzed data; and prepared and revised drafts and the final report.
Ritsuko Kakuma is a PhD candidate in the Department of Epidemiology and Biostatistics at McGill University. She completed an MA in epidemiology and biostatistics, and the International Master's Program in Health Technology Assessment and Management at McGill University. She is McGill University site coordinator for The Canadian Cochrane Network and Centre, and a reviewer for the Cochrane Collaboration. She is a research consultant for AÉTMIS and CCOHTA. She reviewed abstracts and selected articles for inclusion for molecular methods, analytical validity and psychosocial issues; assessed study quality; abstracted and analyzed data; and participated in writing and revising drafts of this report.

Chuong Ho is a research officer at CCOHTA. He assisted with protocol development; contributed to designing the literature search strategy; reviewed abstracts and selected articles for inclusion in the clinical management section; assessed study quality; abstracted and analyzed data; and participated in writing and revising the clinical management section of this report.

Ken Bassett is a senior medical consultant at the University of British Columbia Centre for Health Services and Policy Research, and an associate professor in the Departments of Family Practice, Pharmacology and Therapeutics; Health Care and Epidemiology; and Ophthalmology. He is also a research consultant for CCOHTA. He reviewed abstracts and selected articles for inclusion in the clinical management section; assessed study quality; abstracted and analyzed data; and participated in writing and revising the clinical management section of this report.

Hussein Z. Noorani is a research officer at CCOHTA. He reviewed abstracts and selected articles for inclusion in the ethical issues section; assessed study quality; abstracted and analyzed data; and prepared and revised the ethics section of this report.

Janet Joyce is the director of Library and Information Services at CCOHTA. She designed and executed the literature search strategies and updates; wrote the methods section and associated appendix on literature searching; and verified and formatted bibliographic references.

Judith Allanson is a professor of pediatrics at the University of Ottawa, and chief of the Department of Genetics at the Children's Hospital of Eastern Ontario. She assisted with protocol development; advised on literature identification; provided clinical expertise; and critically reviewed drafts of this report.

Sherry Taylor is an associate professor in the Department of Pathology and Molecular Medicine at Queen's University, and director of the DNA Diagnostic Laboratory at the Kingston General Hospital. She assisted with protocol development; advised on literature identification; provided molecular genetics expertise; assisted with the molecular genetics section; provided Ontario data; and critically reviewed drafts of this report.

Acknowledgements

Ingeborg Blanquaert is a researcher at AÉTMIS. She assisted with protocol development; contributed to developing preliminary drafts of the report, and facilitated review of preliminary drafts by the AÉTMIS Board.

Lucy Boothroyd is a PhD candidate in the joint Department of Epidemiology, Biostatistics and Occupational Health at McGill University; and a research consultant for AÉTMIS. She assisted with protocol development; and contributed to designing the literature search strategy and developing preliminary drafts of the report.

Beverly Ann Lee was a research consultant for AÉTMIS. She contributed to extracting ethical and psychosocial data.

Chantale Lessard is a PhD candidate in public health at the University of Montréal and was a research consultant for AÉTMIS and CCOHTA. She contributed to abstract selection for clinical management.

Eugenia Palylyk-Colwell is a senior scientific associate at Alberta Blue Cross and a consultant editor for CCOHTA. She edited preliminary drafts of this report.

Julie Tranchemontagne is a researcher at AÉTMIS. She assisted with protocol development; contributed to designing the literature search strategy, selecting abstracts for ethical and psychosocial issues, and developing preliminary drafts.

George Wells is a senior scientist in clinical epidemiology at the Ottawa Health Research Institute; professor in the Departments of Medicine and Epidemiology and Community Medicine; chair of the Department of Epidemiology and Community Medicine at the University of Ottawa; and vice-chair of CCOHTA's Scientific Advisory Panel. He provided scientific advice regarding technical challenges encountered in analyzing the analytical validity data.

Conflicts of Interest

Jacques Simard was the director of the Canadian Institute of Health Research (CIHR)-funded interdisciplinary health research team on breast cancer susceptibility. He was listed as the inventor on *BRCA1* and *BRCA2* patents without financial interest.

REPORT *IN BRIEF*

March 2006

BRCA1 and *BRCA2* Predictive Genetic Testing for Breast and Ovarian Cancers: A Systematic Review of Clinical Evidence

Technology

Tests to detect mutations in *BR*east *CA*ncer susceptibility genes *BRCA1* and *BRCA2*

Condition

Some individuals are more likely to have *BRCA1* and *BRCA2* gene mutations. These mutations have been linked to hereditary breast and ovarian cancers, which account for 5% to 10% of the roughly 24,000 new cases diagnosed annually. Individuals diagnosed with hereditary breast cancer have one or both mutations 84% of the time. The prevalence of *BRCA1/2* mutations is between one in 500 and one in 1,000.

Issue

Genetic testing for these mutations is available in Canada, and can be accessed as a clinical laboratory service or through a research study. There is a need to better understand the benefits and harms that are associated with testing, the available tests and how they compare with each other, the social factors that influence testing, and the psychological and ethical issues that are associated with testing.

Methods and Results

Literature was identified through a defined search strategy and selection criteria. The analytical performance of tests was evaluated from 27 unique studies. Sixty-eight reports with quality measures of psychosocial and ethical issues were identified and synthesized. Eighty-four reports that described the clinical outcomes in prophylactic or therapeutic studies were identified and synthesized to assess the benefit and harm of testing.

Implications for Decision Making

- **Other factors need consideration when choosing *BRCA1/2* testing.** There is no clear evidence to suggest testing will lead to decisions that result in long-term health benefits.
- **Psychological and social implications require consideration.** Knowledge about the association of cancer and genetics is limited in the general population. Test results influence individual risk perception, emotional states, and social issues. Counselling reduces the perceived risk and the associated anxiety, and increases the uptake of testing.
- **There is no compelling evidence that one test performs better than another.** Until better information becomes available, other factors such as test availability, ease of implementation, regulatory considerations, and price should be considered in deciding the method used for testing.
- **Decisions regarding *BRCA1/2* testing need to be revisited.** Scientific data are accumulating rapidly. If the expansion of testing and the creation of best practices are pursued, this report should be updated. Decision makers who adopt this technology should consider the value of gathering information that can contribute to future analyses.

This summary is based on a comprehensive health technology assessment available from CCOHTA's web site (www.ccohta.ca): McGahan L, Kakuma R, Ho C, Bassett K, Noorani HZ, Joyce J, Allanson J, Taylor S. *BRCA1 and BRCA2 predictive genetic testing for breast and ovarian cancers: A systematic review of clinical evidence.*

Canadian Coordinating Office for Health Technology Assessment (CCOHTA)
600-865 Carling Avenue, Ottawa ON Canada K1S 5S8 Tel: 613-226-2553 Fax: 613-226-5392 www.ccohta.ca

EXECUTIVE SUMMARY

The Issue

Breast and ovarian cancers are among the leading causes of cancer-related deaths in Canadian women. A range of mutations in the *BR*east *CA*ncer susceptibility genes *BRCA1* and *BRCA2* have been linked to the development of both cancers. Several molecular techniques are available to analyze *BRCA1* and *BRCA2* for mutations that may predispose individuals to disease.

Objectives

The objectives of the collaborative systematic review are to evaluate the analytical and clinical validity of *BRCA1/2* genetic testing; assess the contribution of molecular testing to genetic counselling and clinical management; and to discuss the ethical and psychosocial issues inherent in *BRCA1/2* testing.

To address these objectives, the following questions are examined in this report. What are the molecular techniques used to identify *BRCA1/2* mutations? What values of analytical validity are associated with these techniques? What social factors influence participation in testing? What psychological and ethical issues are associated with testing? What are the benefits and harms associated with surveillance and preventive methods?

Clinical Review

Methods: Published and grey literature were identified in January 2003 for 1994 and onward (updated July 2004) by searching electronic databases, the Internet, trial registries, guidelines databases, and the web sites of health technology assessment agencies. Efforts were made to access unpublished studies by contacting the commercial developer of the *BRCA1/2* tests and primary researchers. A study was included for review if it met the eligibility criteria established a priori by two independent reviewers. Study quality was assessed and data were extracted regarding molecular methods, analytical validity, psychosocial impact, ethical issues, and clinical management.

Results: The analytical performance of *BRCA1/2* mutation testing, primarily in high risk families and founder populations, was examined. For studies of analytical validity of *BRCA1/2* testing, information on each method was extracted, and calculations of sensitivity and specificity were reported. High variability was found between studies. Although most studies used direct sequence analysis (DSA) as a "gold standard," no two tests used the same index test, thereby precluding comparisons of methods. Clinically relevant mutations may be missed if DSA is used as a primary strategy for detecting *BRCA1/2* mutations. As a result, the most analytically valid molecular technique for *BRCA1/2* testing could not be determined.

The contribution of *BRCA1/2* testing to the clinical management of unaffected carriers and affected carriers was examined. Data regarding the influence of testing on clinical management was limited, partly because of the limited treatment options available. Prophylactic surgery was shown to reduce the risk of breast and ovarian cancers in cohort studies, whereas surveillance strategies or chemoprophylaxis have not been shown to offer significant effects for cancer risk.

Health Services Impact

Studies on psychosocial impact and ethical issues were examined. Counselling informs and has an influence on perceived risk, associated anxiety, and uptake of testing. Knowledge about the association of cancer and genetics is limited, based on the studies selected for psychosocial impact. The positive or negative result of the test has an influence on risk perception, psychological impact (e.g., distress, depression, emotional reactions), and social issues (e.g., communication of results to family members). Ethical considerations include the importance of informed consent (or informed refusal), and privacy and confidentiality concerns (e.g., risk of genetic discrimination from insurers, employers, or family members). Unique ethical implications exist for disclosure or the failure to disclose genetic information by health care providers.

Conclusions

BRCA1/2 genetic testing is an emerging practice. The decision to offer *BRCA1/2* testing is based on short-term cohort studies that suggest prophylactic surgery reduces the risk of breast and ovarian cancers for mutation carriers. There is insufficient evidence to suggest that a positive *BRCA1/2* test result will lead to clinical management decisions that reduce long-term mortality and morbidity. Among the options that could be considered by policy and decision makers are conditional reimbursement of *BRCA1/2* genetic testing for selected indications, and restricted use to specific centres with identified protocols or to particular health care providers while more information is gathered.

In the literature identified for this report, there was insufficient evidence to conclude that a particular molecular technique demonstrated overall superior analytical performance compared with another molecular technique. Other factors should be considered in selecting the method used for testing. Future research should seek to overcome the methodological limitations identified in the studies selected for this report, so that quantitative analyses can be conducted and clear comparisons can be made. This applies not only to fundamental research, but also to the monitoring of clinical and technical practices, and to the follow-up of families undergoing genetic counselling and testing to measure outcomes. Scientific data are accumulating rapidly. If the expansion of testing or consensus guidelines are pursued in the future, an update of this report should be considered.

ABBREVIATIONS

AB-2	anti-*BRCA1* (AB-2) monoclonal antibody
AÉTMIS	Agence d'évaluation des technologies et des modes d'intervention en santé
AGE	allele-specific gene expression
ASCO	American Society of Clinical Oncology
ASO	allele-specific oligonucleotide
BIC	Breast Cancer Information Core Database
BRCA1	*BR*east *CA*ncer gene 1
BRCA2	*BR*east *CA*ncer gene 2
BRCAPRO	A statistical model for assessing the probability that an individual carries a germline deleterious mutation of the *BRCA1* and *BRCA2* genes based on family history of breast and ovarian cancer
BSE	breast self examination
Ca125	a tumour-associated antigen proposed for serologic screening of ovarian cancer
CBE	clinical breast examination
CCOHTA	Canadian Coordinating Office for Health Technology Assessment
CDGE	constant denaturant gel electrophoresis
cDNA	complementary DNA
CELI	endonuclease purified from celery
CI	confidence interval
CGSC	Cancer Genetics Studies Consortium
CLIA	Clinical Laboratory Improvement Act/Amendment
CMP	clinical management program
CSGE	conformational sensitive gel electrophoresis
DDF	dideoxy fingerprinting
del	deletion
DGGE	denaturing gradient gel electrophoresis
DHPLC	denaturing high performance liquid chromatography
DNA	deoxyribonucleic acid
DSA	direct sequence analysis
EMD	enzymatic mutation detection
F-CSGE	fluorescent conformation sensitive gel electrophoresis
F-MD	fluorescent mutation detection
FDR	first-degree relative
FMPA	fluorescent multiplexed-PCR analysis
FS	frame shift
FU	follow-up
GLK-2	a monoclonal antibody
HA	heteroduplex analysis
HADS	Hospital Anxiety and Depression Scale
HBOC	hereditary breast and ovarian cancer
HR	hazard ratio
IES	Impact of Events Scale
IHC	immunohistochemistry
LOH	loss of heterozygosity

MLPA	multiplex ligation-dependent probe amplification
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MS-PCR	multiplex mutagenically separated PCR
NA	not applicable
NPV	negative predictive value
NR	not reported
OCGN	Ontario Cancer Genetics Network
OR	odds ratio
PCR	polymerase chain reaction
PPV	positive predictive value
PTT	protein truncation test
REF-SSCP	restriction endonuclease fingerprinting single strand polymorphism analysis
RNA	ribonucleic acid
RR	relative risk
SD	standard deviation
SCCP	single-strand conformational polymorphism analysis
SSCA	single-strand conformation analysis
SSCP	single-strand conformation polymorphism
SSCP-HA	single-strand conformation polymorphism combined with heteroduplex analysis
TDGS	two-dimensional gene scanning
TVU	transvaginal ultrasound

GLOSSARY

Alleles: alternative forms of a gene differing in their nucleotide sequence.

Amino acids: aminocarboxylic acids form the building blocks of protein and peptides for which DNA carries the genetic code.

Amplimer: product of gene amplification where specific DNA sequences are replicated.

Analytical sensitivity of molecular genetic tests: proportion of individuals with the genotype of interest (i.e., the mutations detectable by the test) for which test will be positive; ability of test to detect the mutations that it was designed to detect.

Analytical specificity of molecular genetic tests: proportion of individuals who do not have the genotype of interest (i.e., the mutations detectable by the test) for which test will be negative; ability of test to detect only the mutations that it was designed to detect.

Autosome: any of the 22 pairs of chromosomes other than sex chromosomes.

cDNA: complementary or copy DNA, which is synthetic DNA transcribed from a specific RNA through the action of reverse transcriptase.

Chromosome: genetic material, contained in cell nucleus, consisting of DNA; humans have 46 chromosomes, of which 22 pairs are autosomes and two are sex chromosomes.

Clinical sensitivity of molecular genetic tests: proportion of individuals with the phenotype of the disease (or who will develop the phenotype) for which test will be positive; proportion of individuals with the phenotype of the disease (or who will develop this phenotype) for which mutations detectable by the test are present (i.e., this represents upper limit of clinical sensitivity when analytical sensitivity is 100%).

Clinical specificity of molecular genetic tests: proportion of individuals who do not have the phenotype of the disease (and who will not develop the phenotype) for which test will be negative; probability that test will be negative in individuals who do not develop the disease.

Codon: triplet of three nucleotides or bases in a DNA or RNA molecule that specify one amino acid.

Deletion: loss of one or more consecutive base pairs without a break in the continuity of the DNA molecule.

Deoxyribonucleic acid (DNA): genetic material contained in the chromosomes of cell nucleus and mitochondria; DNA consists of two chains made up of nucleotides that are coiled into a double helix.

Dominant: an allele is dominant when it is expressed in the heterozygous state (i.e., when it is present on one of the two homologous chromosomes); carrier of dominant disorder inherits

mutation from one parent, unless it is a new mutation; each child of an affected parent may inherit a normal or an abnormal gene; probability that a child will be affected is one in two.

Exon: gene sequence where transcript persists in final messenger RNA and is translated into a polypeptide chain; each gene contains several noncontiguous exons that are separated by introns; exons are protein coding DNA sequence of a gene.

First-degree relatives*:* parents, siblings, and children of an individual.

Gene: physical and functional unit of heredity, comprised of a sequence of nucleotides situated at a specific locus on a given chromosome that performs a specific function.

Genetic linkage: co-segregation of two or more alleles over generations because of the physical proximity of their loci on the genome.

Genetic marker: variation in the DNA sequence that creates different alleles at a given locus and can be used to identify this locus.

Genetics: scientific study of heredity; the structure, regulation, expression, transmission, and frequency of genes; and the pathologies associated with structural defects in genes.

Genetic test: test for detecting mutation, defective gene, abnormal protein, chromosomal abnormality or presence of a genetic marker near or in a gene.

Genotype: genetic makeup of an individual, as contrasted with his or her phenotype.

Haplotype: group of alleles from closely linked loci that are usually inherited as a unit.

Heterozygosity: genotypic situation in which two homologous loci in a given chromosome pair each carry a different allele.

Homozygosity: presence of identical alleles on both chromosomes in a given pair; this term may apply to the genotype of individuals who have inherited a double dose of an abnormal allele, whether the mutated version is the same or different on each chromosome.

Index case: affected family member who first draws attention to a pedigree.

Insertion: insertion of one or more consecutive base pairs without a break in the continuity of the DNA molecule.

Intron: DNA sequence transcribed and subsequently eliminated by splicing during RNA processing.

Justice: obligation of fairness in the distribution of benefits and risks.[1]

Kilobase (kb): unit of length for nucleic acids; in the case of DNA: 1,000 base pairs (bp), whereas in the case of RNA: 1,000 bases.

Locus: position of a DNA segment on a chromosome; defined by its information content (gene) or its sequence, whether the latter is polymorphic or not.

Lod score: abbreviation for logarithm of the odds; measure of odds ratio obtained by dividing likelihood that two loci are linked at a specific recombination fraction by likelihood that they are unlinked; acceptable evidence of linkage.[2]

Mendelian trait or disease: characteristic trait or disease due to one gene transmitted by a simple pattern of inheritance (e.g., autosomal dominant, autosomal recessive, or X-linked).

Mutation: **c**hange in DNA sequence that can result in pathological manifestations; if change involves one base, it is a point mutation; if a mutation occurs in a germ cell, it can be passed onto subsequent generations; a gene that has undergone a mutation is called a mutant.

Negative predictive value: probability that individuals with negative results will not get the disease.

Nucleotides: basic units of DNA and RNA structure, consisting of a phosphorylated sugar (i.e., ribose or deoxyribose) linked to a base. In DNA, there are two purine bases: [adenine (A) and guanine (G)] and two pyrimidine bases [cytosine (C) and thymine (T); each strand of DNA consists of a sequence of nucleotide base pairs that pair in a complementary manner with one other to form DNA double helix (i.e., adenosine with thymine and guanine with cytosine); in RNA, thymine is replaced by uracil (U).

Penetrance: probability of a gene or genetic trait being expressed; percentage of individuals with a specific genotype in whom the phenotype associated with the genotype is expressed; alternatively, it is the cumulative risk of the disease (i.e., up to a specific age or over a lifetime), given a carrier genotype; with "complete" penetrance, gene or genes for a trait are expressed in all the population who have genes; "with incomplete" penetrance, trait is expressed in part of the population.

Phenotype: outward manifestation of the makeup of genome in the form of a morphological trait, clinical syndrome or physiological characteristic; for example, a qualitative or quantitative variation in the final product expressed by a gene (e.g., protein or metabolites).

Point mutation: single nucleotide base pair change in DNA.

Polymerase chain reaction (PCR): selective amplification of sequence of double-stranded DNA carried out in vitro by iterative extension of two primers, one on either side of region of interest, using a DNA polymerase; amplification is effected by repeated cycles of denaturation, annealing, and extension, which result in the logarithmic replication of each strand.

Polymorphism: occurrence of two or more alternative genotypes in a population that leads to indistinguishable phenotypes, each with appreciable frequency; a locus is arbitrarily considered to be polymorphic if the rare allele has a frequency of at least 1% in the general population (i.e., heterozygote frequency of at least 2%).

Positive predictive value: probability that individuals with positive test results will get disease.

Prevalence: ratio of number of individuals with a disease divided by number of individuals in a given population at a given point in time.

Primer: DNA sequence of approximately 15 to 30 nucleotides (i.e., oligonucleotide) that serves as an anchor and starting point for the replication of a specific DNA sequence by DNA polymerase during PCR.

Proband: family member through whom the family is ascertained; if affected, this individual may be called the index case.

Probe: nucleic acid sequence that is homologous to a DNA or an RNA sequence, to which it anneals in a stable and specific manner by re-association between complementary nucleotides; probes are usually at least 15 nucleotides in length.

Prognostic factor: characteristic associated strongly enough with a condition's outcome to predict accurately the eventual development of those outcomes.

Restriction enzymes: Bacterial endonucleases that specifically cleave two DNA strands at a particular DNA sequence (i.e., four to eight nucleotides) referred to as a restriction site; mutation in this sequence will alter the pattern of cleavage by that restriction enzyme, and generate a restriction fragment length polymorphism.

Restriction site: double-stranded DNA sequence specifically recognized and cleaved by a given restriction enzyme.

Ribonucleic acid (RNA): nucleic acid formed on a DNA template that contains ribose sugar as opposed to deoxyribose sugar found in DNA.

Second-degree relatives: grandparents, grandchildren, aunts, uncles, nieces, nephews, half-sisters, and half-brothers.

Splice site: region at interface of exon and intron where splicing out of introns and splicing together of exons occurs in generation of mature mRNA from primary transcript.

Third-degree relatives: great-grandparents, great-grandchildren, great-aunts, great-uncles, first cousins, grand-nephews, and grand-nieces.

5' untranslated region: region of mRNA molecule that precedes initiation of translation codon and thus does not encode a polypeptide sequence.

3' untranslated region: region of mRNA molecule that follows the termination of translation codon and thus does not encode a polypeptide sequence; occasionally, mutations in this region, such as polyadenylation site mutations, affect stability of the mRNA molecule, and can lead to functional deficiency or pseudodeficiency of protein even though coding sequence is normal.

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1 INTRODUCTION

1.1 Background

Breast cancer is the second leading cause of death and the most frequently diagnosed cancer among Canadian women. Each year, approximately 21,200 new cases of breast cancer are diagnosed, and 5,200 women die from this disease.[3] The lifetime risk of a woman developing breast cancer is 11.3%, or one in 8.8.[3] A woman's short-term risk of developing breast cancer in a 10-year time period varies with age in years: 0.3% (30 to 39) 1.3% (40 to 49), 2.5% (50 to 59), 3.1% (60 to 69), 3.2% (70 to 79), and 2.6% (80 to 89).[3] One in 10 women develop breast cancer by the age of 80.[4] A woman's risk of death from breast cancer also varies with age in years: one in 2,873 (34), one in 136 (54), one in 39 (75), and one in 26 (85).[5]

Breast cancer occurs as a result of abnormal proliferation of breast cells leading to tumour formation, which may then metastasize. Clinical symptoms can include changes to the breast such as a lump, thickening, or skin change. Non-palpable cancers are often only detected by mammography. Once diagnosed, breast cancer is staged from earliest (0) to advanced (IV), as indicated by tumour size, degree of invasion, and presence or absence of metastases. The histological examination of biopsy tissue is required for tumour grading, which ranges from one to four. The stage of cancer at the time of diagnosis is the most important factor in determining a woman's chance of survival.[6]

Surveillance for breast cancer involves clinical breast examination (CBE) and mammography. The examiner's knowledge and experience are important factors when performing CBE, especially for detecting small breast lumps. The Canadian Task Force on Preventive Health Care recommends screening CBE and mammography for women aged 50 to 69 years, and suggests that there is contradictory evidence regarding the benefits and risks of both. The task force advises that there is fair evidence to exclude asymptomatic women aged 40 to 49 years from these surveillance techniques during a periodic health examination.[7]

Ovarian cancer is the fifth leading cause of death due to cancer, and is the sixth most common cause of cancer among Canadian women.[3] Each year, approximately 2,500 new cases of ovarian cancer are diagnosed, and 1,500 women die from this disease. Epithelial tumours account for 80% to 90% of all ovarian cancers. The cancer typically spreads by direct growth or the sloughing off and implantation of cancerous cells in the peritoneal cavity. Although staging for ovarian cancer ranges from I to IV, most patients (70%) present with advanced disease (stage III or IV). Symptoms may include abdominal discomfort and bloating, followed by vaginal bleeding, and gastrointestinal and urinary tract symptoms.

Clinical abdominal and pelvic examinations are used to screen women for ovarian cancer. The detection techniques for women with suspected ovarian cancer include transvaginal ultrasound (TVU), serum Ca125 determination, paracentesis, magnetic resonance imaging (MRI), laparoscopy and laparotomy, and examination of biopsied tissue.[8] A review of evidence does not

support periodic testing for ovarian cancer in asymptomatic pre- and post-menopausal women in the general population, or in women who do not have one or more first-degree relatives with ovarian cancer.[9]

A proportion of hereditary breast and ovarian cancers can be attributed to the *BR*east *CA*ncer susceptibility genes, *BRCA1* and *BRCA2*. The mode of inheritance of a *BRCA1* or *BRCA2* mutation is autosomal dominant. The probability that *BRCA1/2* mutation carriers will transmit the affected copy of the gene to their offspring is one in two (50%). The *BRCA1/2* genes behave like tumour suppressor genes (i.e., they act as a gatekeeper or a brake to stop cells from multiplying). If one copy of a tumour suppressor gene is altered, a subsequent alteration or mutation to the other copy may result in uncontrolled cell growth or cancer. For breast cancer to develop, the second *BRCA* gene copy must also be altered.[10] There is evidence to suggest that *BRCA1* and *BRCA2* are involved in the DNA repair process and act to stabilize the integrity of the genome (i.e., they act as caretakers that fix DNA; or as the automobile mechanic).[11,12] When stability genes are inactivated, mutations in oncogenes and tumour suppressor genes affect cell growth. The loss of DNA repair function is assumed to lead to the accumulation of additional mutations, and ultimately to carcinogenesis. In the automobile analogy, a defective stability gene is akin to an inept mechanic.[12]

The contribution of *BRCA1* and *BRCA2* to inherited breast cancer was assessed by linkage and mutation analysis of 237 families, each with at least four cases of breast cancer, collected by the Breast Cancer Linkage Consortium.[13] Disease was linked to *BRCA1* in 52% of families, to *BRCA2* in 32% of families, and to neither gene in 16% (95% CI 6% to 28%).[13] The estimated cumulative risk of breast cancer reached 28% (95% CI 9% to 44%) by age 50 and 84% (95% CI 43% to 95%) by age 70. Ovarian cancer risks were 0.4% (95% CI 0% to 1%) by age 50 and 27% (95% CI 0% to 47%) by 70 years.[13] Studies suggest that women whose breast cancer occurred before age 50 had a one in four chance of carrying a *BRCA1/2* mutation if they had any relative (first-, second-, or third-degree) who also developed the disease before age 50.[14] The likelihood of finding a *BRCA1/2* mutation in a woman with breast cancer before age 50, and one relative with ovarian cancer at any age was 35%.[14]

It is reported that approximately 5% to 10% of all breast and ovarian cancers are hereditary.[15] Hereditary breast cancer is clinically distinct from sporadic cancer, in that it occurs at an early age, more often affects both breasts, and is associated with other tumours.[16] Individuals who are affected can be documented over several generations in families with an inherited predisposition to the disease. Factors that make presence of a mutation more likely for inherited breast or ovarian cancer include:

- multiple cases of breast cancer or ovarian cancer
- diagnosis of breast cancer at less than 35 years of age
- family member diagnosed with both breast and ovarian cancer
- breast or ovarian cancer in Jewish families
- family member with primary cancer occurring in both breasts
- family member diagnosed with invasive serous ovarian cancer
- presence of male breast cancer in the family
- family member with an identified *BRCA1* or *BRCA2* mutation
- presence of other associated cancers or conditions suggestive of an inherited cancer syndrome.[17]

Genetic testing is offered to families that meet set criteria for testing and when the risk of carrying a mutation for the individual being tested is greater than 10%.[18] Samples for testing must be accompanied by a three-generation pedigree indicating which of the affected individuals have had their cancer diagnosis confirmed by pathology review.[18]

The following criteria are considered in determining who should undergo testing in individuals affected with breast or ovarian cancer:

At least one case of cancer occurs as follows:

- Jewish ancestry and breast cancer occurring at less than 50 years of age, or ovarian cancer at any age (ethnic-specific testing);
- Breast cancer, less than 35 years of age;
- Male breast cancer (*BRCA2* mutation testing);
- Invasive serous ovarian cancer at any age.

At least two cases of cancer occur on the same side of the family as follows:

- Breast cancer occurring at less than 50 years of age, and a first or second-degree relative with ovarian cancer or male breast cancer;
- Breast and ovarian cancer in the same individual, or bilateral breast cancer with the first tumour occurring at less than 50 years of age;
- Two cases of breast cancer, both occurring at less than 50 years of age, in first or second-degree relatives;
- Two cases of ovarian cancer, any age, in first or second-degree relatives;
- Jewish ancestry and breast cancer occurring at any age, and any family history of breast or ovarian cancer (ethnic-specific mutations, unless other criteria are met).

At least three cases of cancer on the same side of the family:

- Three or more cases of breast or ovarian cancer at any age, in a pattern suggestive of an inherited form of breast or ovarian cancer.[18]

Unaffected individuals undergo testing only when affected individuals are unavailable (deceased), based on the following criteria:

- Relative of an individual with a known *BRCA1/2* mutation (family specific mutation testing)
- Jewish ancestry and first or second-degree relative of an individual with:
    a. Breast cancer occurring at less than 50 years of age; or
    b. Ovarian cancer at any age; or
    c. Male breast cancer; or
    d. Breast cancer occurring at any age, with a positive family history of breast or ovarian cancer (ethnic-specific mutation testing, unless other criteria are met).
- In exceptional cases, testing may be offered to a first-degree relative of an affected individual who has breast or ovarian cancer, and who also has a pedigree strongly suggestive of hereditary breast and/or ovarian cancer.[18]

All common cancers show familial clustering in which the disease is two- to four-fold more common among first-degree relatives of affected persons.[19,20] Twin studies suggest that most of the familial aggregation results from inherited susceptibility rather than lifestyle or

environmental factors.[19-21] This is accounted for in part by specific familial cancer syndromes in which variants of single genes confer a high risk of disease.[21] During the past decade, the discovery of such gene variants has provided insight into aspects of carcinogenesis.[22] *BRCA1* and *BRCA2* genes account for approximately 20% of the heritability of breast cancer, while other rare alleles, such as TP53, ATM and PTEN, account for less than 5%. The number and properties of the genetic variants accounting for the remaining 75% of heritability are unknown.[19] Current data are consistent with a polygenic model involving many genetic variants, each conferring a slight to moderate increase in risk.[20,21] Based on this model, it is estimated that 12% of women have a risk of breast cancer of at least 10% by age 70.[20] This subpopulation accounts for half of the total number of breast cancer cases diagnosed in the general population. By contrast, 50% of women are estimated to have a breast cancer risk of 3% or lower by age 70 and this subpopulation accounts for 12% of all breast cancers.[20] As a result, 88% of all breast cancer cases will be diagnosed in half of the general population of women.[20]

The penetrance of cancer-predisposing mutations is the likelihood of cancer occurring when a mutation is present. For *BRCA1*/*2* mutation carriers, the penetrance for breast and ovarian cancer is incomplete and not all carriers will be affected by cancer.[23] Penetrance is also variable, as differences have been noted in studies of multiple families with identical cancer-predisposing mutations in defined ethnic populations.[16] Initial studies on the penetrance of *BRCA1* mutations involved families with four or more members affected with breast or ovarian cancer at an early age. The cancer risks estimated in these families are high and may over-estimate the risk in all families with *BRCA1* mutations. In *BRCA1* mutation carriers, the lifetime risk of developing breast or ovarian cancer was as high as 85% and 42% to 63% respectively.[16,23,24] In *BRCA2* carriers, the lifetime risk of developing breast or ovarian cancer was as high as 86% and 27% respectively.[15,16]

Cancer risk in carriers has been estimated from less selected families (i.e., more generalized population) and population-based studies.[25] According to a combined analysis of 22 studies involving cases unselected for family history (i.e., not selected for study solely on the basis of family history), by age 70, the average cumulative risk of breast cancer for *BRCA1* mutation carriers was 65%, while that for ovarian cancer was 39%.[26] The corresponding cumulative risks for breast and ovarian cancers among *BRCA2* mutation carriers were 45% and 11% respectively.[26] The relative risks of breast cancer declined significantly with age for *BRCA1* mutation carriers, but not for *BRCA2* mutation carriers.[26]

The prevalence of a mutation may be high as a result of a founder effect. This occurs when a common mutation in a well-defined population group is traceable to a common ancestor. A study of Ashkenazi Jewish families found that individuals with 185delAG or 5382insC mutations in *BRCA1* or 6174delT mutations in *BRCA2,* had a 56% risk of breast cancer and a 16% risk of ovarian cancer by 70 years of age.[15] While most 185delAG carrier families are Ashkenazi, the mutation has been reported in other groups including three United Kingdom families and six families of Spanish or Latin American ancestry.[19] The 5382insC mutation is more widespread, being common in Poland, Russia, and most European countries.[19]

Similarly, a founder effect was observed with the 999del5 mutation in *BRCA2* in Iceland.[16] A study of breast cancer patients, unselected for family history of breast cancer, showed that 56 (10%) of 541 women and 13 (38%) of 34 men carried the 999del5 mutation.[27] Population-based studies showed that Icelandic women with the 999del5 mutation had a 17% risk of breast cancer by 50 years of age and a 37% risk of breast cancer by 70 years of age.[16,27]

A founder effect was also observed in the French Canadian population.[28] While the *BRCA1* founder mutation R1443X (arginine, R is changed to a stop codon X) was probably introduced into the Québec population by a founder couple,[29] the 8765delAG mutation in *BRCA2* was likely introduced to the population more than once by two founders, or there was one introduction followed by a recombination event, which led to two haplotypes.[30] While there is no evidence supporting the existence of any deleterious *BRCA1/2* recurrent genomic rearrangements in the French Canadian population based on Southern blot or multiplex ligation probe amplification analyses, eight additional mutations have been identified by target sequencing.[31] The proportion of *BRCA1/2* positive families among those showing in the first- or second-degree relatives of the index case, a history of three, four or five, and six breast cancer cases was 13.5%, 16%, and 53% respectively.[31] A mutation was found in 47% and 53% of families with one or two ovarian cancer cases respectively and 58% of families with at least one case of male breast cancer.[31] Five common mutations, 444C>T, and 2953delGTAinsC (in *BRCA1*) and 8765delAG, 6085G>T, and 3398delAAAAG (in *BRCA2*), account for approximately 84% of all mutation-positive families of French Canadian descent.[32]

The Interdisciplinary Health Research International Team on Breast Cancer Susceptibility (INHERIT BRCAs) was established in 2001 as part of the Interdisciplinary Health Research Teams program created by the Canadian Institutes of Health Research. One of seven projects undertaken by the INHERIT BRCAs team was to identify mutations in English-Canadians in Alberta.[33] While no common founder mutations were found, 118 different *BRCA1* mutations and 140 different *BRCA2* mutations were identified in the Alberta population. Of the mutations identified, 66 *BRCA1* mutations and 44 *BRCA2* mutations are pathogenic; 30 *BRCA1* mutations and 42 *BRCA2* mutations are missense mutations. Missense mutations are problematic in diagnostic interpretation, because it is not obvious which are pathogenic and which are benign polymorphisms. An additional 20 intronic *BRCA1* mutations and 29 intronic *BRCA2* mutations are undergoing further evaluation. While this probably identifies most *BRCA1/2* mutations in Albertans, approximately 75% of high risk families do not have a mutation in either gene, yet they display pedigree-level characteristics suggestive of a high risk autosomal dominant mutation. Further work remains to identify new genes and the effects of modifier genes in known *BRCA1/2* pedigrees.[33]

Evidence suggests that a dozen other ethnic groups have higher prevalence of specific *BRCA* mutations, up to eight times that of the general population.[6] The prevalence of cancer-predisposing *BRCA* mutations in the general population is estimated to be between one in 500 to one in 1,000.[15] It may be possible to test for specific founder mutations in some countries. The genetic variation in countries with ethnically mixed populations, such as the United Kingdom, Canada, and the United States, is wide.[22] Knowledge of the ethnic background of an individual can direct mutation testing.

Testing for *BRCA1/2* mutation status among Canadians and the subsequent assessment of an individual's risk of developing breast or ovarian cancer brings up complex issues that range from the technical to the psychosocial. AÉTMIS and CCOHTA collaborated to systematically examine the available evidence regarding the analytical and clinical validity of available molecular technologies and review inherent issues associated with testing. The results pertaining to molecular methods, analytical validity, psychosocial impact, ethical implications, and clinical management are presented in this report. The results related to prevalence, penetrance, risk assessment, clinical validity, and genetic counselling will be presented in forthcoming AÉTMIS monographs.

1.2 Overview of the Technology

In 1990, a research team at the University of California at Berkeley identified a common variation in chromosome 17 while studying repeated cases of breast cancer over generations. Subsequently, the location of *BRCA1* on the long arm (q) of chromosome 17 (17q12-21) was identified in 1994. *BRCA1* is one of the largest genes described to date with 22 coding regions dispersed over 100,000 base pairs of DNA that encode a *BRCA1* protein of 1863 amino acids.[34] More than 600 variants of *BRCA1* have been identified, but not all are associated with an increased risk of breast cancer.[15,16]

In assessing the clinical significance of a variant, the following are considered: mutation type, location in the gene, presence or absence of the variant in a control population, co-segregation or lack thereof of the variant and disease in families, co-occurrence with a deleterious mutation, type of amino acid change, conservation of the amino acid across species, and biochemical or functional analysis.[35] For *BRCA1*, frame-shift and nonsense mutations that begin before or at codon 1853 are classified as deleterious.[35] Mutations occurring at cysteines are classified as deleterious and those occurring at the histadine are classified as suspected deleterious.[35] Genetic variants of uncertain significance include missense mutations, mutations that occur in intronic regions whose clinical significance remains to be determined, and frame-shift and nonsense mutations that occur after codon 1853.[35] Genetic variants classified as polymorphisms include neutral variants for which available evidence indicates that variant is unlikely to contribute to cancer risk.[35] At the outset of clinical testing, 9% of patients tested received a *BRCA1* uncertain variant result. According to data from population studies, 4% of patients now receive such test results.[35]

After the identification of *BRCA1*, it became clear that less than 50% of breast-only cancer families showed linkage to this locus. Linkage of many non-*BRCA1* linked families to another locus on 13q12-13 was then established, resulting in the identification of *BRCA2* in 1995. *BRCA2* is large, with 26 coding exons distributed over approximately 70,000 base pairs of DNA that encode the *BRCA2* protein.[36] Similar to *BRCA1*, hundreds of *BRCA2* variants have been identified, although a proportion are of unknown clinical significance.[16]

While *BRCA2* unclassified variants including missense mutations and in-frame deletions and insertions account for 43% of all identified *BRCA2* sequence alterations, the influence of *BRCA2* unclassified variants on *BRCA2* function and cancer risk has not been defined.[37] Several factors are considered in classifying *BRCA2* variants as deleterious or neutral, including the

cosegregation of variants with cancer in families, the co-occurrence of variants with other known deleterious mutations, the sequence conservation of relevant amino acids, and functional assays. In combining the results of functional assays with individual likelihood models and overall odds of causality, it is apparent that the D2723H unclassified variant is a disease-causing mutation in *BRCA2* and is of relevance to 24 families that carry the mutation.[37] Other *BRCA2* unclassified variants may be classified similarly, improving the risk classification for carriers.[37]

BRCA1 and *BRCA2* mutation analysis is a method of genetic testing that evaluates individual risk status for breast or ovarian cancer. It may be considered after studying family history, providing pre-test genetic counselling and education, and after obtaining informed consent. Genetic testing predicts the likelihood of developing cancer and does not mean that the cancer will or will not occur. As a result, appropriate genetic counselling for the individual is imperative.

Genetic counselling provides individuals and their family members with information about the nature, risks, and benefits of genetic testing, the meaning of test results, and clinical management options. Psychosocial support of the individual is needed regardless of positive or negative status. During a counselling session, an individual should be advised if his or her family history is indicative of the presence of a *BRCA1/2* mutation. The individual should receive sufficient, clearly articulated information, so that he or she can provide informed consent or informed refusal of genetic testing. The complexities associated with conveying genetic risk factor information requires that the results be communicated in person.

In Canada, clinical geneticists in each province have developed clinical criteria, based on family or personal history of cancer, which are used to establish an individual's eligibility for *BRCA1/2* mutation screening. In Ontario, individuals who are eligible for mutation screening would have an a priori risk of approximately 10% of being a mutation carrier. The Ontario Physicians' Guide recommends genetic testing when risk factors are present.[17] In Ontario, the ordering physician must ensure that individuals considering testing receive appropriate genetic assessment. Samples for testing must be accompanied by a three-generation pedigree indicating which of the affected individuals have had their cancer diagnosis confirmed by pathology review. The American Society of Clinical Oncology (ASCO) recommends that cancer predisposition testing be offered when the individual has a strong family history of cancer, a family history of very early onset of cancer, when previous test results can be adequately interpreted, when the results will influence medical management, or if clinically justified.[38] To assist with the assessment of breast cancer risk, mathematical models have been developed as tools. There is no unanimously accepted tool for this purpose.[16,39,40]

The susceptibility model (BOADICEA, Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm) takes into account the simultaneous effects of *BRCA1*, *BRCA2* and other genes as a joint multiplicative effect of multiple genes of small effect on breast cancer risk.[41] There is evidence for a modifying effect of other genes on the risks of breast cancer in *BRCA1/2* mutation carriers. Using BOADICEA, breast cancer risk by age 70 years was estimated to be 35.3% for *BRCA1* mutation carriers and 50.3% for *BRCA2* mutation carriers; with corresponding ovarian cancer risks of 25.9% for *BRCA1*mutation carriers and 9.1% for *BRCA2* mutation carriers.[42]

BRCA1 and *BRCA2* mutation testing can be accessed as a clinical service or through ongoing research. The distinction between them involves the source of funding, enrollment of patients in a research protocol, and whether test results are communicated to patients. Clinical tests are those in which specimens are examined and results are reported to the provider for diagnostic, preventive, or treatment purposes. In contrast, research tests are those in which specimens are examined for the purpose of better understanding a condition. In Canada, laboratories performing research testing are obligated to report results of medical significance (positive or negative) to research subjects. In Ontario, the results of research-based *BRCA1/2* testing (as part of the Ontario Cancer Genetic Network or OCGN) are reported to the genetic centres. In Alberta, the results of clinical *BRCA1/2* testing are reported to clinical subjects. Clinical *BRCA* testing in Canada is available through provincially funded molecular genetics laboratories that work with genetics clinics, which also provide risk assessment and pre- and post-test genetic counselling. In all areas of Canada, an accreditation system exists for laboratories and is mandated by provincial governments. In the United States, laboratories conducting clinical genetic testing must be approved under the Clinical Laboratory Improvement Act/Amendment (CLIA) whereas research laboratories are not subject to this legislation.

The interpretation of genetic test results is a complex task. A situation may arise where a positive test result for variants of uncertain clinical significance is obtained. This translates into a dilemma for clinical management. Options are available to assess the clinical significance of the mutation. These include family studies to identify whether the mutation segregates with affected family members, allele frequency analysis to determine if the allele has a higher frequency in cancer patients as opposed to the general population, or protein function assays to measure the effect of the mutation on protein function.[16] Studies that combine the results of functional assays with data from the analysis of cosegregation of unclassified *BRCA1/2* variants with cancer, co-occurrence of unclassified variants with other deleterious mutations, and interspecies sequence variation help to distinguish between disease predisposing and neutral unclassified variants.[37,43] Information about common single nucleotide polymorphisms has also been applied to help determine the clinical significance of genetic variants.[35] A multifactorial likelihood-ratio model has been developed integrating direct epidemiological observations, including cosegregation with disease in families, and degree of family history of the disease, or indirect measures on evolutionarily based comparative genomics evidence from functional assays. This integrated approach may result in a more reliable classification of unclassified variants and improve the clinical utility of *BRCA1/2* genetic tests.[44]

There are also publicly available computer programs (SIFT[45] and POLYPHEN[46]) which can aid in the determination of whether a mutation is tolerated by a protein. It is important to consider the evolutionary conservation of gene sequences among many animal species, because mutations are less likely to be tolerated in areas of a gene that show few differences among species. In contrast, negative test results must be interpreted with caution in individuals with a positive family history of the disease. In the case of breast and ovarian cancer, if an affected individual has no identifiable mutation or is unavailable for testing, all negative *BRCA1/2* test results in other family members should be considered uninformative rather than negative (i.e., this may reflect false negatives). If an individual tests negative for a *BRCA1/2* mutation that has been identified in an affected family member, it is considered to be a true negative result. It is important to consider that a true negative result does not reduce the cancer risk for an individual below that of the general population.[15,16,23]

Once the results of genetic testing are obtained, clinical management of the cancer risk must be addressed. In Canada, consensus approaches have been developed by the OCGN and its committees (e.g., Clinical Research Committee, Genetics Committee, and Clinical Practice Resource Group) and through the contributions of interested practitioners. Consensus approaches are intended to guide decisions regarding the management of breast and ovarian cancer risk in patients who are carriers of mutations, patients who are members of families in whom there appears to be a hereditary factor, but who have unknown mutation status and patients with a family history that is not suggestive of a hereditary factor.[18] The agreed-upon approaches are not evidence-based and are intended to be used as a guide for subsequent intervention in these populations with a view to generating data that will facilitate future evidence-based guideline development.[18]

In the US, the Cancer Genetics Studies Consortium (CGSC), organized by the National Human Genome Research Institute, recommends that women with confirmed *BRCA1* or *BRCA2* cancer-predisposing mutations undergo cancer surveillance in an effort to reduce morbidity and mortality.[47] Breast cancer surveillance techniques recommended by the CGSC include annual mammography starting at age 25 to 35 years. The age at which regular screening is initiated should be determined by the preferences of the individual, the adequacy of mammography imaging, and the feasibility of breast examination. Evidence suggests that surveillance and integrated programs of mammography, clinical breast examination, and ultrasound are effective in detecting breast cancers in women with a family history of breast cancer or *BRCA1/2* mutations.[4] There is controversy regarding the risk of radiation-induced breast cancer. Theoretically, early and frequent radiation exposure through mammography could promote cancer development in mutation carriers.[48] Reviews suggest that the risk of radiation-induced breast cancer is small compared to the benefits of breast cancer detection, and that the margin of benefit over risk is sufficient in women with a family history of breast cancer.[4] Most evidence indicates that women with *BRCA1*-associated tumours have a worse outcome than women with sporadic breast cancers, but for those with *BRCA2*-associated tumours mutations, the situation is less clear.[22] The effect of treatment has rarely been considered, and could be influenced by chemotherapy. One study found that carriers of *BRCA1/2* mutations are more likely to show a complete response to preoperative chemotherapy than non-carriers.[22]

A committee of experts in France recommend MRI as an option for breast cancer screening, while screening for ovarian cancer was not an attractive option. The French position favours prophylactic surgeries to improve quality of life with expected benefit, despite methodological flaws, low power, and short follow-up of surveys.[49] Prophylactic mastectomy and cophorectomy involves the removal of seemingly healthy breasts and ovaries to prevent future cancer.

For ovarian cancer, the CGSC recommends the use of ultrasound with colour flow Doppler and testing for Ca125 annually or semi-annually, also beginning at age 25 to 35 years. The most effective detection modality available to date is TVU, ideally with the addition of colour flow Doppler and a morphologic index.[47] The current recommendations for surveillance are based on expert opinion only.[47] In addition to surveillance, preventive measures, such as prophylactic mastectomy or oophorectomy, may be considered by the individual.

BRCA1/2 genetic testing is associated with unique ethical and psychosocial issues. Key ethical implications include informed consent, privacy, confidentiality, and familial implications. A range of psychological effects may be experienced, regardless of the result of a test (i.e., positive or negative). Furthermore, ethnic and gender issues exist, as does the risk of genetic discrimination faced by mutation carriers seeking insurance, employment, or adoption.[50]

1.2.1 Molecular methods and protocols for *BRCA1/2* mutation testing

There are molecular biological techniques that are available for laboratory detection of *BRCA1* and *BRCA2* mutations. As this area is developing rapidly, the choice of technique used may be influenced by the availability of laboratory resources and biological material, expected nature of the mutation, size of the gene, sensitivity required, and origin of the sample (from an index case or relatives). The advantages and disadvantages of each technique, and relevant clinical issues must be considered before a test is implemented. An example is the difficulty of developing efficient strategies for screening large genes, for which the characterization of the protein and knowledge of its biological function is incomplete. To meet one of the objectives of this review, information on available molecular methods was obtained as part of the search strategy for subject area II (analytical validity) (Appendix 1).

Genetic testing for *BRCA1/2* mutations is most often performed in an effort to reduce the risk of cancer in individuals who, on the basis of family history, are at increased risk for breast or ovarian cancer. A spectrum of mutations has been observed throughout the *BRCA1/2* genes that act to alter their structure or protein products (e.g., missense, nonsense, frameshift, large deletions, duplications, and inversions), and this complicates the search for mutations in these two genes. In families where a mutation has been identified, additional family members may be tested for the identified mutation using techniques that are best suited to identifying that particular mutation type. For at-risk affected individuals where no mutation has been identified in their family, *BRCA1/2* mutation screening in Canadian clinical molecular genetics laboratories is a two-step process. First, the genes are screened for the presence of mutations using a cost-effective technique such as denaturing high performance liquid chromatography (DHPLC) or protein truncation test (PTT).[51,52] Second, if potential mutations are detected during the initial screen, the presence and identity of the mutation are confirmed by direct sequence analysis (DSA). The prevalence of a mutation type may be a factor when choosing a method to detect a mutation in an individual.[53] In some ethnic groups, one or a few mutations of the *BRCA1* or *BRCA2* genes are present at increased frequencies.[19,22,54,55] The ethnic background of the individual being tested is a key piece of information required by the laboratories. Each mutation detection method that can be used to screen for mutations of the *BRCA1/2* genes has its advantages and disadvantages. Typically, more than one method is used to identify mutations. Regardless of the methods chosen, there is no method that can detect 100% of gene mutations.

Genetic testing for mutations can be performed on an individual's DNA or RNA (i.e., expressed sequences from the DNA) that are extracted from peripheral blood leukocytes. Most genetic techniques that are commonly used to analyze DNA or RNA require portions of a gene to be replicated or amplified in vitro using the polymerase chain reaction (PCR). There are advantages and disadvantages to consider when choosing genomic DNA or messenger deoxyribonucleic acid (mRNA) for mutation detection.[56] Although mRNA can be used to scan large stretches of sequence and requires fewer PCRs to be performed, it degrades more readily than DNA, and can

be more difficult to manipulate. In addition, mRNA molecules containing truncating mutations are more likely to be degraded by cells than normal molecules. As a result, the use of RNA may lead to false negative results. While genomic DNA is the preferred substrate for most mutation screening protocols, in this case, it requires more PCR reactions and knowledge of the intron-exon structure. Without RNA, it is difficult or impossible to confirm if a mutation has led to aberrant splicing (processing) of the messenger RNA.[57]

a) DNA amplification

The most frequently used detection techniques rely on PCR amplification of the starting genomic DNA or mRNA before analysis. PCR is based on a specialized polymerase enzyme that can synthesize a complementary DNA (cDNA) strand in a mixture containing four DNA bases and two DNA fragments (i.e., primers of about 20 bases long) flanking the target sequence. The mixture is first heated to separate the strands of DNA containing the target sequence and then cooled. During cooling, the primers locate and bind their complementary sequences on the separated strands and the polymerase extends the primers into new complementary strands. Repeated heating and cooling cycles exponentially multiply the target DNA sequence.

b) Direct sequence analysis (DSA)

Many methods of mutation detection localize the area of the gene encompassing the mutation. DNA sequencing pinpoints the location of the mutation and may give an indication as to what effect the mutation may have on the encoded protein. This technique is helpful in testing members of a family with known mutations. Solid tumour and blood DNA are suitable materials for this method. This technique is applied in a clinical setting when the full sequence is available through a public database, the type and frequency of mutations are well known and a frequently updated catalogue is available. The gene sequences for *BRCA1* and *BRCA2* are available through Genbank (www.ncbi.nlm.nih.gov) and a catalogue of mutations is available through the Breast Cancer Information Core. Cost may be a factor when DSA is used as a primary strategy for detecting mutations if genes are large and the entire gene must be sequenced. Sequencing strategies that use genomic DNA as starting material may be unable to detect large rearrangements in genes (i.e., including deletions, inversions, and duplications). Genomic deletions in *BRCA1* are infrequent, accounting for 5% to 10% of all germline mutations, and these mutations are less common in *BRCA2*.[22] As large genomic rearrangements have been observed in the *BRCA1* and *BRCA2* genes in families with breast and ovarian cancer, clinically relevant mutations may be missed if DSA is used as the only method for screening *BRCA1* and *BRCA2*.

c) Multi-step analysis

Several methods can be used to pre-screen a gene for mutations before DSA to identify a mutation. Single strand conformation polymorphism analysis (SSCP), heteroduplex analysis (HA), and DHPLC, which are commonly used, take advantage of the fact that mutations will alter the migration of a DNA molecule through a gel or matrix. Similarly to DSA, these methods may miss large DNA rearrangements. Detection methods such as SSCP, SSCP/HA, conformation sensitive gel electrophoresis (CSGE) or denaturing gradient gel electrophoresis (DGGE) are often used to test large populations to determine the population frequency of mutations. The direct sequencing of aberrant bands is used to identify the DNA mutation. As single-base changes often cause small changes in these assays, methods like SSCP and DDGE do not detect all mutations.

When used in a competent clinical laboratory, DSA generally detects all mutations other than the following three cases, mostly due to limitations of the PCR process preceding the sequence analysis: large intron-intron deletions or whole gene deletions; intron-intron inversion mutations, and point mutations that are masked by second mutations in cis that affect a primer binding site (null alleles due to primer-binding site variation). DHPLC generally detects all mutations other than those that fail to make a difference to the melting profile of the segment under the DHPLC conditions used and those identified regarding DSA. When using DSA and DHPLC, one must be certain that both alleles are fully represented in the analysis. Detection of heterozygosity means that both alleles have been detected; however, apparent homozygosity (two identical alleles) is potentially, hemizygosity (one allele opposite a deletion or an artefactual null allele). The most common source of artefactual null alleles is primer-binding site variation. As the expected outcome in most *BRCA1/2* assays is homozygous normal, it is essential to ensure that these are true negatives and not false negatives (Dr. Peter Bridge, Director, Molecular Diagnostic Laboratory, Alberta Children's Hospital, Edmonton: personal communication, 2005 July 16).

Three approaches may be used to minimize the impact of the three types of mutations not readily detectable by DSA. One approach would be to sequence through all primer binding sites in a large number of controls using external flanking primers to ensure the rate of polymorphism at these sites was as close to zero as possible and then to repeat the whole process with more distal primers. Another option would be to compare the degree and positions of hetrozygosity at a large number of known intragenic polymorphic sites to that expected, as too little heterozygosity may signify a deletion or null alleles. Lastly, using long-range PCR in the initial amplification procedure, long segments of DNA, often containing several exons and intervening introns, can be amplified. Sequence primers can be placed such that each primer site would be close enough to the next that the sequence derived from one reads through the binding site of the next. One can deliberately overshoot each exon and sequence well into, or right through the intron in search for polymorphic heterozygous sites (Dr. Peter Bridge: personal communication, 2005 July 16).

Using the dual testing strategy of HA and PTT, a mutation detection frequency of 10% to 14% has been reported for the *BRCA1* gene.[58] A hierarchical mutation screening strategy has been applied for detection of a diversity of mutations in a heterogeneous population in New Zealand.[58] The strategy consisted of two tiers: multiplex heteroduplex and exon 13 duplication analysis, and exon amplification and DSA. This approach allows the division of analytical tools to achieve both low- and high-resolution mutation screening in a sensitive and rapid assay with reduced labour costs and handling errors.[58]

A different two-stage screening procedure has been developed for *BRCA1/2* mutation screening from blood spot paper.[59] For this strategy, stage one screening implies detection of common mutations by adapting an assay to heterozygote screening for common disease associated sequence variants. Stage two screening uses CSGE adapted to automate HA of *BRCA1* and *BRCA2*. In this study, it was concluded that pre-screening for common mutations was a relatively effective first-line analysis, and subsequent analysis by fluorescent conformation sensitive gel electrophoresis (F-CSGE) and fragment sequencing was a sensitive alternative to full nucleotide sequencing.[59]

d) Protein analysis

Further analysis of *BRCA1* led to the use of PTT to screen for nonsense mutations in the gene transcript. The protein truncation test (PTT) can be performed on DNA or RNA samples. The gene proteins are synthesized (i.e., mimicking what happens in the cell) and the size of the synthesized protein is compared to that of the normal protein. A shorter protein product indicates the presence of a disease causing mutation that alters protein structure and function. Once an abnormal protein product is produced, sequencing is performed to identify the nature of the mutation. While the assay is complex to perform, it has the advantage of allowing larger regions of the gene to be screened at one time. Only those mutations that alter protein structure and function will be detected by PTT, but it is quick, efficient, and can be used to detect protein-truncating mutations that are present outside the coding region of the gene. It can also readily detect large deletions and rearrangements of the gene that affect the coding region. In terms of cost, a combination of PTT on exon 11 and HA on the remaining 21 exons of the *BRCA* gene was found to be cost-effective in terms of the lowest cost per mutation detected; however, a high false negative rate was identified.[60] Most mutations result in protein truncations that are thought to be detectable by immunohistochemical (IHC) analysis with commercially available antibodies. Antibodies directed against the amino and carboxy terminals demonstrate a quantitative reduction in reactivity in tissue carrying a mutation relative to normal tissue.[61] The stop codon assay is also used to identify protein truncating mutations in *BRCA1* and *BRCA2*.[62]

Lastly, southern hybridization, which does not require PCR, may be added to the analysis to detect some mutation types, including large rearrangements.[63] A Southern blot is a filter to which DNA has been transferred after restriction enzyme digestion and gel electrophoresis to separate DNA molecules. The filter is hybridized with specific probes.[64] Modifications in the intensities or location of the bands on Southern blots after radiography signal changes such as deletions and insertions.[57] Appendix 1 includes details about the molecular methods.

e) Technical limitations and emerging technologies

One shortcoming of most available detection technologies is that they are not sensitive to large deletions or splice mutations that remove entire exons.[23] While rare, splice mutations that are outside of the amplicons examined will be missed, even by DSA. Although Southern blotting may be used to detect large deletions, insertions, and other rearrangements, it is time-consuming and requires a large amount of DNA. DSA will miss large gene rearrangements, including inversions, deletions, and duplications. Large deletions may also be detected through quantitative PCR,[23] for example, by MLPA, and haplotype analysis of *BRCA1* has revealed rearrangements and large deletions.[65] The increased resolving power of new gels enable the identification of short insertions and deletions in *BRCA1*.[66] Other detection strategies such as allele specific oligonucleotide (ASO), DGGE, and SSCP are efficient, yet they suffer from allele specificity or lack of sensitivity.
In the section of this report on analytical validity, DSA is considered to be the "gold standard" when selecting studies for review, but there continues to be no ideal method that will identify all the mutations in a gene. The detection of *BRCA1/2* mutations presents a challenge for the achievement of speed and accuracy. Mutation clustering at a limited number of sites in *BRCA1* led to the development of a (MLPA) assay to screen exons 2, 11A, 11B, and 20 for mutations in a simple, rapid manner.[58] This technique was developed to detect large genomic deletions and duplications in *BRCA1,* and now in *BRCA2*.[67] MLPA kits are available to detect copy number changes in *BRCA1* and to confirm deletions and duplications.[68] The MLPA kit for *BRCA2* contains probes for most coding exons of the *BRCA2* gene.[68] MLPA is a PCR-based technique

that allows relative quantification of many different sequences in a single reaction.[69] Furthermore, it is designed to detect unusual copy numbers of exons or genes, so results require confirmation with a second technique, or a second independent reanalysis, if a single exon deletion is detected.[70].

In addition, the technique of colour bar coding *BRCA1* on combed DNA (DNA that has been stretched using a technique called "molecular combing") is another strategy for detecting large gene rearrangements. The *BRCA1* bar code may be useful for identifying under-reported rearrangements such as inversions and insertions.[71] Four large *BRCA1* rearrangements and a 17 kb *BRCA1* duplication encompassing exons three to eight have been detected using colour bar coding.[71] Rearrangements as small as two to six kb with respect to the normal size of the fragment can be achieved when the *BRCA1* region is divided into 10 fragments.[71] Southern blot and colour bar coding are low throughput techniques that can take several days to perform.[63] Southern blot analysis requires substantial amounts of DNA, while colour bar coding requires high quality genetic material.[63]

Oligonucleotide microarrays are emerging tools that allow alterations in the transcript level of entire genomes to be assayed simultaneously.[72] Microarray technology has been used to scan *BRCA1* coding sequences. In *BRCA1* exon 11 mutation screens, subsets of heterozygous mutations were detected by observing increased hybridization signals to mutation-specific probes. Frameshift mutations in repetitive sequences remain a challenge for oligonucleotide microarray-based mutation detection.[73] Single nucleotide polymorphisms, deletions and insertions in *BRCA1* have been identified using microelectronic DNA assay.[74] A fluorescent DNA microarray assay has been used to rapidly and simultaneously screen for rearrangements along the *BRCA1* gene.[75] Capillary and microchip electrophoresis based methods are emerging as a means of simple, automated, high-throughput mutation detection after allele-specific amplification.[76]

A gene dosage assay, based on real-time PCR, has been developed that calculates the copy number of each *BRCA1* exon to detect one, two, and three or more copies of *BRCA1* target exons.[77] In a series of 91 French families at high risk of carrying *BRCA1* mutations, seven large rearrangements of the *BRCA1* gene were detected using real-time PCR. The semiautomated real-time quantitative PCR assay is an alternative technique to Southern blot, bar code analysis on combed DNA, quantitative MLPA of short fluorescent fragments, and cDNA length analysis for the detection of large rearrangements.[77]

Single nucleotide polymorphism haplotype pair analysis and gene amplification with dispersed primer sets has been used to identify a multi-exonic *BRCA1* deletion.[78] Using this method, a 26 kb deletion of *BRCA1* exons 14 thorough 20 has been detected in 15 North American families with hereditary breast and ovarian cancer.[79]

f) Availability, cost, and utilization of testing in Canada

In most provinces, clinical assessment, counselling and screening for *BRCA1/2* mutations are available. There are regional genetic laboratories in Alberta, British Columbia, Manitoba, Ontario, Quebec, and Nova Scotia. While not all provinces have a clinical molecular genetics laboratory to perform the testing, arrangements for out-of-province testing can be made.

g) Availability and cost of out-of-country testing

Proprietary forms of *BRCA1/2* testing are available. Myriad Genetics Inc. of Salt Lake City, Utah, holds a series of US and Canadian patents awarded from October 2000 to April 2001. Myriad offers genetic analysis and information services to health care providers, and Myriad Genetic Laboratories is accredited by the United States Department of Health and Human Services under CLIA (Clinical Laboratory Improvement Act/Amendment), the College of American Pathologists, and the New York State Department of Health under the Clinical Laboratory Evaluation Program.[65] Myriad has exclusive marketing agreements with laboratories in Canada (MDS Laboratory Services, Toronto ON), Japan (FALCO Biosystems Ltd., Kyoto) and the United Kingdom and Ireland (Rosgen Ltd, Roslin, Midlothian, Scotland).[66] Three types of analyses are offered by Myriad:

- Comprehensive BRACAnalysis®: a full sequence analysis of the protein-coding regions of *BRCA1* and *BRCA2* designed for patients who do not have relatives with a known mutation. The analysis also includes detection of five *BRCA1* rearrangement mutations.[82] Approximately 3% of the time, Ashkenazi Jewish individuals sent for multisite analysis carry other mutations that can be identified by a Comprehensive BRACAnalysis®. A Rapid BRACAnalysis® can be done in seven to 10 days.
- Single Site BRACAnalysis®: a sequence analysis of a small portion of a patient's DNA to determine whether the individual is a carrier of a specific mutation that is present in an affected family member. [83]
- Multisite 3 BRACAnalysis®: an analysis that detects three specific mutations in *BRCA1* and *BRCA2* that are most common in the Ashkenazi Jewish population: 187delAG, 5385insC, and 6174delT.

The Comprehensive BRACAnalysis® (Appendix 2), full sequencing of the gene, is conducted for a fee of US$2,580 to US$2,600 (C$3,850).[81,84] The fee for Single Site BRACAnalysis®, used to analyse for a known family mutation, is US$295 (C$525).[81,84] Multisite 3 BRACAnalysis® for the three common Ashkenazi Jewish mutations is conducted for a fee of US$450 (C$600).[81,84] Patients who have never undergone *BRCA* testing in their family would undergo full gene sequencing as the index case of both *BRCA* genes. If a mutation is found, other family members may be tested using the Single Site BRACAnalysis® to determine whether they carry the known family mutation.

1.2.2 Test performance

According to the United States Task Force on Genetic Testing, clinical use of a genetic test must be based on evidence that the gene is associated with the disease state, that the test has analytical and clinical validity, and that the results of the test are useful to those tested.[85] The technical and clinical performances of molecular genetic tests are evaluated as analytical and clinical validity.

a) Analytical validity

Analytical validity reflects a comparison between test result and genotype, and addresses the ability to detect mutations present in an individual's gene. The analytical validity of a test is determined by its sensitivity (ability to detect the mutations it was designed to detect) and

specificity (ability to detect only those mutations it was designed to detect). Reliability is determined by whether the same results occur each time the test is run. While DSA was considered as the "gold standard" and was used as a reference for comparison purposes in the Analytical Validity section (5.1) of this review, various factors (instruments, techniques, interpretation) influence test validity and DSA is not a suitable method to detect large rearrangements. A study designed to document all aspects of analytical validity would yield data for all the cells indicated in the 2x2 table in Table 1.

Table 1: Ideal study design for assessment of analytical validity

	Genotype + (mutation)	**Genotype –**
Test +	True positive* (TP)	False positive (FP)
Test -	False negative (FN)	True negative (TN)

*DSA may miss some TPs that are large rearrangements; sensitivity=TP/(TP+FN); specificity=TN/(TN+FP)

b) Clinical validity

Clinical validity reflects a comparison between test result and phenotype or clinical development of breast or ovarian cancer. It is affected by analytical validity, the contribution of detectable mutations to "at risk" genotypes, and the relation between mutations and phenotype (penetrance). Clinical validity is determined by the clinical sensitivity and specificity, and the positive and negative predictive values (PPV and NPV) of a test. For *BRCA1* and *BRCA2*, the clinical sensitivity is the probability of a positive genetic test result in people that develop breast or ovarian cancer. Clinical specificity is the probability that the test will be negative in individuals who do not develop cancer. PPV is the probability that people with positive test results will develop the disease, whereas NPV is the probability that individuals with negative results will not get cancer.[85] Clinical validity is also affected by genetic heterogeneity (the fact that cancer can result from any of several variants of the same gene or different genes) and penetrance (the cumulative risk of cancer given a carrier genotype) indicated by the PPV of a test. In the case of

BRCA1/2, heterogeneity reduces clinical sensitivity and incomplete penetrance reduces the PPV of a positive test result. This is complicated by the fact that current technology may not detect all cancer-related mutations, and other factors may influence the development of disease.

Before any genetic test can be accepted into clinical practice, data should exist to demonstrate the benefits and risks (i.e., clinical and psychological) from positive and negative results. If clinical benefit is anticipated, the safety and efficacy of available intervention methods should be established before the test is made available for clinical use. The United States Task Force on Genetic Testing recommends that the clinical use of a genetic test be based on evidence that the gene examined is associated with the disease in question, that the test has analytical and clinical validity, and that the test results will be useful to people being tested.[86] As a result, establishment of the analytical and clinical validity of the test is paramount.

2 THE ISSUE

Breast and ovarian cancers are among the leading causes of cancer-related deaths in Canadian women. Mutations in *BRCA1* and *BRCA2* genes have been linked to the development of both cancers. Technologies for genetic testing for these mutations are available in Canada, and can be accessed as a clinical laboratory service or through a research study.

It follows that an examination of the analytical and clinical validity of available molecular technologies and a comprehensive overview of the issues associated with *BRCA1/2* mutation testing will help patients, health care providers, hospitals, health regions, and governments make informed decisions. Clinical management, psychosocial impact, and ethical implications associated with *BRCA1/2* mutation testing will be important in managing individuals who undergo testing. The financial and legal implications associated with genetic testing are important; other initiatives are underway in Canada to address these issues.

3 OBJECTIVES

The objectives of the collaborative systematic review are to:

- evaluate the analytical and clinical validity of *BRCA1* and *BRCA2* genetic testing
- assess the contribution of molecular testing to genetic counselling and clinical management
- discuss the ethical and psychosocial issues inherent in *BRCA1* and *BRCA2* testing.

To address these objectives, the following questions are addressed in this report:

- What are the molecular techniques used to identify *BRCA1* and *BRCA2* mutations?
- What values of analytical validity are associated with these techniques?
- What social factors influence participation in testing?
- What psychological, social, and ethical issues are associated with testing?
- What are the benefits and risks of surveillance and preventive methods?

4 CLINICAL REVIEW

4.1 Methods

4.1.1 Literature search strategy

A comprehensive search strategy was designed to identify published, grey, and unpublished literature for each subject area. Searches were limited to human studies with no language restrictions. Extensive scoping searches were developed a priori to test the recall and precision of draft search strategies. The search strategy with subject headings, textwords, and logic can be found in Appendix 3.

Electronic databases searched included PubMed®, Cochrane Library, and a Dialog® OneSearch® on MEDLINE®, CANCERLIT®, EMBASE®, Biosis Previews®, PASCAL, and PsycINFO® (III only). Searches for subject areas II to IV covered the publication period 1994 to January 2003. A revised search for subject areas II and III was performed in March 2003. An updated search was performed for all subject areas in July 2004. As a result of reviewers' comments on a preliminary draft, an expanded search was performed for the ethics component in mid-July 2004.

Grey literature was identified through searches of the web sites of the International Network of Agencies for Health Technology Assessment and related health agencies, clinical trial registries, clinical practice guidelines, and other specialized databases. The web sites of relevant societies and associations on the Internet were searched for conference abstracts. Hand searching of abstract booklets, proceedings from conferences, and meetings was also performed. The reference lists of relevant studies, review articles, and reports were examined to identify any relevant citations missed in other sources. Lastly, efforts were made to access unpublished studies by contacting the commercial developer of the *BRCA1/2* tests and primary researchers recommended by the clinical experts consulted.

4.1.2 Selection criteria and methods

a) Selection criteria

A study was eligible for inclusion if it fulfilled all the selection criteria for topics identified in subject areas II to IV.

Subject Area II: Analytical Validity and Molecular Methods

1. Study design:
    a. Primary study in a research or clinical setting
    b. Sample size of $\geq$20 patients
2. Population: individuals at risk for inherited breast or ovarian cancer
3. Intervention:
    a. Molecular method to detect a *BRCA1* mutation
    b. Molecular method to detect a *BRCA2* mutation
4. Outcome: analytical validity measures of sensitivity or specificity
    a. Comparison of test result with genotype
    b. Comparison of test with sequence analysis
    c. Comparison of more than one test
    d. Any new technique for *BRCA* analysis described in the literature

Subject Area III: Genetic Counselling, Psychosocial Impact and Ethical Issues

1. Study design:
    a. Primary study in a research or clinical setting
    b. Sample size of $\geq$ 20 patients
2. Population: individuals at risk for inherited breast or ovarian cancer
3. Intervention: *BRCA1* or *BRCA2* genetic testing
4. Outcome: qualitative measures
    a. Contribution of testing to counselling
    b. Psychosocial implications
    c. Ethical implications

Subject Area IV: Clinical Management

1. Study design: any study design
2. Population: individuals at risk for inherited breast or ovarian cancer with:
    a. Multiple cases of breast or ovarian cancer
    b. Age <35 years at diagnosis of breast cancer
    c. Family member diagnosed with both breast and ovarian cancer
    d. Breast or ovarian cancer in Jewish families
    e. Family member with primary cancer occurring in both breasts
    f. Family member with an identified *BRCA1* or *BRCA2* mutation
    g. Presence of male breast cancer in family
    h. Presence of associated conditions suggestive of an inherited cancer syndrome
3. Intervention:
    a. Molecular method to detect a *BRCA1* mutation
    b. Molecular method to detect a *BRCA2* mutation
4. Outcome: any clinical outcome, prophylactic or therapeutic purposes

b) Methods

For each subject area, two reviewers developed and tested abstract selection forms before independently reviewing citations identified by the searches (Appendix 4). The individual subject area reviewers correspond to those in Table 1 with the exception that LM, JT, RK, and HN were involved with subject area III. The decision to order the full article from a citation was based on title and abstract, when available. In cases of insufficient information to make an informed decision on inclusion, the article was ordered for more information. Two reviewers independently made the final selection of relevant studies to be included in the systematic review based on the selection criteria. Degree of agreement between reviewers was noted and differences were resolved by consensus.

4.1.3 Data extraction and abstraction strategy

The following methods apply to each subject area unless otherwise specified. Two reviewers independently extracted data for each article selected for inclusion using the data extraction forms (Appendix 4). The individual subject area reviewers correspond to those in Table 1 with the exception of subject area III, where the three topics were distributed among five researchers as follows: RK or LM with BAL and JT for genetic counselling, RK or LM with BAL for psychosocial issues, and BAL and HN for ethical issues.

For subject area II, if multiple reports were identified that were based on one study, the most recent publication was used as the primary citation for the study. The technique of DSA was considered to be the "gold standard" for selection of studies to evaluate analytical validity. It was acknowledged that this method does not identify all mutations, particularly large rearrangements. Alternative methods identified from the selected studies were reviewed, and the technical aspects, advantages, and disadvantages of each method were considered.

4.1.4 Strategy for quality assessment

For each subject area, the reviewers assessed the quality of each study selected for inclusion, using the study summary and quality assessment forms (Appendix 5). Attempts were made to

evaluate the robustness of the study design, conduct, analysis and interpretation of each study reviewed for this report. Studies with methodological flaws were not necessarily excluded, but their limitations were described. After independent assessment, the data sets were compared and disagreements were resolved by consensus.

For subject area II (analytical validity), there were additional quality assessment elements that the reviewers considered when evaluating studies. These elements were based on the guideline, Standards for Reporting Diagnosis Accuracy, or the "STARD" statement:[87]

- Clearly stated objectives to assess the accuracy of a particular molecular test in relation to an identified "gold standard" or to compare accuracy between tests or across participant groups
- Ideal study design being a systematic prospective approach with clear eligibility criteria and transparent methods of sample collection and analysis. The best selection procedure is the inclusion of all subjects meeting the eligibility criteria (consecutive sampling) and the second best is a random sample of eligible subjects. Any other method of selection is considered to be susceptible to bias.
- The generalizability of the study results (e.g., information on study subjects, such as age, ethnicity, family history of breast or ovarian cancer, mutation carrier status (if known), and presence of breast or ovarian cancer); context information (e.g., geographic location, single- versus multi-site study); and study setting, to determine the comparability and independence of the studies identified.
- A description of reference technique and rationale for its selection (e.g., DSA).
- Testers should be blinded to results so as to exclude measurement bias. They should have adequate training and demonstrate high intra-observer reliability. In studies with multiple testers, inter-observer reliability should be ensured to facilitate comparison of tests between multiple laboratories.
- All study subjects should be tested by the index and reference tests; if this did not occur, then the numbers and reasons for not receiving a particular test should be assessed for potential bias and described.
- A description of and rationale for the unit of analysis (i.e., results are reported as detection of unique mutations, individuals, samples or fragments; choice of unit will affect interpretation).
- Details regarding the technical specifications of the genetic material and molecular methods used to assess their appropriateness (i.e., the primers used, how the primers were prepared, which instruments were used, and modifications made).
- Study start and end dates, because of the fast rate of technological advances in this area, and the potential time lag between when the study was conducted and when results were published.
- Statistical methods used in the assessment of diagnostic accuracy (e.g., confidence intervals or (ideally) cross-tabulations of the number of individuals and sample mutations by the reference test with those of the index test).
- A statement of the source of funding, and conflict of interest declarations.

4.1.5 Data analysis methods

For this report, only subject area II was amenable to the calculation of measures of effect. Analytical sensitivity and specificity values were calculated for those studies reporting sufficient data. If feasible, subgroup analysis was performed by population, technique, or mutation type.

4.2 Results

4.2.1 Subject Area II: Analytical Validity

a) Quantity of research available

The original electronic search strategy for subject area II identified 765 citations. The updated literature search yielded 116 citations (Figure 1). The degree of agreement between reviewers was calculated as kappa=0.78 (original search) and kappa=0.57 (update search). Many studies were excluded due to the index and reference techniques not being applied to all samples. For example, it was often found that only those samples that had aberrant results detected by the index test were subsequently tested with the reference test to confirm the presence of a mutation. As a result, those tests that were not identified as aberrant by the index test, would not be tested with the reference, thereby leading to the possibility of missing results that were undetected by the index test. This design may lead to overestimation of the sensitivity of the results and verification bias.[88]

b) Trial characteristics

Selected trials were primarily single-site and hospital-based, in individuals with unknown mutation status and a family history of breast or ovarian cancer. One report presented three studies in non-peer reviewed format, providing little information regarding patient and study characteristics.[89] Two studies of 27 reported how the subjects were sampled.[62,90] Studies were conducted in populations spanning a variety of ethnic groups. The age of subjects was not reported in any study. A substantive amount of information that was initially sought was unavailable in the studies, thereby preventing a proper assessment of the validity and comparability of the results. Furthermore, the degree of heterogeneity across studies precluded any meaningful synthesis of the data extracted. An account of the individual study characteristics can be found in Table 1 Appendix 6. A summary of overall trial characteristics and corresponding numbers of articles is provided in Table 2.

c) Data analysis and synthesis

An account of the quality assessment of selected articles can be found in Table 2 Appendix 6. A detailed description of the molecular techniques for index and reference tests used in the selected studies can be found in Table 3 Appendix 6.

Many articles reported high variability in the mutations tested, tests examined, and the reference test used. With the exception of one report,[89] all other studies adequately described the index and reference tests. Issues were identified with the reporting of sensitivity and specificity of the methods used. Eight of the 27 individual studies reported these values correctly.[56,62,89,91-93] Other issues with blinding, intra- and inter-observer reliability, and sources of bias were also identified.

Figure 1: Selected material for analytical validity (Subject Area II)

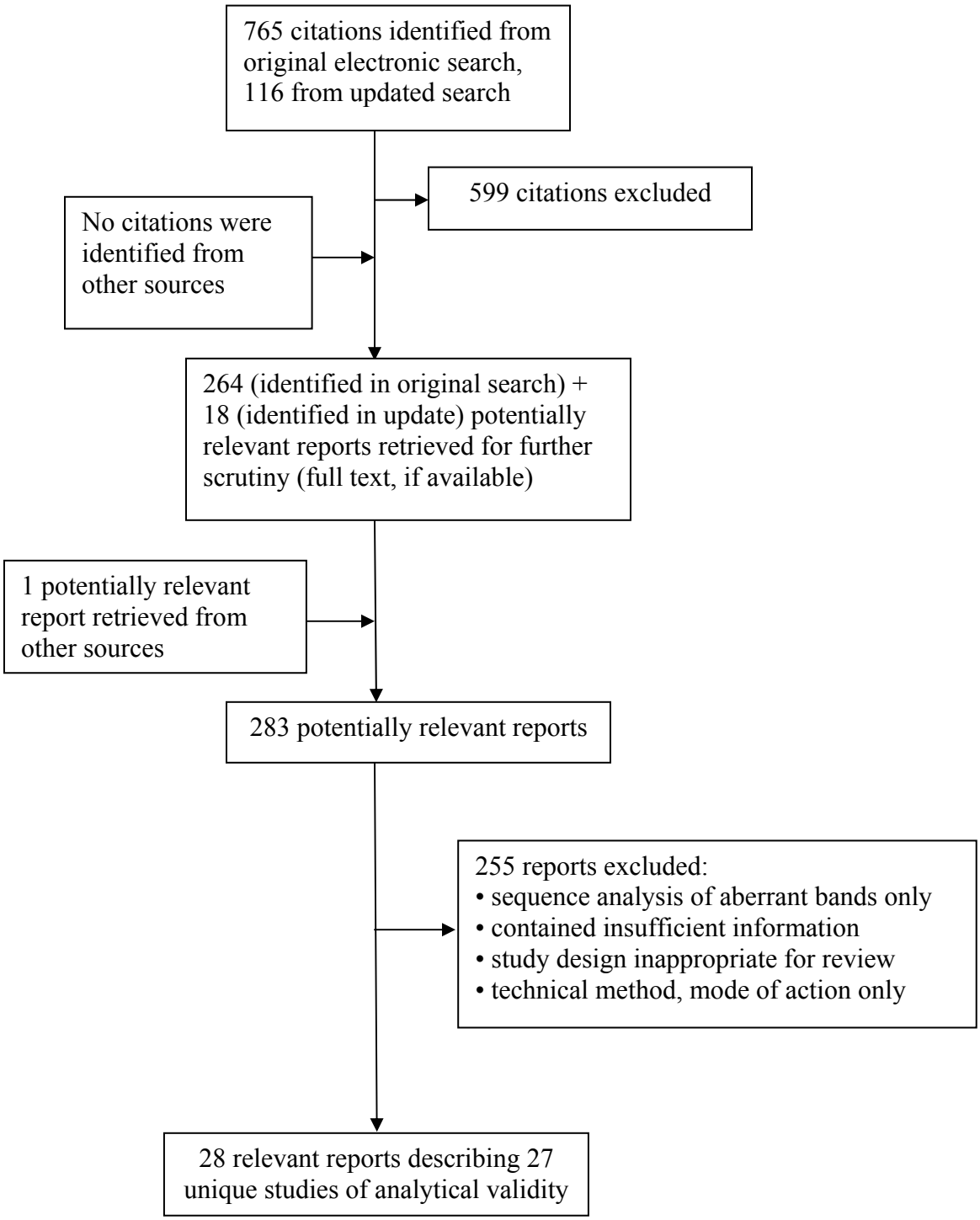


Table 2: Trial characteristics of studies for analytical validity

Study Characteristic	Details	Number of Studies
Site	Single-site	14
	Multi-site	6
	Not reported	7
Study setting	Hospital	10
	Clinic	2
	Registry	2
	Referral process	1
	Community	1
	Other	4
	Not reported or unclear	7
Mutation status	Carrier	5
	Both carrier and non-carrier	8
	Unknown	14
Family history of breast or ovarian cancer	Yes	15
	Not reported	12

Study Characteristic	Details	Number of Studies
Mutations associated with	Breast cancer	6
	Ovarian cancer	2
	Both	16
	Unclear	3
Sampling method	Consecutive	2
	Not reported	25
Ethnicity (studies may have included more than one ethnic group)	Ashkenazi Jewish	2
	German	2
	Norwegian or Swedish	3
	French Canadian	1
	Italian	1
	Polish	1
	Japanese	2
	Cypriot	1
	Multinational	1
	Not reported	14

A summary of the results from the 27 studies selected for the review of this section can be found in Table 3. There was a high degree of variability between studies in mutations tested, tests examined, and the reference test used. A number of differences can be attributed to the gene, genetic region, and mutation under study. Many studies tested for all *BRCA1* regions, whereas others focused on specific exons in *BRCA1* (e.g. exon 11). One study tested *BRCA2* only,[94] whereas 11 studies tested *BRCA1* and *BRCA2*.[56,62,89,93,95-101] Lastly, a few studies concentrated on the identification of particular mutations, e.g., Chan *et al.* examined the accuracy of multiplex mutagenically separated PCR (MS-PCR) in detecting three common mutations: 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*.[95]

With the exception of one report,[89] all other studies adequately described the reference and index test assessed. In 21 of the 27 studies, the reference test selected was considered to be adequate. The most common reference test used was DSA, and in the 14 studies that used this technique, four studies combined DSA with additional tests such as SSCP;[92] HTA;[95] CSGE; DHPLC and PTT;[94] and DGGE, PTT, and SCCP.[99] Alternative reference tests included genotype,[93,96,102] SSCP,[90] SSCP or PTT,[103] single-strand conformation analysis (SSCA), or dideoxy fingerprinting (DDF),[104] SSCP and PTT,[98] PTT alone or with partial nucleotide sequencing,[105] PTT or DGGE for Norwegians, PTT only for Swedes,[106] or CSGE.[107,108] Although DSA was used exclusively as the reference test in 10 studies, no

two studies used the same index test and the same unit of analysis. Studies presented their results in terms of the detection (or lack thereof) of the number of individuals (nine studies), samples (10 studies), unique mutations (four studies), fragments (two studies), DNA changes (one study), and carcinoma (one study). The different units of analyses precluded any direct comparison of the data, so the reviewers were unable to conduct any meaningful statistical analyses of the synthesis of these data.

Details on the sensitivity and specificity of tests that are shown in Table 2 and for each study are provided in Table 4 Appendix 6. A difficult issue encountered by the reviewers was the inconsistency in the method in which polymorphisms and mutations were treated among studies. While some studies combined known mutations and sequence "variants" of unknown clinical significance, others differentiated them, and reported results for both or focused on mutations. In a few studies, the reference test was not clearly specified, so the accuracy of the reported sensitivity and specificity values must be interpreted with caution. In several studies, the sensitivity and specificity values were provided without any information about actual numbers detected or missed. In studies that focused on subjects or samples with mutations only, specificity values could not be calculated.[93,96,104,107,108] Depending on the performance of the reference test, it was necessary to classify some mutations as false positives. Such false positives may truly be mutations and thus undetectable by the reference test. This leads to the inability to differentiate between the results that are true false positives and those that have been labelled as such on account of the accuracy of the reference test. This underscores the lack of a "gold standard" test that demonstrates 100% sensitivity and specificity for all mutations.

In the studies that incorporated statistical analyses, none reported confidence intervals. Most studies reported the number of mutations or polymorphisms detected by each test or provided the sensitivity or specificity values. Despite this, reviewers were able to calculate these values for a few of the studies. The study by Geisler *et al.* [103] required recalculation of the sensitivity and specificity values. The authors reported these values, but an inappropriate reference test procedure was used. Although DSA was used as the reference test, only those results that were identified as being aberrant underwent DSA, and thus the sensitivity and specificity values may be overestimated. The reviewers opted to recalculate these values using each of the index tests (i.e., SSCP and PTT) as a reference for the other, because these were the two tests that were known to have been carried out on all samples. Other studies revealed additional ambiguities in reference testing, such as failure to report the proportion of individuals who tested negative by the index test and were tested by the reference test.[53,97,102] Other limitations were identified during the review of the selected studies. Although blinded analyses were carried out for most of the reference tests, it was unclear in three studies if this was done.[97,104,108] Nine studies stated that the index test was carried out under blinded conditions,[52,53,91-93,97,105,109,110] whereas other studies either did not report or did not require conditions of blinding. Most studies did not assess intra- and inter-observer reliability. Inter-observer reliability was reported in only two studies,[92,97] and intra-observer reliability (i.e., by laboratory) in two.[94,106] Clinical information pertaining to the subjects was known in 11 of the 27 studies, but was unknown in two, and the remaining 14 failed to report whether this information was known. Pedigree information was known for 15 studies and not reported for 12 studies. While control samples were used to ensure quality of test methods in these studies, the mutation carrier status of the sample was known to testers in 15, unknown in two, and not reported in the remaining 10 studies. Lastly, 11 studies reported a time lag between the reference and index tests, whereas nine did not have a lag time, and seven

studies did not report this adequately. A time lag may have a bearing on study results, as the quality of genetic material may degrade over time.

Sources of bias were also examined. Measurement bias can occur during the testing process if the tester is aware of the mutation status of the subject. In the selected studies, the presence of measurement bias was unclear in six,[53,56,89,99,101] probable in five,[90,91,94,104,107] and unlikely in 16 studies. The quality and age of a sample may differ by test or by mutation status, which could influence the validity of results (i.e., sample handling bias). A total of 15 studies provided no evidence of such bias, whereas in nine, it was unclear,[53,89,90,94,95,99,104] and in three, there appeared to be potential bias due to sample handling.[91,105,107] In 19 studies, there was insufficient information to judge for selection bias, although it was probable in seven studies[53,89-91,98,105,107,108] and unlikely in one study.[106] All but one study[97] accounted for the entire number of study subjects or samples in the results, so attrition was not identified as an issue.

The analysis of cost implications was not an objective of this assessment. Four of the 27 studies addressed the issue of cost of the tests in their analyses.[53,93,94,105] The first of these studies reported the cost of fluorescent mutation detection (F-MD) to be approximately 0.07 U per fragment, and estimated that 14 fragments could be screened for the price of one DSA.[94] Furthermore, the entire *BRCA2* gene could be screened for the cost of approximately three DSA. For the reference technique, DSA was the most costly method of screening. In the second study, costs of mutation analysis were calculated in two ways.[53] The first calculation method took into account the cost of consumable supplies on a per sample basis, whereas the second derived a "universal cost equivalent" in an attempt to analyze each method in terms of labour, quantities of supplies, and run times necessary to perform an analysis. In the third study, it was reported that two-dimensional gene scanning (TDGS) costs approximately US$70, and the reference technique (PTT alone or with partial sequencing) costs in the order of US$2400.[105] The source of this information was not reported. Lastly, high-throughput mutation detection scanning of *BRCA1* and *BRCA2* based on HA by capillary array electrophoresis was estimated to cost eightfold less than that of direct sequencing.[93]

In Europe and Canada, the technique of denaturing high performance liquid chromatography (DHPLC) has gained favour in many laboratories. As a result, the methodological limitations of five studies in which this technique was used warrants further mention.[52,53,56,99-101,109] The sample sizes of the studies were relatively small, ranging from 20 blood samples[56] to 238 fragments[52] to 30 to 46 individuals as units of analyses.[100,109] With regard to objectivity in testing, in one of the five studies, the DHPLC test was carried out without knowledge of clinical information. In four of the five studies, pedigree information was known. None of the studies mentioned reliability assessment of the test, and one study reported having carried out both DSA and DHPLC techniques at the same time. It follows that the potential for bias cannot be ruled out, and the possibility of an overestimation in test performance should not be dismissed.

d) Summary points for analytical validity

- Several studies were excluded because index and reference techniques were not applied to all samples. This design may lead to an overestimation of the sensitivity of the results and verification bias.

- Information is provided on study characteristics and results for selected studies used in the review of the analytical validity of available molecular *BRCA1/2* detection techniques. In addition, sensitivity and specificity values were computed by the reviewers for each study.
- Selected trials were primarily single-site, hospital-based, in individuals with unknown mutation status and a history of breast or ovarian cancer. While two out of 27 studies reported how subjects were sampled, age was not reported in any study.
- A substantive amount of information that was initially sought was unavailable in the studies, thereby preventing a proper assessment of the validity and comparability of the results. Regarding the BRACAnalysis® information provided by Myriad Genetic Laboratories, Inc., index and reference tests were inadequately described, and the potential for bias cannot be ruled out. The possibility of an overestimation in test performance should not be dismissed.
- Studies reported high variability in mutations tested, tests examined, and reference test used (i.e., attributed to gene, genetic region, and mutation under study). The degree of heterogeneity across studies precluded any meaningful synthesis of the data extracted.
- The most common reference test used was DSA. Although a large number of studies used DSA exclusively as the reference test, no two studies used the same index test and the same unit of analysis. This precluded any direct comparison of the data, and reviewers were unable to conduct any meaningful statistical analyses of the data.
- Eight of the 27 studies reviewed correctly presented sensitivity and specificity values.
- Methodological limitations of studies in which the technique of DHPLC was used are discussed. The potential for bias cannot be ruled out, and the possibility of an overestimation in test performance should not be dismissed.
- While the information retrieved for the technologies in this section was informative and afforded the opportunity to compare and contrast the methodologies used in each study, the diversity between studies precluded any quantitative analysis of the evidence. As a result, it is not possible to draw strong conclusions as to the most analytically valid molecular technique for the detection of *BRCA1* and *BRCA2* mutations.

4.2.2 Subject Area IV: Clinical Management

a) Quantity of research available

The original electronic search strategy for subject area IV identified 416 citations in addition to 72 citations from other sources (Figure 2).

b) Trial characteristics

Upon review of the selected studies, it became evident that there are no controlled studies of genetic testing and treatment programs (i.e., studies where a program of *BRCA1/2* testing and treatment has been compared with no such program in a single population or between populations). In addition, there are no comprehensive uncontrolled studies of populations subjected to testing (i.e., no studies follow the total exposed population to consider false positive and false negative test results or women who refuse to undergo testing). The available studies are limited to patient cohorts that have undergone testing and subsequent treatment. They include cohort studies of women with breast or ovarian cancer stratified by *BRCA1/2* status (e.g., prospective or retrospective), or women with *BRCA1/2* mutations stratified as being primary (i.e., asymptomatic mutation carrier) or secondary (i.e., breast or ovarian cancer).

Table 3: Results from selected studies for analytical validity

Author ID	Setting	Cancer	Gene	Reference Technique	Number	Unit of analysis	Molecular Technique	Sensitivity	Specificity
Andersen[90]	NR	Both	*BRCA1*	SSCP	48	Individuals	CDGE for overall	100%	82.93%
							CDGE for frameshift	100%	85.71%
							CDGE for substitutions	100%	97.87%
							CDGE for insertions	100%	97.83%
							CDGE for deletions	100%	88.64%
Andrulis[56]	Referral criteria and process	Both	*BRCA1* *BRCA2*	DSA	20	Samples	DHPLC	100%	100%
							EMD	100%	100%
							TDGS	87.5%	100%
							PTT	75%	100%
							SSCP	62.5%	100%
Arnold[109]	Hospital	Both	*BRCA1*	DSA	46	Individuals	DHPLC	100%	100%
Blesa[107]	NR	Both	*BRCA1*	CSGE	24	Mutations	F-CSGE	100%	NR
Byrne[92]	Hospital	Ovarian	*BRCA1*	SSCP and DSA	10	Samples	IHC D20 antibody	100%	100%
							IHC C20 antibody	100%	100%
Campbell[108]	Hospital	Breast	*BRCA1*	DHPLC	29	Samples	CSGE	100%	NR
Chan[95]	Hospital	Both	*BRCA1* *BRCA2*	HA and DNA sequencing	66	Individuals	MS-PCR all mutations	100%	100%
							MS-PCR for 185delAG	100%	100%
							MS-PCR for 5382insC	100%	100%
							MS-PCR for 6174delT	100%	100%
Edwards[94]	Clinic	Breast	*BRCA2*	BIC, various for sequencing, CSGE, DHPLC, PTT	9	Samples	F-MD	100%	0%
							F-CSGE	50%	100%
Eng[53]	Other	Both	*BRCA1*	DSA	66	Mutations	SSCP	64.71%	93.33%
					60		CSGE	60%	100%
					71		TDGS	91.07%	80%
					73		DHPLC	100%	100%
Estaban-Cardenosa[93]	Clinic	Breast	*BRCA1* *BRCA2*	CSGE	57	DNA changes	Capillary-based HA	100%	100%
Geisler[103]	Hospital	Ovarian	*BRCA1*	SSCP or PTT	94	Carcinomas	SSCP	52.63%	96%
					94		PTT	76.92%	88.89%

Author ID	Setting	Cancer	Gene	Reference Technique	Number	Unit of analysis	Molecular Technique	Sensitivity	Specificity
Gross[52]	Hospital	Both	*BRCA1*	DSA	212	Fragments	SSCP	94%	98.21%
					238		DHPLC	100%	100%
Hadjisavvas[111]	Hospital	Breast	*BRCA1*	DSA	13	Mutations	SSCP	92.31%	NR
Jugessur[106]	NR	Both	*BRCA1*	PTT or CDGE for Norwegian, PTT for Swedish	25	Samples	REF-SSCP for Norwegians	90%	80%
					20		REF-SSCP for Swedish	100%	37.5%
Kashima[102]	Hospital	Both	*BRCA1*	Genotype	44	Individuals	IH with GLK-2	100%	90%
							IH-AB-2 antibody	87.5%	100%
Kozlowski[96]	Registry	Both	*BRCA1* *BRCA2*	Genotype	31	Mutations	SSCP/HA versus genotype	100%	N/A
							SSCP versus genotype	90.32%	N/A
							HA	80.65%	N/A
Kringen[110]	Hospital	Both	*BRCA1*	DSA	292	Fragments	REF-SSCP	100%	98.89%
Kuperstein[97]	Registry	Breast	*BRCA1* *BRCA2*	DSA	60	Ashkenazi Jewish Samples	FMPA: 60 Ashkenazi Jewish samples	100%	100%
					30	Ashkenazi Jewish women	FMPA: 30 Ashkenazi Jewish women with BRCA1/2 founder mutation	NR	NR
					56	French Canadian samples	FMPA 56 French Canadian samples	100%	100%
					120	French Canadian women with an identified BRCA1/2 mutation	FMPA 120 French Canadian women with identified BRCA1/2 mutation	100%	100%
Lancaster[104]	Hospital	Both	*BRCA1*	SSCA or DDF	17	Samples	DDF	100%	N/A
					21		SSCA	80.95%	N/A
Montagna[98]	Community	Both	*BRCA1* *BRCA2*	SSCP and PTT	44	Individuals	AGE	100%	100%
Oleykowski[91]	Clinic	Both	*BRCA1*	DSA	19	Samples	CEL I	100%	100%
Sakayori[62,112]	Hospital	Breast	*BRCA1* *BRCA2*	DSA	29	Individuals	Stop Codon Assay	100%	99%

Author ID	Setting	Cancer	Gene	Reference Technique	Number	Unit of analysis	Molecular Technique	Sensitivity	Specificity
Van Orsouw[105]	NR	Both	*BRCA1*	PTT alone or with partial nucleotide sequencing	60	Individuals	TDGS	100%	100%
Wagner *et al*[99-101]	NR	Both	*BRCA1* *BRCA2*	Combined DGGE, PTT, SCCP, DSA	180	Mutations	DHPLC for 180 mutations	99.44%	NR
					30	Individuals	DHPLC for 30 individuals; reference=DSA	100%	NR
					Unclear	(3 with mutations, non-mutation, unknown)	DHPLC for 41 individuals, reported by independent mutations only; reference=DGGE	100%	NR
BRACAnalysis® Information, Myriad Genetic Laboratories, Inc. 2003[89] (3 studies)	Other	NR	*BRCA1* *BRCA2*	Allele specific oligonucleotide hybridization or radioactive sequencing	55 samples (sensitivity) 46 samples (specificity)		Myriad's high-throughput robotic fluorescent sequencing system	98.18%	100%
	Other	NR	*BRCA1* *BRCA2*	Myriad's gel-based sequencing	128 samples (sensitivity) 910 samples (specificity)		Myriad's capillary-based sequencing	100%	100%
	Other	NR	*BRCA1*	Genotype	85 samples with no known large rearrangements, 10 samples with large rearrangements		Myriad's BRACAnalysis® Large Rearrangements test	100%	100%

c) Data analysis and synthesis

An evaluative framework was developed to organize selected reports from the strongest (1) to the weakest (4) study design (Table 4).

The evaluative framework includes the determination of the effect of *BRCA1/2* testing on clinical decision-making, treatment, and ultimately patients' health. Health impact is measured in a hierarchy with mortality at the highest level, other serious adverse events (e.g., hospitalization, extension of hospital stay, serious disease sequelae) and last, quality of life. For testing to have a beneficial effect on patient health, the three components (i.e., *BRCA1/2* testing, clinical decisions, and treatment options) must move in a positive direction. Testing must accurately sort individuals into those who do and those who do not have the mutation. Results, whether positive or negative, must result in a predictable change in clinical management, which in turn, must have an effective, accessible treatment option.

The available *BRCA1/2* testing and treatment program range from population-based screening to staging of women already diagnosed with cancer. There are two options that are relevant to this report. The first is testing women considered a priori to be at high risk of breast or ovarian cancer and offering subsequent treatment options; or second, testing women already diagnosed with breast or ovarian cancer to tailor available treatment options.

We found little evidence regarding women at risk for carrying *BRCA1 /2* gene mutations who declined testing. One study from the Netherlands[113] provides insights into the motives for declining testing among 13 women at 25% and 50% risk to be a *BRCA1* or *BRCA2* mutation carrier. Compared to the tested group (N=85), the group of non-tested women had similar distress levels, but a higher education level, were more often childless, showed more reluctance towards prophylactic surgery, were younger when first aware of afflicted relatives, and were longer aware of the genetic nature of the disease. Due to the lack of published information, this group of women will not be discussed further in this review.

Management of unaffected BRCA1/2 mutation carriers

There are no "gene replacement" therapies for the missing gene products, so women who test positive for *BRCA1/2* are limited to choosing between prevention via surgery or drugs or intensive surveillance for early detection of cancer.

Prophylactic surgery: Prophylactic surgery (e.g., mastectomy and oophorectomy, with or without salpingectomy) is an option for mutation carriers through a few programs.[114-116] Several cohort studies have examined the efficacy and safety of prophylactic surgery or chemoprevention.

Mastectomy: The strongest evidence of efficacy to date is from a prospective study conducted by researchers in Rotterdam examining mastectomies and surveillance among 139 women with pathogenic *BRCA1* (84% to 89%) and *BRCA2* mutations.[117] Mean age at entry was 37.7 and 39.9 years for the mastectomy and surveillance groups respectively. At entry, 58% of the mastectomy group had undergone pre-menopausal oophorectomy compared with 38% of the surveillance group. Of the 76 women who underwent prophylactic mastectomy, there were no cases of breast cancer after a mean period of 2.9 years. In contrast, eight cancers were found in the 63 women who chose not to undergo prophylactic mastectomy [p = 0.003; hazard ratio: 0 (95% CI: 0 to 0.4)].

Figure 2: Selected material for clinical management (Subject Area IV)

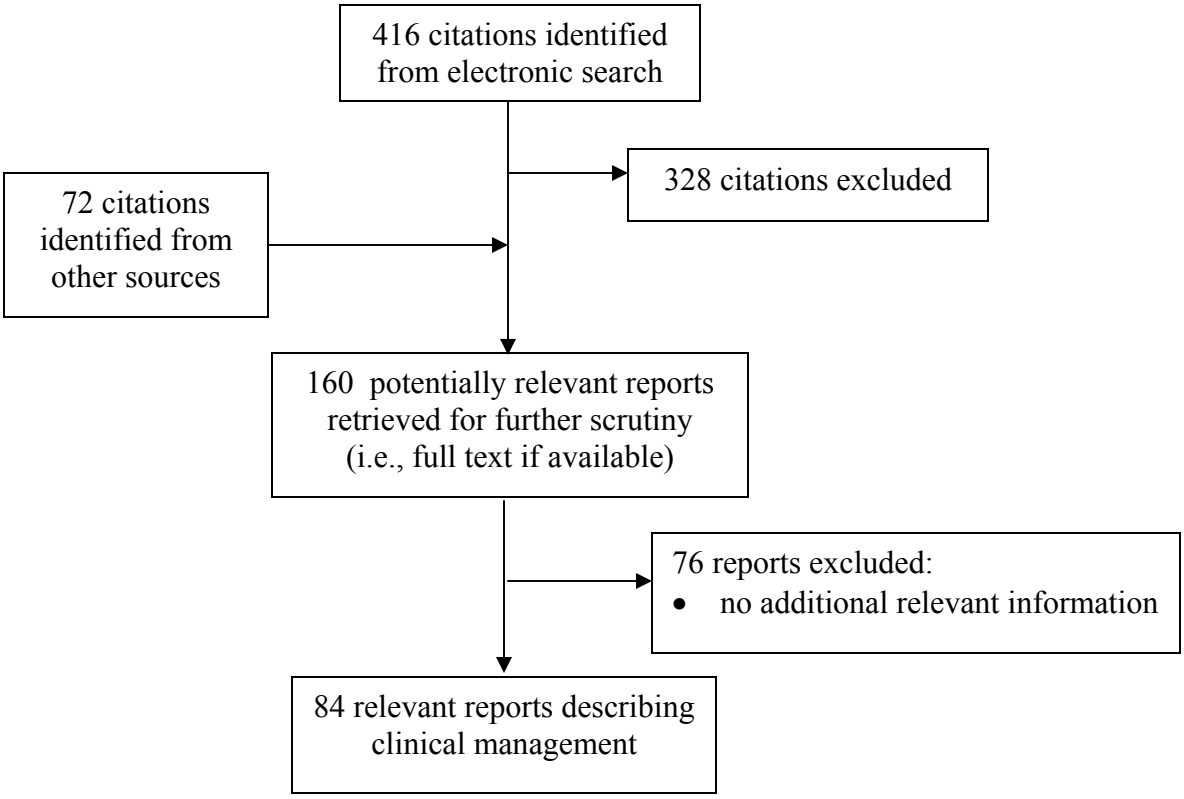


Table 4: Evaluative framework for selected studies on clinical management

Study Design	Details
1. Randomized controlled trial of population impact	Women prospectively randomized to a CMP that includes *BRCA1/2* testing or one that does not, and five to 10 year follow-up to determine impact of testing on morbidity, mortality and quality of life; stratified according to baseline risk category and age; follow-up of entire cohort, including those who refuse testing and those who are *BRCA1/2* negative after testing
2. Non-randomized, controlled (concurrent or historical) trial	Prospective observational study of all women in a country (or province) that includes *BRCA1/2* testing compared with matched control population from a jurisdiction without testing
3. Prospective, uncontrolled, observational cohort trial	Patients with a high risk of breast or ovarian cancer enrolled in a CMP that includes *BRCA1/2* counselling and testing, and followed to determine impact of *BRCA1/2* testing on service utilization and cost; follow-up of entire cohort, including those who refuse testing and those who are *BRCA1/2* negative after testing; patients diagnosed with breast or ovarian cancer, usually from cancer treatment programs with *BRCA1/2* status determined prospectively or retrospectively, and follow-up as above
4. Retrospective, uncontrolled, observational case-control trial	Patients diagnosed with breast or ovarian cancer stratified by *BRCA1/2* status or patients with *BRCA1/2* mutation stratified as to primary (i.e., asymptomatic mutation carrier) or secondary (i.e., breast or ovarian cancer)

CMP=Clinical Management Program

A retrospective study examined the efficacy of bilateral prophylactic mastectomy in a case-control sample drawn from an historical cohort.[118] Women with germline disease associated *BRCA1/2* mutations were identified from 11 North American and European institutions. Researchers compared women undergoing mastectomy to matched controls (i.e., matched for *BRCA1/2* status, age, and institution) without mastectomy or breast cancer at the time of surgery for the matched subjects. The mean age at time of surgery for all subjects was 38.1 years. Follow-up began at a mean age of 38.1 years for women undergoing surgery versus 36.3 years for controls and continued for 5.5 years (surgery) and 6.7 (controls). Of the 105 mastectomy cases reported, two subjects (1.9%) were diagnosed with breast cancer after surgery compared with 184 (48.7%) of controls. Other authors have raised concerns regarding the relative incidence of breast cancer in the control group of this study.[119] The original study authors selected controls who did not have cancer at the time that their matched cases had surgery, but who could have had cancer at the time that they visited the clinic. Potential bias could be reduced by considering a prospective cohort from the time of the first clinic visit as controls could still visit the clinic if a suspicious lesion proves to be cancerous.

The benefit of prophylactic mastectomy is supported by a retrospective study of 639 women at moderate and high risk of breast cancer.[120] The authors did not report *BRCA1/2* status. For women considered to be high risk (n=214) based on family history criteria for first- and second-degree relatives, three cases (1.4%) had a diagnosis of breast cancer. The median age of the high risk women was 42 years including 13% nulliparous and median age of first birth equal to 21 years. Results were compared with 430 sisters who had not undergone prophylactic mastectomy in which 156 (39%) had a diagnosis of breast cancer. The result was a 90% to 94% reduction in the risk of breast cancer (95% CI: 0.70 to 0.98). In a subsequent publication by the same researchers, blood samples from 176 of 214 high risk women who underwent prophylactic surgery showed that 26 had alterations in *BRCA1* and *BRCA2,* and none of the women developed breast cancer after a median follow up of 13.4 years.[121]

While consistent data on the magnitude of risk reduction provided by prophylactic mastectomy for prevention of breast cancer are starting to accumulate, there remains a paucity of data regarding the magnitude of mortality reduction, proper timing of surgery, and use of adjunctive hormones or chemotherapy. Randomized trials, while ideal for the determination of the magnitude of benefit, are unfeasible as women are unlikely to accept randomization to a prophylactic mastectomy group. Prospective studies, while reducing selection or survival bias, will require several years to complete. Cohort studies risk bias as a result of confounding by indication and competing events. Confounding by indication could affect results if the reasons for undergoing mastectomy are related to the risk of breast cancer. Competing events, especially ovarian cancer, could also affect the characteristics of the sample.

Ongoing international prospective studies may provide answers to these key morbidity and mortality questions, for example, The International *BRCA1/2* Carrier Cohort Study: (IBCCS) (www-gep.iarc.fr/ , or in Goldgar *et al.* 2000).[122]

In addition, databases are being established to support research activities. For example, a multinational collaboration has established an infrastructure for studying the genetic epidemiology of familial breast cancer.[123] As of September 2003, 9,116 population-based and

2,834 clinic-based families have been enrolled. Data include epidemiological questionnaires for affected probands and relatives with or without a personal history of breast or ovarian cancer and a biospecimen repository that contains blood or mouthwash samples.

Oophorectomy: The strongest evidence for a benefit of prophylactic salpingo-oophorectomy is reported in a prospective study of 170 *BRCA1/2* mutation carriers comparing surgery and surveillance over a two-year period.[124] The mean age of women in each group at the time of genetic testing was 47.5 (surgery) and 45.5 (surveillance) years. *BRCA1* mutations were present in 57% (surgery) and 67% (surveillance) of the women. Of these, 70% (surgery) versus 68% (surveillance) had a history of breast cancer. Ovarian or peritoneal cancer developed in 6.9% (five of 72) of women who elected surveillance. In the 98 women who underwent prophylactic salpingo-oophorectomy, three had early stage tumours discovered at the time of surgery (3.1%) and one patient developed peritoneal cancer (1%). For women who did not undergo prophylactic mastectomy, breast cancer developed in 12.9% (eight of 62) in the surveillance group and 4.3% (three of 69) women in the surgery group. The hazard ratio for subsequent breast cancer or *BRCA*-related gynecological cancer in the salpingo-oophorectomy group was 0.25 (95% CI: 0.08 to 0.74).

In a retrospective study of prophylactic oophorectomy in *BRCA1/2* mutation carriers,[125] a benefit was demonstrated by reductions in ovarian and breast cancer. These results support those of an earlier publication based on results from the initial patients in this study.[126] The mean ages of women at the time of surgery were 42 (surgery) and 41 (controls) years. *BRCA1* mutations were present in 85% (surgery) and 82% (controls) of the women. More women in the surgery group (48%) versus controls (20%) used hormone therapy at any time (p<001). A total of 3.1% of women undergoing prophylactic oophorectomy were diagnosed as having cancer at the time of surgery or during the eight-year follow-up compared with 19.9% of women who did not undergo surgery. The hazard ratio is 0.04 (95%CI: 0.01 to 0.16). In those women who underwent surgery, breast cancer developed in 21% versus 42% in the control group (hazard ratio 0.47; 95% CI: 0.29 to 0.77).

The number of cases of fallopian tube cancer reported in different studies of *BRCA1/2* mutation carriers has lead some authors to recommend bilateral salpingo-oophorectomy for surgical prophylaxis.[127,128] This recommendation is supported by a retrospective study by Olivier *et al.*[129] The authors report that of the 38 women who underwent bilateral oophorectomy (mean follow-up 45 months), three of 26 *BRCA1* mutation carriers developed peritoneal papillary serous carcinoma. Of the 58 *BRCA1* mutation carriers who underwent salpingo-oophorectomy (mean follow-up 12 months), no cases of peritoneal papillary serous carcinoma have occurred. The authors note that the difference in papillary carcinoma could be explained by the difference in the duration of follow-up.

Utilization: The utilization of prophylactic surgery varies significantly among countries where it is offered. A Dutch study showed that in a sample of unaffected women with an identified mutation, 51% opted for bilateral mastectomy and 64% for oophorectomy.[130] This rate of uptake is similar to that reported in other European test centres,[131] but higher than in US test centres.[132] A Canadian study reported that of 263 Ontario women who underwent prophylactic

oophorectomy between 1992 and 1998, 16 women had *BRCA1/2* mutations.[133] It was impossible to determine the acceptance rate, as the authors did not report the total number of women with *BRCA1/2* mutations who were offered surgery.

A population-based study of 357 multiple-case breast cancer families from Australia reports a low use of prophylactic mastectomy.[134] They report 49 of 2,107 women (2%) underwent prophylactic mastectomy (21% or 43% were *BRCA1* or *BRCA2* mutation positive). This observational cohort includes all of the Australian population and indicates the relative size of the high risk population versus the more limited number relevant to genetic testing and willing to undergo prophylactic mastectomy.

In an American setting, Schwartz *et al.*[135] reported that among 289 high risk women who underwent testing for *BRCA1/2* genes, 27% of the 79 mutation carriers versus 2% of non-carriers received a bilateral prophylactic oophorectomy in the year after testing.

In Poland, Menkiszak *et al.*[136] reported that among 72 women over the age 40 who carried a *BRCA1* mutation, 43 (60%) women had undergone prophylactic oophorectomy after a mean follow-up of 19 months.[136]

Chemopreventive therapy: Chemopreventive studies investigated the role of endocrine intervention in healthy women (i.e., non-*BRCA1/2* mutation carriers) at an increased risk of breast cancer.[137] These studies have primarily investigated the antiestrogenic drug, tamoxifen. In the US, tamoxifen is licensed for use in the prevention of primary breast cancer, although this role is controversial.[138] Studies have so far examined the effects of tamoxifen on the incidence of breast cancer and not its impact on overall breast cancer mortality. Conflicting evidence exists as to whether endocrine intervention, particularly with tamoxifen, is as effective in reducing the incidence of breast cancer in *BRCA1/2* mutation carriers compared with other women who are at increased risk of breast cancer.[139] One concern is that approximately 80% of *BRCA1*-related breast cancers are estrogen-receptor negative, putting into doubt the efficacy of tamoxifen, because its mechanism of action is to target estrogen receptors.[138-140]

The Breast Cancer Prevention trial, a randomized double-blind study of 13,338 high risk but cancer-free women (i.e., including those with *BRCA1/2* mutations), compared tamoxifen and placebo over a five-year period.[141] In a post-hoc analysis of the data, it was found that of 288 breast cancer cases, 19 (6.6%) had *BRCA1/2* mutations. In 11 patients with *BRCA2* mutations, three had received tamoxifen and eight placebo, resulting in a risk ratio of 0.38 (95% CI: to 0.06 to 1.56). In eight patients with *BRCA1* mutations, five had received tamoxifen and three placebo, resulting in a risk ratio of 1.67 (95% CI: 0.32 to 10.70). Tamoxifen reduced the incidence of breast cancer among estrogen receptor-positive *BRCA2* mutation carriers by 62%, similar to the reduction observed among estrogen receptor positive breast cancer in all women in the Breast Cancer Prevention Trial. The differences favouring tamoxifen were not statistically significant. Because of the potential for harmful effects associated with its use, and the lack of statistically and clinically significant morbidity and mortality data, the overall health benefit of tamoxifen has not been established for healthy women or for younger women with *BRCA1/2* mutations.

In a case control study by the Hereditary Breast Cancer Clinical Study Group,[142] the authors report that tamoxifen resulted in a 50% reduction in the risk of bilateral breast cancer among women with *BRCA1* and *BRCA2* mutations. This study involved 209 carriers of *BRCA* mutations who had bilateral breast cancer and 384 *BRCA1/2* heterozygotes with unilateral breast cancer. The protective effect of tamoxifen was greater in carriers of *BRCA1* mutations (OR 0.38, CI: 0.19 to 0.74) compared to *BRCA2* mutations (OR 0.63, CI:0.20 to 1.5). These results seem to be contradictory to the fact that *BRCA*- associated tumours are more estrogen-receptor negative than positive.[143] Foulkes *et al.* using a similar case-control method, albeit on a smaller sample, also reported that the estrogen receptor negative status of breast cancers (that occur in *BRCA1* mutation carriers) may not have the same effect on the response to tamoxifen as it does for women with breast cancer in the general population.[144]

Both these case control studies lend evidence to the recognized reduction in breast cancer risk in women with *BRCA1/2* mutation carriers by blocking the activity of endogenous estrogens.[142,144] However, prospective studies are needed to determine if the observed differences in the risk of contra-lateral breast cancer are due to tamoxifen or systematic differences in the cases with bilateral breast cancer and controls.

Early cancer detection (surveillance) programs: Women who are *BRCA1* mutation carriers are advised to undergo earlier screening for breast and ovarian cancer (i.e., compared with the unaffected population). Those with *BRCA2* mutations are advised to seek early breast but not ovarian cancer screening.[25] Early screening is not recommended for other cancers associated with *BRCA1* (e.g., prostate or colon cancer), despite the increased relative risk among these individuals.[47] Large randomized trials of older women have shown that breast cancer screening in the non-*BRCA1/2* mutation carrying population reduces morbidity or mortality risk due to earlier diagnosis and effective therapy. In the absence of such trials to address early screening and intervention in young *BRCA1/2* carriers, the results from studies in other populations must be applied, although their relevance remains debatable. Thompson *et al.* determined the risk of other cancers in *BRCA1* mutation carriers in a cohort study of 11,847 individuals from 699 families segregating a *BRCA1* mutation.[145] *BRCA1* mutation carriers were at a statistically significant increased risk for several cancers, including pancreatic cancer (RR=2.26, 95% CI=1.26 to 4.06) and cancer of the uterine body and cervix (uterine body RR=2.65, 95% CI =1.69 to 4.16 and cervix RR=3.72, 95% CI=2.26 to 6.10. The overall increased risk in cancer is unseen in men.[145]

Breast cancer surveillance

Mammography: Mammographic screening for women over age 50 is supported by randomized trials that show a reduction in mortality in this age group.[146] *BRCA1/2* mutation carriers are at high risk for early onset breast cancer, and over half of the risk is known to occur at an age before the start of most routinely recommended screening programs (i.e., 50 years). This risk increases the likelihood that mammography screening provides benefit, although there are no data to support this view.[47] Any potential benefits of early mammography screening must be weighed against issues associated with false positive results and exposure of breast tissue to radiation. It is unknown whether tumours in *BRCA1/2* mutation carriers have different sensitivities to DNA-damaging agents, such as radiation.[47] One study explored the relative merits of annual mammography and a semi-annual physical examination among *BRCA1/2* positive and

negative women.[147] In a combined retrospective and prospective study of 621 high risk and 128 *BRCA1/2* mutation carriers (mean age 38 years), researchers found that surveillance detected a greater percentage of cancer in the *BRCA1/2* mutation carriers (33 per 1,000 person years; 95% CI: 17 to 63) than in high risk non-carrier women [8.4 per 1,000 person years (95% CI: 5.4 to 13.2)].[147] The importance of these findings is unknown, because the surveillance findings were not linked to subsequent treatment or health outcome.

One study of *BRCA* mutation carriers retrospectively reviewed the charts of all mutation carriers who were followed by a centre in New York between 1995 and 2002.[148] They reported that of the 13 patients who chose to undergo close surveillance at their institution, three patients did not develop breast cancer, four developed breast cancer detected at the time of annual screening, and six developed palpable interval malignancies in less than 12 months (a mean of five months after a normal screening result). While acknowledging the small sample size, they recommend considering more frequent screening of *BRCA1/2* mutation carriers. [148]

Magnetic resonance imaging (MRI): Several studies confirmed earlier and preliminary studies[149-152] suggesting the superior sensitivity of MRI screening versus mammography and clinical breast examination in *BRCA1/2* mutation carriers and women of unknown mutation status from high risk families. Hartman *et al.*[153] conducted a pilot screening study of clinical breast examination, mammography, breast MRI, and ductal lavage in 41 women at high risk of breast cancer. Twenty-four women (58.5%) were positive for a *BRCA1* or *BRCA2* mutation. The median age of patients was 42 years. Twelve patients (29%) had a history of breast carcinoma, and three patients (7%) had a history of ovarian carcinoma. Eleven patients (27%) had undergone bilateral salpingo-oophorectomy. Abnormal MRIs were seen in 25 (60%) patients, with 14 recommended to have repeat MRI within six months and 11 to have a biopsy. Four of 41 patients (10%) had ductal carcinoma in situ or high risk findings, including atypical lobular hyperplasia and radial scars. At the time of reporting, 16 women had received a follow up MRI, resulting in no biopsies. Trecate *et al.*[154] reported similar high sensitivity for MRI screening versus mammography among 23 women with *BRCA* mutations or at high risk for *BRCA1/2* mutations. MRI identified four breast cancers among women found negative on mammography. In this instance, 17 of 23 MRI examinations conducted were considered to be negative.

In the largest study to date, Kriege *et al.*[155] prospectively screened 1,909 women, 358 of whom were germ-line mutation positive (*BRCA1*, *BRCA2*, PTEN, and TP53 mutations) with biannual breast self-examination and annual MRI and mammography. After a mean follow up of 2.9 years, 19 breast cancer cases (16 invasive) were detected in the mutation carrier group. The authors do not report sensitivity or specificity for the genetic mutation carrier group. The overall sensitivity of clinical breast examination, mammography, and MRI for detection of invasive breast cancer was 17.9%, 33.3%, and 79.5% respectively, and the specificity was 98.1%, 95.0%, and 89.8% respectively. Of the 32 cancers detected by MRI in the total screened population, 22 of these were invisible on mammography. MRI missed 13 cancers, eight of which were visible on mammography. This suggests that there is value in using multiple concurrent screening modalities. Screening by MRI led to twice as many unneeded additional examinations as did mammography (420 versus 207), and three times as many biopsies (24 versus seven). The study is limited, because a "gold standard" test for early detection of breast cancer is unavailable.

Instead, the authors calculated sensitivity by comparing one screening method versus the others, meaning that a test result is a false negative when a proven cancer (based on histology) is detected in the interval or by one or the other methods. The study is of insufficient length to determine accurate sensitivity (true false negative rates) or the impact of the program on morbidity or mortality.

Warmer *et al.* studied 236 women between the ages of 25 and 65 years (mean age 46), all of whom had *BRCA1* and *BRCA2* mutations (30% with a history of breast cancer) for one to three years of annual screening examinations with MRI, mammography or ultrasound, and clinical breast examination biannually. A total of 22 cancers were detected (16 invasive and six carcinoma in situ). Of these, 17 (77%) were detected by MRI versus eight (36%) by mammography, seven (33%) by ultrasound, and two (9.1%) by CBE. The sensitivity and specificity (based on biopsy rates) were 77% and 95.4% for MRI, 36%, and 99.8% for mammography; 33% and 96% for ultrasound; and 9.1% and 99.3% for CBE. This analysis remains limited, similar to Kriege,[155] because sensitivity was defined as "the number of cancers detected by a given modality (or combination of modalities) divided by the total number of cancers detected by all four modalities plus interval cancers during the three year study period." Specificity was defined as "the number of true-negative divided by the sum of true-negative results and false-positive results (i.e., examinations leading to a negative biopsy)."

The MARIBS[156] study group in the UK found a similar sensitivity and specificity of annual MRI and mammography in a prospective study of 649 women between the ages of 35 and 49 years (median age 40) at high risk of developing breast cancer (82% or 13% *BRCA1* and 36% or 6% *BRCA2*).[156] They diagnosed 35 cancers in the 649 screened women, 19 by MRI only, six by mammography only, and eight by both, with two interval cancers. Sensitivity was significantly higher for MRI (77%, 95% CI: 60 to 90, p=0.01) than for mammography (40%, 95% CI: 24 to 58) and was 94% (95% CI: 81 to 99) when both methods were used. Specificity was 93% (95% CI: 92 to 95) for mammography, 81% (95% CI: 80 to 83) for MRI (p<0001), and 77% (95% CI: 75 to 79) for both methods. The difference between MRI and mammography sensitivities was pronounced in *BRCA1* carriers (13 cancers; 92% versus 23% respectively, p=0.004).[156]

Lehman *et al.*[157] conducted a larger, more recent study showing the superior sensitivity of screening MRI versus mammography. This study does not specify the genetic testing status of the population.

Patients have not been followed to determine the false negative rate for MRI. The authors appropriately considered these findings as preliminary in terms of MRI as a screening modality. They conclude that a large, multicentre trial is needed to determine the MRI interpretation criteria for screening high risk women.

Breast Self Examination (BSE): The impact of teaching breast self-awareness has not been studied in the *BRCA1/2* population. It is taught and practised in many breast clinics that manage *BRCA1/2* mutation carriers.

Observational studies in the general population have shown that BSE downstages nodal status in tumours at presentation, but that it does not reduce mortality.[158] Two meta-analyses[159,160]

concluded that, based on two randomized trials of the general population (totalling 388,535 women), BSE has no mortality benefit versus no intervention. Almost twice as many biopsies (3,406) with benign results were performed in the screening group compared to the control group (1,856) (RR=1.88 95% CI 1.77 to 1.99). There are no randomized trials of clinical breast examination for the general population or for the *BRCA1/2* population.

Ultrasound: Warner *et al.*[150] studied ultrasound, MRI, mammography, and clinical breast examination in 236 women with *BRCA1* and *BRCA2* mutations for one to three years. A total of 22 cancers were detected (16 invasive and six carcinoma in situ). Of these, 17 (77%) were detected by MRI, eight (36%) by mammography, seven (33%) by ultrasound, and two (9.1%) by CBE. The sensitivity and specificity (based on biopsy rates) were 77% and 95.4% for MRI, 36% and 99.8% for mammography, 33% and 96% for ultrasound, and 9.1% and 99.3% for CBE. The study is limited because a "gold standard" test for early detection of breast cancer is unavailable. Instead, the authors calculated sensitivity by comparing one screening method versus the others, meaning that a test result is a false negative when a proven cancer (based on histology) is detected in the interval or by one of the other methods. The study is of insufficient length to determine accurate sensitivity (true false negative rates) or the impact of the program on morbidity or mortality.

Simmons notes the utility of ultrasound in assisting with diagnosis using biopsy but raises the issue of who should receive ultrasound screening in addition to traditional mammography. The problem she highlights is that the high sensitivity of ultrasound results in unnecessary biopsies of many non-cancerous lesions.[161]

Compliance: Compliance with breast cancer surveillance recommendations was assessed in one prospective, uncontrolled, observational study of 251 women.[162] It was found that, subsequent to genetic counselling and testing positive for *BRCA1/2* mutations, the frequency of cancer surveillance (i.e. physical examinations and imaging studies) was significantly increased.[162] Similar results were reported in another study where *BRCA1/2* positive women had significantly higher rates of mammograms (68%) compared to high risk non-mutation carriers (44%) at one year after genetic testing.[163] The authors note that the adherence rate in mutation carriers was the same as that before testing, thereby suggesting that the relative difference is due to non-adherence in non-mutation carriers.

Ovarian cancer surveillance: In contrast to breast cancer screening, no controlled trial evidence exists to support the benefits of any screening technique for ovarian cancer, although there are trials underway in the general population to investigate this.[25] In practice, annual or semi-annual screening of *BRCA1* carriers using TVU is often started between 25 and 35 years of age. To date, TVU is the most effective modality for the detection of ovarian cancer.[47] Surveillance is also an option for *BRCA2* mutation carriers, but their lower risk reduces the likelihood of benefit.[164] The use of TVU in *BRCA1/2* mutation carriers is more controversial than the use of mammography, because ovarian screening in high risk women has not been shown to reduce mortality. In addition, the use of tumour markers such as Ca125 in *BRCA1/2* mutation carriers is based on little supporting data. It is recommended that testing for Ca125 be done annually beginning at age 25 to 35 years; this is based only on expert opinion.[25]

Jacobs *et al.*[165] reviewed tumour markers that may have a role in ovarian carcinoma and highlighted prospective trials underway to test strategies that screen high risk, usually younger, women. Screening younger women is problematic because of physiological (menstrual cycle variations) and benign conditions (endometriosis, ovarian cysts). They offer optimism for earlier detection of ovarian cancer based on advancements in proteome analysis of human serum. Mor *et al.*[166] for example, report encouraging findings using a multiplex of serum proteins to distinguish patients with epithelial ovarian cancer and controls, including stage I and stage II disease. The authors note that the serum markers are not specific for ovarian cancer.[166]

Management of affected *BRCA1/2* mutation carriers

The management of *BRCA1/2* mutation carriers affected with cancer is based on such features as tumour pathology, survival differences, radiosensitivity, chemosensitivy, and screening for second primary cancers.

Two studies documented that *BRCA* testing at the time of breast cancer diagnosis had a significant impact on subsequent surgical decision making.[167,168] In one series,[167] all patients with a *BRCA* mutation (N=7) opted for bilateral mastectomy, whereas 20 of 22 patients with negative test results chose stage-appropriate treatment. In the second series,[168] 48% of women who were found to have a *BRCA1/2* mutation chose bilateral mastectomy as their definitive breast cancer surgery. In contrast, 24% of women at 10% *a priori* risk of breast cancer, but who tested negative for *BRCA1/2* mutations, opted for bilateral mastectomy.

A prospective study from the Netherlands reported the use of *BRCA1/2* testing in all women with a primary breast or ovarian cancer from a consecutive series of 112 high risk families in which *BRCA1/2* was eventually identified.[169] A total of 192 of 220 (87%) underwent genetic testing. Among eligible women, 35 of 101 (35%) requested bilateral or contralateral mastectomy, and 47 of 95 (49%) requested oophorectomy.

Tumour pathology and impact on survival: Breast tumours in *BRCA1* mutation carriers tend to be of higher grade, have a higher proportion of atypical medullary cancer, have a lower proportion of carcinoma in situ, and are estrogen-receptor negative as opposed to those in non-mutation carriers. Cancers associated with *BRCA1* mutations tend to have higher mitotic counts, a greater proportion of the tumour with a continuous pushing margin, and more lymphocytic infiltration.[170-173] Breast tumours in *BRCA2* mutation carriers exhibit a higher score for tubule formation (i.e., fewer tubules), a higher proportion of the tumour perimeter with a continuous pushing margin, and a lower mitotic count than control cancers.[172] Despite these poor prognostic features, survival studies of *BRCA1/2* mutation carriers have provided conflicting results. In one study, 49 Dutch patients with *BRCA1* mutations were compared with 196 patients with sporadic cancer.[173] Disease-free survival at five years was 49% (95% CI: 33 to 64) for *BRCA1* and 51% (95% CI: 43 to 59) for sporadic patients (p=0.98). The overall survival at five years was 63% (95% CI: 47 to 76) and 69% (95% CI: 62 to 96) respectively for *BRCA1* and sporadic cancer patients respectively (p=0.88).[171]

A study of 278 women over 10 years demonstrated a trend toward a worse prognosis for *BRCA1* mutation carriers in whom tumours over-express the tumour suppressor gene, p53.[174] A retrospective analysis that combined this study with another cohort of Ashkenazi Jewish women

undergoing breast conservation surgery for invasive cancer concluded that *BRCA1*, but not *BRCA2* mutations, are associated with decreased survival.[175] At a mean of 116 months, breast cancer specific survival rate was worse in 91 women with *BRCA1/2* mutations than those without (i.e. 62% versus 86% respectively at 10 years; $p<0.05$). The poor prognosis associated with *BRCA1* mutation status may be mitigated by adjuvant chemotherapy. In contrast, a retrospective analysis of 92 women (including 30 women with breast cancer and *BRCA1/2* mutations) who developed breast cancer before age 42 found no difference between *BRCA* and non-*BRCA* associated cancers in five-year relapse-free survival or overall survival.[176] Results were 65% (*BRCA*) versus 69% (non-*BRCA*) for relapse-free survival at five years. The conclusions of this study are weakened, because of the inclusion of prevalent cases that likely include a prognostic factor positively affecting short-term outcome. Such cases may bias the result. Patients are in favour of a good outcome due to good prognostic factors enabling the prevalent cases to survive and be available for study. A similar finding was reported in a Swedish study of 71 *BRCA1* associated breast or ovarian cancer patients compared with a population-based cohort (n=7,011) of all other invasive cancers.[177] It was reported that survival in *BRCA1*-associated breast or ovarian cancers was similar to, or worse than, sporadic cancer (i.e., disease that is apparently not hereditary) survival, although differences were not statistically significant.[177] Lastly, a retrospective cohort study from France reported that in 40 patients with *BRCA1* associated breast cancer among 183 patients with invasive breast cancer, overall survival was worse for the *BRCA1* group at a median follow-up of 58 months. At five years, the rates were 80% versus 91% for *BRCA1* mutation carriers and non-mutation carriers respectively ($p=0.002$). When the authors limited the analysis to 110 patients whose diagnosis to counselling interval was less than 36 months, the overall survival difference increased (i.e., 49% for mutation carriers versus 85% for non-carriers).[178]

Lakhani *et al.*[143] studied the immunohistochemical profiles of tumours arising in patients with *BRCA1/ 2* gene mutations. They conclude that *BRCA1* mutations have a distinct morphology and immunohisotochemistry phenotype that can be used to predict the risk of a young person harbouring this germline mutation.

Lakhani *et al.* also studied the pathological features of ovarian cancers in 178 *BRCA1* and 29 *BRCA2* carriers, and 235 controls.[179] Tumours in *BRCA1* mutation carriers were more likely than tumours in age-matched controls to be invasive serous adenocarcinomas (odds ratio 1.84 95% CI:1.21 to 2.79). Tumours in *BRCA1* carriers were of a higher grade ($p<0.0001$), had a higher percentage solid component ($p=0.001$), and were more likely to stain strongly for p53 ($p=0.018$). The distribution of pathological features in *BRCA2* carriers was similar to that in *BRCA1* carriers. The authors suggest that the use of pathological features may improve the targeting of predictive genetic testing.

Radiotherapy: One selected study compared 71 women with *BRCA1/2* mutations and breast cancer (i.e., stage I or II) who received breast-conserving therapy, with 213 women with sporadic cancer. There was no evidence of any adverse effects of radiotherapy (i.e., in skin, subcutaneous tissue, lung, or bone) in women with *BRCA1/2* mutations compared with sporadic cases.[117,121,124,125,180]

Risk of developing second primary cancers: The risk of a second primary breast cancer after the first in women is 64%, and the lifetime risk in *BRCA1/2* mutations carriers is 56%.[13] An

observational study of 164 patients reports an association between the age at diagnosis of the first *BRCA1*-associated breast cancer and the risk of developing cancer in the contralateral breast at 10 years follow-up.[181] It was found that 40% of 124 with *BRCA1* mutations patients diagnosed with breast cancer before age 50 had developed contralateral breast cancer versus 12% of patients over age 50 at first diagnosis (p=0.02).

Metcalfe *et al.*[182] looked at the risk of developing contralateral breast cancer using a retrospective chart review of 491 women with stage I or stage II breast cancer (mean age 41 at the time of diagnosis) for whom *BRCA1* (327 of 491) or *BRCA2* (152 of 491) mutation had been identified in the family. They do not report how many individual women were *BRCA1* or *BRCA2* positive in the study cohort. The subjects were treated with breast-conserving therapy (39%) or with unilateral mastectomy (52%) or bilateral mastectomy (9%). One hundred and six women had contralateral preventive mastectomy at various times after initial surgery. After a mean follow up of 9.2 years, one contralateral breast cancer (in the chest wall) occurred among the 146 women treated with bilateral, prior, or delayed contralateral mastectomy. In contrast, 97 contralateral breast cancers occurred among the 336 women who retained the contralateral breast (HR, 0.03 p=0.0005). Significant risk factors included presence of *BRCA2* versus *BRCA1* mutations (hazard ratio 0.73 95% CI 0.47 to 1.15), age 50 years and older (≤49 HR, 0.63 95% CI: 0.36 to 1.10) use of tamoxifen (HR, 0.59 95% CI: 0.35 to 1.01) and history of oophorectomy (HR, 0.44 95% CI: 0.21 to 0.91). They concluded that the risk of contralateral breast cancer is about 4% per year or 40% over 10 years.[182]

Using the same observational cohort, Metcalfe *et al.*[183] estimated the risk of ovarian cancer after breast cancer. At the time of breast cancer diagnosis, 42 women had a bilateral oophorectomy, and were eliminated from the study cohort. After diagnosis with breast cancer, 40 women (8.9%) developed ovarian cancer after a mean of 8.1 years. The 10-year actuarial risk of ovarian cancer was 12.7% for *BRCA1* mutation carriers and 6.8% for *BRCA2* mutation carriers. Neither tamoxifen nor chemotherapy use had a significant impact on the risk of ovarian cancer. The authors note that 25% of women with stage I breast cancer died of ovarian cancer.[183]

A US study examined *BRCA1/2* status among 52 breast cancer patients who had undergone lumpectomy and radiation, and subsequently developed ipsilateral breast tumour recurrence.[184] These patients were compared with the same number of breast cancer patients treated with lumpectomy and radiation who did not develop ipsilateral recurrence. *BRCA1/2* mutations were found in eight of 52 patients with ipsilateral recurrence, and six of 15 patients with recurrence under age 40. In the under age-40 group, one of 15 matched control patients without ipsilateral recurrence had a *BRCA1/2* mutation. It was concluded that because of the time to recurrence (median time 7.8 years) and the histological features, these constituted new primary tumours occurring in the same breast.

Weitzel *et al.* studied the degree of concordance with bilateral breast cancer, using a retrospective review of 286 women with *BRCA1* (211) and *BRCA2* (75) mutations.[185] The mean interval between first and second tumours was 5.1 years. The tumours were found concordant for estrogen receptor status and grade, but not for histology. Age, menopausal status, oophorectomy, and tamoxifen use were not predictive of the estrogen status of the second tumour. The authors were not conclusive as to whether the tumours reflected a preneoplastic lesion common to both mutation types.

Drug therapy: There was little evidence found among selected articles pertaining to chemoprophylaxis among *BRCA1/2* mutation carriers with cancer. In a case-control study, tamoxifen used up to four years was found to have a statistically significant protective effect on the risk of contralateral breast cancer for *BRCA1* mutation carriers [odds ratio 0.38 (95% CI: 0.19 to 0.74)].[142] The study also recorded a non-significant increase in risk of contralateral breast cancer in the multivariate analysis for *BRCA1* mutation carriers who had used tamoxifen for over four years [odds ratio 1.53 (95% CI: 0.44 to 5.27)].

One study provided early supportive evidence for the use of the antineoplasic drug docetaxel, by studying 25 *BRCA1/2* mutation positive women with locally advanced (13) or locally recurrent (12) breast tumours.[186] It was concluded that low *BRCA2* mRNA levels predict a good response to docetaxel for the treatment of breast cancer patients.[186] A Canadian study (Montréal) provided retrospective data on the initial responses to neoadjuvant (i.e., preoperative) chemotherapy for hereditary breast cancer patients.[187] Subjects included *BRCA1* (n=7) and *BRCA2* (n=4) mutation carriers and 27 non-carriers with breast cancer who received neoadjuvant treatment. Patients were matched for age, tumour grade, and estrogen receptor status. After three to four chemotherapy cycles, a complete clinical response was noted in 93% (10 of 11) *BRCA1/2* carriers compared with 30% (eight of 27) of non-carriers (p=0.0009). A complete pathological response was noted in 44% (four of 9) of *BRCA1/2* patients evaluated versus 4% (one of 27) of non-carriers (p=0.009). This difference diminished when the cases and controls were matched for tumour stage and grade. As noted in the study, these are preliminary but encouraging findings and may be subject to bias because of the retrospective case study design and the small number of patients selected using various diagnostic criteria. The response rate in carriers could have been overestimated if women receiving adjunctive therapy died before testing could be offered.

Models of *BRCA1/2* testing and treatment programs

Clinical management strategies that include *BRCA1/2* testing and treatment have been modelled and compared to strategies that exclude such testing. In doing so, there are key assumptions that must be made: the baseline population prevalence of *BRCA1* and *BRCA2* mutations, the proportion of women in the clinical cohort who accept *BRCA1/2* mutation testing, and the proportion of women who test positive and who undergo prophylactic surgery.

Cancer prevention models: One study considered clinical outcomes based on a simulated cohort of 30-year-old healthy women who tested positive for *BRCA1/2* mutations.[188] The extent (in years) that a woman could prolong her life beyond that associated with surveillance alone, was affected by the use of the following preventive measures at age 30: tamoxifen alone (1.8), prophylactic oophorectomy alone (2.6), tamoxifen and prophylactic oophorectomy (4.6), prophylactic mastectomy (3.5), and both prophylactic surgeries (4.9).

Prophylactic surgery models: An analysis selected for review reports that, based on the results of a Markov decision analytic model, prophylactic surgery at a young age improves the survival in women with *BRCA1/2* mutations.[189] Improvement in survival was found to be greater for prophylactic mastectomy than prophylactic oophorectomy. Authors of another analysis modelled different prophylactic surgical strategies for *BRCA1* mutation carriers from high and low cancer risk populations, beginning at age 30 years.[190] It was assumed that prophylactic mastectomy reduces the risk of breast cancer by 90%, and that prophylactic oophorectomy

reduced the risk of ovarian cancer by 95%. The authors report that for a woman at age 30, prophylactic mastectomy and oophorectomy are the most effective ways to increase life expectancy (i.e., by 11.7 years) if oophorectomy is performed before age 40. In the high risk group, they estimate that survival is increased by 9.5 years with prophylactic oophorectomy, and 4.9 years for prophylactic mastectomy.

A decision analytic model has also been applied to a specific population (i.e., Ashkenazi Jewish women).[191] The model examined the effects of genetic screening for three *BRCA1/2* mutations on survival and cost-effectiveness. It was assumed that the prevalence of *BRCA1/2* mutations is 2.5%, and that the associated cancer risks are breast cancer (56%) and ovarian cancer (16%). The sensitivity and specificity of the tests were assumed to be 98% and 99% respectively. It was also assumed that bilateral prophylactic oophorectomy would reduce ovarian cancer risk by 95%, and that bilateral prophylactic mastectomy would reduce breast cancer risk by 90%. The model predicted that beginning with women at age 30, genetic testing followed by any of the three prophylactic surgical strategies (oophorectomy, mastectomy or both surgeries) for women who tested positive, would improve survival by 11 days (95% CI: 3 to 8), 33 days (95% CI: 18 to 43), and 38 days (95% CI: 22 to 57) respectively. All three prophylactic strategies were found to be cost-effective compared with surveillance alone (e.g., genetic testing, physical examination, gynecologic examination, ultrasound, Ca125 measurement, mammogram, or Pap test).

Chemoprophylaxis models: One systematic review of trials of tamoxifen (given for at least three years) estimated that the prophylactic effect was a 13% reduction in the risk of breast cancer diagnosis in *BRCA1* mutation carriers, and a 27% reduction in *BRCA2* mutation carriers.[192]

Prophylactic surgery and chemoprophylaxis models: Other researchers have used decision analysis to examine the life expectancy of a woman 30 years of age with *BRCA1/2*-associated breast cancer, and facing decisions pertaining to secondary cancer prevention. Seven strategies were compared with surveillance alone including tamoxifen for five years, prophylactic oophorectomy, prophylactic contralateral mastectomy, and combinations of the aforementioned strategies. Findings were dependent on the penetrance of the *BRCA1/2* mutation, with the least benefit for women with low penetrance mutations. The results were that patients could expect to gain 0.4 to 1.3 years of life with tamoxifen compared to prophylactic oophorectomy (0.2 to 1.8 years), or prophylactic contralateral mastectomy (0.6 to 2.1 years).[193]

Hormone Replacement Therapy after Prophylactic Oophorectomy: Armstrong *et al.*[194] constructed a Markov decision analytic model to assess the expected outcomes of prophylactic oophorectomy with or without hormone replacement therapy in women with *BRCA1* and *BRCA2* mutations. The model is based on data about mutation carriers in which bilateral salpingoophorectomy results in approximately 90% and 50% reductions in the dominant risks of ovarian and breast cancers respectively. The effects of HRT were taken from large randomized studies in post menopausal women. The authors conclude that oophorectomy increased lifespan by between 3.34 and 4.65 years, irrespective of whether HRT was used after oophorectomy. They did not predict significant gain or loss in life years from HRT.[194] In an accompanying editorial, Garber *et al.*[195] criticize one assumption in the model by Armstrong, noting that Armstrong may be underestimating the maximum relative risk of HRT on breast cancer in women with

BRCA1/2.[195] They advise caution, noting that many aspects of HRT on breast cancer risk remain unknown.

General summary: The assumption behind screening high risk women for genetic cancer susceptibility is that, in those individuals affected, the cancers anticipated can be prevented or their negative health impact reduced by early diagnosis. In the case of *BRCA1/2* mutations, specific therapies to mediate against gene function deficits have not been developed, and current therapy primarily consists of surgical removal of the potentially cancerous tissue (i.e., breast, ovaries, and fallopian tubes).

Testing a cohort of women for *BRCA1/2* mutations at high risk of or diagnosed with cancer identifies a subset of women in terms of age of primary cancer diagnosis, recurrence rate, and perhaps prognosis.[176,177] In a cohort of such women, *BRCA1/2* mutation testing also labels some women as "negative" for genetic testing. This label can prove to be problematic for clinicians because of false negative findings for the mutations, and because it may provide women at high risk of cancer from other mutations or factors with false reassurance of their cancer risk, despite counselling. The clinical challenge is to encourage the latter individuals to comply with rigorous surveillance measures. Similar difficulties may arise if a genetic variant of unknown significance is found in a family.

The use of prophylactic surgery for *BRCA1/2* mutation-positive versus mutation-negative women illustrates a primary difference in the clinical management strategy. Prophylactic surgery, while logical in terms of pathogenesis, has not been nor is it likely to be subjected to a properly controlled study. Prophylactic mastectomy has remained controversial for three reasons: concern about the psychological effect of surgery, the fact that breast cancer does not develop in all carriers, and the public and professional belief that early detection of cancer, through intense surveillance, leads to morbidity and mortality benefit, and that cancer can be effectively treated. In contrast, prophylactic oophorectomy is more commonly recommended after childbearing, even though the risk of ovarian cancer in *BRCA1/2* mutation carriers is lower than that of breast cancer. This may be attributed to the absence of reliable methods for the early detection of ovarian cancer and the lethality of advanced stage disease. Most prospective and retrospective observational studies confirm the effectiveness of prophylactic surgery in preventing cancer occurrence.[196]

Additional difficulties arise when trying to conduct prospective cohort studies of women undergoing *BRCA1/2* testing. Because individuals decide to test their mutation status for a variety of reasons, it has been impossible to develop a cohort that will include all women undergoing *BRCA1/2* testing. Such studies also face an inherent bias because mutation detection must be performed on blood, so the women with cancer have to be alive to be tested. In light of this, the retrospective use of stored pathologic specimens obtained at the time of diagnosis may offer a more complete analysis of a patient population.[175]

d) Summary points for clinical management

- Information is provided on the clinical management of unaffected *BRCA1/2* mutation carriers (i.e., prophylactic surgery, chemopreventive therapy, and early cancer detection programs) and affected carriers (i.e., tumour pathology, impact on survival, radiotherapy, risk of developing second primary cancers, and drug therapy). In addition, models of *BRCA1/2* testing and treatment programs are discussed.

- Data regarding the influence of *BRCA1/2* mutation detection on clinical management are limited, partly because treatment options are limited. There are no gene or drug therapy substitutes for the missing gene products. The only options available are early detection of cancer through surveillance, or prophylaxis through surgery or drug therapy.
- Prophylactic surgery has been shown, in cohort studies, to reduce the risk of breast and ovarian cancer. For mastectomy, the strongest evidence from a prospective study shows that of 76 women who had prophylactic surgery, no one developed breast cancer after a mean follow-up of 2.9 years (this is too brief a follow-up period to establish serious morbidity and mortality effects). For oophorectomy, a prospective study of 170 women found that the risk of cancer was reduced from 6.9% to 3.1%, but the follow-up in these cases was limited to a mean of two years.
- Surveillance strategies or chemoprophylaxis have not been shown to offer significant effects on cancer risk.

5 ECONOMIC ANALYSIS

The financial and legal implications associated with genetic testing are important. An economic analysis was not conducted as part of this review, as other initiatives are underway in Canada to address these issues.

In Canada, AÉTMIS plan to present an economic analysis of *BRCA1/2* genetic testing as part of their upcoming monograph series. A Markov analysis is also underway to compare prophylactic strategies for *BRCA1/2*-positive and unknown mutation carrier status women (Julia Witt, University of Guelph, Guelph, ON: personal communication, 2005 April 05).

6 HEALTH SERVICES IMPACT

6.1 Subject Area III: Psychosocial Impact

6.1.1 Quantity of research available

The original electronic search strategy for subject area III identified 236 citations. The literature search update yielded 76 citations (Figure 3). Of the 236 studies identified in the original search and 76 identified in the update, 158 were retrieved for full-text review. The degree of agreement between reviewers was kappa=0.45 (for the original search) and kappa=0.50 for the updated search. There were 59 relevant articles that were used in the review of psychosocial issues for this report. It is unclear if the selected articles report on 59 unique and independent studies, as there was insufficient information to determine whether there was an overlap in study populations.

6.1.2 Trial characteristics

Selected trials were primarily single-site, cross-sectional studies where testing for *BRCA1/2* was carried out in populations for which the mutation carrier status was unknown at the onset of the study. Most studies used selective sampling procedures in a clinical setting. Most trials studied US or Canadian populations, or a combination of both. The individual study characteristics and study

population characteristics can be found in Tables 5 and 7 in Appendix 7. A general summary of overall trial characteristics and corresponding numbers of articles is provided in Table 5.

Figure 3: Selected material for psychosocial impact (Subject Area III)

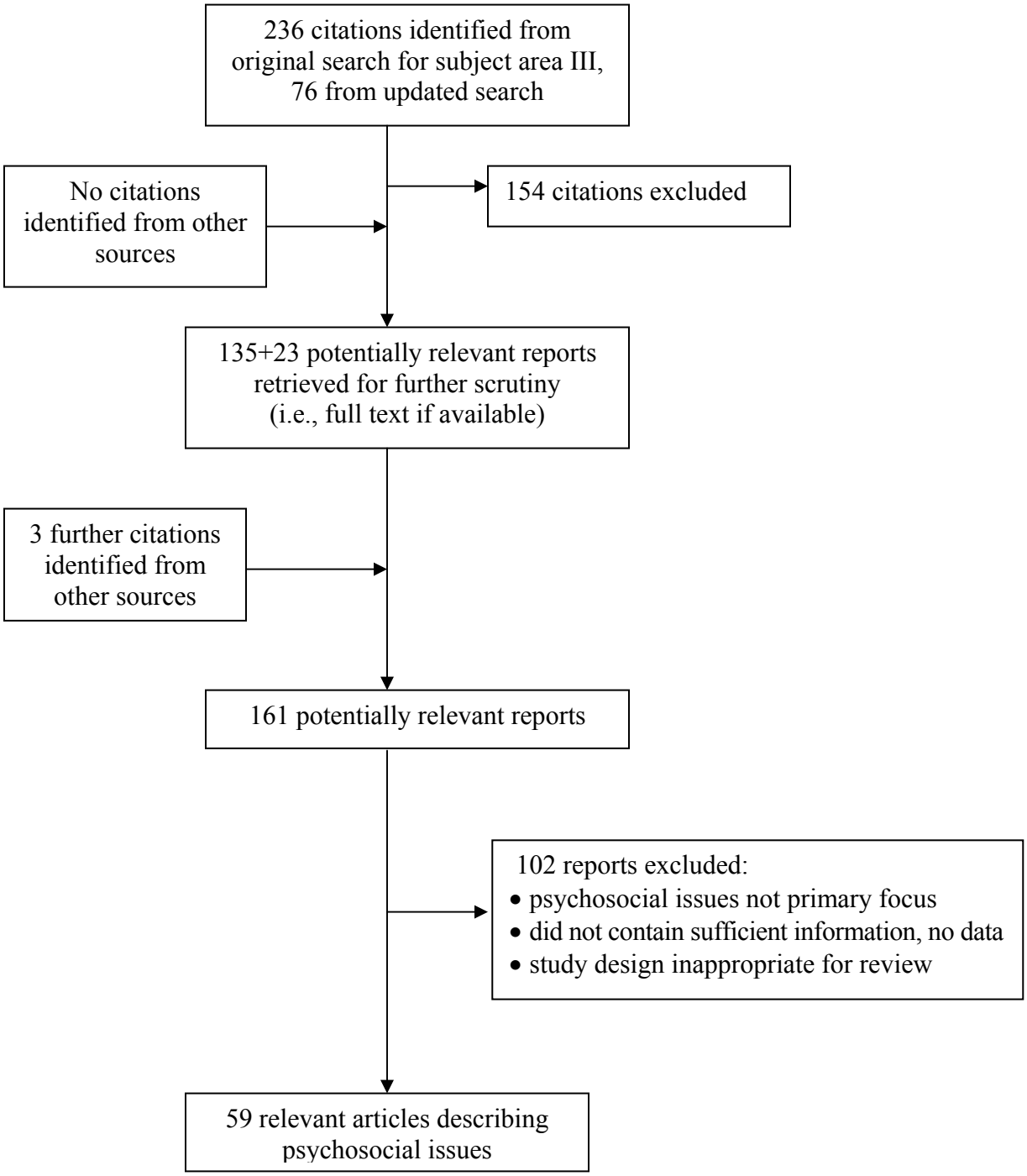


Table 5: Trial characteristics of studies for psychosocial impact

Study Characteristic	Details	Number of Articles
Site	Single-site	34
	Multi-site	21
	Unclear	4
Study setting	Clinic	29
	Community	7
	Hospital	11
	Registry	6
	Referral criteria or process	2
	Other or unclear	4
Design	Case-control	3
	Cohort	13
	Cross-sectional	36
	Descriptive	3
	Focus group	1
	Other	2
	Randomized controlled	1
Sampling procedure	Consecutive	16
	Random	1
	Selective	28
	Other	2
	Unreported	12

Study Characteristic	Details	Number of Articles
Gene	*BRCA1*	19
	BRCA1 and *2*	39
	Not reported	1
	Unclear	
Mutation status	Carrier	4
	Carrier and non-carrier	18
	Unknown	34
	Unclear	3
Geographic location	Australia	2
	Belgium	2
	Canada	5
	France	2
	Germany	2
	Israel	2
	Netherlands	3
	Norway	1
	United Kingdom	3
	US	33
	US and Canada	4

6.1.3 Data analysis and synthesis

An account of the quality assessment of selected articles can be found in Appendix 7 Table 6. Many of the articles reported results from questionnaires, surveys, interviews, or a focus group, and did not involve a follow-up. In the articles where follow-up was reported, 11 reported adequate follow-up, whereas four did not.[197-200] All study subjects were accounted for in most studies, with the exception of one study that did not,[201] two studies that did not report this information,[202,203] and one study for which this was inapplicable.[204] In total, 90% of the articles reported appropriate statistical analysis of study results, of which 90% reported on associated uncertainty. Results of sub-group analyses were reported in 70% of the articles. In 50% of the articles, study participants were either self-referred or volunteers that were part of a program that offered genetic counselling and testing at no cost. This potentially contributed to selection bias,

as these individuals would not be representative of the general population. Measurement bias may have occurred in four studies where the tester may have had prior knowledge of the status of study participants.[205-208] The potential for bias due to the attrition of study participants was reported in 15 articles and was unclear in nine articles. A summary of study quality parameters and corresponding numbers of articles is provided in Table 6.

Reported outcomes of the selected trials for psychosocial impact were organized into four categories: knowledge and risk perception, interest in and attitudes towards genetic testing, psychological issues, and social issues (Tables 8 to 11 in Appendix 7).

a) Knowledge and risk perception (Table 8 in Appendix 7)

Knowledge of the association between breast or ovarian cancer and genetics
Eleven studies examined the overall knowledge of study participants regarding genetic involvement in the onset of breast or ovarian cancer.[132,197,204,207,209-215] Participants' knowledge was limited in most studies. In the study by Kinney *et al.*,[211] participants correctly answered 3.2 of nine items pertaining to breast and ovarian cancer genetics.[211] A similar lack of knowledge was demonstrated in another study, where participants correctly answered questions pertaining to breast cancer, cancer genetics and inheritance, 42.5%, 45.4%, and 55%, of the time respectively.[212] With regard to the type of knowledge, 56% of participants in the study by Bluman *et al.* were unaware that a father could pass a mutation to his children.[209] Furthermore, 43% did not know that there is a 50% chance of passing a mutation to a child and 14% knew that the prevalence of gene alterations in *BRCA1* or *BRCA2* is not one in 10. A total of 62% knew that a woman could get breast cancer after having a prophylactic mastectomy; 23% thought that prophylactic oophorectomy would not be completely protective against ovarian cancer. Subjects answered correctly 51% of the time.

The amount of knowledge about the genetics of breast and ovarian cancer was assessed in one study.[210] Self-referred respondents showed higher mean knowledge scores than those recruited through referrals. The amount of knowledge was found to be significantly higher among Caucasian and married respondents who reported affluent household incomes. Those individuals with an education beyond high school demonstrated higher knowledge scores. Ethnicity had a significant independent association with knowledge (i.e., African American women had significantly lower levels of knowledge). Ethnicity was also examined in one other study, where it was found that 44% of participants had heard of the term "breast cancer gene," and 16% of these participants knew anything beyond name recognition.[213] Knowledge differed by family history and by ethnicity with results demonstrating Ashkenazi Jewish women to be more knowledgeable (67%) than European American or African American women (both 43%).

Knowledge of genetic testing and sources of information
Three studies addressed issues pertaining to the knowledge of genetic testing and sources of information for *BRCA* testing.[216-218] In these studies, about half of study participants were aware of genetic testing for breast or ovarian cancer. The proportion of participants who were knowledgeable about genetic testing was greater among high risk groups than the general population. All three studies indicated that the media was the most frequently cited source of informaiton (43% to 68%), then family friends and acquaintances (10% to 28%), and the least cited source of information was the attending physician (6% to 16%).

Table 6: Quality assessment of studies for psychosocial impact

Quality Parameter	Details	Number of Articles
Adequate statistical procedures	Yes	53
	No	1
	Not applicable	4
	Unclear	1
Sub-group analysis	Yes	41
	No	18
Bias:		
Selection	Yes	30
	No	20
	Unclear	9
Performance	Yes	4
	No	49
	Unclear	6
Measurement	Yes	4
	No	46
	Unclear	9
Attrition	Yes	15
	No	35
	Unclear	9
Results applicable to target population	Yes	31
	No	2
	Unclear	26

Quality Parameter	Details	Number of Articles
Adequate follow-up	Yes	11
	No	4
	Not applicable	44
All subjects accounted for	Yes	55
	No	1
	Not applicable	1
	Not reported	2
Representative of population eligible for genetic testing	Yes	32
	No	8
	Unclear	19
Uncertainty quantified	Yes	48
	No	8
	Not applicable	3

Risk perception of cancer and mutation status

Nine studies examined the perception of their own cancer risk in study participants.[217-225] Among study participants were individuals who had no personal history of breast cancer and who had tested positive for *BRCA1/2* mutations; and men who had tested positive for a *BRCA1/2* mutation. Two of these studies were carried out in individuals who were members of families with increased risk (i.e., identified as having a first-degree relative with breast or ovarian cancer).[219,221] In all studies, participants demonstrated a perception of elevated risk. Three studies compared the perceived risk of individuals having a personal history of breast or ovarian cancer with those at risk but without such a history.[217,218,220] In one study, women in a high risk group demonstrated a higher overall perceived risk of developing cancer when compared to low risk groups; however, differences in perceived risk of developing cancer, breast cancer, or an inherited form of cancer disappeared once education was controlled for.[217] The high risk group did perceive their risk as being higher than that of the general population in all cases. In the study by Mehnert *et al.*, healthy women who were at risk of breast cancer due to hereditary disposition estimated their own risk to be 47% (median value). The general risk of disease in women of comparable age ranges from 10% to 13%. Sociodemographic characteristics were not found to vary significantly with subjective risk perception in this study. In a study of spousal

risk perception, the rank of the husbands' perceptions of the chance of mutation was significantly correlated with the ranks of their wives' perceptions of risk.[207] Husbands of wives who underwent testing tended to perceive a higher likelihood that their wives had a mutation.[207]

Three studies examined perceived cancer risk among those with a personal history of cancer.[222-224] All three studies demonstrated that increased risk perception was associated with positive genetic test results. The study by Liede *et al.* examined the risk perception among men with family histories of breast or ovarian cancer.[225] Results demonstrated that most unaffected men thought that they were at increased cancer risk, and more than half the respondents thought they had increased susceptibility to prostate cancer. The perception of increased risk was greatest in men with an affected mother (97%) and in those whose mother had died from breast or ovarian cancer (96%) compared with those with unaffected mothers (70%).

The perceived risk of being a *BRCA1* or *BRCA2* mutation carrier was addressed in 10 studies.[201,209,211,213,216,226-230] One study compared the perception of carrier risk of an individual with that estimated by a predictive model (i.e., BRCAPRO).[209] The BRCAPRO model was used to examine the risk of *BRCA1/2* mutations in women diagnosed with breast or ovarian cancer, and the risk of being a mutation carrier was found to be 36%.[209] More than 75% of the women had overestimated their risk, and approximately 25% had underestimated their risk. Women with at least three first- or second-degree relatives were one third more likely to overestimate their risk of having a mutation compared with women who had fewer affected relatives, after controlling for age, race, and previous testing in the family.

In the remaining studies, eight of nine reported that most subjects overestimated their risk of being a mutation carrier. In the one study that differed, female subjects with breast cancer diagnosed before age 50 and in the preceding two-year period who had received treatment did demonstrate a relatively accurate perceived risk (5% to 10%) when compared with women 18 to 50 years of age without a personal history of breast cancer (i.e., 10% to 25%).[216]

b) Interest and attitudes to genetic testing (Table 8 in Appendix 7)

Interest and uptake

In many studies, genetic counselling or testing was offered to examine the participants' interest in their mutation status. Most participants in these studies were from high risk families. In three studies, participants were not offered genetic testing, but were asked if they would be interested in genetic testing if available.[211,213,231] Most participants (70% to 82%) expressed their interest. In most studies, participants requested and underwent genetic testing. In those studies where genetic counselling was offered, interest in testing was consistently greater after the counselling sessions than before.

One study asked participants who had been tested for a *BRCA1* mutation and had underage children (<18 years) whether they would be interested in testing their children;[232] 17.3% expressed the desire to have their children tested. No significant differences were noted between carriers and non-carriers. Furthermore, there was no significant difference in support of participants for testing children in general, as compared with their own children (p=0.58). Of individuals who endorsed testing for minors in general, 7.7% did not endorse it for their own children; approximately 5% of individuals unsupportive of testing minors in general wanted their

own children tested. Men were more likely to support *BRCA1* testing in children, but because this mutation confers greater risks of cancer on women than men, men may not have perceived the risk to be as personally threatening. This study only included individuals of northern European descent, and demographic factors may have contributed to the findings.

An individual's reasons for and against testing were also examined in many studies. Reasons for testing included familial advantages, assessment of risk for children, assistance with prophylactic decision making, access to testing, and curiosity. Reasons against testing included familial disadvantages, anxiety and psychological burden, medical insurance discrimination and confidentiality (Table 8 in Appendix 7). Predictors of uptake and genetic counselling were also addressed in some studies.

Satisfaction

Six studies examined participants' satisfaction with the services that they received during the genetic counselling and testing process.[199,207,220,225,233,234] Experiences were positive, with over 90% of participants in one study reporting satisfaction with the clinical services that they received with the exception of the wait for test results.[220] Similarly, all participants who had tested positive in a second study, and had undergone genetic counselling and testing reported mainly satisfactory results with a mean response of 4.2 on a five-point Likert scale.[225] A third study reported that 90% of the participants were glad to have undergone testing, 8% were unsure (half mutation carrier, half non-carrier), and 1% regretted it (one mutation carrier woman).[233] In the fourth study, 95% of participants were satisfied or very satisfied with their decision regarding testing, 1% were dissatisfied, and 4% were unsure;[233] 57% reported that the counselling session helped them make a decision about testing, and 87% reported feeling more confident after counselling. In one study, 64% of participants found the counselling process to be helpful for future medical decisions.[199] The most useful aspect was thought to be the multidisciplinary counselling effort by the genetic counsellor and oncologist, whereas assistance with communicating with family members could have been improved. A survey of spousal preferences for programs and services showed that 38% would like to have more written information about testing and an educational session for children of different ages.[207] Among spouses, 25% reported an interest in talking with a genetic counsellor again, meeting with other spouses involved in the program, and talking with a professional counsellor.[207]

c) Psychological issues (Table 10 in Appendix 7)

Distress

A total of 27 studies focused on the anxiety component of genetic testing. A variety of instruments were used to measure distress, but the most commonly used was the Impact of Events Scale (IES).[206,211,212,219,227,230,235-241] Distress levels were highest in participants undergoing genetic testing and in those who had received positive results. Higher distress levels were found among women with breast cancer as compared with the general population; some differences were noted between studies.[216] In the study by Wood *et al.*,[199] a significant reduction in anxiety levels was reported after the disclosure of test results, irrespective of the results. Meiser *et al.*[233] found that carriers showed a significantly greater level of breast cancer distress at seven to 10 days, and 12 months post-notification than untested women. The same carriers also showed significant reductions in anxiety levels at 12 month post-notification, as did non-carriers at seven to 10 days post-notification. In another study, the mean levels of general and cancer-related distress levels before

and after disclosure were not significantly different from non-mutation carriers with a prior risk of 25%.[242] These results are corroborated in the study by Schwartz *et al.,*[223] where investigators failed to find a difference between general or cancer specific distress at baseline for carriers versus non-carriers or any change. After adjusting for baseline scores and employment status, those with negative results had significantly decreased general and cancer-specific distress. Furthermore, accounting for familial clustering confirmed that negative results were significantly associated with cancer-specific distress and reduced general distress.

Age may also play a role in cancer-specific worry, as demonstrated in the study by Foster *et al.*[221] The authors found that women (<50 years) expressed more cancer-specific worry than do older women ($p<0.001$). Compared with the older women, younger women worried more often and considered it more of a problem. Cancer-related worry was not associated with a higher level of risk management activity.

A study by Hagoel *et al.*[243] showed that probands and non probands, and carriers and noncarriers did not differ regarding demographic characteristics, health behaviours, distress experienced, or social integration. Being a mutation carrier was not considered to be a psychosocial risk factor, nor did it affect mutation carrier resources and lifestyle.[243] Women affected by cancer had a lower sense of coherence than nonaffected individuals.[243]

Depression

Depression among individuals faced with decisions relating to genetic testing was addressed by six studies.[132,197,211,227,237,244] In one study, the average level of depression was found to be comparable to that in the general population.[227] A later study by the same authors reported that despite having found no significant difference at baseline between non-carriers, carriers, and non-decliners, differences in depression levels were significant at follow-up one month later.[132] A third study, also by the same authors, found that among participants with high baseline stress levels, depression increased among test decliners, decreased among non-carriers, and remained the same among carriers at follow-up one and six months later.[237] In a study by Kinney *et al.,* 45.6% of those intending to test and 23.5% of those not intending to test had depressive symptoms that did not differ by gender or cancer status.[211] In the Lodder *et al.* study that included males, no significant evidence of depression was found.[244] Men with higher scores on the optimism scale were significantly less likely to have high levels of pre-test depression than non-optimistic men, and those with daughters had significantly higher depression than those without.

Emotional reactions

Four studies addressed positive or negative reactions to genetic test results.[201,225,245,246] Participants experienced a range of emotional reactions in these trials. In one trial, some of the women who received positive results expressed surprise in having their suspicions confirmed, whereas others were pleased to have information that ended their uncertainty.[246] In women who received inconclusive results, there was a range of responses: relief, elation, disbelief, acceptance, disappointment, anger, and frustration. Lastly, some women expressed disappointment in the inadequacy of the technology to identify a mutation at a given time, as this left them in an uncertain position. A similar report of the range of emotions experienced by those receiving positive test results can be found in the study by Liede *et al.*[225] In women who received negative results, emotions reported were feeling happiness and relief (80%), surprise

(8%), survival guilt (4%), and no apparent reaction (10%). One study examined the anticipated reactions to test results.[245] Subjects were asked to respond to a hypothetical disclosure of negative test results before they received the results. Their responses were associated with higher levels of anticipated favourable emotions than those after positive results. The mean levels of unfavourable responses (i.e., sadness, anger, and worry) after positive results were significantly higher than those after negative results. Post-disclosure guilt ratings were low, suggesting that there was no strong guilt after disclosure of either type of result.

Support and cosping

Four studies reported on the coping and support mechanisms used by participants.[218,220,225,234] In one study, it was found that prayer was the most common technique used (57%), followed by talking with a friend (45%), relaxation or other tension-reducing techniques (20%), change in the amount of exercise (19%), speaking to physicians (13%), and change in eating habits (12%).[234] Younger women (<50 years) and those with a college degree were more likely to use relaxation or other similar techniques to reduce tension, whereas women who did not have a college education were more likely to use prayer. In the study by Liede *et al.*,[225] genetic counsellors were reported to be a main source of psychosocial support, followed by physicians, spouses, and family members. In another study, one third of women with a personal or family history of cancer expressed the desire for psychological support during the decision phase before genetic testing, whereas 54% desired psychological support in the event of a positive result.[244] In a study examining the results from a focus group, participants favoured a regular support group scenario, with most being content with a peer-led group or a professionally led group.[220] Other participants expressed interest in a support group or advised that they were supported by family and friends. The effects of counselling were considered on parameters such as sleeplessness, moodiness, tension, and anxiety. Most study participants (92%) indicated an interest in follow-up with the genetic counselling team for updates on new research studies or treatments, or for the opportunity to have their psychological well-being assessed.[220] These results support the notion that genetic counselling and testing have a negligible effect on long-term psychosocial parameters.

d) Social issues (Table 11 in Appendix 7)

A total of 16 studies dealt with issues concerning the communication of genetic test results to family and friends.[198-200,204,208,218,220,222,225,226,244,246-250] Many issues were raised in these studies, ranging from the burden and anxiety after disclosure of test results, uncertainty as to which family members to tell and the best means of communication with close and distant relatives. A theme in many studies was the concern pertaining to the communication of a participant's test result to family members for a variety of reasons (e.g. to do so was problematic, an unspoken topic, family member too young, geographic distance, denial).[199,218,220,246]

In the study by Tercyak *et al.*,[200] 47% of participants shared information on their mutation status with their children. Carriers disclosed with approximately the same frequency as those who did not disclose (i.e. 53% versus 47%), whereas in non-carriers, the rates were almost reversed (i.e. 43% versus 57%). The disclosure rates were 49% among parents with older children (14 to 18 years) and 37% with younger children (<14 years). Mothers were more likely to disclose than fathers. Baseline general distress was found to be significantly associated with communication, after controlling for gender, mutation status, and cancer history (i.e. OR: 3.45; 1.32, 8.96). In another study by the same authors, 53% of participants who disclosed their results to their

children indicated that the primary reason for doing so was the child's right to know (50%).[198] For those participants who did not disclose, the primary reason for not doing so was that the child was too young to understand (47%). Factors associated with disclosure were child's age, number of maternal health conversations with children, interest in pediatric genetic testing, and stronger intentions to share results. The age of the child or family member seems to be a key factor in the decision to disclose test results, as seen in many studies.[220]

The issue of whom to tell was also considered in some studies.[222,226,247] Most carriers and non-carriers communicated their test results to a sibling or offspring more than 18 years old.[226] Communication with close and distant relatives was the subject of a study by Claes *et al.*[222] Close relatives, such as children, siblings, and parents, were usually informed of the diagnosis; distant relatives were rarely informed. An evaluation of the process and content of communication between sisters regarding *BRCA1/2* testing results was reported by Hughes *et al.*[247] Test results of *BRCA1/2* status were communicated to 85% of sisters. Carriers communicated their results to their sisters 96% of the time, whereas those with uninformative results did so 76% of the time. Test results were communicated to 25% of sisters on the same day as disclosure, and results were communicated to 70% of sisters within one week of receiving the test results. An evaluation of male *BRCA1* carriers revealed that most men discussed their results with a family member.[225] In another study evaluating the attitudes and psychological implications of genetic testing in men, it was found that all participants intended to postpone informing children of the possible risk for several years if they tested positive.[244]

e) Summary points for psychosocial impact

- Given the diversity in study designs, target populations, outcome measures, and quality of studies, it was not possible to conduct meaningful comparisons of the data from selected studies.
- Knowledge of the availability of genetic testing, and of the risk of developing breast or ovarian cancer if a mutation is present, is critical for an individual to make an informed decision about genetic testing. While there are many reasons an individual may choose to be tested or not, the availability of genetic counselling provides an opportunity to discuss their questions and concerns. The decision to undergo genetic counselling and testing is a personal choice. A multi-disciplinary system of support can ensure that an individual has access to support and tools that will help him or her deal with the psychological impact and social issues that arise.
- In general, high risk individuals (i.e., hereditary disposition) and those who received positive genetic test results demonstrated an increased risk perception of developing breast or ovarian cancer. Most overestimated their risk of being a mutation carrier.
- Interest and uptake in testing revealed that most individuals from high risk families expressed interest in being tested (i.e., 70% to 82%). In studies where genetic counselling was offered, interest in testing was consistently greater after the counselling sessions than before.
- The psychological impact was assessed by examination of distress, depression, emotional reactions, and support or coping strategies. Distress levels were highest in participants who received positive results, yet anxiety levels 12 months after the disclosure of test results were reduced irrespective of the results received. Participants experienced a range of emotions. The mean levels of unfavourable responses after positive results were significantly higher than those after negative results. Common coping techniques included prayer, talking with their physician and others, tension-reducing techniques, and lifestyle changes.
- The social issues explored were communication of test results to family members (i.e., including offspring, close and distant relatives) and friends. Participants were concerned about communicating their test results to family members for a variety of reasons. Close relatives, such

as children, siblings, and parents, were informed about results most often. Carriers communicated their results to their sisters 96% of the time, while those with uninformative results did so 76% of the time. Most men discussed their results with a family member.

6.2 Subject Area III: Ethical Issues

6.2.1 Quantity of research available

The original electronic search strategy for subject area III identified 236 citations, and 103 citations were excluded (Figure 4). Nine relevant articles were used for ethical issues. Concern was expressed among reviewers over the inclusion of one study.[251] Although this study acknowledged the potential for harm and violation of patient privacy in the context of genetic testing, it mainly assessed the harms caused by the actions of the study investigators. Nonetheless, the decision was made to include the study.

6.2.2 Trial characteristics

Selected trials primarily examined the ethical issues of informed consent, privacy, confidentiality and familial implications specific to *BRCA1/2* genetic testing (Appendix 8). Eight studies reported on family cohorts with breast or ovarian cancer, whereas the remaining study reported results from a survey of informed consent forms from seven US *BRCA1/2* testing centres for breast cancer.[252] All studies were observational. The study design was quantitative in six studies[251-256] and qualitative in three studies.[229,257,258] Common study objectives pertained to informed consent, privacy, and confidentiality issues (Table 7).

Details of the characteristics of patients included in these studies can be found in Appendix 8. In the eight studies reporting on family cohorts, the sample size ranged from 30 to 636, and the mean age of the patients ranged from 44 to 65 years (i.e., results from five studies). Participants were considered to be at moderate (i.e., at least one affected first-degree relative) or high (i.e., those with *BRCA1/2* mutations, or two or more affected first-degree relatives) risk of developing breast or ovarian cancer. Two studies included women of Ashkenazi Jewish descent[229,253] and three studies reported results from male participants (i.e., 7% to 31% of the study population).

The ethical considerations examined in each study are detailed in Appendix 8. Seven studies reported on privacy and confidentiality, six on familial implications, and five on informed consent. Most participants thought that test results should be kept confidential from third parties, including insurers, employers, and other family members. In two studies, the potential issues arising from denial of access to *BRCA1/2* testing were reported. The issues examined were denial of access to testing of a participant if their physician recommended against testing and denial of family members access to testing if participants prefer not to undergo testing. Two studies reported the benefits of testing accrued to family members in the context of positive and negative test results. In one study, participants reported a conflict when undergoing *BRCA* testing,as they felt a moral duty to undergo testing to benefit family members; but they were burdened by the dilemma of potentially causing harm when relating "bad news" to those family members who did not want to know their genetic status. The limitations of the selected studies and the policy recommendations that are made are summarized in Table 8.

Table 7: Study objectives of selected studies for ethical implications

Author (Country)	Objective(s) of Study
Armstrong *et al.* (US)[253]	To evaluate impact of breast cancer risk information on purchase of life insurance, impact of concerns about life insurance discrimination on use of *BRCA1/2* testing, and incidence of life insurance discrimination after participation in breast cancer risk assessment and *BRCA1/2* testing
Benkendorf *et al.* (US)[254]	To assess attitudes pertaining to confidentiality and autonomy regarding *BRCA1/2* genetic testing
Durfy *et al.* (US)[252]	To survey informed consent forms and other protocol materials for minimal information being given to patients; to assess risks and benefits being cited in relation to *BRCA1/2* genetic testing
Goelen *et al.* (Belgium)[257]	To describe moral concerns of patients undergoing genetic counselling and testing
Hallowell *et al.* (United Kingdom)[258]	To gain insight into ethical issues surrounding informed consent for *BRCA*1/2 genetic testing (i.e., disclosing test results to family members and other third parties), and into information and support needs of women involved in genetic testing process
Lehmann *et al.* (US)[255]	To assess knowledge and attitudes about genetic testing
Peterson *et al.* (US)[256]	To determine whether concerns about cost, confidentiality, and insurance discrimination pose barriers to gene testing among individuals at risk for hereditary breast or ovarian cancer; to determine why and how these concerns affect test uptake and subsequent cancer prevention and screening options; to report experiences of patients interacting with insurers
Phillips *et a.l* (Canada)[229]	To examine factors that influenced decision to undergo genetic testing for *BRCA1/2* mutations in Canadian Jewish women with breast cancer
Winter *et al.*[251]	To determine impact of risk notification of family history of breast cancer

6.2.3 Data analysis and synthesis (Appendix 8)

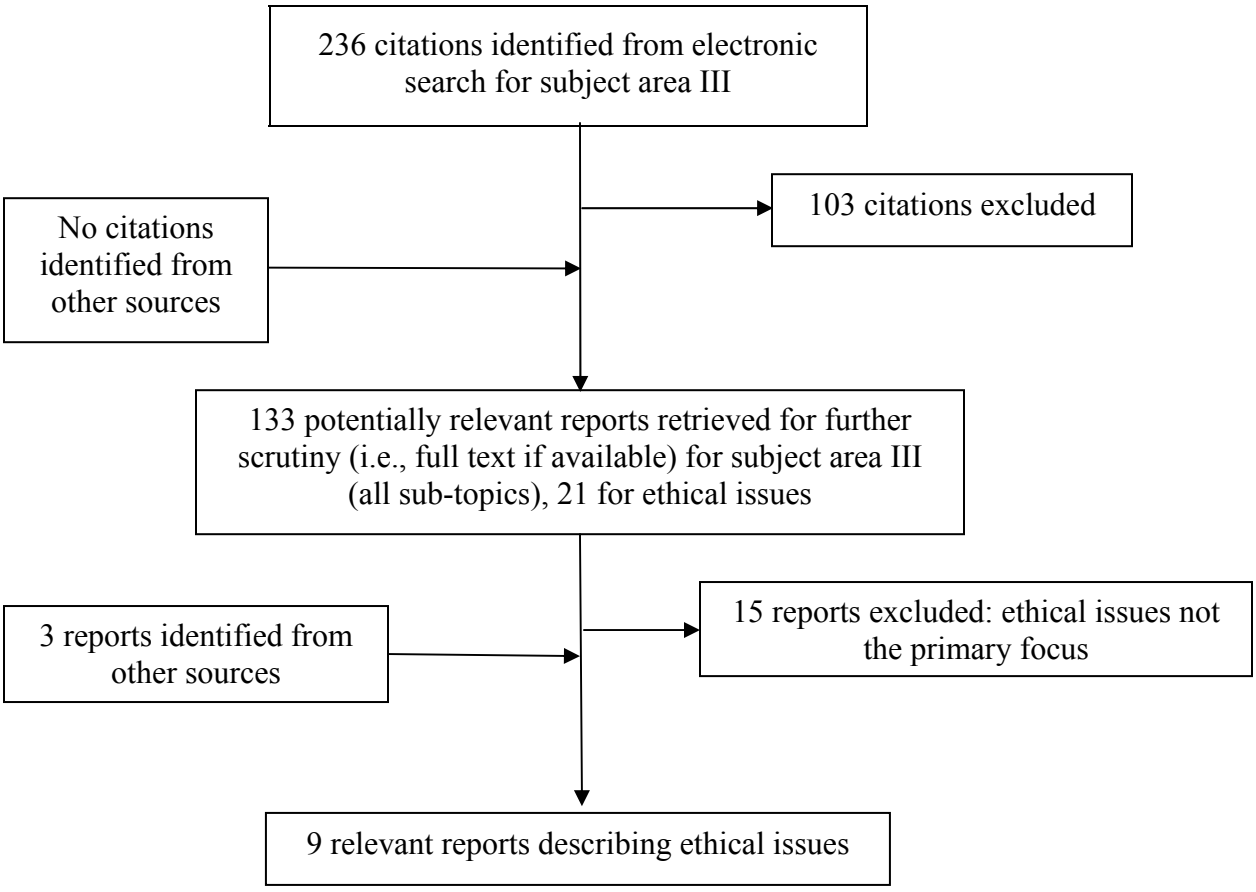
a) Informed consent

The purpose, nature, and effectiveness of informed consent forms for genetic testing require review. Such forms to be used before *BRCA1/2* testing must be complete and readable, so that individuals understand the documents that they are signing. Five of the nine selected studies reported on the ethical implications of informed consent. One study, in which the informed consent forms used by seven *BRCA1/2* testing centres in the US were reviewed, revealed inconsistencies in content and organization, and found that a limited number of topics were covered in the informed consent process.[252]

Informed consent for *BRCA1/2* testing must encompass the medical and non-medical risks and benefits (familial and social consequences) of choosing to be tested or not (i.e., informed refusal).[259-261] Given the familial nature of genetic information, the informed consent process should encourage the participant to consider the implications of testing for other family members, to discuss the associated issues with them and to involve them, in the genetic counselling process whenever possible. Communication is a critical element, especially given the potential for conflict in a family concerning participation in genetic testing.[262] The informed consent process for *BRCA1/2* testing is compounded by factors that make it difficult for individuals and for the health professionals who advise them, to determine morally appropriate responses to the availability of such testing.[263] These factors include the multifactorial causes of breast cancer, variability in gene penetrance with particular mutations, and for those who do test

positive, the lack of available therapeutic options that offer prevention of breast and related cancers. Coupled to this is a risk of discrimination pertaining to access to life and health insurance, an employment of the individual and biological relatives.

Figure 4: Selected material for ethical issues (Subject Area III)



In consideration of the complexities involved with the provision of informed consent, in 2004 ASCO recommended the discussion of the following topics during the informed consent process:

- information on the specific test being performed
- implications of positive and negative tests
- possibility that the test will not be informative
- options for risk estimation without genetic testing
- risk of passing a mutation to children
- technical accuracy of the test
- fees involved in testing and counselling
- risks of psychological distress
- risks of insurance and employer discrimination
- confidentiality issues
- options and limitations of medical surveillance and screening after testing
- importance of sharing genetic test results with at-risk relatives.

It is evident that informed consent obtained before genetic testing is a critical element of *BRCA1/2* genetic testing.

b) Privacy and confidentiality

The concept of privacy has evolved from a right to privacy to the personal right to be left alone and ultimately to a fundamental right based on human dignity and respect for the individual, the latter notion understood in terms of self-determination.[1] As such, genetic information is protected by the ethical and legal principle of confidentiality that exists in the patient-physician relationship.

Seven of the nine studies addressed the issues of privacy and confidentiality of genetic testing for breast and ovarian cancer. Most participants indicated that they wished to keep test results confidential from employers, insurers, and other family members. As genetic material is shared by biological relatives, identifying a causative link, such as that through a *BRCA1/2* mutation, has implications beyond the individual.[1,259-261,264,265] Misuse of genetic information could have implications for an individual in terms of employment, or obtaining life or health insurance.[259-261,264,265] This "genetic discrimination" has received considerable media coverage.[266] As a result, the actual or perceived threat of genetic discrimination may affect an individual's decision to undergo genetic testing. These factors underscore the importance of the legal and policy implications of disclosure of genetic information and confidentiality of results. The suggestion has been made that health professionals should advocate that *BRCA1/2* and other mutation testing be used constructively to modify health care choices, rather than to stigmatize individuals or deprive them of appropriate care.[261,265]

In many of the studies reviewed, insurance discrimination was cited as a barrier to *BRCA1/2* testing. The issue of life insurance and genetics has been debated in Canada and elsewhere.[253,267,268]

Several international jurisdictions do not have legislation or guidelines pertaining to insurance for individuals who, because of a genetic predisposition, may be at an increased risk of cancer. In France, a moratorium on the use of test results for insurance purposes exists for up to five years, whereas in the Netherlands the moratorium is indefinite. In the United Kingdom, the Human Genetics Commission published a statement in May 2001 that includes interim recommendations for the use of genetic information for insurance purposes. Submissions to the Australian Law Reform Commission and the Australian Health Ethics Committee provide support to the fact that a perceived fear of genetic discrimination with regards to obtaining life insurance has caused individuals to avoid genetic testing.[267] In the US, the Health Insurance Portability and Accountability Act prevents genetic test results from being used as a pre-existing condition to deny coverage to those seeking new or continued group health insurance coverage. At the state level, many jurisdictions have enacted laws to prohibit or limit the use of genetic information by health insurers in underwriting decisions.

c) Familial implications

The personal yet familial information provided by genetic testing raises questions about the moral and legal obligations of health care professionals to disclose genetic information to at-risk relatives. Six of the nine studies addressed issues pertaining to familial implications of genetic testing. In the study by Lehmann *et al.*, 97% of respondents believed that patients should inform at-risk family members, 83% believed that physicians should inform their patients of the familial implications, and 22% believed that physicians should inform at-risk family members against a

patient's wishes. Five studies, including two of note,[255,258] examined the issues of family obligations and disclosure where a women's autonomy is regarded as "substantially compromised" by the biological implications of *BRCA1/2* testing for her kin and by social obligations towards family members. A reasonable conclusion would be for health care professionals to raise these issues before *BRCA1/2* testing. In keeping with the ASCO recommendations, the pre-test session should include a discussion on the importance of sharing genetic test results with at-risk relatives. Inherent in this are the obligations of the individual and associated health care professionals. The health care professional should offer assistance with the process of disclosure and support for the individual and their family members after testing.

It is believed that genetic information should be protected by the ethical and legal principle of confidentiality that exists in the patient-physician relationship.[1] The principle of confidentiality is not considered to be absolute. Therefore, ethical, legal, and statutory obligations may permit health professionals to disclose otherwise confidential information on an exceptional basis. According to the Social Issues Subcommittee on Familial Disclosure convened by the American Society of Human Genetics in 1998, disclosure of genetic information should be permitted in the following scenarios:

- attempts to encourage disclosure on the part of the patient have failed
- harm is highly likely to occur, and is serious, imminent, and foreseeable
- the at-risk relative(s) is identifiable
- the disease is preventable or treatable; or medically accepted standards indicate that early monitoring will reduce the genetic risk.

If these conditions are met, a health professional may warn at-risk family members (based on professional duties or "privilege") in cases where the information reveals that the relative is at a substantially higher risk of suffering from a serious and otherwise undetected genetic disorder, and when prevention or treatment is available. The harm from failing to disclose is thought to outweigh the harm from disclosure, and at a minimum, the health professional should be obliged to inform the patient of the implications of her or his genetic test results and the potential risks to family members.

Many international jurisdictions and organizations, such as the World Health Organization, have examined questions relating to genetic privacy and confidentiality, and have formulated recommendations that try to balance the privacy expectations of participants with the right of other parties to know sensitive genetic information. Most are in favour of permitting limited disclosure of genetic test results (i.e., without the consent of the individual) in cases where the harm to at-risk relatives is serious and imminent. A limited number of jurisdictions maintain that confidentiality and the wishes of participants with regard to non-disclosure be respected at all times.

Testing children at risk

Two studies explored parental decisions to undergo *BRCA1/2* testing on behalf of minor children. This is a contentious issue, and there is an ongoing debate as to whether parents have the authority to verify the gene status of their child for hereditary breast or ovarian cancer (or other late-onset genes) that do not manifest until adulthood, without the child's consent.[261,265,269] Because test results may "stigmatize" an otherwise normal child for the rest of his or her life (e.g., potential burdens of altered self-concept, differential treatment in the family), a limit on

parental authority and autonomy may be justified.[259,265] On the other hand, the serious health implications of *BRCA1/2* mutations suggest that interventions, including the early application of preventive measures or gene correction, may make it desirable to know the individual's genetic status at a young age.[265] To complicate the issue, unique ethical implications arise in situations where children who are at risk of inheriting a mutation for an adult-onset disease such as breast or ovarian cancer have been relinquished for adoption.[270] Because of the limitation of interventional strategies, the accepted ethical opinion is that genetic testing should not be made on children, with or without parental consent.[259,264,265]

Testing males at risk

In the studies reviewed, men were in the minority among participants. Males from high risk families may be candidates for testing for several reasons, including the association of male breast cancer with *BRCA2* mutations, and the fact that *BRCA1* mutations are found to be associated with a marginally increased risk of colon and prostate cancer.[259,265] In the former situation, although the absolute risk is low, because of the rare nature of the disease, the heightened awareness may lead to early detection and treatment. In the latter situation, both colon and prostate cancer pose a significant morbidity and mortality burden in Canada.[3] This area warrants further study, given the complexity associated with genetic *BRCA1/2* testing for men.[271]

6.2.4 Summary points for ethical issues

- Information is provided on the ethical issues of informed consent, privacy, and confidentiality, and familial implications (e.g., testing in children, males at risk) for *BRCA1/2* genetic testing.
- It is crucial that informed consent forms are complete and readable, so individuals understand the documents that they are signing to provide informed consent for or informed refusal of testing. Informed consent must encompass the medical and non-medical risks and benefits (i.e., familial and social consequences) of being tested or not.
- Most participants in the studies selected thought that genetic test results should be kept confidential from third parties including insurers, employers, and other family members.
- The personal yet familial nature of information provided by genetic testing raises profound questions about the moral and legal obligations of health care professionals to disclose information to at-risk relatives. While genetic information should be kept confidential, there may be circumstances when otherwise confidential information is disclosed on an exceptional basis (e.g., attempts to encourage disclosure by patient have failed, harm is likely to occur, at-risk relatives are identifiable and the disease is preventable and treatable, or early monitoring may reduce the genetic risk of the disease).
- Additional familial implications of testing, such as parental decisions to undergo *BRCA1/2* genetic testing on behalf of minor children and testing males at high risk, are contentious issues that present unique ethical considerations.

Other ethical implications, such as cost-effectiveness and patent issues, were not addressed in the selected studies. These issues warrant further exploration.

Table 8: Study limitations and policy recommendations from studies for ethical implications

Author (Country)	Study Limitations	Policy Recommendations
Armstrong *et al.* (US)[253]	Patients drawn primarily from one clinical site, reliance on self-reported information for insurance purchasing, small sample size resulting in inability to provide reliable estimates of magnitude of change in life insurance benefit amount in those who changed policy size, point estimates likely understate changes observed over time as women who entered the program earlier had more opportunity to change life insurance coverage and those who entered late may change them in the future	Larger and more longitudinal assessments of behaviour are required, combined with actuarial models of impact of these behaviours on life insurance pricing and subsequent demand
Benkendorf *et al.* (US)[254]	Primarily Caucasian, middle-class, more self-referrals, survey administered before education session, most had only one affected first-degree relative	When tailoring and eliciting consent, genetic professionals must be cognizant of the influences of ethnicity, life experiences, personality, and coping styles
Durfy *et al.* (US)[252]	No evaluation made of the structural organization of forms (which may play a role in patients' perception and understanding); no evaluation was made of wording of forms, their tone, and manner in which concepts were framed	Authors favour emphasizing educational function of informed consent documents: information in document should be more detailed and extensive; document should serve as reference for individuals (and relatives) considering testing, and help those who have already received test results, thereby supplementing counselling and information physicians can provide; readability grade level should accommodate half of all Americans whose reading skills are $\leq$9th grade level; the information most relevant to individuals considering testing should be determined
Goelen *et al.* (Belgium)[257]	Other moral concerns may exist among family members who were not part of the study or among other types of health care settings	Further studies needed to assess whether BRCA test is most effective means of addressing patients' concerns
Hallowell *et al.* (United Kingdom)[258]	Not reported	Genetic testing process includes: informing participants of their role in disseminating information in the family; explaining which family members may be at risk; offering advice on how and when to pass on information; providing advice and information about implications and potential problems of not sharing test results; and providing time for participants to reflect on implications of testing before making decisions.
Lehmann *et al.* (US) [255]	Generalizability of results may be limited, as study was conducted among Jewish women, whose ethical sensibilities may be influenced by their background	A national debate is required about confidentiality of genetic information, to develop public policy that reflects concerns of health professionals and general public
Peterson *et al.* (US)[256]	Data derived from chart reviews of participants who had been seen over a 30-month period (1997-1999), one researcher (not blinded to the study) reviewed patient charts for data, amount of time between initial visit and survey may have affected responses, responses were retrospective and may not accurately capture feelings and experiences	Long-term follow up required to evaluate whether loss of confidentiality or discriminatory practices ensue over time
Phillips *et al.* (Canada)[229]	Generalizability to other ethnic groups of results, given study's focus on Jewish women, small sample size, attitudes of women who did not undergo genetic testing, were not examined	Influence of altruistic factors and psychological benefits need to be considered when deciding on coverage for *BRCA1/2* testing
Winter *et al.* (US)[251]	Variable response to notification of cancer family history in close versus distant relative, participants not selected based on strong family history but based on single proband with disease	Understanding privacy and psychosocial issues of family members who are informed of family history of breast cancer may aid in developing appropriate guidelines for notification of results

7 DISCUSSION

Genetic testing for *BRCA1/2* mutations is integrated into clinical practice in many Canadian centres specializing in the care of patients with hereditary cancers. This has been accomplished under a variety of conditions and at different rates of uptake across Canada. The transition from research testing into clinical practice underscores the heterogeneity that exists in the precise testing, indications and analytical techniques used, the organization of services accompanying testing and the regional availability of health care professionals and resources to provide these services. Part of this variability reflects differences in mutation distribution across populations. Other sources of variability can be attributed to gaps in knowledge, and to the research and clinical care environments from which these practices have emerged. The interpretation of the available scientific data is challenging, given the complexity of the questions, the scarcity of good quality data, and the high level of uncertainty. As a result, many questions pertaining to "best" practices remain unresolved.

Clinical decisions regarding genetic testing depend partly, on available expertise and resources. To meet the need, research facilities have partially driven the development of testing, the transition of testing from the research setting into clinical practice, and the framework and organization of genetic and cancer services. In many centres, access to information, counselling, and support services were put in place for patients and families in the context of research. These have inspired the delivery of clinical care, but these modalities have often been adapted to the available resources in clinical practice. The demand for access to genetic services, mainly from high risk families, has been another driver for integration of testing into clinical practice, and clinicians have been sensitive to the needs of these groups as they often constitute most of a clinician's practice. The demand among moderate- to low-risk groups for access to genetic testing is growing. This is likely reflective of direct to consumer advertising (e.g., advertising campaigns, and web site access to testing and information).

Given the progression of genetic testing in Canada, few jurisdictions have organized testing services at a regional level. AÉTMIS and CCOHTA collaborated to systematically examine the evidence regarding the analytical and clinical validity of molecular technologies, and review the issues associated with testing. This report describes available molecular techniques (and associated analytical validities) used to identify *BRCA1/2* mutations, discusses psychosocial and ethical issues inherent to testing, and the benefits and risks of surveillance and preventive methods. From the perspective of the policy maker, the questions to be addressed go beyond the balance of risks and benefits at the individual and family level, or the local constraints on health care delivery and available resources. Issues pertaining to resource planning and training, costs, and implications for the health care system need to be examined. An attempt was made to examine several issues through systematic review of the literature, to provide support for policy or decisions at a regional or national level regarding the provision of *BRCA1/2* genetic testing.

How should testing be carried out?

When examining the performance of molecular tests, several questions must be addressed: whether it is appropriate to use a particular technique for all analyses and detection of all mutation types, what technique(s) should be given preference and under what circumstances, in

what proportion of individuals would erroneous results occur, how often analyses are inconclusive, and to what extent test results contribute to a more definite and precise risk assessment, in terms of personal and familial risk.

The molecular analyses of the *BRCA1* and *BRCA2* genes are complicated by their length, the distribution of mutations throughout, and the diversity of the mutation types. No available technique will detect all mutation types. The sequencing of all exons and exon-intron junctions (i.e., DSA) has traditionally been considered as the "gold standard" for the detection of point mutations, and of small insertions and deletions in these parts of the genes. This approach is costly and labour-intensive, motivating researchers to develop alternative approaches that are as valid yet affordable. Several of these have been described and some adopted by clinical laboratories, as an alternative to DSA or as a first step ("pre-screen") followed by confirmation of positive results by targeted sequencing. The review of information for analytical validity revealed that although DSA was commonly used as a reference test in many investigations, no two studies used the same index test, thus precluding direct comparisons between methods. DSA is an inappropriate technique to detect large rearrangements (e.g., large deletions or insertions or mutations, in non-coding regions), particularly for *BRCA1*. The proportion of mutations that are not detected by DSA has been a topic of research, especially in relation to founder effects for large deletions. One technique that has been adopted in many laboratories, in Europe and in Canada, is DHPLC. DHPLC has been compared to DSA in several small studies. Although the results appear promising (i.e., reported analytical sensitivity of 100% in all studies), there were methodological limitations in these studies.

Given that no one molecular technique will detect all mutation types, the question as to which technique or combination of techniques should be used depends on several factors. These include the prevalence and distribution of mutations in the target population, clinical sensitivity of the technique(s) for that population, technical (analytical) performance of the technique(s), the availability of laboratory resources and biological material, and cost. The acceptability and consequences of inconclusive or erroneous results must be taken into account. Issues such as available resources and expertise must be weighed in the decision about how to offer testing.

The quality of data on the technical performance of available molecular tests for the detection of *BRCA1/2* mutations is lacking, but the choice of laboratory technique used in a testing environment is often based on these data. Unpublished data may have contributed to decision-making in particular instances. As molecular tests are conducted once in a lifetime, an erroneous result can result in serious long-term consequences. This is compounded by the lack of systematic implementation of external quality control for molecular testing. In Europe, early experience with external quality control for *BRCA1/2* testing revealed significant rates of technical errors, and of errors associated with the misinterpretation of results. The long-term follow-up of testing practices (e.g., recording discrepancies between techniques or familial data, subsequent clinical data, and results of further research on negative test specimens) would provide valuable information.

Once a gene is cloned and clinically relevant mutations are identified, there is a rapid demand to implement testing as a service. Often, this swiftly becomes a standard of care, leaving little or no time for in-depth evaluations to facilitate decision making on how to do the test or how to manage

the patients. The clinical service and the knowledge of what mutations are seen, how best to test for them, and how best to manage the patients develops together and is not sequential. In addition, many at-risk individuals need to be tested to find true mutation carriers, so it takes a long time to acquire knowledge about the mutation profile of a gene. A decade would be considered a short time to do this.

There are gaps in our knowledge about the appropriateness of a particular test for mutation in most populations. Because of the gaps in our knowledge of mutation prevalence, and analytical and clinical validity results of available tests, techniques may be used or introduced with uncertainty. These gaps also affect the quality of the information that can be provided via genetic counselling to patients and families during the pre-test session (which affects the provision of fully informed consent) or the post-test session (which affects counselling of the individual with a residual risk after a negative test result).

Who should be considered for testing?

In Canada, there are no guidelines for genetic testing or consensus as to best practice. The American Society of Clinical Oncology recommends cancer predisposition testing be offered when the individual has a strong family history of cancer, a family history of very early onset of cancer, when test results can be adequately interpreted, when the results will influence medical management, or if clinically justified.

The Ontario Physicians' Guide recommends genetic testing only when certain risk factors are present (e.g., multiple affected family members, young age at diagnosis, ovarian cancer, bilateral or male breast cancer, ethnic-specific risk criteria), and samples must be accompanied by a three-generation pedigree indicating which of the affected individuals have had their cancer diagnosis confirmed by pathology review. To assist with the assessment of breast cancer risk, different statistical models have been developed as tools. There is, however, currently no unanimously accepted tool for this purpose. Most guidelines and models have been based on heterogeneous populations, which may not be as suitable for application in founder populations. The lack of consensus regarding a "best" testing guideline or risk assessment model fosters variability in clinical practice.

While there is a need for flexibility, and for clinical judgment and independence, it is necessary to examine the implications for the health care system. A decision to integrate these specialized tests into a public health care system does not imply an acceptance of widespread use. Modalities of conditional acceptance or reimbursement have to be delineated by individual jurisdictions. Organization of service delivery, and the recruitment and training of professionals must be planned accordingly. For instance, the professionals who are to assume a gate-keeping role must be trained in sufficient numbers to ensure accessibility and quality of care. Requirements and accountability for quality of care become evident, and should be linked to the need to accrue further data to refine the information that is provided to individuals during counselling. This raises issues pertaining to linkages between newly established services and research, and the financial involvement of the health care system, research funding bodies, and industry.

An additional implication for planning and policy arises from the fact that if testing indications are flexible, it will be more difficult to make projections of testing and service needs, and may yield wider confidence intervals. If greater consideration is given to individual demand, then the impact on the health care system will be partially driven by the individual's risk perception, which in turn is influenced by knowledge, family history, closeness to an affected family member, and personal experience with cancer. The individual may also be influenced by information presented to the public by the media and industry.

What do test results imply?

The provision of a test result by a laboratory is one aspect of the testing process. The interpretation of the test result and the discussion of the implications in terms of familial risk and personal management options are two essential steps, because of the complexity and degree of uncertainty associated with the result. Interpretation of test results should take into account the pedigree information, the clinical validity of the technique used for the relevant target population, and the nature of the detected mutation. Interpretation of test results is different for members of a family in which a deleterious mutation has been detected, as opposed to the first person in the family to be tested (i.e., the index case).
When a mutation has been previously detected in a family, the interpretation is usually easier, regardless of whether the result is positive or negative. An individual in whom the test is found to be negative is at comparable risk of developing cancer as the general population. Similarly, their offspring are not at increased hereditary risk. When a test result is found to be positive, the personal risk of developing cancer is elevated but cannot be precisely estimated. Instead, a range of probabilities is given to the individual. More precise estimates can be provided in founder populations. First-degree relatives can be informed of their one in two (50%) probability of having inherited the same mutation. This information may have psychosocial consequences, and result in modification of their risk perception, and an interest in counselling and testing.

When a mutation has not been previously detected in a family, three outcomes are possible. If a mutation is detected that is known in other families to be deleterious, the interpretation of the test result does not pose any particular problems, because it should be considered as a true positive result. In contrast, when a mutation of unknown clinical significance is detected, the a priori risk estimate remains applicable as long as the clinical significance of the sequence variation has not been clarified. The information should not be used to guide decision-making about surgical prophylaxis. In the meantime, tests are usually not offered to other family members. To clarify whether the sequence variation has an impact on protein production and function, sophisticated investigations usually performed in research laboratories may be required. For the genetic counsellor, this situation is particularly complex, whereas for the family, this situation is unlikely to relieve anxiety. The third possible outcome, a negative test result, should be presented to the family as an "undetermined negative" result. The interpretation of this result is complex, because, depending on the family history, the probability of developing cancer will be reduced (but not to the general population level) or remain unchanged. In the latter case, decisions regarding clinical management would likely be based on the familial risk assessment.

Test results have implications for clinical management strategies, even when the interpretation of the test result is straightforward (e.g., for unaffected mutation carrier and non-carrier members of a family in which a deleterious mutation has been previously detected). For non-carriers, surveillance can be relaxed to the standards applied to the general population. Not only will these individuals suffer less anxiety, but they will have to comply with less cumbersome follow-up. Their risk does not fall to zero, and they should follow the standard surveillance procedures for their age group.

For individuals found to be carriers, the test result does not allow for the precise prediction of their risk of developing cancer, or predict the site or age of onset of the cancer. The fact that these individuals belong to a moderate to high risk group, previously ascertained on age and pedigree information, is confirmed. Histopathological features of tumours in *BRCA1* carriers are suggestive of a worse prognosis, but this has not been confirmed by survival data, which show conflicting results. The balance between the benefits and risks of the available clinical management options may be considered differently once the molecular information is obtained. Evidence on the effectiveness of early detection programs and preventive measures for *BRCA1/2* mutation carriers is still being collected, and the field is in an early stage of development. No surveillance measures, be it through BSE, CBE, mammography, MRI, TVU, or Ca125 monitoring, have been shown to be efficacious specifically in *BRCA1/2* mutation carriers. In practice, recommendations based on experts' opinion suggest that there be surveillance for breast cancer in *BRCA1/2* carriers and surveillance for ovarian cancer for *BRCA1* carriers, beginning as early as age 25. The effect of chemoprophylaxis on cancer incidence in *BRCA1/2* carriers remains uncertain and conflicting results have been reported. The biological plausibility of a beneficial effect of chemoprophylaxis in *BRCA1* carriers is questionable, because most tumour tissues in *BRCA1* carriers are estrogen-receptor negative (i.e., tamoxifen is an antiestrogenic compound).

The only preventive measure for which the evidence of efficacy for *BRCA1/2* mutation carriers has been documented is surgical prophylaxis. Prophylactic mastectomy has been shown in cohort studies to reduce the incidence of breast cancer. All carriers do not readily accept this invasive procedure and psychosocial studies are only beginning to explore the long- term consequences. Prophylactic oophorectomy has been shown to reduce ovarian and breast cancer incidence, and is recommended as early as age 40 years (Europe) or 35 years (US). The appropriateness of hormone replacement therapy after oophorectomy remains unclear. Furthermore, there are no long-term follow-up studies to document the impact of prophylactic mastectomy and oophorectomy on mortality. Decision analyses have been conducted, but these rely on multiple assumptions. Life expectancy gains could be expected for women carrying mutations with a relatively high penetrance and who undergo prophylactic surgery at a young age. Once quality of life is considered, these estimated benefits decrease.

Large-scale studies are necessary to facilitate better understanding of the contribution of the preventive measures to the management of *BRCA1/2* mutation carriers. Some types of trials will raise ethical issues. The appreciation of benefits and risks related to preventive measures and to testing, is likely to differ between individuals. This underscores the importance of individual decision-making informed by current information on all factors that are likely to play a role in decision-making. In addition, the decision-making process may be a distressing experience, and providing appropriate genetic counselling and psychosocial support is crucial.

For individuals who are affected by cancer, a positive test result raises different questions in terms of the impact of the information on prognosis and management. Despite the fact that the probability of occurrence of second primary breast cancers (i.e., ipsilateral and contralateral) is higher in *BRCA1/2* mutation carriers than in non-carriers, no consistent difference has been demonstrated in terms of survival. With regard to clinical management, no indications were found to indicate widespread changes pertaining to radiotherapy or chemotherapy protocols for *BRCA1/2* mutation carriers. As far as surgical strategies are concerned, some authors defend the appropriateness of standard conservative approaches, and insist on the presence of the additional psychological burden resulting from the need for decision-making about bilateral mastectomy at the time of diagnosis. Others have suggested that early bilateral mastectomy could be proposed as an initial surgical procedure after cancer diagnosis. This would require early genetic counselling and rapid molecular testing, which in turn would cause organizational and economic constraints on the health care system. Scientific evidence in this area is evolving. It is beyond the objectives of this report to consider testing in cancer patients without a family history of cancer, with the objective of adapting surgical procedures.

What conditions or services should be made available with testing?

The potential benefits that can be derived from knowledge about an individual's *BRCA1/2* mutation status if the individual belongs to a family at increased risk of breast or ovarian cancer have been explored. These include reduced anxiety and surveillance requirements for non-carriers, and adapted surveillance and preventive measures for carriers. The advantages for other family members of a more precise risk assessment should not be neglected. Risks will be appreciated differently according to the individual's prior risk, risk perception, knowledge, and personal exposure to cancer, in addition to the test result and contemplated preventive options. In contrast to the physical risks related to most preventive measures, the risks associated with test results are mainly psychosocial. Psychological distress, anxiety, and depression may occur while awaiting test results or after a positive result. This may lead to inappropriate surveillance behaviour if the test result is misinterpreted.

Even without considering the psychosocial consequences of knowing one's *BRCA1/2* mutation status, the testing process and the choices that have to be made as a result of the testing warrant special services being put in place to support patients and families. Because of the amount and complexity of the information that must be conveyed to ensure informed choices are made, qualified personnel and ample time must be dedicated to providing this information. The baseline knowledge among patients and family members is often limited. These individuals should have the opportunity to ask questions and reach decisions in their own time, which necessitates that several information sessions may be required. Choices are difficult to make, not only because of the amount of information that must be integrated, but also because of the uncertainty surrounding several dimensions, such as the individual risk of developing cancer (i.e., which is related to penetrance of *BRCA1/2* mutations). Uncertainties are also due to the limited evidence and at times questionable quality of the data. Test performance and efficacy of surveillance and preventive measures are examples. Several choices need to be made with respect to undergoing the test, disseminating the information to family members, and choosing among surveillance and preventive options. These choices can have an impact, not only on the individual, but also on his or her offspring and close relatives. They can affect family dynamics,

personal health and survival, quality of life, self-perception, psychological functioning, reproduction, and sexuality. If the long-term acceptance of the consequences of one's choices is the goal, then such personal, sensitive, and consequential decisions must be made on the basis of complete and understandable information and in full accordance with one's values. The quality of the relationship between the professional providing counselling and the patient and family members is paramount.

Because of the potential for risks, and the difficulties that individuals and families have to face during testing, conditions should be modified to guarantee the quality of service delivery and the minimization of risks. It is accepted that testing for hereditary breast and ovarian cancer should be preceded and followed by a genetic counselling session conducted by qualified personnel. Genetic counselling has been shown to improve knowledge and risk perception, without affecting the interest in testing.

Testing for *BRCA1/2* mutations raises issues that are common to testing for other hereditary conditions. These include concerns about social consequences, such as stigmatization and discrimination; the need to respect privacy and confidentiality; the issues of disclosure to family members and testing in children; the cost; and the accessibility to services (associated with the granting of patent rights). While these issues are a concern for patients, families, health professionals, others have an impact on the health care system, and entail the organization of services, oversight mechanisms, and protection of the citizen.

What are the implications for the health care system?

This report was not designed to examine organizational and economic issues related to testing for *BRCA1/2* mutations. As a result, many of the policy issues arising from the dissemination and use of this technology cannot be answered. A comprehensive overview in the form of a systematic review of the issues associated with testing for *BRCA1/2* mutations was thought to be of value as supporting information to policy and decision makers. The information compiled in this report will assist with identifying gaps in current knowledge, variability in technical and clinical practice, and uncertainties regarding the benefits and risks associated with the clinical management options.

The absence of clear testing indications for *BRCA1/2* mutation detection and evolving conceptions about who is in the best position to benefit from testing render difficult the estimation of need for tests and services. The demand for testing and counselling is partly driven by public awareness of the condition and by the availability of testing, both of which may be influenced by the media and industry. Furthermore, the uptake of testing and the adoption of preventive measures are likely influenced by cultural factors.

A lack of estimates for need and demand complicates the estimation of costs and implications for the health care system. The quality of care, particularly the quality of information provided and the quality of the provider-patient relationship, is important. Modalities for organizing services should support the delivery of quality care and access to counselling services. There is a shortage of qualified health care providers in genetic testing and services. Primary health care professionals should be kept current, so that they can manage referrals to specialized care

appropriately. Cost implications are not negligible, not only because the cost of a test is substantial (depending on the choice of technique), but also because the potential demand for genetic testing could increase if expanded indications are adopted. Furthermore, second-order costs could arise if testing is conducted outside the public health care system, and preventive measures (i.e., prophylactic surgery, chemophylaxis, and hormone replacement therapy after prophylactic surgery) are requested in the system. In light of the variability of mutation prevalence and distribution, and consequently of the clinical validity of testing, consideration could be given to the tailoring of testing methods and targeted mutations according to geographic regions and ethnic groups (i.e., regional coordination of services).

8 CONCLUSION

The integration of *BRCA1/2* mutation testing into the health care system has occurred under a variety of conditions and at different rates of uptake. This underscores the heterogeneity in the analytical validation of test methods, and the efficacy of surveillance and preventive measures. Variation also exists in the organization of services accompanying testing (i.e., genetic counselling), and the regional availability of health care professionals and resources to deliver these services. Ideally, the clinical use of testing in an individual should be based on solid evidence that the gene is associated with the disease, that the test has analytical and clinical validity, and that the result will be useful to the individual and clinician for decision-making. The intent of this systematic review of the literature was to identify evidence for *BRCA1/2* mutation testing and issues associated with the adoption of testing into clinical practice.

The analytical performance of *BRCA1/2* mutation testing, primarily in high risk families and founder populations, was reviewed. High variability was found between studies. Alhough most studies used DSA as a "gold standard," no two tests used the same index test and unit of analysis, thereby precluding comparisons of methods. Clinically relevant mutations may be missed if DSA is used as a primary strategy for detecting *BRCA1/2* mutations. As a result, the most analytically valid molecular technique for *BRCA1/2* could not be determined.

The contribution of *BRCA1/2* testing to the management of unaffected and affected carriers was examined. Data regarding the influence of testing on clinical management are limited, partly because of the limited treatment options available. Prophylactic surgery was shown to reduce the risk of breast and ovarian cancers in cohort studies, whereas surveillance strategies or chemoprophylaxis have not been shown to have a significant effect on cancer risk.

Studies on psychosocial impact and ethical issues were examined. Counselling informs the patient and has an influence on perceived risk, associated anxiety, and uptake of testing. Based on the studies reviewed for psychosocial impact, the public's knowledge of the association of cancer and genetics is limited. The positive or negative result of the test has an influence on risk perception, psychological impact (e.g., distress, depression, emotional reactions) and social issues (e.g., communication of results to family members). Ethical considerations include the importance of informed consent (or informed refusal), and privacy and confidentiality concerns

(e.g., risk of genetic discrimination from insurers, employers, or family members). Unique ethical implications exist for disclosure or the failure to disclose genetic information by health care providers.

Requirements for quality health care service delivery, and the moral obligation to provide optimal conditions for informed consent, create a responsibility to pursue research in these areas. Information is required to address timely issues in all the areas that were reviewed. Future research should seek to overcome the methodological limitations identified in the studies selected for this report, so that quantitative analyses can be conducted and comparisons made. This need applies not only to fundamental research, but also to the monitoring of clinical and technical practices, and to the follow-up of families undergoing genetic counselling and testing, to measure outcomes.

The need for additional research is symptomatic of an emerging practice. As a result, each jurisdiction will likely be required to handle this situation differently, in accordance with its own regulatory mechanisms, resources, and health care delivery abilities. Among possible options to be considered by policy and decision makers are conditional acceptance or reimbursement of *BRCA1/2* genetic testing for selected indications (i.e., testing under certain criteria or quality measures, reimbursement for individuals who are high risk), and restricted use to specific centres with identified protocols or to particular health care providers.

This report has systematically examined the literature for *BRCA1/2* mutation testing, primarily in high risk families and founder populations. Scientific data are accumulating rapidly, so if expansion of testing or consensus guidelines are pursued, an update to this report should be considered.

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APPENDICES

APPENDIX 1: Molecular Methods

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Allele-Specific Gene Expression Analysis (AGE)[98] relies on detection of functional effect of mutation at RNA level, known as nonsense-mediated RNA decay. Target polymorphisms are amplified from genomic or cDNA using direct incorporation primers. Amplification cycles occur for *BRCA1*. SSCP is carried out. Electrophoresis is performed, gels are dried and autoradiographed. Allele-specific band intensities are quantified by densitometric analysis. Allele ratios are calculated.	*BRCA1*, and truncating mutations	Identifies cis-regulatory mutations that are missed by other techniques	Performed for preliminary screening of high risk individuals. A slight imbalance prompts further investigation. Real-time PCR and mass spectrometry may be used for precise quantification of nucleic acids, improving precision of this approach.
A nuclease CEL I,[91] from celery, that is specific for DNA distortions and mismatches. A simple method of enzyme mutation detection using CEL I identifies mutations and polymorphisms. Exons of gene are amplified by PCR using primers labelled with two fluorescent dyes. PCR products are annealed forming heteroduplexes and subjected to CEL I incision. In an automated sequencer, two independent incision events, one in each strand, produce truncated fragments of two colours that complement each other to confirm position of mismatch.	*BRCA1*, and mutations and polymorphisms in various exons, including deletions, point mutations, and insertions. CEL I is most active on mismatch substrates.	CEL I mutation detection identifies mutations by different principles than DNA sequencing and SSCP. In genes such as *BRCA1*, where mutations are numerous, ability of CELI to detect mismatch at one or more nucleotide positions without prior knowledge of mutation provides promise as screening method. Ease of set up and performing CEL I mutation detection should allow it to be established quickly in most laboratories.	
Constant Denaturant Gel Electrophoresis (CDGE).[90] DNA fragments are separated based on size as they denature when run through gel containing chemicals that break down DNA. Position in gel where fragment melts depends on nucleotide sequence in melted region. Method is useful for separating DNA fragments of same size having different sequences.	*BRCA1*, and frameshift deletions, insertions, and single nucleotide substitutions	CDGE identified twice as many *BRCA1* sequence alterations as SSCP screening by Castilla *et al.*, 1994[90,272]	CDGE failed to detect rare sequence alteration that was identified by SSCP[90]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Conformational Sensitive Gel Electrophoresis (CSGE)[94] is based on altered mobility of heteroduplexed strands in gel matrix caused by annealing of mutant and wild type strands. Mutated strands that form heteroduplexes with wild type strands manifest as band shifts from homoduplexes. This technique differs from other heteroduplex based methods in that mildly denaturing solvents are used. This technique can be enhanced with fluorescence.[107]	*BRCA1* and *BRCA2*; and single base and frameshift mutations in *BRCA1* coding region and mismatches in *BRCA2*	CDGE precludes separate optimizations for each amplimer, as it is less affected by temperature and pH. CSGE allows detection of single-base mismatches in *BRCA2* that can be missed using conventional gels. Resolving power is increased using fluorescent electrophoresis platform. Flourescent CDGE is as sensitive and specific as manual CSGE for frameshift mutations and single base substitutions.[94] Variant of fluorescent CSGE includes modifications in gel conditions, reducing running time to three hours for fragments of 500 base pairs. Three PCR fragments in one lane of an ABI377gel can be run, each labelled with different coloured dye. This method is amenable to processing large sample sets with acceptable sensitivity. All primers are labelled with same dye, which is useful for PCR amplification and sequencing. This variant allows for changing strategy of multiplex PCR at no additional cost. Throughput can be increased by second loading of the same gel two hours after first. Electrophoresis with two loads is complete in five hours.[107]	Limits amount of PCR product loaded on gel. With an excess of DNA, there is a plateau effect on top of respective peak, which cannot be corrected by fragment analysis software. This can be avoided by reducing number of cycles in PCR to 30, rather than by making dilutions of samples.[107] Some mutations could not be analyzed, because failure to amplify by PCR. Four mutations were missed, because sequence analysis failed to confirm mutation after observation of abnormal gel mobility. Missed mutations included base substitution and three frameshift deletions.[53] CSGE showed lower sensitivity compared to DHPLC and TDGS.[53] CSGE tends to miss single nucleotide base substitutions.[53]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Dideoxy Fingerprinting (DDF) [109] is a combination of a Sanger sequencing reaction with multiple fragment SSCP. Dideoxy nucleotides lack the 3' hydroxyl group necessary for chain elongation and therefore generate series of discrete fragments. These fragments form multiple sequence-specific secondary structures when electrophoresed under nondenaturing conditions. Sequence variants form unique single-stranded conformational structures with altered electrophoretic mobility detected by autoradiography.	*BRCA1;* and deletions and insertions	DDF is reported to be more sensitive than SSCP, yet labour intensive.[109]	Detection of such mutations is possible by DDF in one direction only, if electrophoresis is performed at less than room temperature. Varying use of ddA, ddT, ddC, and ddG agents for amplification, could alter sensitivity of technique, depending on position of sequence change. Differences in sensitivity are difficult to determine. Changes must be further investigated by DNA sequencing. Purifying primary DNA product before DDF produces more distinct bands and reduces incidence of false positives.[109]
Denaturing High Performance Liquid Chromatography (DHPLC)[56] is based on heteroduplex formation. Detection is carried out after DNA fragments of interest are amplified by PCR. Fragments are denatured and slowly cooled to allow hybridization of DNA fragments. Samples that are heterozygous for single-nucleotide substitutions, small deletions, or insertions hybridize to produce mixture of hetero and homoduplexes. These fragments are resolved after being passed through DNA separation column in DHPLC machine. Samples showing a different pattern than known homozygous wild-type samples or samples with multiple peaks on DHPLC analysis are assumed to contain sequence variants and undergo sequence analysis.[56]	Detects most mutations other than: large intron-intron deletions or whole gene deletions; intron-intron inversion mutations; point mutations that are masked by second mutations in cis that affect a primer binding site; and any mutation that fails to make a difference to melting profile of segment under DHPLC conditions used.	After PCR setup and amplification, little further processing is needed. Samples are placed into machine and automatically loaded into column. If abnormal fragments are discovered, remaining aliquot can be used for sequencing. In comparison with direct sequencing, costs for analysis of a fragment are 10 times lower by DHPLC. DHPLC is eight times faster than direct sequencing, and results can be obtained in one day. Evaluation of results is effortless, because investigator has only to compare elution profiles.[109] Although initial capital investment is required, combination of low running costs and reduced effort of sequencing make DHPLC a suitable method for mutation detection.[273]	Each fragment injected into separation column requires custom temperature profile that matches sequence melting temperature. This may hinder post-PCR mixing of samples and restrict sample throughput. Purchase costs of a DHPLC system are significant.[94] A disadvantage of DHPLC is that because it does not detect every nucleotide change, for common polymophisms, most samples will also need to undergo sequence analysis. PCR primers designed to exclude intronic polymorphisms that are of no interest may be necessary to reduce number of samples that need sequencing.[56] Amplicons with extreme G-C content require resolution by running analysis under two temperatures or by incorporating 7-deaza-GTP in the PCR mix.[109] Highest cost versus SSCP, CSGE, or TDGE.[53]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Direct Sequence Analysis (DSA) is considered to be optimum method, and most sensitive of available methods of mutation detection.[56] DSA pinpoints location of mutation and may provide indication of effect a mutation may have on encoded protein. Sequencing technologies use gel electrophoresis to produce high resolution separation of DNA molecules. Fragments differing in size by a nucleotide can be resolved.	Most mutations except large intron-intron deletions or whole gene deletions; intron-intron inversion mutations; and point mutations that are masked by second mutations in cis that affect a primer binding site.	Favoured method in detecting point mutations found 38% of frameshift mutations and 58.5% of point mutations[56]	Large costs in equipment and reagents make this method untenable for average investigator. DSA is most expensive method for screening, because of commercial charges levied for sequencing kits. Sceening of large number of samples exacerbates problems. Approximately 14 fragments of *BRCA2* can be screened by F-MD for price of one DSA.[56] It is impossible to detect large gene rearrangements such as exon 22 using PCR-based DNA methods, because primers cannot anneal to mutant strand when DNA is absent.[56] Because of limitations of PCR process preceding sequence analysis: large intron-intron deletions or whole gene deletions; intron-intron inversion mutation; and point mutations that are masked by second mutations in cis that affect a primer binding site (null alleles due to primer-binding site variation) may be missed.
Enzymatic Mutation Detection (EMD) uses resolvase, endo VII, which has high specificity for insertions, deletions, and base-substitution mismatches. PCR is used to amplify normal and mutant alleles of target sequence. Forward PCR primers are labelled a blue fluorescent dye, and the reverse primers with a green fluorescent dye. Upon denaturing and renaturing, normal and mutant alleles in mixture form mismatched heteroduplexes. For each base change, two mismatches are formed. The endo VII enzyme scans double-stranded DNA, until it detects structural distortion, either bubble caused by single base pair mismatches or a heteroduplex loop formed by hybridizing wild-type allele with mutant allele containing insertion or deletion.	EMD has high specificity for many types of alterations including insertions, deletions, and base substitution mismatches.[56] This technology is well suited to detecting mutations in large genes, mutated in unpredictable locations. This technique is not widely used for *BRCA1* or *BRCA2*.	EMD is simple, specific, and easy to use. Enzyme cleaves all possible miss-pairings, but efficiency of cleavage varies, depending on mismatch and local nucleotide. EMD is more efficient for heterozygous mutation screening than DSA, as some point mutations that are ambiguous in the automated sequencing results are readily detected by EMD. This test allows for detection of multiple sequence variants in same PCR product reaction, even those separated by only several base pairs. Multiplexing can be performed to	EMD methods lack the sensitivity and specificity of the chemical cleavage of the mismatch method[109]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Enzyme cleaves within six base pairs on 3' side of mutation, forming two shorter fragments, one blue and one green. DNA product is analyzed on automated sequencer and mobility of each fragment is evaluated.[56]		reduce overall cost of screening large genes.[56]	
Fluorescent Mutation Detection (F-MD) Fast, automated method for screening large genes based on HA, adapted for high throughput by combining fluorescent technology of automated sequencers and robotic sample handling.[94]	*BRCA2;* F-MD can detect point mutations more reliably and robustly than commonly used conformation gel based band shift assay CSGE; and F-MD will detect alterations as reliably as DHPLC and DSA.	This approach allows entire *BRCA2* gene to be screened with appropriate overlaps in four lanes of an ABI377 gel. This method relies on band shift detection. Sensitivity has been increased in that every fragment loaded in gel has to migrate through entire gel to be detected. This differs from conventional gels, wherein mix of fragments migrate with larger ones travelling short distance into gel to retain shorter fragments for visualization. Additional distance travelled allows conformational variants to be resolved. Screening rate compares favourably with DHPLC and DSA, where approximately seven to eight fragments per hour can be screened. F-MD costs about 0.07U per fragment; 14 fragments can be screened for price of one DSA. Entire *BRCA2* gene can be screened for cost of approximately three DSA.[94] Many laboratories may operate an automated analyzer, and would be able to modify existing machine to conduct F-MD screening.[94]	Fragments do not migrate according to molecular weight in mutation detection electrophoretic (MDE) gel matrix, because of inherent conformation and secondary structure of different sequence stretches. On rare occasions, heteroduplexes produced by some larger fragments do not emerge from gel in run times available, is in contrast to conventional gels where whole gel is visualized for band shifts. Fluorescent MDE gel is capable of resolving homoduplexes efficiently. Restricting fragments to 200 to 300 base pairs for *BRCA2*, while increasing detection sensitivity, would increase number of fragments needed for screening from 45 to 65.[94]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Heteroduplex Analysis (HA) detects base changes in double-stranded DNA subjected to electrophoresis in non-denaturing conditions. PCR-amplified DNA fragments are denatured and re-annealed to give mixture of four duplexes consisting of two homoduplexes and two heteroduplexes in heterozygote samples. Heteroduplexes have aberrant, distorted structure with bubbles or bulges at sites of mismatched bases, and move more slowly in gel than homoduplexes.[96]	Insertions and deletions	Enhancements in HA include improvement of sensitivity by running gels in mildly denaturing conditions, multiplex analysis, and adaptation of method to fluorescent platform	Mutation detection rate of HA is about 80%, and PCR products are usually of similar length as that of SSCP, or longer. Among drawbacks of this method is its lower sensitivity in detecting base substitutions compared to detection of insertions and deletions.[96]
Immunohistochemistry (IHC): most mutations result in protein truncations that are thought to be detectable by IHC analysis with commercially available antibodies. Antibodies directed against amino and carboxy terminals demonstrate quantitative reduction in reactivity in tissue carrying mutation relative to normal tissue.[61]	*BRCA1;* and protein truncating mutations	IHC is less expensive and less labour intensive than DNA analysis[92]	This technique may not be widely used in a clinical setting
Microarray Based Detection: microarrays are systematic arrays of cDNAs or oligonucleotides of known sequence that are printed or synthesized at discrete loci on glass or silicon surface.[72] Microarrays allow alterations in transcript level of genomes to be simultaneously assayed.	*BRCA1;* and designed to screen entire coding sequence of gene for all possible sequence changes including single nucleotide insertions and deletions[73]	Microarray-based mutational analysis is well suited for targets with non-repetitive sequence composition	Sensitivity and specificity are issues when a frameshift mutation occurs in context of short repeated sequence[73]
Multiplex Ligation-Dependent Probe Amplification (MLPA) determines relative copy number of all *BRCA1* exons simultaneously with high sensitivity and throughput.[67] MLPA kits are available to detect copy number changes in *BRCA1* and confirm deletions and duplications.[68] The MLPA kit for *BRCA2* contains probes for most coding exons of *BRCA2* gene.[68] MLPA is PCR based and allows relative quantification of many DNA sequences in one reaction.[274]	*BRCA1* and *BRCA2*; single or multiple exon deletions and amplifications	MLPA can be an inexpensive and effective method of dosage analysis	When MLPA shows one exon deletion, results should be confirmed with a second dosage technique.[70] MLPA is sensitive to experimental conditions and template contaminants.[70]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Multiplex Mutagenically Separated PCR (MS-PCR): a simple, rapid method for simultaneous detection of common mutations. Three allele-specific oligonucleotide primers used for each mutation (common, mutant and wild-type). Mutant and wild type primers contain mismatch base sequence that generates mutagenized PCR product that is refractory to cross amplification by competing primer, ensuring specificity of reaction. Heteroduplexes may be formed from short and long products, but mutant and wild type product separate mutagenically. [95]	*BRCA1* and *BRCA2;* used to detect common founder mutations: *BRCA1*; 185delAG; 5382insC; *BRCA2*: 6174delT	Because at least one of two allelic PCR products is present, MS-PCR provides an intrinsic quality control against false negatives or PCR refractory conditions. Presence of wild-type and mutant allelic products allows easy and objective interpretation of test results. This assay eliminates need for radioisotopes, endonuclease digestion, and high resolution electrophoresis.[95]	MS-PCR requires careful optimization of each reaction condition, including magnesium concentration co-solvents, and length and temperature of cycling stages. Concentration of primers must be determined empirically to give equal amplification of wild-type and mutant alleles. Minimum of three bands (absence of any mutant allele) and maximum of six bands (all three mutations) can be detected.[95]
Protein Truncation Test (PTT) PPT provides mutational analysis of complete coding region using RNA and DNA as PCR templates. Protein synthesis is achieved from PCR template using coupled transcription-translation in vitro system and radio-labelled protein products. Shorter protein products are detected by autoradiography. Complete PTT analysis of *BRCA1* requires RNA and DNA as a template. Exon 11 of *BRCA1* covers 60% of coding region.[56]	*BRCA1;* in addition to coding region mutations, PTT can detect protein truncating mutations present outside coding region, including internal intronic sequence changes causing splice sites errors. Alterations resulting from genomic deletions or insertions represent mechanism of mutations in *BRCA1*, resulting in exclusion of entire exons, causing frameshift and premature termination.	PTT has advantages over conventional mutation detection. It allows analysis of larger regions of coding sequences compared to conventional methods. While exon 22 deletion requires confirmation with additional methods, PTT detects this deletion at mRNA level. PTT with 5' sequencing efficiently detects deleterious mutations in *BRCA1* that may be missed by other detection methods, including DSA.[56] Sensitivity in detecting deleterious mutations is high, although this is influenced by design and application.	PTT only identifies sequence alterations leading to truncated protein; missense mutations, inframe deletions and insertions are not detected.[109] As it is unknown whether most sequence alterations leading to truncated protein (missense mutations, inframe deleterious mutations and insertions) affect function of protein, PTT is clinically feasible to detect deleterious mutations. Mutations in 5' region of gene result in protein products that are too small to be detectable by PTT alone; therefore, one can use PTT with complementary sequencing of exons 2,3,5, and 6. There is decreased sensitivity in detecting mutations within first few hundred base pairs of designed PTT fragment, partly because of instability of shorter truncated proteins and insensitivity of gel systems used in detecting these short proteins. This can be overcome by increasing overlapping regions between PTT fragments and by developing more sensitive gel systems.[56]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Restriction Endonuclease Fingerprinting Single Strand Conformation Polymorphism Analysis (REF-SSCP) is based on repeated detection of DNA sequence variants in different restriction endonuclease fragments. REF-SSCP involves digestion of PCR products with restriction endonucleases before polyacrylamide gel electrophoresis.[106]	*BRCA1* exon 11; and deletions, insertions, frameshift and nonsense mutations	REF-SSCP has been reported to increase capacity and efficiency of mutation detection compared to SSCP.[106] In contrast to PTT, REF-SSCP is capable of detecting truncating mutations and single nucleotide changes in several sequence contexts created by using multiple restriction endonucleases, increasing statistical probability of detecting mutation.[106]	This technique missed a mutation that was in a sample from Norway. Extended exposure may increase sensitivity of REF-SSCP; optimization may be achieved by combination of and electrophoresis of different RE digestion reactions in one lane.[106]
Single-Strand Conformation Analysis/Polymorphism (SSCA/SSCP) relies on change in conformation in one DNA strand to identify sequence alterations. This technique exploits conformational changes caused by mutations and has been used to detect alterations in RNA and single stranded DNA.[96] In SSCP, PCR product is denatured, and separated strands adopt folded structures determined by their nucleotide sequences. A single base alteration is detected when folding of single strand changes sufficiently to alter its electrophoretic mobility.	SSCP is used to detect frameshift, deletions, insertions and missense mutations.[103] SSCP detects frameshift mutations that involve length difference in addition to base alterations. Most *BRCA* sequence variants are frameshift in nature.	Recent improvement of approach used in SSCP was development of heteroduplex analysis to detect mutations in double stranded DNA using CSGE.[94] Capillary electrophoresis (CE) offers higher analysis speed and lower reagent consumption compared to slab-gel electrophoresis. Automation of SSCP analysis by CE makes this method attractive for clinical genetic laboratories. With advent of multicapillary systems, instruments no longer have a lower throughput than slab gels. SSCP analysis is economical and simple.[109]	Temperature and pH significantly affect sensitivity of this method and conditions must be optimized for each amplimer.[94,109] Maximum sensitivity is obtained by running fragments of 200 base pairs under various conditions of time, temperature, and gel composition. Samples must be run under multiple conditions, decreasing efficiency of technique. Common use of SSCA templates larger than 200 base pairs decreases sensitivity of technique and may result in significant number of alterations being missed. SSCP required 41 PCR reactions with gel analysis under various conditions for each tumour, while PTT required five PCR reactions using three PCR conditions for each tumour.[103] SSCP has lower mutation detection rate compared to DHPLC and TDGS.[53] SSCP failed to detect several variants resulting from substitutions,[53] some protein truncating mutations, splice mutations, and a large deletion.[56,90]

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
SSCP and Heteroduplex analysis (HA): novel combined SSCP/duplex analysis adapted to modern capillary electrophoresis, which takes advantage of multicolour labelling of DNA fragments and laser-induced fluorescence detection[96]	*BRCA1* and *BRCA2* mutations, polymorphisms, and variants; a highly efficient method of detecting insertion and deletion mutations	Simple, low cost technology. Sensitivity of mutation detection in SSCP portion alone was 90% that in duplex portion, was 81% in single conditions of electrophoresis. Advantages most visible when large genes are scanned for scattered unknown mutations or when large number of DNA samples are screened for specific mutations. Potential for application to analyze pooled genomic DNA samples and multiplex analysis of amplicons from different gene fragments, which may reduce costs of analysis; attractive for large scale application in single nucleotide polymorphism scanning and screening. This method has advantage of single primer labelling, which allows multi-colour labelling of gene fragments for multiplex analysis. Short time of analysis makes it attractive for clinical laboratories.	
Stop Codon Assay: PCR-amplified DNA fragment from patient's cDNA or genomic DNA is recombined into specific gap vector harbouring yeast URA3 gene. Inability to express URA3-fusion protein depends on whether a protein-truncating mutation exists in inserted PCR fragment.[62]	*BRCA1* and *BRCA2* truncating mutations	Assay detected all protein truncating mutations in examined DNA fragments. Cost of stop codon assay for screening plus DNA sequencing only for stop codon assay-positive fragments was significantly lower than that for full-length DNA sequencing in all specimens.[62]	A false positive was noted in first screening.[62] Stop codon assay has not been compared to PTT in large number of clinical samples.

Technique and Description	Gene and Mutation Type	Advantages	Disadvantages
Two-Dimensional Gene Scanning (TDGS):[56] customized computer program is used to design optimal primer combinations for PCR amplification, DGGE and DNA-fragment distribution in the 2-D gel. When results of 2-D gel show aberrant patterns or missing DNA-spots, specific fragment is re-examined in 1-D gel and aberrant fragments are sequenced to identify DNA alteration. TDGS is based on parallel processing of mutational target fragments using combination of extensive multiplex PCR amplifications and 2-D DNA electrophoresis in denaturing gradient gels. Mutational variants are detected on basis of aberrant melting behaviour similar to DHPLC.[56]	*BRCA1;* test allows detection of mutations and polymorphisms in *BRCA1* coding regions and splice site mutations. TCGS detects point mutations, and small insertions and deletions that are in-frame or frameshift.	Different mutations or polymorphisms in same fragment can be recognized on basis of spot configuration. Advantages of TDGS include simultaneous localization of mutations to specific exons of entire *BRCA1* gene in one assay on basis of DNA fragment size and melting temperature characteristics, and possibility to recognize recurrent mutations or polymorphisms on basis of unique 4-spot configurations, which obviates need for sequence confirmation of all variants detected. These features increase test sensitivity, reduce labour, and permit screening of large numbers of samples at relatively low cost.[56] TDGS reported three mutations otherwise not identified by any other technique and has high throughput capability.[53,275]	TDGS missed two truncating mutations out of 15.[56] TDGS missed large exon22 deletion, 2985del15 and the Y1463X mutations, which were missed initially due to design flaws. Small deletion was not identified because heteroduplex molecules were so slow in passing through 2-D electrophoresis that they fell outside scanned region. By enlarging the scanning region, mutation was detected the second time. Y1463 X was missed because of suboptimal primer design, but was detected after primers for exon 14 were redesigned and optimized.[53] Using TDGS, two mutations could not be identified because of failure of sequence analysis; three false positives were reported after sequence analysis. Each missed mutation (L246V; IVS17+1G>T; Y1463X; 3171ins5 and 4510del3insTT) appeared to be a result of misinterpretation of the 2-D gel.[53] Interpretation of complex spot patterns may be source of error for false positive results.[53]

APPENDIX 2: BRACAnalysis® Technical Specifications

BRAC*Analysis*® Technical Specifications, Myriad Genetic Laboratories, March 19, 2004

Test results should be used only after review of following specifications:

1. Description of Analysis

***Comprehensive BRACAnalysis*®**

BRCA1: Full sequence determination in both forward and reverse directions of approximately 5,400 base pairs comprising 22 coding exons and approximately 750 adjacent base pairs in the non-coding intervening sequences (introns). Exons 1 and 4, which are non-coding, are not analyzed. The wild-type *BRCA1* gene encodes a protein comprised of 1863 amino acids.

BRCA2: Full sequence determination in both forward and reverse directions of approximately 10,200 base pairs comprising 26 coding exons and approximately 900 adjacent base pairs in the non-coding intervening sequence (intron). Exon 1, which is non-coding, is not analyzed. The wild-type *BRCA2* gene encodes a protein comprised of 3418 amino acids.

The non-coding intronic regions of *BRCA1* and *BRCA2* that are analyzed do not extend more than 20 base pairs proximal to the 5' end and 10 base pairs distal to the 3' end of each exon.

This analysis also includes detection of the following five specific large genomic rearrangements of the *BRCA1* gene: a 3.8-kb deletion of exon 13 and a 510-bp deletion of exon 22 described in individuals of Dutch ancestry[276], a 6-kb duplication of exon 13 described in individuals of European (particularly British) ancestry (The BRCA1 Exon 13 Duplication Screening Group. The Exon 13 duplication in the BRCA1 gene is a founder mutation present in geographically diverse population [277], a 7.1-kb deletion of exons 8 and 9 described in individuals of European ancestry[278]; 28:300-307), and a 26-kb deletion of exons 14-20.[89]

Single Site BRACAnalysis*®*: DNA sequence analysis for a specified mutation in *BRCA1* and/or *BRCA2*. Analysis for a specified large genomic rearrangement includes analysis for all five rearrangements described above.

Multisite 3 BRACAnalysis*®*: DNA sequence analysis of specific portions of *BRCA1* exon 2, *BRCA1* exon 20 and *BRCA2* exon 11 designed to detect the mutations 187delAG and 5385insC in *BRCA1* and 6174delT in *BRCA2*.

2. Description of Method

Blood samples are assigned a unique bar-code for robotic specimen tracking. DNA is extracted and purified from white cells isolated from each sample. Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification (35 reactions for *BRCA1*, 47 reactions for *BRCA2*). The amplified products are each directly sequenced in forward and reverse directions using fluorescent dye-labeled sequencing primers. Chromatographic tracings of each amplicon are analyzed by a proprietary computer-based review followed by visual inspection and confirmation. Genetic variants are detected by comparison with a consensus wild-type sequence

constructed for each gene. All potential genetic variants are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above. Genomic rearrangements are detected by recombination-specific PCR using primers specific for the normal gene as well as for the rearrangement.

3. Performance Characteristics

Analytical specificity: The incidence of a false report of a genetic variant or mutation resulting from technical error is considered negligible because of independent confirmation of all genetic variants (see above). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%).

Analytical sensitivity: Failure to detect a genetic variant or mutation in the analyzed DNA regions may result from errors in specimen handling and tracking, amplification and sequencing reactions, or computer-assisted analysis and data review. The rate of such errors is estimated from validation studies to be less than one percent (<1%).

Overall test accuracy: For a patient with at least a 10% probability of a positive test based on a personal or family history of cancer, the chance of an incorrect test result is less than one percent.

Limitations of method: There may be limited portions of either *BRCA1* or *BRCA2* for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare polymorphisms under primer sites. Other than the five specific large genomic rearrangements specified above, this assay will not detect genomic rearrangements or some types of errors in RNA transcript processing. The proportion of clinically significant defects in *BRCA1* and *BRCA2* attributable to such abnormalities is estimated to be approximately 15%[279].

4. Description of Nomenclature

All mutations and genetic variants are named according to the convention of Beaudet and Tsui.[280] Nucleotide numbering starts at the first transcribed base of *BRCA1* and *BRCA2* according to GenBank entries U14680 and U43746, respectively. (Under these conventions, the two mutations commonly referred to as "185delAG" and "5382insC" are named 187delAG and 5385insC, respectively.)

5. Interpretive Criteria

"Positive for a deleterious mutation": Includes all nonsense and frameshift mutations that occur at or before amino acid 1853 and 3308 of *BRCA1* and *BRCA2,* respectively (based on documentation of deleterious mutations in *BRCA1* and *BRCA2*). In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high risk families, functional assays, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.

"Genetic variant, suspected deleterious": Includes genetic variants for which the available evidence indicates a likelihood, but not proof, that the mutation is deleterious. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

"Genetic variant, favor polymorphism": Includes genetic variants for which available evidence indicates that the variant is highly unlikely to contribute substantially to cancer risk. Includes missense mutations in *BRCA2* that occur at and distal to amino acid 3326. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

"Genetic variant of uncertain significance": Includes missense mutations and mutations that occur in analyzed intronic regions whose clinical significance has not yet been determined, as well as nonsense and frameshift mutations that occur distal to amino acid position 1853 of *BRCA1* and between amino acid positions 3309 and 3325 of *BRCA2*.

"No deleterious mutation detected": Includes non-truncating genetic variants observed at an allele frequency of approximately one percent of a suitable control population (providing that no data suggest clinical significance), as well as all genetic variants for which published data demonstrate absence of substantial clinical significance. Includes truncating mutations in *BRCA2* that occur at and distal to amino acid 3326. Also includes mutations in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of the gene that have been demonstrated to have no deleterious effect on the length or stability of the mRNA transcript. Data on polymorphic variants are available upon request.

There may be uncommon genetic abnormalities in *BRCA1* and *BRCA2* that will not be detected by BRACAnalysis® (see Limitations of method). This analysis, however, is believed to rule out the majority of abnormalities in these genes, which are believed to be responsible for most hereditary susceptibility to breast and ovarian cancer.

"Specific variant/mutation not identified": Indicates that specific and designated mutations or variants are not present in the individual being tested. If one (or rarely two) specific deleterious mutations have been identified in a family member, a negative analysis for the specific mutation(s) indicates that the tested individual is at the general population risk of developing breast or ovarian cancer.

Change of interpretation and issuance of amended reports: If and whenever there is a change in the clinical interpretation of a specific reported variant, an amended test report will automatically be provided by Myriad Genetic Laboratories.

APPENDIX 3: Literature Search

In Dialog®
de = descriptor, i.e. Medical Subject Heading (a controlled vocabulary, or thesaurus, term)
ti = title (i.e. word has to occur in title field of the bibliographic record)
ab = abstract (i.e. word has to occur in abstract field of bibliographic record)
! = explode; picks up narrower terms as well, i.e. terms which are conceptually subsets of a broader term
F1$ = a large MeSH category, e.g. Behavior and Behavior Mechanisms, which is exploded to pick up all terms related to behavior and behavior mechanisms, as defined by the National Library of Medicine, i.e. about 400 MeSH terms
()= words must be adjacent
(2w) = words a maximum of two words apart in either direction
? = truncation symbol
dt= publication type
Set 22: Set 23 = Set 22 OR Set 23

In PubMed
[MeSH Term] = Medical Subject Headings (a controlled vocabulary, or thesaurus term)

[Title/Abstract Term] = word must appear in title or abstract of record

DATABASE	LIMITS	KEYWORDS
Dialog® OneSearch® (including MEDLINE®, CANCERLIT®, EMBASE®, Biosis Previews®, PASCAL, PsycINFO®	Human	
MEDLINE® (File 154), CANCERLIT® (File 159)	Subject Area I: 1st search Jan.28, 2003 for 1994 to2002; Update search on July 12, 2004 for 2003/2004.	**Subject Area I: Prevalence and Penetrance/Clinical Validity/ Indications, Risk Assessment/Guidelines for Testing** 1. (genes, brca1 OR genes, brca2 OR brca1 protein OR brca2 protein)/de 2. [brca OR brca1 OR brca2 OR 17q21 OR 13q12 OR 185delag OR 5382insc OR 999del5 OR breast()cancer()susceptibility()gene]/ti,ab 3. Set 1:Set 2 4. [breast neoplasms OR ovarian neoplasms/de] OR [breast()cancer? OR ovarian()cancer?]/ti,ab 5. Set 3 AND Set 4 6. (prevalence OR gene frequency OR penetrance/de OR probability! 7. [cumulative()risk? OR lifetime()risk? OR prevalence OR genotype()frequency OR mutation()frequenc? OR carrier()frequenc? OR allele()frequenc? OR population()based]/ti,ab

DATABASE	LIMITS	KEYWORDS
		8. "sensitivity and specificity"! OR reproducibility of results/de 9. [test()validity OR clinical()validity OR detection()rate? OR test()performance OR clinical()utility OR sensitivit? OR specificit? OR predictive()value? OR reproducibility()result?]/ti,ab 10. models, statistical! 11. risk()assessment OR model? OR Gail OR Claus OR Couch OR BRCAPRO OR FHAT OR myriad()genetics/ti,ab 12. {[test?] AND [guidelines! OR guideline?/ti,ab] OR {[test()criteria] OR [test()indication]}?/ti,ab 13. Set 6: Set 12 14. Set 5 AND Set 13 15. Limit Set 14/human 16. Limit Set 15/1999:2004 17. Set 16 from 154 (MEDLINE) 18. Set 16 from 159 (CANCERLIT)
	Subject Area II: 1st search from 1994 to 2002: Revised search performed March 5, 2003; Update search performed July 2, 2004 from 2003/2004	**Subject Area II:** **Molecular Methods, Analytical Validity** 19. [genetics, biochemical! OR (molecular diagnostic techniques OR polymorphism, single-stranded conformational)/de OR sequence analysis! OR blotting, southern/de OR polymerase chain reaction! OR cytogenetic analysis! OR chromatography, liquid! OR electrophoresis! 20. genetic()test? OR molecular()test? OR molecular()diagnostic()technique?/ti,ab OR molecular()diagnos?s/ti,ab OR [allele()specific()olignonucleotide() OR ASO OR protein()truncation()test? OR PTT OR conformation()sensitive()gel()electrophoresis OR CSGE OR constant()denatur?()gel()electrophoresis OR CDGE OR single()strand()conformation?()polymorphism() OR SSCP OR heteroduplex()analysis OR genetic()linkage OR sequencing OR denaturi?()gradient()gel()electrophoresis OR DGGE OR polymerase()chain()reaction OR PCR OR non()isotopic()RNA()cleavage()assay? OR NIRCA OR southern()blot? OR microarray OR DHPLC OR densitometry OR RNA OR quantitative()PCR OR missense OR frameshift OR nonsense OR truncating OR deletion OR duplication OR inversion OR splice()site OR splice()variant OR insertion OR

DATABASE	LIMITS	KEYWORDS
		multiplex()ligation()dependent probe amplification OR MLPA/ti,ab 21. Set 19 :Set 20 22. "sensitivity and specificity" OR reproducibility of results OR false negative reactions OR false positive reactions/de 23. [analytic?()valid? OR sensitivit? OR specificit? OR diagnostic()error? OR accurate OR accuracy OR reliable OR reliability OR predictive()value?()test? OR test()performance OR detection OR art?fact? OR false()positive? OR false()negative?]/ti,ab 24. Set 22:Set 23 25. Set 5 AND Set 21 AND Set 24 26. Set 25/human 27. Set 26/2003:2004 28. Set 27 from 154 (MEDLINE) 29. Set 27 from 159 (CANCERLIT)
	Subject Area III: 1st search Jan.28, 2003 for 1994 to2002; Update search on July 12, 2004 for 2003/2004.	**Subject Area III: Genetic Counselling, Psychosocial and Ethical Issues** 30. confidentiality! OR informed consent OR genetic counseling OR privacy! OR ethics! OR "behavior and behavior mechanisms!"{F1$} OR "psychological phenomena and processes!" OR "mental disorders!"{F3$} OR "behavioral disciplines and activities"!{F4$} 31. [(genetic(2w)counsel?ing OR privacy OR informed()consent? OR psychological OR psychosocial OR ethics OR ethical OR discrimination OR test()perception OR patient?()attitude]/ti,ab 32. Set 30:Set 31 33. Set 5 AND genetic(3w)test?/ti,ab AND Set 32 34. Set 33/human 35. Set 34/2003:2004 36. Set 35 from 154 (MEDLINE) 37. Set 35 from 159 (CANCERLIT)
	Subject Area IV: 1st search Jan.28, 2003 for 1994 to2002; Update search on July 12, 2004 for 2003/2004.	**Subject Area IV: Clinical Management** 38. (surgery OR ovariectomy)/de OR chemoprevention! OR "prevention and control"/de OR tamoxifen! OR diagnostic imaging! OR guidelines! 39. [clinical()management OR mammograph? OR mammogram? OR mastectom? OR oophorectom? OR ovariectom? OR tamoxifen OR hemoprevention OR MRI OR magnetic()resonance()imag? OR prophylactic OR

DATABASE	LIMITS	KEYWORDS
		prophylaxis OR guidelines?}/ti,ab 40. Set 38: Set 39 41. Set 5 AND (Set 21 OR genetic(3)test?) AND Set 4 42. Set 41/human 43. Set 42/2003:2004 44. Set 43 from MEDLINE 45. Set 43 from CANCERLIT
	See above	**Subject Area I:** **Prevalence and Penetrance/Clinical Validity/ Indications, Risk Assessment/Guidelines for Testing** 46. (brca1 protein or brca2 protein)/de 47. breast cancer! OR ovary cancer/de] OR [breast()cancer? OR ovarian()cancer?]/ti,ab 48. (Set 2 OR Set 46) AND Set 47 49. (reproducibility OR probability OR prevalence OR receiver operating characteristic OR reliability OR gene frequency OR segregation analysis OR penetrance)/de OR risk! 50. Set 7 OR Set 9 OR Set 11 OR Set 12 OR Set 49 51. molecular genetics! OR blotting! OR nucleic acid analysis OR liquid chromatography! OR gel electrophoresis! OR genetic analysis! 52. Set 20 OR Set 51 53. Set 48 AND Set 50 AND Set 52 54. Set 53/human 55. Set 54/1999:2002 56. Set 55 from 72 (EMBASE)
	See above	**Subject Area II: Molecular Methods, Analytical Validity** 57. (diagnostic error OR reproducibility OR probability)/de 58. Set 23 OR Set 57 59. (Set 2 OR Set 46) AND Set 52 AND Set 58 60. Set 59/human 61. Set 60/1994:2003 62. Set 51 from 72 (EMBASE)
	See above	**Subject Area III: Genetic Counselling, Psychosocial and Ethical Issues** 63. (genetic counseling OR privacy OR ethics! OR mental function! (F1$) OR behavior! (F2:90$) OR mental disease! (F3$) OR "psychological and psychiatric procedures, techniques and concepts" (F4$)

DATABASE	LIMITS	KEYWORDS
		64. Set 48 AND genetic(3w)test?/ti,ab AND (Set 63 OR Set 31) 65. Set 64/human 66. Set 65/1994:2002 67. Set 66 from 72 (EMBASE)
	See above	**Subject Area IV: Clinical Management** 68. (mastectomy OR ovariectomy OR chemoprophylaxis OR mammography OR diagnostic imaging OR tamoxifen OR nuclear magnetic resonance imaging) OR Set 39 69. Set 48 AND Set 52 AND Set 68 70. Set 69/human 71. Set 70/1994:2002 72. Set 71 from 72 (EMBASE)
Biosis Previews® (File 55)	Human 1998+ See above	**Subject Area I:** **Prevalence and Penetrance/Clinical Validity/ Indications, Risk Assessment/Guidelines for Testing** 73. (BRCA1 OR BRCA1-gene OR BRCA1 gene OR BRCA2 OR BRCA-2 gene OR BRCA2 gene)/de 74. (breast cancer OR breast carcinoma OR ovarian cancer OR ovarian carcinoma)/de 75. (Set 2 OR Set 73) AND Set 74 76. (sensitivity OR specificity OR reproducibility OR validity OR reliability OR risk OR risk analysis OR risk assessment OR penetrance OR prevalence OR gene frequency OR population-based study OR population studies)/de 77. Set 76 OR Set 7 OR Set 9 78. (molecular genetics OR molecular diagnosis OR protein truncation test OR heteroduplex analysis OR sequencing OR denaturing gradient gel electrophoresis OR polymerase chain reaction OR polymerase chain reaction analysis OR Southern blot OR Southern blot analysis OR high performance liquid chromatography OR polymerase chain reaction-single strand conformational polymorphism OR protein truncation test OR genetic linkage OR genetic linkage analysis)/de 79. Set 78 OR Set 20 80. Set 75 AND Set 77 AND Set 79 81. Set 80/human 82. Set 81/1999:2002 83. Set 82 from 55 (Biosis Previews)

DATABASE	LIMITS	KEYWORDS
	See above	**Subject Area II: Molecular Methods, Analytical Validity** 84. (sensitivity OR specificity OR diagnostic accuracy OR reproducibility)/de 85. Set 7 OR Set 84 86. (Set 2 OR Set 73) AND Set 77 AND Set 85 87. Set 86/human 88. Set 87/1994:2003 89. Set 88 from 55 (Biosis Previews)
	See above	**Subject Area III: Genetic Counselling, Psychosocial and Ethical Issues** 90. (genetic counseling OR genetic counselling OR confidentiality OR informed consent OR ethics OR psychiatric symptoms OR psychological distress OR psychological stress OR psychological well-being OR psychosocial function OR psychosocial factors OR psychosocial stress OR psychosomatics OR psychotherapy OR emotional OR emotional behaviour OR emotional distress OR emotional response OR emotional stress OR emotions)/de 91. Set 90 OR Set 31 92. Set 75 AND genetic(3w)test?/ti,ab AND Set 91 93. Set 92/human 94. Set 93/1994:2002 95. Set 94 from 55 (Biosis Previews)
	See above	**Subject Area IV: Clinical Management** 96. (chemoprevention OR tamoxifen OR practice guidelines OR diagnostic imaging OR mammography OR mammogram OR ovariectomy OR oophorectomy OR prognosis)/de 97. Set 39 OR Set 96 98. Set 75 AND Set 77 AND Set 97 99. Set 98/human 100. Set 99/1994:2002 101. Set 100 from 55 (Biosis Previews)
PASCAL (File 144) (Textword searching only)	Human 1998+ See above	**Subject Area I:** **Prevalence and Penetrance/Clinical Validity/ Indications, Risk Assessment/Guidelines for Testing** 102. (Set 2) AND [breast()cancer? OR ovar?()cancer? OR breast()neoplasm? OR ovarian()neoplasm?]/ti,ab 103. Set 102 AND (Set 7 OR Set 9 OR Set 11 OR Set 12)

DATABASE	LIMITS	KEYWORDS
		104. Set 103 AND (human? OR m?n OR wom?n)/ti,ab 105. Set 104 from 144 (PASCAL)
	See above	**Subject Area II:** **Molecular Methods, Analytical Validity** 106. Set 2 AND Set 20 AND Set 23 107. Set 106 AND (human? OR m?n OR wom?n)/ti,ab 108. Set 107/1994:2003 109. Set 108 from 144 (PASCAL)
	See above	**Subject Area III: Genetic Counselling, Psychosocial and Ethical Issues** 110. Set 102 AND genetic(3w)test?/ti,ab AND Set 31 111. Set 110 AND human? OR m?n OR wom?n)/ti,ab 112. Set 111/1994:2002 113. Set 112 from 144 (PASCAL)
	See above	**Subject Area IV: Clinical Management** 114. Set 102 AND Set 20 AND Set 39 115. Set 114 AND (human? OR m?n OR wom?n)/ti,ab 116. Set 115/1994:2002 117. Set 116 from 144 (PASCAL)
PsycINFO® (File 11)	Subject III only Human 1998+ See above	**Subject Area III: Genetic Counselling, Psychosocial and Ethical Issues** 118. [breast neoplasms OR (neoplasms AND ovaries)/de] OR [(breast()cancer? OR ovarian()cancer?]/ti,ab 119. Set 2 AND Set 118 120. Set 119 AND genetic(3w)test?/ti,ab 121. Set 120/human 122. Set 121/1994:2002 123. Set 122 from 11 (PsycINFO)
	Eliminate duplicate references from Dialog® OneSearch®	**Subject Area I**: **Prevalence and Penetrance/Clinical Validity/ Indications, Risk Assessment/Guidelines for Testing** 124. Set 17 OR Set 18 OR Set 56 OR Set 83 OR Set 105 125. Rd (reduce duplicates) Set 124 126. type Set 125 all from 154,159,73,55,144

DATABASE	LIMITS	KEYWORDS
		Subject Area II: Molecular Methods, Analytical Validity 127. Set 28 OR Set 29 OR Set 56 OR Set 89 OR Set 109 128. Rd (reduce duplicates) Set 127 129. Type S128/all from 154,159,73,55,144 **Subject Area III: Genetic Counselling, Psychosocial and Ethical** 130. Set 36 OR Set 37 OR Set 67 OR Set 95 OR Set 113 OR Set 123 131. Rd(reduce duplicates) Set 130 132. type Set 131/4/all from 154, 159,73,55,144,11 **Subject Area IV: Clinical Management** 133. Set 44 OR Set 45 Set 72 OR Set 101 OR Set 117 134. Rd (reduce duplicates) Set 133 135. Type Set 132/4/all from 54,159,73,55,144
PubMed	Human 1999+ 1st search performed Jan. 29, 2003 Update search performed June 23, 2004	**Subject Area I: Prevalence and Penetrance/Clinical Validity/ Indications, Risk Assessment/Guidelines for Testing** 1. (genes, brca1 OR genes, brca2 OR brca1 protein OR brca2 protein)[MeSH Terms] 2. brca OR brca1 OR brca2 OR 17q21 OR 3q12 OR 185delag OR 5382insc OR 999del5 OR "breast cancer susceptibility gene" [Title/Abstract] 3. Set 1:Set 2 4. [breast neoplasms OR ovarian neoplasms] [MeSH Terms] OR ["breast cancer" OR "breast neoplasm?" OR "ovarian cancer?" OR "ovarian neoplasm][Title/Abstract Term] 5. Set 3 AND Set 4 6. [prevalence OR gene frequency OR penetrance OR probability] [MeSH Terms] 7. ["cumulative risk" OR "lifetime risk" OR prevalence OR "genotype frequency" OR "gene frequency" OR "mutation frequency" OR "carrier frequency" OR "allele frequency" OR population based"][Title/Abstract] 8. Set 6: Set 7 9. ["sensitivity and specificity" OR "reproducibility of results"][MeSH Term] 10. ["test validity" OR "clinically valid" OR "clinical validity" OR "detection rate" OR "test performance" OR "clinical utility" OR specificit?

DATABASE	LIMITS	KEYWORDS
		OR sensitivit? OR "reproducibility of results" OR "risk assessment" OR model? OR gail OR claus OR couch OR brcapro OR fhat OR "myriad genetics"] [Title/Abstract Term] 11. {test? [Title/Abstract] AND [guidelines [MeSH Term] OR guideline? [Title/Abstract Term] OR {test indication?" OR "test criterion" OR "test criteria" [Title/Abstract Term]} 12. Set 9:Set 11 13. Set 5 AND Set 8 AND Set 12/human
	See above; Revised Subject II search performed March 6, 2003	**Subject Area II: Molecular Methods, Analytical Validity** 15. [genetics, biochemical OR molecular diagnostic techniques OR polymorphism, single-stranded conformational OR sequence analysis OR blotting, southern OR polymerase chain reaction OR cytogenetic analysis OR chromatography, liquid OR electrophoresis!][MeSH Terms] 16. ["genetic test?" OR "molecular test?" OR"molecular diagnostic technique?" OR "molecular diagnos?s" OR "allele specific oligonucleotide" OR ASO OR "protein truncation test?" OR PTT OR"conformation sensitive gel electrophoresis" OR CSGE OR "constant denatur? gel electrophoresis" OR CDGE OR "single strand conformation? polymorphism" OR SSCP OR "heteroduplex analysis" OR MHA OR "genetic linkage" OR sequencing OR "denaturi? gradient gel electrophoresis" OR DGGE OR "polymerase chain reaction" OR PCR OR "non isotopic RNA cleavage assay?" OR NIRCA OR "southern blot?" OR microarray OR DHPLC OR densitometry OR RNA OR "quantitative PCR" OR missense OR frameshift OR nonsense OR truncating OR deletion OR duplication OR inversion OR " splice site" OR "splice variant" OR insertion OR brcapro][Title/Abstract Terms] 17. Set 15: Set 16 18. ("predictive value of tests" OR "sensitivity and specificity" OR false negative reactions OR false positive reactions)[MeSH Terms] OR ["analytic?valid?" OR sensitivit? OR sensitivity OR sensitive OR specificit? OR specificity OR specific OR "false positive reaction?" OR "false negative reaction?" OR "diagnostic error?" OR accurate OR accuracy OR reliable OR reliability OR "predictive value? test? OR 'test performance"

DATABASE	LIMITS	KEYWORDS
		OR detection OR "art?fact?"] [Title/Abstract Term] 19. Set 3 AND Set 17 AND Set 18/human
	See above	**Subject Area III: Genetic Counselling, Psychosocial and Ethical Issues** 21. (confidentiality OR informed consent OR genetic counseling OR privacy OR ethics OR "behavior and behavior mechanisms" OR "psychological phenomena and processes" OR "mental disorders" OR "behavioral disciplines and activities")[MeSH Terms] OR ["genetic counsel?ing"OR privacy OR "informed consent?"OR psychological OR psychosocial OR ethics OR ethical OR discrimination OR"test perception" OR "patient atttitude?"] [Title/Abstract Term] 22. Set 5 AND "genetic test?"[Title/Abstract Term] AND Set 21 23 Set 22/human
	See above	**Subject Area IV: Clinical Management** 24. (surgery OR ovariectomy OR chemoprevention OR tamoxifen OR diagnostic imaging OR "prevention and control")[MeSH Terms] 25. ("clinical management" OR cancer management" OR mammograph? OR mammogram? OR mastectomy? OR tamoxifen OR chemoprevention OR MRI OR "magnetic resonance imaging" OR prophylactic OR prophylaxis)[Title/Abstract Terms] 26. Set 24: Set 25 27. Set 5 AND Set 17 AND Set 26 28. Set 27/human
PubMed Additional search	Performed Feb. 16, 2004	**Subject Area II: Molecular Methods, Analytical Validity** 1. "multiplex ligation-dependent probe amplification"/all fields 2. MLPA/all fields
Cochrane Collaboration and Update Software The Cochrane Library on CD-ROM	See dates from Dialog searches	Same strategy as for PubMed, i.e. Medical Subject Headings and textwords, using the syntax and system features particular to Cochrane Library on CD-ROM
Dialog® OneSearch® (including MEDLINE®, CANCERLIT®,	Ethics search performed July 28, 2004	1. (genes, brca 1 OR genes, brca1 OR genes, brca2 OR brcai protein OR brca2 protein)/de 2. (brca OR brca1 OR brca2 OR 17q21 OR 13q12

DATABASE	LIMITS	KEYWORDS
EMBASE®, Biosis Previews®, PASCAL, PsycINFO®)		OR 185 delag OR 5382insc OR 999delag)/ti,ab OR breast()cancer()susceptibility()gene/ti,ab OR breast()cancer()susceptibility()genes/ti,ab 3. Set 1 OR Set 2 4. (breast neoplasms OR ovarian neoplasms)/de OR breast()cancer?/ti,ab OR ovarian()cancer?/ti,ab 5. Set 3 AND Set 4 6. (confidentiality! OR privacy! OR ethics! OR informed consent)/de 7. (privacy OR ethics OR ethical OR ethic OR moral OR morals OR morality OR confidential?)/ti,ab OR informed()consent?/ti,ab 8. Set 5 AND (Set 6 OR Set 7) 9. Set 8/human 10. Set 9/2000:2004 11. Set 10 from 155 12. Set 11 from 159 13. (brca1 protein OR brca2 protein)/de 14. breast cancer! OR ovary cancer/de OR breast()cancer?/ti,ab OR ovarian()cancer?/ti,ab 15. Set 13 OR Set 2 16. Set 14 And Set 15 17. ethics! OR (ethicist OR confidentiality OR privacy OR genetic privacy OR morality)/de 18. Set 16 AND (Set 7 OR Set 17) 19. Set 18/human 20. Set 19/2000:2004 21. Set 20 from 72 22. (brca1 OR brca1-gene OR brca1 gene OR brca2 OR brca2-gene OR brca2 gene)/de 23. (breast cancer OR ovarian cancer OR ovarian carcinoma OR breast carcinoma)/de 24. (Set 22 OR Set 2) AND (Set 23 OR breast()cancer?/ti,ab OR ovarian()cancer?/ti,ab OR breast()neoplasm?/ti,ab) 25. (confidentiality OR informed consent OR ethics OR morality)/de 26. Set S24 AND (Set 7 OR Set 25) 27. Set 26/human 28. Set 27/2000:2004 29. Set 28 from 55 30. Set 2 AND Set 4 AND Set 7 31. Set 30 AND (human OR humans OR man OR men OR woman OR women)/ti,ab 32. Set 31/2000:2004 33. Set 32 from 144 34. breast neoplasms/de OR (neoplasms AND ovaries)/de OR breast()cancer?/ti,ab OR ovarian()cancer?/ti,ab 35. Set 2 AND Set 34

DATABASE	LIMITS	KEYWORDS
		36. (ethics OR morality OR professional ethics OR informed consent OR privacy OR privileged communication OR anonymity)/de OR Set 7 37. Set 35 AND Set 37 38. Set 37/human 39. Set 38/2000:2004 40. Set 39 from 11 41. Set 11 OR Set 12 OR Set 21 OR Set 29 OR Set 33 OR Set 40 42. Rd Set 42
PubMed	Ethics Search	
Cochrane	Ethics Search	
"CCOHTA HTA Checklist"		Includes websites of the International Network of Agencies for Health Technology Assessment (INAHTA) and related agencies such as the NHS National Institute for Clinical Excellence (NICE)(UK); trial registries such as the Clinical Trials Database (US National Institutes of Health); clinical practice guidelines databases such as the Canadian Medical Association Infobase; and specialized databases such as those of the University of York Centre for Reviews and Dissemination.
Internet searching		Google™ and AlltheWeb search engines
Society and association websites		Relevant websites such as the American Association of Cancer Research for conference abstracts.

APPENDIX 4: Abstract Selection Forms for Subject Areas II-IV

Abstract

Other subject areas

BRCA Project Abstract Review
Study inclusion or exclusion form

Subject area II analytical validity
Title
First author and year
Reviewer **L. McGahan**_____ **R. Kakuma**_____

Inclusion Criteria

1. Population yes_____ no_____ cannot tell_____
   Individuals at risk for inherited breast or ovarian cancer.

2. Intervention yes_____ no_____ cannot tell_____
   - molecular method to detect a *BRCA1* mutation
   - molecular method to detect a *BRCA2* mutation

3. Study design yes_____ no_____ cannot tell_____
   - primary study in a research or clinical setting
   - sample size (n≥20 patients)

4. Outcome measures yes_____ no_____ cannot tell_____
   - comparison of test result with genotype
   - comparison of test with sequence analysis
   - comparison of more than one test
   - any new technique for *BRCA* analysis described in literature

---

- "yes" (1 to 4 inclusive), include study and order full paper_____
- "cannot tell" (any of 1 to 4), order full paper _____
- "no" (any of 1 to 4), exclude study _____

- agreement between reviewers yes_____ no_____
- decision by reviewer 3 if disagreement include ___ exclude_____

Other topic(s) covered

SA1: prevalence, penetrance, clinical validity, risk assessment	yes_____	no_____
SA3: psychosocial and ethical, counseling	yes_____	no_____
SA4: clinical management	yes_____	no_____

Other comments (review, editorial, comment)

BRCA Project Abstract Review
Study inclusion or exclusion form

Abstract

Other subject areas

Subject area III genetic counselling, psychosocial, and ethical issues
Title
First author and year
Reviewer **L. McGahan_____** **J. Tranchemontagne_____**
R. Kakuma_____ **H.Z. Noorani_____**

Inclusion Criteria

1. Population yes_____ no_____ cannot tell_____
   Individuals at risk for inherited breast or ovarian cancer

2. Intervention yes_____ no_____ cannot tell_____
   - *BRCA1* or *BRCA2* genetic testing

3. Study design yes_____ no_____ cannot tell_____
   - primary study in a research or clinical setting (note reviews/editorials)
   - sample size (n≥20 patients)

4. Outcome measures (qualitative): yes_____ no_____ cannot tell_____
   - contribution of testing to counselling
   - psychosocial implications
   - ethical implications

- "yes" (1 to 4 inclusive), include study and order full paper_____
- "cannot tell" (any of 1 to 4), order full paper _____
- "no" (any of 1 to 4), exclude study _____

- agreement between reviewers yes_____ no_____
- decision by a third reviewer if disagreement include ___ exclude_____

Other topic(s) covered
SA1: prevalence, penetrance, clinical validity, risk assessment yes_____ no_____
SA2: analytical validity yes_____ no_____
SA4: clinical management yes_____ no_____

Other comments (review, editorial, comment)

BRCA Project Abstract Review
Study inclusion or exclusion form

Abstract
Other subject areas

Subject area IV clinical impact of BRCA testing
Title
First author and year
Reviewer C. Ho_____ C. Lessard_____ K. Bassett_____

Inclusion Criteria

1. Population yes_____ no_____ cannot tell_____
   Individuals at risk for inherited breast or ovarian cancer:
   - multiple cases of breast cancer or ovarian cancer
   - <35 years of age at diagnosis of breast cancer
   - family member diagnosed with both breast and ovarian cancer
   - breast or ovarian cancer in Jewish families
   - family member with primary cancer occurring in both breasts
   - family member with an identified *BRCA1* or *BRCA2* mutation
   - presence of male breast cancer in family
   - presence of associated conditions suggestive of inherited cancer syndrome

2. Intervention yes_____ no_____ cannot tell_____
   - molecular method to detect a *BRCA1* mutation
   - molecular method to detect a *BRCA2* mutation

3. Study design yes_____ no_____ cannot tell_____
   - any study design

4. Outcome measures yes_____ no_____ cannot tell_____
   - any clinical outcome, prophylactic or therapeutic purposes

---

- "yes" or "cannot tell" (1 to 4 inclusive): order full paper_____
- "no" (any of 1 to 4), exclude study _____
- agreement between reviewers yes_____ no_____
- decision by a third reviewer if disagreement include ___ exclude_____

APPENDIX 5: Study Summary and Quality Assessment Forms for Subject Areas II to IV

BRCA Project (Subject Area II) Study Summary and Quality Assessment

Date	**Reviewer Initials**	**ID**
Article identification (author, year): Full citation: Geographic location: Time period: Setting: (e.g., hospital-based, clinic-based, community-based registry, referral criteria and process) Declared conflict of interest: Source(s) of funding:		
Study Characteristics Purpose and objective(s) of study (include among whom):		
Design (cohort, case-control, cross-sectional, case-series, pedigree-based, other): Sample size (families, individuals, samples): Participation rate:		
Subject Characteristics Sampling procedure (consecutive, selective, random, unreported, other):		
Inclusion criteria (characteristics of subjects): Age: Ethnicity: Family history: Carrier status (carrier, non-carrier, unknown): Cancer (breast, ovarian):		
Exclusion criteria:		

Test Description **Mutation Testing Technique**	**Reference**
Description of the technique: Details (primers, probes):	Description of the technique: Details (primers, probes):
Modifications (if any):	Modifications (if any):
Alleles or mutations tested for:	Alleles or mutations tested for:
Nucleic acid source and type (DNA, RNA):	Nucleic acid source and type (DNA, RNA):
Setting, manufacturer, normal range (if applicable):	Setting, manufacturer, normal range (if applicable):
Regulatory status:	Regulatory status:
Cost:	Cost:
Failure rate of test (plus reasons if available; if not specified, verify number of subjects with test results):	Failure rate of test (plus reasons if available; if not specified, verify number of subjects with test results):
Sensitivity:	Sensitivity:
Specificity:	Specificity:
Notes (calculations, if any):	Notes (calculations, if any):

Data Extraction: Analytical Validity Data

2.1 Analytical sensitivity (proportion test positive among genotype positive)
Analytical specificity (proportion test negative among genotype negative)

Sensitivity=True (+)/ True (+) + False (–)
Specificity=True (–)/ True (–) + False (+)

BRCA1/2	Genotype (+)	Genotype (–)
Test (+)	True (+)	False (+)
Test (–)	False (–)	True (–)

*many mutations and alleles may be tested for

BRCA1	**Genotype (+) or Referenced**	**Genotype (-) or Referenced**
Molecular test (+)		
Molecular test (–)		
Sensitivity		
Specificity		

BRCA1	**Genotype (+)**	**Genotype (–)**
Comparison test (+)		
Comparison test (–)		
Sensitivity		
Specificity		

BRCA2	**Genotype (+) or Referenced**	**Genotype (–) or Referenced**
Molecular test (+)		
Molecular test (–)		
Sensitivity		
Specificity		

BRCA2	**Genotype (+)**	**Genotype (–)**
Comparison test (+)		
Comparison test (–)		
Sensitivity		
Specificity		

Assessment of Study Quality	
Disease or Test Verification e.g., molecular test used for confirmation, biopsy, linkage, statistical model	
Reference (ref) standard used	
description of reference adequate for replication yes no	% tested with ref of those + on other test
ref analyzed while blind to other test results yes no	% tested with ref of those – on other test
ref acceptable as a gold standard yes no unclear	test negatives assumed disease/mutation free yes no
Test Results (not reference)	
description adequate for replication yes no	clinical info used in test assessment/interpretation yes no
test analyzed while blind to reference results	pedigree info used in test assessment/interpretation

yes no	yes no
intermediate or uninterpretable results presented yes no	reproducibility verified by repeating same test yes no
agreement tested between different observers yes no *results (e.g., kappa):*	if more than 1 test used, validity of testing estimated for the group of tests yes no validity of testing estimated for each test separately yes no
Timing	
adequate length of follow-up (FU) yes no not applicable	testing method varied over time yes no unreported
potential for lead time bias yes no not applicable (e.g., in prognostic study, did follow-up begin sooner for some subjects?)	population selection varied over time yes no unreported (pop'n=population)
comparable FU for cases and controls yes no not applicable	time lag between test and ref yes no not applicable
Analysis	
all eligible subjects accounted for yes no	uncertainty quantified (give 95% CIs) yes no
test for representativeness completed yes no (comparison of eligible and analyzed subjects)	appropriate case or control comparison yes no not applicable
statistical procedures adequate yes no unclear	subgroup analysis performed yes no
Notes:	

Potential Biases **(mark with √ or ? if it may apply)**

Selection	**Performance**	**Measurement**	**Attrition**
Systematic (non-random) differences between those selected for the study and those not selected	Systematic differences in the study in how interventions were delivered (subject areas 3 or 4 only)	Systematic differences in the study in how variables were measured (or how subjects were classified)	(loss to follow-up) systematic differences between those analyzed and those who withdrew or were lost from study

Describe potential biases and their estimated impact on results:
Reporting of study details (mark) complete incomplete notes:
Completeness of clinical information (mark) complete incomplete (e.g., pathological confirmation of cancer) notes:
Limitations of study: (e.g. regarding test sensitivity or specificity, uninterpretable data, study design, potential biases, surprising results)
Population targeted by authors: Results appear applicable or generalizable to authors' target: yes no unclear Results appear applicable or generalizable to another target: yes no unclear if so, which target(s):

Conclusions made by authors based on data (in words, related to objectives on p.1) Consistent with data or analysis? (mark) yes no
Policy Recommendations made by authors (in words): Consistent with data or analysis? (mark) yes no

BRCA Project (Subject Area III) Study Summary and Quality Assessment

Date	**Reviewer Initials**	**ID**
Article identification (author, year): Full citation: Geographic location: Time period: Setting (e.g., hospital-based, clinic-based, community-based registry, referral criteria or process): Declared conflict of interest: Source(s) of funding:		
Study Characteristics Purpose or objective(s) of study (include among whom):		
Setting (hospital-based, clinic-based, community-based, registry, referral criteria or process): Design (cohort, case-control, cross-sectional, case-series, pedigree-based, other): Sample size (families, individuals): Participation rate:		
Subject Characteristics Sampling procedure (consecutive, selective, random, unreported, other):		
Inclusion criteria (characteristics of subjects): Age: Ethnicity: Family history: Carrier status (carrier, non-carrier, unknown): Cancer (breast, ovarian):		
Exclusion criteria		

Data Extraction

1.0 Genetic Counselling

1.1 Description of Pre-Test Genetic Counselling

Factors	
Description (content)	
Venue (face-to-face, CD-ROM, video)	
Provider	
Duration	
Population	
Indications for attendance	
Contribution of genetic testing (with known mutation, with no known mutation, with variant of unknown significance)	
Notes	

1.2 Description of Post-Test Genetic Counselling

Factors	
Description (content)	
Venue (face-to-face, CD-ROM, video)	
Provider	
Duration	
Population	
Indications for attendance	
Contribution of genetic testing *(with known mutation, with no known mutation, with variant of unknown significance)*	

2.0 Ethical Implications

2.1 Ethical Implications

Prima facie Concepts	**Application to *BRCA1* and *BRCA2* Genetic Testing**
Privacy and confidentiality (sensitive information handling)	
Justice (eligible persons receive access regardless of factors like geography)	
Autonomy (respect for persons) (informed consent, informed decision)	
Beneficence or non-maleficence (duty to benefit others, do no harm)	
Notes (other ethical issues, e.g., testing adolescents, children)	

3.0 Psychosocial Implications

3.1 Study Description and Implications of Testing

Study Description	**Reference *(if any)***
Description of technique (questionnaire, interview, focus group): Details*:* Modifications (if any): Factors assessed: Setting:	Description of technique: Details*:* Modifications (if any): Factors assessed: Setting:

Psychosocial Implications			
Pre-test (interest, knowledge, anxiety, attitude regarding prevention):			
Uptake of testing:			
Post-test notification of status (knowledge, anxiety, attitude, communication of results to family):			
Impact of known mutation: Impact of no known mutation: Impact of sequence variant of unknown significance: Impact of true negative result:			
Other psychological issues:			
Social issues:			
Assessment of Study Quality			
Timing			
Adequate length of follow-up (FU)	yes	no	not applicable
Population selection varied over time	yes	no	not applicable
Comparable follow-up for cases and controls	yes	no	not applicable
Analysis			
All eligible subjects accounted for	yes	no	
Representative (eligible versus analyzed compared)	yes	no	
Uncertainty quantified (give 95% CIs)	yes	no	
Statistical procedures adequate	yes	no	unclear
Subgroup analysis performed	yes	no	unclear
Notes:			

Potential Biases **(mark with √ or ?)**			
selection	**performance**	**measurement**	**attrition**
systematic (non-random) differences between those selected for the study and those not selected	systematic differences in study regarding how interventions were delivered (subject areas 3 or 4 only)	systematic differences in the study regarding how variables were measured (or how subjects were classified)	(loss to follow-up) systematic differences between those analyzed and those who withdrew or were lost from study
Describe potential biases and estimated impact on results:			
Reporting of study details (mark):		complete	incomplete
Notes:			
Completeness of clinical information (mark):		complete	incomplete
(e.g., pathological confirmation of cancer)			
Notes:			
Limitations of study: (e.g., regarding test sensitivity or specificity, un-interpretable data, study design, potential biases, surprising results)			
Population targeted by authors:			
Results appear applicable or generalizable to authors' target:		yes no	unclear
Results appear applicable or generalizable to another target:		yes no	unclear
if so, which target(s):			
Conclusions made by authors based on data (in words, related to objectives):			
Consistent with data or analysis? (mark) yes no			
Policy Recommendations made by authors (in words):			
Consistent with data or analysis? (mark) yes no			

BRCA Project (Subject Area IV) Study Summary and Quality Assessment

Study title		
Reference		
Methods		
Study design		
Study duration		
Diagnosis		
Eligibility criteria		
Country of origin		
Industry sponsorship	☐ Yes ☐ No ☐ Unknown	
	***BRCA* Positive**	***BRCA* Negative**
Surveillance or treatment strategy		
Sample size		
Baseline characteristics of study participants		
Age		
Concomitant therapies		
Other		
Other		
Outcomes		
Comments		

APPENDIX 6: Analytical Validity Studies

Table 1: Study characteristics

Author	Geographic Location	Centre	Setting	Ethnicity	Family History of Cancer	Carrier Status	Cancer	Gene	Number of Techniques Studied (including reference)
Andersen[90]	NR (US)	NR	NR	Norwegian*	yes	unknown	both	*BRCA1*	3
Andrulis[56]	NR (Canada, US, and Australia)	NR	referral criteria or process	NR	yes	known carrier and non-carrier	both	*BRCA1 and 2*	7
Arnold[109]	Germany	single	hospital-based	German	yes	unknown	both	*BRCA1*	2
Blesa[107]	Spain	single	NR	NR	NR	carrier	both	*BRCA1*	3
Byrne[92]	USA	single	hospital-based	NR	NR	unknown	ovarian	*BRCA1*	2
Campbell[108]	United Kingdom	single	hospital-based	NR	NR	carrier	breast	*BRCA1*	2
Chan[95]	Canada	NR	hospital-based	Ashkenazi Jewish	yes	unknown	both	*BRCA1 and 2*	2
Edwards[94]	UK	single	clinic-based	NR	NR	known carrier and non-carrier	breast	*BRCA2*	3
Eng[53]	international	multiple	Other	NR	NR	unknown	both	*BRCA1*	5
Esteban-Cardenosa[93]	Spain	NR	NR	NR	NR	carrier	breast	*BRCA1 and 2*	2
Geisler[103]	US	single	hospital-based	NR	NR	unknown	ovarian	*BRCA1*	2
Gross[52]	Germany	multiple	hospital-based	German	yes	unknown	both	*BRCA1*	3
Hadjisavvas[111]	Cyprus	single	hospital-based	Cypriot	yes	unknown	breast	*BRCA1*	2
Jugessur[106]	Norway and Sweden	NR	NR	Norwegian and Swedish	yes	unknown	both	*BRCA1*	3
Kashima[102]	Japan	multiple	hospital-based	Japanese	yes	unknown	both	*BRCA1*	2
Kozlowski[96]	Poland	single	registry	Polish	yes	carrier	both	*BRCA1 and 2*	3
Kringen[110]	Norway	multiple	hospital-based	Norwegian	yes	unknown	both	*BRCA1*	2
Kuperstein[97]	Canada	NR	registry	Ashkenazi Jewish and French Canadian	NR	known carrier and non-carrier	breast	*BRCA1 and 2*	2
Lancaster[104]	US	single	hospital-based	NR	NR	carrier	both	*BRCA1*	2

Author	Geographic Location	Centre	Setting	Ethnicity	Family History of Cancer	Carrier Status	Cancer	Gene	Number of Techniques Studied (including reference)
Montagna[98]	Italy	single	community-based	Italian	yes	unknown	both	*BRCA1 and 2*	2
Oleykowski[91]	US	single	clinic-based	NR	yes	unknown	both	*BRCA1*	2
Sakayori[62,112]	Japan	NR	NR	Japanese	yes	unknown	breast	*BRCA1 and 2*	2
Van Orsouw[105]	US	multi	NR	NR	yes	known carrier and non-carrier	both	*BRCA1*	2
Wagner *et al*.[99-101]	international	Multi	NR	International	yes	known carrier and non-carrier	both	*BRCA1 and 2*	5
BRACAnalysis® Information, Myriad Genetic Laboratories, Inc.[89] (three studies)	US	Single	Other	NR	NR	known carrier and non-carrier	NR	*BRCA1 and 2*	3
	US	Single	Other	NR	NR	known carrier and non-carrier	NR	*BRCA1 and 2*	2
	US	Single	Other	NR	NR	Known-both carrier and non-carrier	NR	*BRCA1*	2

*Unsure whether all subjects are Norwegian; NR=not reported

Table 2: Quality assessment

Author	Reference: Good Gold Standard	Test Method	Blind Analysis of Tests	Reliability of Tests	Pedigree Known when Tested	Lag between Reference and Test	Selection Bias	Sample Handling ** Bias	Measurement Bias	Attrition Bias
Andersen[90]	yes	CDGE	reference only	NR	yes	yes	yes	unclear	yes	no
Andrulis [56]	yes	multiple	reference yes, test NR	NR	yes	yes	unclear	no	unclear	no
Arnold[109]	yes	DHPLC	yes	NR	yes	NR	unclear	no	no	no
Blesa[107]	yes	fluorescent CSGE	neither	NR	NR	no	yes	yes	yes	no
Byrne[92]	yes	IHC C20 and D20	yes	intra NR, inter yes	NR	no	unclear	no	no	no
Campbell[108]	yes	CSGE	NR	NR	NR	no	yes	no	no	no
Chan[95]	yes	MS-PCR	reference yes, yes NR	NR	yes	yes	unclear	unclear	no	no
Edwards[94]	unclear	F-CSGE and F-MD	reference only	intra yes, inter NR	NR	yes	unclear	unclear	yes	no
Eng[53]	yes	SSCP, CSGE, TDGS, DHPLC	yes	NR	NR	NR	yes	unclear	unclear	no
Esteban-Cardenosa[93]	yes	HA	yes	NR	NR	yes	unclear	no	no	no
Geisler[103]	yes	SSCP and PTT	NR	NR	NR	no	unclear	no	no	no
Gross[52]	yes	SSCP	yes	NR	yes	no	unclear	no	no	no
Hadjisavvas[111]	yes	SSCP	reference yes, test NR	NR	yes	no	unclear	no	no	no
Jugessur[106]	yes	REF-SSCP	reference NR,test NR	intra yes, inter NR	yes	no	no	no	no	no
Kashima[102]	yes	GLK-2 and Ab-2 antibodies	reference yes,test NR	NR	yes	NR	unclear	no	no	no
Kozlowski[96]	yes	SSCP, SSCP/HA, HA	reference yes,test NR	NR	yes	NR	unclear	no	no	no
Kringen[110]	yes	REF-SSCP	yes	NR	yes	no	unclear	no	no	no
Kuperstein[97]	unclear	FMPA	test yes, reference NR	intra NR, inter yes	NR	NR	unclear	no	no	no

Author	Reference: Good Gold Standard	Test Method	Blind Analysis of Tests	Reliability of Tests	Pedigree Known when Tested	Lag between Reference and Test	Selection Bias	Sample Handling ** Bias	Measurement Bias	Attrition Bias
Lancaster[104]	unclear	DDF and SSCA	reference NR, test no	NR	NR	NR	unclear	unclear	nes	no
Montagna[98]	yes	AGE	NR	NR	yes	no	yes	no	no	no
Oleykowski[91]	yes	CEL I	yes	NR	yes	yes	yes	yes	yes	no
Sakayori[62,112]	yes	stop codon assay	yes	NR	yes	unclear	unclear	no	no	yes
Van Orsouw[105]	yes	TDGS	yes	NR	yes	yes	yes	yes	no	no
Wagner *et al.*[99-101]	unclear	DHPLC	NR	NR	yes	yes	unclear	unclear	unclear	no
BRACAnalysis® Information, Myriad Genetic Laboratories, Inc.[89] (3 studies)	unclear	Myriad's high-throughput robotic fluorescent sequencing system	unclear	unclear	NR	yes	unclear	unclear	unclear	no
	yes	Myriad's capillary-based sequencing	unclear	unclear	NR	yes	unclear	unclear	unclear	no
	unclear	Myriad's BRACAnalysis Large Rearrangements	unclear	unclear	NR	yes	unclear	unclear	unclear	no

* See text for description of sample handling bias; NR=Not Reported

Table 3: Test and reference techniques

Author	Molecular Technique	Reference Technique	Alleles or Mutations Tested for		Nucleic Acid Source and Type	
			Molecular Technique	Reference Technique	Molecular Technique	Reference Technique
Andersen[90]	CDGE	SSCP	exons 2,11,13-16,20,24 of *BRCA2*		DNA and mRNA	DNA
Andrulis[56]	EMD, TDGS, PTT, PTT+, SSCP, DHPLC	DSA	SSCP: 22 coding exons of B*RCA1* in addition to intronic splice donor and acceptor regions	NR	DNA for all, PTT also used RNA	DNA
Arnold[109]	DHPLC	DSA	*BRCA1* exons: mutations: G300T, 962del4bp, 1246delA, C1806T, C2457T, G3238A, 3600del11bp, A4071G, 3875del4bp, 3819del4bp, C4302T, G4304A, 4419insA, G4654T, G5075A, 5382insC, 5433delT, 5611delC, T5628C	NR	DNA	
Blesa[107]	fluorescent CSGE	CSGE	18 single base and six frameshift mutations in *BRCA1* coding region previously identified by DNA sequencing; alterations in exons 2, 7, 8, 10, 11, 13, 16 and 18; 185delAG; IVS7-34C>T; IVS8-58delT; IVS10-49delT; A356R; 1623del5; D693N; 2201C>T; 2274insA; 2430T>C; P871L; E1038G; S1040N; K1183R; N1236K; 3875del4; 4077T>C; 4427T>C; 4808C>G; 4952C>T/S1613G; S1613G; M16521; A1708E; IVS18+66G>A		DNA	
Byrne[92]	IHC	SSCP and DSA	protein truncating mutations: two *BRCA1* mutations identified in ovarian tumours and matched uninvolved tissue included exon 12 G insert at nucleotide site 4167 and exon 15-two C insertions at nucleotide sites 54325 and 54328	NR	DNA	
Campbell[108]	CSGE	dHPLC	*BRCA1* exon 11		DNA	
Chan[95]	MS-PCR	HA and DSA	*BRCA1*: 185delAG; 5382insC and *BRCA2*: 6174delT		DNA	
Edwards[94]	F-CSGE and F-MD	BIC, DSA, CSGE, DHPLC, PTT	eight point mutations and three frameshift *BRCA2* mutations; (exon sub-fragment; ex10.03 1742T>C; ex11.16 6893A>G; ex11.11 5416A>T; ex11.12 5868T>G; ex11.05 4035T>C; ex11.15 6631A>CCC; ex22 9179C>G; ex11.12 5972C>T; ex11.13 6174delT; ex11.13 5909insA; ex11.15 6630delTAACT)		DNA	
Eng[53]	SSCP, CSGE, TDGS, DHPLC	DSA	65 samples: 58 mutations established; 15 additional samples in which no mutation had been identified; positive samples included 20 frameshift mutations (17 deletions, three insertions); 18 nonsense mutations, 15 mis-sense mutations, and five mutations occurring in non-coding regions adjacent to beginning or end of exon		DNA	

Author	Molecular Technique	Reference Technique	Alleles or Mutations Tested for		Nucleic Acid Source and Type	
			Molecular Technique	Reference Technique	Molecular Technique	Reference Technique
Esteban-Cardenosa[93]	capillary-based HA	CSGE (previously detected)	57 DNA changes, 11 insertions or deletions, 46 single-nucleotide substitutions in *BRCA1*(exons 2 to 24) and 32 in *BRCA2* (exons 2 to 27) *BRCA1 exon 7 excluded in analysis because three frequent insertion/deletion polymorphisms are located downstream of 3' end of exon 7		DNA	based on BIC database and published literature
Geisler[103]	PTT	SSCP	*BRCA1* mutations, frameshift, and nonsense resulting in truncated protein		RNA	DNA
Gross[52]	SSCP, DHPLC	DSA	sequence variations in *BRCA1*		DNA	
Hadjisavvas[111]	SSCP	DSA	entire *BRCA1* coding region		DNA	
Jugessur[106]	REF-SSCP	PTT or CDGE for Norwegians, PTT only for Swedish	*BRCA1* exon 11		DNA	
Kashima[102]	IHC	genotype	*BRCA1*		DNA	
Kozlowski[96]	SSCP/HA, SSCP, HA	genotype	31 *BRCA1* and *BRCA2* mutations, polymorphisms, and variants in 24 *BRCA1* and *BRCA2* fragments; 22 base substitutions and nine insertions or deletions; mutations: *BRCA1* frag 2 185 delAG; frag 5 300T/G; frag7 433A/G; frag 8 C/T; frag 9 delT; frag 11.04 1186A/G; frag11.11 2201C/T; frag 11.13 2430T/C; frag 11.19 3232A/G; frag11.22 3667A/G; frag11.22 3667A/c; frag11.22 3667A/T; frag11.22 3667C/G; frag11.22 3667C/T; frag11.22 3667G/T; frag11.26 4153 delA; frag13 4427T/C; frag17 G/A; frag 18 A/G; frag20 5382insC; frag20 ins12bp; frag22 5465G/A; *BRCA2* frag3.02 426A/G; frag10.01 1342C/A; frag11.04 3624A/G; frag11.11 6886delGAAAA; frag14.02 7470A/G; frag16' delTAG; frag16 7883del4bp; frag25.2 9599A/T; frag 25.2 9630delC		DNA	
Kringen[110]	REF-SSCP	DSA	*BRCA1* exon 12		DNA	
Kuperstein[97]	FMPA	DSA	Jewish 185delAG, 5382insC and 6174delT; French Canadian *BRCA1* Ex112953del3+C; Ex11 3768insA; *BRCA2* Ex11 2816insA; Ex11 6503delTT; ex20 8765delAG		DNA	
Lancaster[104]	DDF	SSCA	breast cancer information core database 21 mutations nt 185 del AG (FS); nt 332-11 T→G (ins59, stop); nt1136 insA (FS); nt 1294 del 40 bp (FS); nt 1505 delG (FS); nt 2073 delA (FS); nt 2325 delG (FS);nt2430T→C (PM); nt2575 delC (FS); nt3232 A→G (PM); nt3450 del4bp (FS); nt3667 A→(PM); nt3867 G→(NS); nt 3875 del4bp (FS); nt4184 del4bp (FS); nt4446 C→T (NS); nt5085 del19bp (FS); nt 5242 C→A (MS); nt5382 insC (FS); nt5443 T→A (MS); nt5438 insC (FS)		DNA	
Montagna[98]	AGE	SSCP and PTT	*BRCA1* and *BRCA2* mutations		RNA	
Oleykowski[91]	CEL I	DSA	*BRCA1*		DNA	
Sakayori[62,112]	stop codon assay	DSA	*BRCA1* and *BRCA2* protein truncating mutations		DNA	

Author	Molecular Technique	Reference Technique	Alleles or Mutations Tested for		Nucleic Acid Source and Type	
			Molecular Technique	Reference Technique	Molecular Technique	Reference Technique
Van Orsouw[105]	TDGS	PTT alone or with partial nucleotide sequencing	*BRCA1* mutations		DNA	
Wagner *et al*.[99-101]	DHPLC	combination of DGGE, PTT, SCCP, DSA	*BRCA1* and *BRCA2* mutations		DNA	
BRACAnalysis® Information, Myriad Genetic Laboratories, Inc.[89] (3 studies)	Myriad's high-throughput robotic fluorescent sequencing system	allele specific oligonucleotide hybridization or radioactive sequencing	previously analyzed known BRCA1 and BRCA2 mutations		DNA	
	Myriad's capillary-based sequencing	Myriad's gel-based sequencing	genetic variations in BRCA genes		DNA	
	Myriad's BRACAnalysis Large Rearrangments	genotype	large rearrangements, either deletions or duplications ranging 510 bp to 26 kb in BRCA1		DNA	

Table 4: Sensitivity and specificity of tests

Author	Gene	Number	Unit of Analysis	Test	Sensitivity	Specificity	Comments
Andersen[90]	*BRCA1*	48	individuals	CDGE for overall	100%	82.93%	CDGE was not done in two individuals; SSCP failed to detect 3 of 7 (43%) deletions, 1 of 3 (33%) insertions and 6 of 8 (75%) base substitutions detectable by CDGE; insufficient data available to cross check analysis of SSCP with CDGE as reference.
				CDGE for frameshifts	100%	85.71%	
				CDGE for substitutions	100%	97.87%	
				CDGE for insertions	100%	97.83%	
				CDGE for deletions	100%	88.64%	
Andrulis[56]	*BRCA1 and 2*	20	samples	DHPLC	100%	100%	DHPLC missed exon22del.
				EMD	100%	100%	2494delC not detected on first pass by EMD but detected on second for SSCP, IVS5-11T>G missed in two samples; IV-5-12A-G; 185delAG; exon22del; Y1463X; K679X missed in two samples
				TDGS	87.5%	100%	TDGS missed large exon22 deletion, 2985del15, and Y1463X mutation initially because of design flaws
				PTT	75%	100%	PTT missed IVS5-11T>G (missed in two samples), IVS-5-12A-g, and 185delAG
				SSCP	62.5%	100%	SSCP: IVS5-11T>G missed in two samples; IV-5-12A-G; 185delAG; exon22del; Y1463X; K679X missed in two samples
Arnold[109]	*BRCA1*	46	individuals	DHPLC	100%	100%	
Blesa[107]	*BRCA1*	24	mutations	F-CSGE	100%	NR	
Byrne[92]	*BRCA1*	10	samples	IHC (D20 antibody)	100%	100%	
				IHC C20 antibody	100%	100%	
Campbell[108]	*BRCA1*	29	samples	CSGE	100%	NR	Insufficient information to calculate specificity
Chan[95]	*BRCA1 and 2*	66	individuals	MS-PCR all mutations	100%	100%	Evaluated three mutations only
				MS-PCR for 185delAG	100%	100%	
				MS-PCR for 5382insC	100%	100%	
				MS-PCR for 6174delT	100%	100%	
Edwards[94]	*BRCA2*	9	samples	F-MD	100%	0%	Two samples classified as "equivocal" F-CSGE missed (exon/subfragment; ex10.03 1742T>C; ex11.16 6893A>G; ex11.11 5416A>T; ex11.12 5868T>G; ex11.05 4035T>C; ex11.15 6631A>CCC).
				F-CSGE	50%	100%	

Author	Gene	Number	Unit of Analysis	Test	Sensitivity	Specificity	Comments
Eng[53]	*BRCA1*	66	mutations	SSCP	64.71%	93.33%	SSCP: Seven samples could not be analyzed because of insufficient DNA for sequence analysis; these contained mutations; false positive noted in one negative sample not from technical error, but laboratory sample switch that accounted for one false negative result
		60		CSGE	60%	100%	CSGE: 13 mutations could not be analyzed because of failure to amplify by PCR; four mutations missed because of failure of sequence analysis to confirm mutation after observation of abnormal gel mobility; one was base substitution, T to G at cDNA nt 855; other three were small frameshift deletions: 2072del4, 2080delA, and 2594delC; administrative errors led to three false negative results
		71		TDGS	91.07%	80%	TDGS: Two mutations could not be identified because of failure of sequence analysis; three false positives were reported after sequence analysis; each missed mutation (L246V; IVS17+1G>T; Y1463X; 3171ins% and 4510del3insTT) appeared as result of misinterpretation of the 2D gel; TDGS reported three mutations otherwise not identified by any other technique
		73		DHPLC	100%	100%	
Esteban-Cardenosa[93]	*BRCA1 and 2*	57	DNA changes	capillary-based HA	100%	NR	All 57 mutations detected, and two additional single-nucleotide substitutions (1186>G of *BRCA1* and 3624 A>G of *BRCA2*) previously unresolved by CSGE; Insufficient information to calculate specificity
Geisler[103]	*BRCA1*	94	carcinomas	SSCP	52.63%	96%	Each test reference for other; for exons 2 and 23, SSCP analysis done to supplement PTT data; problem for analysis because SSCP=reference for PTT.
		94		PTT	76.92%	88.89%	
Gross[52]	*BRCA1*	212	fragments	SSCP	94%	98.21%	Fragments of exon 16 exhibited heterozygous polymorphism A4956G, but failed to reveal base pair substitution G5075A
		238		DHPLC	100%	100%	
Hadjisavvas[111]	*BRCA1*	13	mutations	SSCP	92.31%	NR	13 variants in 12 sample.
Jugessur[106]	*BRCA1*	25	samples	REF-SSCP for Norwegians	90%	80%	Use of PTT as gold standard led to low specificity (high "false positives") For Norwegians, frameshift or nonsense mutation missed
		20		REF-SSCP for Swedes	100%	37.5%	

Author	Gene	Number	Unit of Analysis	Test	Sensitivity	Specificity	Comments
Kashima[102]	*BRCA1*	44	individuals	IH with GLK-2	100%	90%	
		44		IH-AB-2 antibody	87.5%	100%	
Kozlowski[96]	*BRCA1 and 2*	31	mutations	SSCP/HA versus genotype	100%	NR	SSCP missed BRCA1 4153delA; BRCA2 3134delC.
		31		SSCP versus genotype	90.32%	NR	
		31		HA	80.65%	NR	
Kringen[110]	*BRCA1*	292	fragments	REF-SSCP	100%	98.89%	
Kuperstein[97]	*BRCA1&2*	60	ashkenazi Jewish samples	FMPA 60 Ashkenazi Jewish samples	100%	100%	
		30	Ashkenazi Jewish BRCA women	FMPA 30 Ashkenazi Jewish BRCA women	NR	NR	
		56	French Canadian samples	FMPA 56 French Canadian samples	100%	100%	
		120	French-Canadian BRCA women	FMPA 120 French Canadian BRCA women	100%	100%	
Lancaster[104]	*BRCA1*	17	samples	DDF	100%	NR	Mismatch in mutation between text and Table 1 (text indicated nt5242C→A undetected by SSCA whereas Table 1 indicated nt5382insC was undetected) possible typing error; SSCA missed base substitutions: nt 3867G→T(ns); nt 5242C→A (MS)[text] versus5382 insC (FS) [table]; nt 332-11T→G ins59,stop
		21		SSCA	80.95%	NR	
Montagna[98]	*BRCA1 and 2*	44	individuals	AGE	100%	100%	
Oleykowski[91]	*BRCA1*	19	samples	CEL I	100%	100%	
Sakayori[62,112]	*BRCA1 and 2*	29	individuals	stop codon assay	100%	99%	One fragment that was positive in first screening had no protein truncating mutation (false-positive case); of five protein truncating mutations detected by DSA, two mutations could not be compared with SC assay because no RNA was available
Van Orsouw[105]	*BRCA1*	60	individuals	TDGS	100%	100%	5 of 19 "variants" not counted in analysis because we focused on "mutations"

Author	Gene	Number	Unit of Analysis	Test	Sensitivity	Specificity	Comments
Wagner *et al.*[99-101]	*BRCA1 and 2*	180	mutations	DHPLC for 180 mutations	99.44%	NR	
		30	individuals	DHPLC for 30 individuals; reference= direct sequencing	100%	NR	
		unclear	3 with mutations, non-mutation unknown	DHPLC for 41 individuals, reported by independent mutations only; reference=DGGE	100%	NR	
BRACAnalysis® Information, Myriad Genetic Laboratories, Inc.[89] (3 studies)	*BRCA1 and 2*	55 samples (sensitivity), 46 samples (specificity)		Myriad's high-throughput robotic fluorescent sequencing system	98.18%	100%	Single false negative reported to be result of insufficient DNA for sample, after specimen volume tracking and storage procedural changes, performance reassessed on >35,000 patient specimens, each containing ≥1 variants at various nucleotide positions; 100% of variants correctly detected, giving a 95% confidence interval for analytic sensitivity that exceeds 99.9%
	BRCA1 and 2	128 samples (sensitivity), 910 samples (specificity)		Myriad's capillary-based sequencing	100%	100%	
	BRCA1	85 samples with no known large rearrangements, 10 samples with large rearrangements		Myriad's BRACAnalysis Large Rearrangments	100%	100%	

APPENDIX 7: Psychosocial Impact Studies

Table 1: Study characteristics

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Armstrong *et al.*[208]	US	multiple	hospital	case-control	consecutive	unknown	breast	*BRCA1 and 2*	To investigate relationships between risk of breast cancer, risk of *BRCA1/2* mutation, sociodemographic factors, and use of *BRCA1/2* testing between 1996 and 1997
Audrain *et al.*[219]	US	multiple	community	cross-sectional	selective	unknown	neither	*BRCA1 and 2*	To characterize psychological status of women with family history of breast or ovarian cancer who self-refer for genetic counselling and *BRCA1* testing; and to identify demographic, personality, and appraisal factors that contribute to cancer-specific distress and general distress in this group of women
Biesecker *et al.*[281]	US	unclear	community	cohort	selective	unclear	both	*BRCA1 and 2*	To identify factors affecting genetic testing decisions in cohort of hereditary breast and ovarian cancer families presented with choice to undergo testing
Blandy *et al.*[215]	France	single	clinic	cross-sectional	selective	carrier	both	*BRCA1 and 2*	To describe diffusion of information by affected women in whom mutation has been identified (index case) to their families and testing participation among high risk relatives; to asses information recall and understanding by index cases and satisfaction with the testing process; and to determine factors associated with higher or lower testing decision in family
Bluman *et al.*[209]	US	unclear	clinic	cross-sectional	selective	unknown	both	*BRCA1 and 2*	To examine baseline knowledge, beliefs, and risk perceptions among group of women with breast or ovarian cancer who participated in a trial designed to improve decision making about genetic testing
Bluman *et al.*[207]	US	single	referral criteria and process	cross-sectional	selective	unknown	both	*BRCA1 and 2*	To determine whether there were associations between responses of women with breast or ovarian cancer considering *BRCA1/2* testing and their spouses, and to examine knowledge and attitudes regarding genetic testing for breast cancer susceptibility, perceptions of likelihood that their wives had mutation, pros and cons of testing, spouses' satisfaction with their involvement in decision-making process and additional resources they would find helpful

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Brandt *et al.*[203]	US	single	clinic	cross-sectional	other	unknown	both	*BRCA1 and 2*	To analyze how patients' attitudes regarding genetic testing vary with respect to previous breast cancer diagnosis to distinguish prime motivators and concerns of affected population seeking breast cancer risk assessment and genetic testing services
Cappelli *et al.*[216]	Canada	single	hospital	cross-sectional	selective	unknown	breast	*BRCA1 and 2*	To examine demand for breast cancer genetic testing and counselling among Canadian women diagnosed with breast cancer under the age of 50, with some factors predicting their intentions to be tested and degree to which they act on their intentions
Cappelli *et al.*[217]	Canada	single	hospital	cross-sectional	other	unknown	breast	*BRCA1 and 2*	To examine social, psychological, and demographic factors associated with intentions to have breast cancer genetic testing based on high risk FDRs of breast cancer patients and women from general population
Claes *et al.*[222]	Belgium	single	clinic	cross-sectional	selective	mixed	both	*BRCA1 and 2*	To gain insight into psychological aspects of diagnostic testing and formulate practical recommendations for counselling
Claes *et al.*[239]	Belgium	single	clinic	case-control	consecutive	mixed	both	*BRCA1 and 2*	To evaluate how cancer patients who had diagnostic genetic test for hereditary breast or ovarian cancer looked back on pre-test period and to gain insight into psychological impact of genetic test result
Clark *et al.*[234]	US	single	clinic	cross-sectional	selective	unclear	both	*BRCA1 and 2*	To examine motivation, satisfaction, coping, and perceptions of genetic counselling and testing among women who underwent pretest counselling and made testing decision.
Croyle *et al.*[235]	US	single	unclear	cross-sectional	selective	mixed	both	*BRCA1*	To examine predictors of distress after *BRCA1* mutation testing
Di Prospero *et al.* [220]	Canada	multiple	clinic	other	selective	carrier	both	*BRCA1 and 2*	To explore how genetic testing affected people found to have *BRCA* mutation and their families, and to determine whether there was interest in peer-support group
Dicastro *et al.*[282]	Israel	single	hospital	cross-sectional	selective	mixed	both	*BRCA1 and 2*	To retrospectively evaluate self-reported distress and anxiety symptoms before and after counselling, and retention of relevant information, one and three years after initial consultation

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Dorval *et al.*[245]	US	unclear	hospital	case-control	selective	mixed	both	*BRCA1*	To examine ability of individuals undergoing genetic testing for cancer susceptibility in two structured research protocols to accurately anticipate emotional reactions to disclosure of test result; study explored whether accuracy of emotional anticipation was associated with post-disclosure psychologic adjustment
Foster *et al.*[221]	United Kingdom	multiple	clinic	cross-sectional	selective	unknown	both	*BRCA1 and 2*	To examine attributes of cohort offered predictive genetic testing for breast or ovarian cancer predisposition and evaluate mental health, perceived risk of developing cancer, preferred risk management options, and motivation for genetic testing
Foster *et al.*[249]	United Kingdom	multiple	clinic	cross-sectional	consecutive	unknown	neither	*BRCA1 and 2*	To examine attributes of group of individuals offered predictive genetic testing for breast or ovarian cancer predisposition who did not proceed with testing at time of entry into study
Hagoel *et al.*[243]	Israel	single	clinic	cross-sectional	selective	mixed	both	*BRCA1 and 2*	To examine whether being a *BRCA1/2* mutation carrier affects aspects of life, and if so, how
Hallowell *et al.*[246]	United Kingdom	single	unclear	cross-sectional	selective	mixed	both	*BRCA1 and 2*	To investigate impact of *BRCA1/2* mutation searching on women previously diagnosed with breast or ovarian cancer, including motivation for testing, perceptions, information and support needs, and reactions to test results
Hamann *et al.*[232]	US	single	clinic	cross-sectional	selective	carrier	both	*BRCA1*	To identify attitudes toward *BRCA1* testing for children, among individuals who have received test results for family-specific *BRCA1* mutation
Hughes *et al.*[210]	US	multiple	hospital	cross-sectional	selective	unknown	neither	*BRCA1*	To describe levels of knowledge about inheritance of breast cancer and *BRCA1* testing and attitudes about benefits, limitations, and risks of testing in women with family history of breast and ovarian cancer; to determine whether knowledge and attitudes about benefits, limitations, and risks of testing differ for African American and Caucasian women, and to determine whether knowledge and attitudes are associated with previous exposure to genetic testing
Hughes *et al.*[226]	US and Canada	multiple	hospital	cross-sectional	selective	mixed	unclear	*BRCA1 and 2*	To evaluate likelihood and determinants of communication of *BRCA1/2* test results to at-risk family members

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Hughes *et al.*[247]	US and Canada	multi	hospital	cross-sectional	selective	mixed	both	*BRCA1 and 2*	To describe motivations for communicating or not communicating *BRCA1/2* test results to sisters and to describe specific topics that were discussed, and to evaluate whether carrier status of proband had influence on communication motivations and content
Hughes *et al.*[250]	US	single	referral criteria and process	cohort	selective	unknown	mixed breast cancer and healthy subjects	*BRCA1 and 2*	To describe associations between cultural beliefs and values, and participation in genetic risk assessment and testing among African American women at high risk for having *BRCA1/2* gene alteration
Jacobsen *et al.*[231]	US and Canada	multiple	clinic	cross-sectional	selective	unknown	unaffected	*BRCA1 and 2*	To explore relation of perceived breast cancer risk, and medical and demographic factors to readiness to undergo genetic testing
Julian-Reynier *et al.* [283]	France	multiple	clinic	cross-sectional	selective	mixed	both	*BRCA1 and 2*	To investigate attendance and uptake of cancer genetic testing in first or second degree relatives after *BRCA1* mutation was found in family and first mutation carrier had been informed
Kinney *et al.*[211]	US	single	unclear	cross-sectional	selective	unknown	both	*BRCA1*	To assess counselling and testing needs from perspective of adult members of large African-American kindred with *BRCA1* mutation
Lee *et al.*[205]	US	single	hospital	other	selective	unknown	both	*BRCA1 and 2*	To assess rate and pattern of *BRCA1/2* genetic test utilization and examine utilization of genetic testing and associated factors among high risk women
Lerman *et al.*[228]	US	single	clinic	descriptive	consecutive	unknown	ovarian	*BRCA1*	To evaluate interest in and expectations about impact of potential genetic test
Lerman *et al.*[227]	US	single	clinic	descriptive	consecutive	unknown	breast	*BRCA1*	To evaluate interest in and expectations about impact of potential genetic test among FDRs of breast cancer patients
Lerman *et al.*[132]	US	multiple	registry	cohort	unreported	mixed	both	*BRCA1*	To identify predictors of use of *BRCA1* genetic testing and to evaluate subsequent outcomes
Lerman *et al.*[206]	US	multiple	registry	cohort	consecutive	unknown	both	*BRCA1*	To examine association between psychological distress and use of *BRCA1* testing by 149 high risk individuals from hereditary cancer families
Lerman *et al.*[237]	US	multiple	registry	cohort	unreported	mixed	ovarian	*BRCA1 and 2*	To identify members of hereditary breast and ovarian cancer families at risk of adverse psychological effects of genetic testing

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Lerman *et al.*[236]	US	multiple	clinic	RCT	consecutive	unknown	both	*BRCA1*	To examine racial differences in response to two pretest education strategies for *BRCA1* genetic testing: education versus education, and counselling
Liede *et al.*[225]	US and Canada	multiple	clinic	cross-sectional	selective	carrier	breast	*BRCA1 and 2*	To evaluate needs and to describe men's experiences with genetic counselling and testing, and to compare information with experience of female carriers of *BRCA1/2* mutations in these families
Lodder *et al.*[244]	Netherlands	single	clinic	cross-sectional	selective	mixed	unclear	*BRCA1 and 2*	To assess levels of psychological distress in men requesting *BRCA1/2* testing and their partners, and investigate the level of intrusive thoughts and feelings about breast and ovarian cancer, and tendency to avoid these
Lodder *et al.*[242]	Netherlands	single	clinic	cross-sectional	selective	mixed	unaffected	*BRCA1 and 2*	To study distress and problems regarding body image and sexuality up to one year after disclosure of test outcome for mutation carriers undergoing mastectomy, for mutation carriers opting for surveillance, and non-mutation carriers, and analyze whether women opting for prophylactic mastectomy differed from those opting for close surveillance with respect to biographical characteristics, experiences with cancer in relatives and personality
Lynch *et al.*[201]	US	multiple	registry	descriptive	unreported	unknown	both	*BRCA1*	To describe process of *BRCA1* testing and genetic counselling, and participants' reactions to results
Mehnert *et al.*[218]	Germany	unclear	community	cross-sectional	random	unknown	mixed breast and unaffected	*BRCA1 and 2*	To investigate processes of undergoing predictive genetic testing in a region of Germany where no multicentric research exists, and to provide empirical knowledge for implementation of *BRCA* testing and genetic counselling
Meiser *et al.*[233]	Australia	multiple	clinic	cohort	unreported	carrier and non-carrier	both	*BRCA1 and 2*	To determine long-term psychological impact of genetic testing in carriers and non-carriers; times at which negative outcomes are most likely; factors likely to facilitate or hinder psychological adjustment and potential moderating influence of individual information-seeking styles
Patenaude *et al.* [202]	US	single	other	cross-sectional	unreported	unknown	breast	*BRCA1*	To examine factors contributing to acceptance or refusal of genetic testing
Phillips *et al.*[229]	Canada	multiple	hospital	cross-sectional	consecutive	unknown	breast	*BRCA1 and 2*	To examine factors that influence testing decisions for *BRCA1* and *BRCA2* in Canadian Jewish women with breast cancer

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Press *et al.*[213]	US	single	community	cross-sectional	consecutive	unclear	breast	NR	To assess women's attitudes toward and hypothetical interest in genetic susceptibility testing for breast cancer
Randall *et al.*[197]	Australia	multiple	clinic	cohort	unreported	unknown	breast	*BRCA1 and 2*	To explore baseline knowledge of breast cancer genetics and psychological adjustment in women from high risk breast cancer families who have had a previous diagnosis of breast cancer and are seeking genetic counselling and possibly testing compared to those not seeking counselling and changes examined over time
Reichelt *et al.*[238]	Norway	single	clinic	cross-sectional	consecutive	unknown	breast	*BRCA1*	To report on uptake of genetic testing in Norwegian families with demonstrated mutations in *BRCA1* who have been offered testing, their compliance with psychosocial questionnaires, their prevalence of mental distress and levels of anxiety and depression when they are offered a test
Richards *et al.*[214]	US	single	community	cross-sectional	consecutive	unknown	both	*BRCA1*	To assess interest, educational effectiveness, and implications of testing for common *BRCA1* mutation, 185delAG, in Ashkenazi Jewish women
Schwartz *et al.*[223]	US	single	clinic	cohort	unreported	mixed	both	*BRCA1 and 2*	To examine long-term psychological impact of receiving *BRCA1/2* test results in clinic-based testing program
Schwartz *et al.*[224]	US	single	clinic	cohort	consecutive	unknown	both	*BRCA1 and 2*	To assess rate and predictors of test use among individuals from newly ascertained high risk families who have self-referred for genetic counselling and testing, with particular interest in spiritual faith and psychological factors
Sheridan *et al.*[240]	Canada	single	community	cross-sectional	unreported	mixed	both	*BRCA1 and 2*	To determine how genetic testing has affected lives of individuals belonging to *BRCA* mutation positive family
Tercyak *et al.*[198]	US	single	clinic	cohort	unreported	unknown	both	*BRCA1 and 2*	To evaluate likelihood and effect of parent-child factors on communicating maternal genetic test results for breast and ovarian cancer risk
Tercyak *et al.*[200]	US	single	registry	cohort	unreported	unknown	both	*BRCA1 and 2*	To evaluate likelihood, correlates, and psychological impact of parental communication to children of parents' *BRCA1/2* genetic test results for breast cancer risk

Author	Geographic Location	Centre	Setting	Design	Sampling Procedure	Carrier Status	Cancer	Gene	Objectives and Purpose
Tessaro *et al.*[248]	US	multiple	hospital	focus groups	unreported	unknown	both	*BRCA1*	To better understand women's knowledge, concerns about testing, and potential influences and support needs in making a decision about genetic testing for susceptibility to breast cancer
Thompson *et al.*[212]	US	single	clinic	cross-sectional	consecutive	unknown	breast	*BRCA1*	To investigate predictors of use of genetic counselling and testing for breast cancer susceptibility in this population
Valdimarsdottir *et al.* [230]	Netherlands	multiple	clinic	cross-sectional	consecutive	unknown	breast	*BRCA1*	To examine role of demographic variables, objective risk, perceived risk, and cancer-specific distress in women's decision to undergo genetic testing
Velicer *et al.*[204]	US	single	registry	cross-sectional	unreported	unknown	both	*BRCA1 and 2*	To identify *BRCA1/2* knowledge, genetic testing intentions, and communication patterns in breast cancer survivors
Wood *et al.*[199]	US	single	clinic	cohort	consecutive	unknown	both	*BRCA1*	To better understand impact of genetic testing and counselling in a group of women who had early breast cancer (age<50) or ovarian cancer and a family history of cancer
Worringen *et al.*[284]	Germany	single	clinic	cross-sectional	consecutive	unknown	neither	*BRCA1 and 2*	To examine intention to be tested for *BRCA1/2* mutations and uptake of test among a consecutive sample of non-affected family members
Wylie *et al.*[241]	US	single	community	cohort	selective	mixed	unclear	*BRCA1*	To test whether persons who are mutation carriers and who perceived their spouses to be unsupportive and anxious will have higher levels of post-test distress than carriers whose spouses were perceived as supportive or not anxious

Table 2: Quality assessment

Author	Adequate Follow-up	Representative?	Uncertainty Quantified? (95%CI)	Appropriate Comparison	Statistical Procedure Adequate?	Subgroup Analysis Performed?	Potential Selection Bias?	Potential Performance Bias?	Potential Measurement Bias?	Potential Attrition Bias?	Results Applicable to Target Population
Armstrong *et al.* [208]	N/A	yes	yes	yes	yes	no	no	no	yes	no	unclear
Audrain *et al.*[219]	N/A	no	yes	N/A	yes	yes	yes	yes	unclear	yes	no
Biesecker *et al.* [281]	yes	no	yes	N/A	yes	yes	yes	no	no	yes	no
Blandy *et al.* [215]	N/A	yes	yes	yes	yes	yes	yes	no	no	no	yes
Bluman *et al.*[209]	N/A	no	yes	N/A	yes	yes	yes	no	unclear	yes	unclear
Bluman *et al.*[207]	N/A	yes	no	yes	yes	yes	yes	no	yes	no	yes
Brandt *et al.* [203]	N/A	no	yes	N/A	unclear	yes	yes	no	unclear	unclear	unclear
Cappelli *et al.* [216]	N/A	unclear	yes	yes	yes	yes	yes	no	unclear	yes	unclear
Cappelli *et al*[217]	N/A	unclear	yes	N/A	yes	yes	yes	no	unclear	yes	unclear
Claes *et al.* [222]	N/A	yes	yes	N/A	yes	yes	yes	no	no	yes	yes
Claes *et al.* [239]	yes	yes	yes	yes	yes	yes	yes	no	no	no	yes
Clark *et al.* [234]	N/A	yes	yes	N/A	yes	yes	yes	yes	no	yes	unclear
Croyle *et al.* [235]	N/A	no	yes	N/A	yes	yes	yes	yes	no	yes	unclear
Di Prospero *et al.*[220]	N/A	yes	yes	N/A	yes	yes	yes	yes	no	yes	unclear
Dicastro *et al.*[282]	N/A	yes	yes	N/A	yes	yes	yes	unclear	no	yes	yes
Dorval *et al.*[245]	N/A	yes	yes	N/A	yes	yes	yes	unclear	no	yes	yes
Foster *et al.* [221]	N/A	yes	yes	N/A	yes	yes	yes	unclear	no	yes	yes
Foster *et al.* [249]	N/A	unclear	no	yes	yes	no	unclear	no	no	unclear	yes
Hagoel *et al.*[243]	N/A	yes	yes	N/A	yes	yes	yes	no	no	no	yes
Hallowell *et al.*[246]	N/A	yes	N/A	N/A	N/A	yes	yes	unclear	no	no	yes
Hamann *et al.*[232]	N/A	no	yes	N/A	yes	yes	yes	unclear	no	no	yes
Hughes *et al.*[210]	N/A	yes	yes	no	yes	yes	yes	no	no	yes	unclear
Hughes *et al.*[226]	N/A	yes	yes	N/A	yes	yes	yes	no	no	no	yes
Hughes *et al.*[247]	N/A	yes	N/A	N/A	N/A	yes	yes	unclear	no	no	unclear
Hughes *et al.*[250]	N/A	yes	yes	yes	yes	yes	yes	no	no	no	yes
Jacobsen *et al.*[231]	N/A	unclear	N/A	no	yes	yes	no	no	no	no	unclear
Julian-Reynier *et al*[283]	N/A	unclear	yes	N/A	yes	yes	yes	no	no	yes	unclear
Kinney *et al.* [211]	N/A	unclear	yes	N/A	yes	yes	unclear	no	no	no	unclear
Lee *et al.*[205]	N/A	yes	yes	N/A	yes	yes	yes	no	yes	no	yes

Author	Adequate Follow-up	Representative?	Uncertainty Quantified? (95%CI)	Appropriate Comparison	Statistical Procedure Adequate?	Subgroup Analysis Performed?	Potential Selection Bias?	Potential Performance Bias?	Potential Measurement Bias?	Potential Attrition Bias?	Results Applicable to Target Population
Lerman *et al.* [228]	N/A	unclear	yes	N/A	yes	no	no	no	no	no	unclear
Lerman *et al.* [227]	yes	unclear	yes	N/A	yes	no	no	no	no	no	unclear
Lerman *et al.* [132]	yes	yes	yes	yes	yes	yes	no	no	no	no	unclear
Lerman *et al.* [206]	yes	unclear	yes	yes	yes	no	no	no	yes	no	yes
Lerman *et al.* [237]	yes	yes	yes	yes	yes	yes	unclear	no	no	unclear	unclear
Lerman *et al.* [236]	yes	unclear	yes	yes	yes	no	yes	no	no	unclear	unclear
Liede *et al.*[225]	N/A	yes	yes	N/A	yes	yes	no	no	no	no	yes
Lodder *et al.* [244]	N/A	yes	yes	N/A	yes	yes	yes	no	unclear	unclear	yes
Lodder *et al.* [242]	N/A	yes	yes	N/A	yes	yes	yes	no	unclear	yes	yes
Lynch *et al.* [201]	N/A	unclear	no	N/A	yes	yes	no	no	no	no	unclear
Mehnert *et al.*[218]	N/A	yes	yes	N/A	yes	yes	yes	no	no	no	yes
Meiser *et al.*[233]	yes	yes	yes	no	yes	no	unclear	no	no	no	yes
Patenaude *et al.*[202]	yes	unclear	no	no	no	yes	unclear	no	unclear	unclear	unclear
Phillips *et al.*[229]	N/A	unclear	no	N/A	yes	no	no	no	no	no	unclear
Press *et al.* [213]	N/A	no	no	N/A	N/A	yes	no	no	no	unclear	yes
Randall *et al.*[197]	no	unclear	yes	no	yes	no	unclear	no	no	no	unclear
Reichelt *et al.*[238]	N/A	no	yes	no	yes	no	no	no	no	no	unclear
Richards *et al.*[214]	N/A	unclear	yes	yes	yes	no	unclear	no	no	no	unclear
Schwartz *et al.*[223]	yes	yes	yes	yes	yes	yes	no	no	no	unclear	yes
Schwartz *et al.*[224]	N/A	yes	yes	yes	yes	no	no	no	no	no	yes
Sheridan *et al.*[240]	N/A	unclear	no	N/A	yes	no	no	no	unclear	no	yes
Tercyak *et al.*[198]	no	yes	yes	yes	yes	no	no	no	no	no	yes
Tercyak *et al.*[200]	no	yes	yes	yes	yes	yes	no	no	no	no	yes
Tessaro *et al.*[248]	N/A	yes	no	N/A	N/A	yes	no	no	no	no	yes
Thompson *et al.*[212]	N/A	unclear	yes	yes	yes	no	unclear	no	no	no	unclear
Valdimarsdottir *et al.* [230]	N/A	unclear	yes	yes	yes	no	unclear	no	no	no	yes
Velicer *et al.*[204]	N/A	yes	yes	yes	yes	yes	no	no	no	no	yes
Wood *et al.*[199]	no	yes	yes	N/A	yes	no	no	no	no	no	yes
Worringen *et al.*[284]	N/A	yes	yes	yes	yes	yes	no	no	no	no	yes
Wylie *et al.* [241]	yes	unclear	yes	yes	yes	no	yes	no	no	unclear	unclear

Table 3: Study population characteristics

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Armstrong *et al.*[208]	Cases 44; controls 52	mean	Cases 95% Caucasian, 27% Jewish; controls: 63% Caucasian, 16% Jewish	for breast cancer: cases 90%; controls 31%	Cases 83% married; controls 48% married	Cases 69% college educated; controls 67% college educated	Cases 74% employed; controls 65% employed
Audrain *et al.*[219]	44 (18 to 75)	mean (range)	90% Caucasian; 10% African American	at least one FDR with breast or ovarian cancer but no personal history of cancer themselves	70% married	95% had greater than high school education	NR
Biesecker *et al.*[281]	40	median	100% Caucasian	yes	51 (30%) single, divorced, separated, or widowed; 121 (70%) married	NR	124 (72%) employed; half of remaining 28% retired; 20 participants (12%) reported an annual income of <$20,000 and 24 (28%) reported >$75,000
Blandy *et al.*[215]	52	mean	NR	participants with breast or ovarian cancer	70% married	60% had greater than high school education	NR
Bluman *et al.*[209]	49	mean	94% of participants were Caucasian; 89% of non-participants were Caucasian	yes	78% married	55% college educated	NR
Bluman *et al.*[207]	50	mean	95% of spouses were Caucasian	women with personal history of breast or ovarian cancer	100% married	58% college educated	NR
Brandt *et al.*[203]	51	mean	93% Caucasian, 2% African, 1% Hispanic, 4% other or unknown ancestry	unclear	NR	53% reported having college or graduate degrees, while 22 of 96 reported having at least some college	NR
Cappelli *et al.*[216]	38.5	mean	NR	women diagnosed with breast cancer before age of 50 within past two years and treated, or women from general population between 18 and 50 years of age who have never been diagnosed with breast cancer	81% (n=48) married, 5% single (n=3), 89% (n=14) divorced, separated, or widowed	21% of breast cancer group and 36% of general population had college diploma	14% of breast cancer group and 25% of general population received income between C$40,000 and C$59, 000

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Cappelli *et al.*[217]	36	mean	primarily Caucasian	at least one FDR diagnosed with breast cancer within past 2 years (high risk group); and volunteers from general population who were never diagnosed with cancer of any type and who had no such family history; all members of high risk group had never been diagnosed with breast cancer	60% of high risk participants and 58% of general population participants married or common-law	22% of high risk participants and 54% of general population participants had received bachelor's degree; women in general population group had significantly higher level of education (±4.38, SD=1) than did women in high risk group (±3.72, SD=1; p≤0.05).	14% of high risk participants and 8% of general population earned income between C$60,000 and C$69,000
Claes *et al.*[222]	52.7	mean	Dutch	participants had personal history of breast or ovarian cancer, family history of these cancers, diagnostic genetic test for HBOC carried out in their centre	78% married	48% of participants had higher education levels	NR
Claes *et al.*[239]	52.7	mean	Dutch speaking Belgians	participants had personal history of breast or ovarian cancer, family history of these cancers, diagnostic genetic test for HBOC was carried out in their centre	78% married	48% of participants had higher education levels	NR
Clark *et al.*[234]	50	mean	96% Caucasian	yes	78% married	55% college educated	NR
Croyle *et al.*[235]	46.5	mean	Utah-based, predominantly Mormon kindred of northern European descent	at risk individuals with high incidence of breast and ovarian cancer among family members	85% (n=51) married, two divorced, and seven single	36.7% (n=22) college graduates	NR

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Di Prospero *et al.*[220]	focus group 51.3, questionnaire group 54.5, non-participants 49.7	mean	88% (n=7) Ashkenazi Jewish focus group attendants and 44% (n=7) Ashkenazi Jewish mail questionnaire responders; 0 other Caucasian focus group attendees and 56% (n=9) other Caucasian mail questionnaire responders, 12% (n=1) other	yes and all participants have positive results for *BRCA1* or *BRCA2*	75% of focus group participants and 94% of mail questionnaire respondents married	NR	NR
Dicastro *et al.*[282]	47.9	mean	65.8% born in Israel; 71% of Ashkenazi or Israeli origin	at least 2 first or SDRs with breast or ovarian cancer, first diagnosed under 50 years; at least 1 FDR with breast cancer diagnosed under age of 40 years, or ovarian cancer diagnosed under 50 years of age; or 1 FDR with breast and ovarian cancer at any age	78.7% married	60% >15 years of education	NR
Dorval *et al.*[245]	most aged 31 to 50 years	other	NR	yes	65% lived with spouse	68.3% greater than high school education	NR
Foster *et al.*[221]	41	mean	85% described themselves as Caucasian, based in England, Scotland, and Northern Ireland	participants from families in which a mutation had been identified, no previous diagnosis of cancer	52% married or common-law	Over one third of participants had college or university degree	75% men and 66% women employed
Foster *et al.*[249]	36 (23 to 62)	median	NR	participants from families in which a mutation had been identified, no previous diagnosis of cancer	76% married	53% university or college training	82% employed
Hagoel *et al.*[243]	49.8 (19 to 81)	mean (range)	90% Ashkenazi Jewish, Hebrew speaking	yes	NR	71% higher education	68% employed
Hallowell *et al.*[246]	53	median	NR	unclear	83% married.	33% of participants had degree or postgraduate education	NR
Hamann *et al.*[232]	46.9	mean	Utah-based kindred of northern European descent	yes	83% married	82.% had post-high school education	82% reported incomes ≥$30,000

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Hughes *et al.*[210]	43 (18 to 75)	mean (range)	76% Caucasian; 24% African American	at least 1 FDR with breast or ovarian cancer, but no personal history of cancer	68% of participants married	88% had greater than high school education	78% of participants employed
Hughes *et al.*[226]	69% <50 years	other	100% Caucasian	yes	82% of participants married	NR	NR
Hughes *et al.*[247]	63% <50 years	other	NR	participants had 10% to 20% probability of having mutation, and were first index family member affected with breast or ovarian cancer to undergo testing and receive results	74% of participants married	74% college graduates	77% of participants employed
Hughes *et al.*[250]	68% <60 years	other	African-American	100% with family history of breast cancer	64% not married	61% college educated	71% employed
Jacobsen *et al.*[231]	44	mean	69 Caucasian, 2 African-American, 1 Hispanic, 1 Asian, 1 other	no prior history of breast or ovarian cancer, have one or more FDRs diagnosed with breast cancer	56% of participants married	80% had college degree	NR
Julian-Reynier *et al.*[283]	>18	other	French families throughout France	yes	NR	NR	NR
Kinney *et al.*[211]	43	mean	African American	yes	44% married (those intending to undergo and not undergo testing)	65.4% of those intending to undergo testing had post-secondary education; 47.1% of those not intending to undergo testing had greater than high school education	47.4% of those intending to undergo testing earned <$30,000; 23.5% of those not intending to undergo testing earned <$30,000
Lee *et al.*[205]	95 patients between 40 to 49 years	other	77 Ashkenazi Jewish; 134 not Ashkenazi Jewish; most were Caucasian	a risk women with >10% chance of families carrying *BRCA1/2* mutation	214 participants were married	98 participants had >16 years of education	NR
Liede *et al.*[225]	53.8 (26 to 83)	mean (range)	100% Caucasian and of Ashkenazi Jewish or other European descent, except 1 man of Pakistani origin	men with *BRCA* mutations	43/59 had some post-secondary education; 33 had received college or technical school diploma or university degree	majority of respondents (43 of 59) had post-secondary education	NR

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Lodder *et al.*[244]	47	mean	Dutch	yes	89% married	32% had greater than high school education	NR
Lodder *et al.*[242]	38.4	mean	Dutch	yes - healthy women with 25% or 50% risk of mutation applying for testing	81% married	9% had greater than high school education	NR
Lerman *et al.*[228]	18 to 75	range	99% Caucasian, 1% Hispanic	subjects were unaffected FDRs of ovarian cancer patients; 95% had 1 affected FDR with ovarian cancer, rest had ≥2	64% married	44.6% had more than high school, 55.4% had less than high school	NR
Lerman *et al.*[227]	30 to 75	range	96% Caucasian, 4% African American	subjects were unaffected FDRs of breast cancer patient; most did not have family histories consistent with hereditary breast cancer (90% had 1 FDR affected with breast cancer)	76% married, 26% unmarried	62% more than high school, 38% less than high school	NR
Lerman *et al.*[132]	43 (14)	mean(SD)	100% Caucasian	at risk individuals	92 (80%) *BRCA1* test requesters and 55 (71%) decliners were married	110 (96%) requesters and 67 (87%) decliners had completed high school	32 (28%) requesters and 17 (22%) decliners were employed
Lerman *et al.*[206]	44 (21 to 84)	mean (range)	100% Caucasian	at risk individuals	76% married	77% had education beyond high school	NR
Lerman *et al.*[237]	45 (18 to 84)	mean (range)	NR	at risk individuals	77% married	69% had education beyond high school	69% employed
Lerman *et al.*[236]	18 to 75; 59% Caucasian and 66% African American, age ≥40	range	228 Caucasian and 70 African American women	76% Caucasian and 86% African American subjects had 1 FDR with breast cancer; 24% Caucasian and 14% African American subjects had 1 FDR with ovarian cancer or ≥2 or more FDRs with breast or ovarian cancer	69% Caucasian and 41% African American, married	82% Caucasian and 33% African American college graduate	NR
Lynch *et al.*[201]	42 (19 to 84)	mean (range)	NR	HBOC families	NR	NR	NR

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Mehnert *et al.*[218]	46.7	mean	German	personal or family history of cancer	64% married	36% attained entrance qualification for university degree	63% of participants had working experience, 50% of participants worked full time
Meiser *et al.*[233]	40 (SD=11.1)	mean (SD)	NR	participants were unaffected women with family history of breast or ovarian cancer	66% married or living together	72% had post-school qualifications	NR
Patenaude *et al.*[202]	NR		NR	50% or 25% risk of carrying gene	NR	NR	NR
Phillips *et al.*[229]	59 (32 to 87)	median (range)	Canadian Ashkenazi Jewish 100%	all had personal history of breast cancer	69% married, 3% single, 29% divorced or widowed	67% post-secondary	NR
Press *et al.*[213]	48 for African American, European American, and native American; 44 for Ashkenazi Jewish	mean	African American, European American, native American, Ashkenazi Jewish	positive, negative, and at risk	NR	limited to those who completed high school degree to completion of college degree	NR
Randall *et al.*[197]	25 to 35 (11.7%), 36 to 45 (30%), 46 to 55 (41.7%), 56 to 65 (15%), 66+ (1.7%)	% by age category	NR	mixed: women with previous diagnosis of breast cancer and family history	among cases tested, 7% single, 78% married, 12% single/divorced/ widowed; among controls, 14% single, 68% married, 15% single/divorced/ widowed	among cases, 52% prior to or in receipt of school certificate, 4% high school certificate or certificate of leaving, 41% tertiary; among controls, 21%, 25%, 54% respectively	NR
Reichelt *et al.*[238]	NR		Norwegian	families with demonstrated mutation of *BRCA1*	NR	NR	NR
Richards *et al.*[214]	mean age 47.7±11.83 for women, mean age 50.6±13.4 for men	mean (SD)	Ashkenazi Jewish	67% negative, 26% positive family history, 11% with personal history of breast or ovarian cancer	NR	5% finished high school, 45% attended college, 50% completed graduate education	NR

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Sheridan *et al.*[240]	NR	NR	Canadian	100% from families with *BRCA* mutation	NR	NR	NR
Schwartz *et al.*[223]	74% positive proband, 88% uninformative proband, 54% positive relative, 55% negative relatives were 40 years old	n/a	86% positive proband, 96% uninformative proband, 97% positive relative, 100% negative relatives Caucasian, rest = "other"	ersonal history of breast or ovarian cancer, and family history of these cancers	79% positive proband, 76% uninformative proband, 66% positive relative, 76% negative relatives were married, rest were unmarried	79% positive proband, 75% uninformative proband, 69% positive relative, 67% negative relatives had college education or more	51% positive proband, 55% uninformative proband, 71% positive relative, 52% negative relatives had full time job, and rest did not
Schwartz *et al.*[224]	31% <45	% category	95% Caucasian, 5% African American	breast cancer patients, 42% with relatives affected with breast or ovarian cancer	73% married, 27% not married	74% at least college graduates	NR
Tercyak *et al.*[200]	39.8 (8.6)	mean (SD)	90% Caucasian	<25% affected with cancer	91% married	72% had education beyond high school	NR
Tercyak *et al.*[198]	44.2 (4.7)	mean (SD)	88% Caucasian	12 (29%) unaffected; remainder had positive breast or ovarian cancer history	88% married	81% college graduates	NR
Tessaro *et al.*[248]	49.2 (24 to 77) for affected 40.4 (23 to 62) for unaffected	mean (range)	among affected, 69% Caucasian, 29% African American, 1 Hispanic woman; among unaffected, 68% Caucasian, 16% African American, 13% native American, 1Asian woman.	among affected, 54% had family history of breast or ovarian cancer in FDR and 46% diagnosed with *BRCA* mutation in past 2 years; among unaffected, 45% had history of >1 family member with breast or ovarian cancer	NR	57% of affected and 45% of unaffected had college degree	NR
Thompson *et al.*[212]	mean 43.4 (standard error 1,1; range 21.6 to 68.5)	mean (SE; range)	100% African American.	at risk women with at least 1 FDR diagnosed with breast cancer	41% married or common-law	68% greater than high school education	NR
Valdimarsdottir *et al.*[230]	Mean 45.1 (standard deviation 9.3; range 21 to 72)	mean (SD, range)	91% Caucasian	at risk women from HBOC families	61% married	75% attended college	NR

Author	Age (years)	Age unit	Ethnicity	Family History	Marital Status	Education	Employment
Velicer *et al.*[204]	26% between 45 to 49, 51% between 50 to 54, 23% between 55 to 69	% category	92.4% Caucasian, 2.4% African American, 4.7% Asian, 0.5% native American; 1.4% Hispanic; 1.4% Ashkenazi Jewish	women with breast cancer or ductal carcinoma in situ 5 to 10 years before study, diagnosed between ages 40 and 49; 26% with at least 1 FDR with breast cancer, 6.9% with at least 1 FDR with ovarian cancer	79.3% married or in relationship	21.7% high school or less, 36.8% some college or technical school, 29.8% graduated from college, 21.7% graduate studies	76.2% employed
Wood *et al.*[199]	46 (25 to 73)	mean (range)	97% Caucasian, 19% Jewish	personal or family history of breast or ovarian cancer; women with early (<50) breast cancer or ovarian cancer diagnosed at any age	NR	89% completed >1 year of college	NR
Worringen *et al.*[284]	37(11) 31% age 18 to 30, 34% age 31 to 40, 22% age 41 to 50, 10% age 51 to 60, 3% age 61 to 69	mean (SD) other	NR	100% at risk (family member with mutation)	60% married; 80% in firm partnership	43% professionals, or college or university graduates	40% employed full time
Wylie *et al.*[241]	45.27 (13.67) total; 43.04 (13.56) for carriers; 46.14 (13.66) for non-carriers	mean (SD)	Caucasian and north European descent	100% with family history	100% married	mean years of schooling (SD): 14.16 (1.92) total; 13.91 (1.69) carriers; 14.25 (2) non-carriers	NR

Table 4: Knowledge and risk perception

Author	Sample Size	Risk Perception	Knowledge
Audrain *et al.*[219]	256	For breast cancer risk, compared to women their age; 43% perceived personal risk as much higher, 36% perceived personal risk as a little higher, 14% perceived personal risk to be about the same, 3% perceived personal risk to be a little lower, and 4% perceived personal risk to be much lower; for ovarian cancer risk, 14% of participants perceived risk as much higher; almost half thought risk was about the same	NR
Blandy *et al.*[215]	30	Three participants overestimated breast cancer risk for woman with mutation and indicated correct percentage for overall population; 7 participants underestimated cancer risk for women with mutation; 5 gave same answer, "50% for breast cancer risk and risk of transmission of mutation"	General lack of knowledge (74%) regarding information given by geneticist; 2 participants gave correct answers to questions about breast cancer risk in overall population and for woman with *BRCA1/2* gene mutation
Bluman *et al.*[209]	200	Average risk of being mutation carrier, as determined by BRCAPRO, was 36% for participants and 34% for non-responders; compared with model estimates, >75% of women overestimated risk and approximately 25% underestimated their risk. Neither interest in testing nor time since most recent cancer diagnosis was associated with overestimation of risk. Women with at least 3 first or second degree relatives were one third (95% CI: 0.2; 0.6) as likely to overestimate risk of having mutation compared with women with fewer affected relatives when controlling for age, race, and previous testing in family	56% participants did not know that father can pass mutation to his children, and 43% did not know there is 50% chance of passing mutation to child; 14% knew that prevalence of gene alterations in *BRCA1* or *BRCA2* is not 1 in 10; 62% knew that woman could get breast cancer after having prophylactic mastectomy; 23% said that prophylactic oophorectomy would not be completely protective against ovarian cancer; subjects gave correct responses to 51% (SD=20) of items
Bluman *et al.*[207]	40	Spouses' knowledge about *BRCA1* and *BRCA2,* and risk associated with mutations in these genes was limited; among husbands who did not attend counselling, 14% knew that prevalence of *BRCA1/2* mutations is not 1 in 10; 38% of wives knew that *BRCA1* or *BRCA2* mutation prevalence is not 1 in 10. most spouses believed there was moderate (36%), likely (33%), or very likely (15%) chance that their wives had an altered gene; husbands' perceptions of chance of mutation was significantly correlated with ranks of their wives' perceptions of risk at time of survey conducted after receiving information	71% of spouses incorrectly reported that one-half of all breast cancers are caused by *BRCA1/2* mutations; 43% of wives answered question incorrectly; 38% of spouses and 67% of wives felt that many genes cause breast cancer; among spouses who did not attend counseling, the percentage of spouses who responded correctly to individual knowledge items ranged from 14% to 100%; corresponding figures for wives were 29% and 95%; spouses who read wives' printed material or sought additional information did not score any better on knowledge items when compared to rest of spouses; spouses who attended genetic counselling correctly answered significantly higher proportion of knowledge items than those who did not attend (71% versus 58%, p=0.02)
Cappelli *et al.*[216]	110	Women with breast cancer thought risk to be, on average, between 5% and 10% (an accurate overall estimate), whereas mean response of women in general population was between 10% and 25%	54% of participants had heard of breast cancer gene testing before filling out survey; most frequently cited source was media (68%); 10% heard about test from a friend; 8% heard about it from their doctor; 32% of general population group and 58% of breast cancer group had previous knowledge of the test

Author	Sample Size	Risk Perception	Knowledge
Cappelli *et al.*[217]	108	Women in high risk group had higher overall perceived risk of getting cancer; 3 items differed significantly between groups: perceived risk of getting cancer ($p<0.001$), perceived chance of getting breast cancer ($p<0.001$), and perceived risk of getting inherited form of cancer ($p<0.05$); group difference on items disappeared once education was controlled for; in all cases, high risk group perceived risk as higher than those in general population	Before filling out survey, 56% of participants had not heard about breast cancer gene testing; 34% of general population group and 52% of high risk population group had heard of genetic testing; most frequently cited source was media (43%), whereas 28% heard about test from family member, 17% friend or colleague, 6% from their doctor, 6% from scientific literature; none of general population heard about test from family member; 28% of high risk group had; none of general population group had heard about test from their doctor; 6% of high risk group had
Claes *et al.*[222]	64	20% of conclusive group and 29% of inconclusive group provided risk figure within acceptable interval for breast cancer and ovarian cancer in female carriers; between 6% and 12% of participants gave risk estimation within acceptable interval for breast cancer and for ovarian cancer in female non-carriers	NR
Claes *et al.*[239]	62	Some misinterpreted the genetic test result as revealing absence of genetic predisposition; others were relieved but aware of increased risk, whereas last group experienced continuing uncertainty and felt less in control	NR
Clark *et al.*[234]	159	Eligible women had estimated 10% or greater risk of carrying *BRCA1* or *BRCA2* mutation; for each participant, BRCAPRO statistical model was used to assess probability of being *BRCA1* or *BRCA2* mutation carrier; risk estimates categorized into quartiles and provided by genetic counsellor at each counselling session	NR
Di Prospero *et al.*[220]	27	For 18 subjects with previous cancer diagnosis, mean recall estimated risk for acquiring second cancer was 46% (range 10% to 90%) before genetic counselling and 57% (range 30% to 100%) after test results were received ($p<0.001$), for 6 subjects who did not have previous diagnosis of cancer, mean recalled lifetime risk estimate was 27.5% (range 10% to 50%) before genetic counselling; and 47.5% (range 15% to 80%) after test results ($p<0.01$)	NR
Dicastro *et al.*[282]	155	19% of participants found to have 1 of the predominant Jewish mutations in *BRCA1/BRCA2* genes and 81% were non carriers	Among carriers, 61.5% reported that they received novel information regarding possibilities of prevention or surveillance that was more than they knew before genetic counselling. Only 30.8% of non-carriers reported the same benefit from genetic counselling ($p=0.01$)
Dorval *et al.*[245]	53	Subjects had at least 12.5% risk or higher of carrying familial mutation based on position in pedigree	NR

Author	Sample Size	Risk Perception	Knowledge
Foster *et al.*[221]	315	Compared with average women, 88% and 69% thought they were at higher or much higher risk of developing breast and ovarian cancer respectively; 14% and 32% of women considered it not very likely that they would develop breast and ovarian cancer respectively; self-referred women had higher perceived risk of breast cancer with 97% of women reporting higher than average risk compared to 81% of other referral groups; younger women have higher perceived risk of breast (p=0.0005; MW) and ovarian (p=0.05; MW) cancer than older women; most (75%) were uncertain about having a mutation; 22% were certain; 3% were certain they did not have mutation; higher educational status associated with accurate figures for population breast cancer risk; 49% of college or university educated participants were correct compared to 33% of those who were school educated	NR
Hamann *et al.*[232]	218	Women in this kindred have high risk of ovarian cancer (approximately 90% by age 80) and later age of onset of breast cancer; 49 women and 29 men had deleterious *BRCA* mutation, all were tested	Non-carriers more likely to permit *BRCA1* testing availability for minors; among non-carriers, personal experience of positive emotions may have been associated with belief that others, including children, would also benefit from testing; individuals who experienced genetic counselling and testing may be more aware of possible consequences and limitations of testing than those who have not been counselled and tested.
Hughes *et al.*[210]	407	NR	Average knowledge score for sample was 6.0 of 11 (SD=2.15, range 0 to 11); respondents recruited through self-referrals had higher mean knowledge scores than those recruited through patient referrals (6.1 versus 5.3, p<0.001); knowledge significantly higher among Caucasian, married respondents who reported household incomes of ≥$50,001; respondents with education beyond high school had higher knowledge scores; only ethnicity had a significant independent association with knowledge (African American women had significantly lower levels of knowledge); average benefits to testing score 17.7 (SD=3.0)
Hughes *et al.*[226]	163	Among non-carriers, perceived risk of having mutation associated significantly with communication of test results to a brother and to offspring aged 18 or older, non-carriers with higher risk perceptions significantly more likely to communicate their test result to a brother and a child aged ≤18 years than non-carriers with lower risk perceptions	NR
Jacobsen *et al.*[231]	74	Average participant estimated to have 18% probability of developing breast cancer by age 79 (SD=8.91, range 11 to 41) because of family history of breast cancer	NR

Author	Sample Size	Risk Perception	Knowledge
Julian-Reynier *et al.*[283]	506	Among 49 families, 506 living adult FDRs and SDRs listed, corresponding on average to 10.3 at risk cases per family (SD=09.6)	NR
Kinney *et al.*[211]	95	31% of participants rated their likelihood of being a carrier at least 50%; 56% did not know	Knowledge about breast and ovarian cancer genetics limited; average knowledge score 3.2 (SD=2.1; range 0 to 7) out of 9; 67% wanted to discuss risk factors with a health care provider; two-thirds of participants indicated that they wanted to learn more about familial risk
Lerman *et al.*[228]	121	Chance of having altered gene perceived to be very likely in 15%, somewhat likely in 44%, less likely in 34%	NR
Lerman *et al.*[227]	105	Perceived chance of having altered gene high for 16%, moderate for 52%, small for 34%, and none for 3% of participants	NR
Lerman *et al.*[132]	192	NR	Subjects answered correctly about 55% of items (11 items) regarding knowledge of inheritance
Liede *et al.*[225]	59	36 of 45 unaffected men felt they were at increased cancer risk; more than half of respondents felt increased susceptibility to prostate cancer, one-third of *BRCA2* carriers felt increased susceptibility to breast cancer; 22% of *BRCA1* carriers specified increased risk of colorectal cancer; 97% (29/30) of men with an affected mother felt increased risk relative to men with unaffected mothers (70%); 96% (23 of 24) of men with a mother having died from breast or ovarian cancer felt increased risk; 2 men with previous history of cancer said they were at increased risk of all types of cancer; more than half of respondents said they had increased susceptibility to prostate cancer	NR
Lynch *et al.*[201]	181	Of 123 women counselled who were at 50% risk of *BRCA1* based on their position in the pedigree, 55 (45%) anticipated increased risk, whereas 16 (13%) believed their risk was decreased relative to their risk based on pedigree analysis; remainder did not respond or believed their risk level was 50/50	NR
Mehnert *et al.*[218]	100	19 of unaffected and 24 of breast cancer affected (43%) women were at risk of hereditary disposition; at the time of interview, 23 women had used genetic counselling; of these, 8 women had been tested and 2 had been diagnosed as positive; healthy women's estimates of their risk of breast cancer showed median of 47%; which is higher than general risk of disease (10% to 13%) for women of comparable age; women who met indication criteria assessed their risk to be higher by average of 53%, but they do not vary significantly from women with less risk laden history; sociodemographic characteristics do not vary significantly with subjective risk perception; subjective perception of risk of getting cancer by women in healthy group perceived as "medium to somewhat" threatening (mean=3.4, SD=1.4); women with breast cancer estimated chance of recurring cancer or relapse, median value of 3.0, lower than healthy women	Source of information on *BRCA* testing: 62% of women named print and television sources, 16% said the attending physician, 22% friends and acquaintances; 28% deliberately sought information because of case of cancer in family or worry about their health; 24% of women talked with their attending gynecologist about *BRCA* genetic testing, whereby physician advised for or against in case of 14 patients; 43% of women in group who had not received counselling were aware of possibility of genetic counselling; most women did not know what institution or professional group offered genetic counselling

Author	Sample Size	Risk Perception	Knowledge
Phillips *et al.*[229]	102	Perceived likelihood of having abnormal gene: certain not to have it (7%), fairly certain not to have it (7%), tend to think not to have it (23%), uncertain (35%), think she might have it (19%), fairly certain of having it (7%), certain of having it (2%)	NR
Press *et al.*[213]	246	Most women overestimated lifetime risk of *BRCA* gene mutation; majority overestimated risk at 40%, which varied by family history and ethnicity	44% had heard of "breast cancer gene," of whom 16% knew anything beyond name recognition; knowledge differed by family history and by ethnicity; Ashkenazi Jewish (67%) more knowledgeable than European American or African American (both 43%)
Randall *et al.*[197]	60	NR	At baseline, there was a trend in total knowledge about testing, with cases averaging 5.4 correct answers and controls 4.4 (out of nine); all subjects increased knowledge over time, but cases increased significantly more than controls at short-term follow-up (also true from baseline to long-term follow-up, but not from short-term to long-term follow-up); younger women with greater education had higher knowledge scores
Richards *et al.*[214]	309	NR	Baseline knowledge did not differ by age, sex, or risk category; on average, knowledge scores improved by approximately 3 additional correct responses post-education session; most participants aware of current screening and prevention options for breast and ovarian cancer pre- and post-session; significant overall improvement in knowledge after education; before and after education, no apparent difference between requesters and decliners of genetic testing
Schwartz *et al.*[223]	279	No baseline or follow up differences found between positive and uninformative on breast cancer perceived risk or ovarian cancer perceived risk; among relatives, no baseline differences found between positives and negatives on breast or ovarian cancer perceived risks; after adjusting for baseline perceived risk and employment status, test results strongly associated with perceived breast and ovarian cancer; accounting for familial clustering confirmed that negative results were significantly associated with decreased perceived breast and ovarian cancer risk	NR
Schwartz *et al.*[224]	290	For breast cancer: 52% high risk, 48% low; for ovarian cancer: 51% high, 49% low	NR
Tessaro *et al.*[248]	66	NR	Women affected and unaffected by cancer knew little about genetic testing for breast cancer; 5% of women with breast cancer likely to have *BRCA1* mutation; most know what they know from media

Author	Sample Size	Risk Perception	Knowledge
Thompson *et al.*[212]	76	NR	On average, participants were correct on 42.5% (SD=18.2, range 12.5 to 87.5) of questions on breast cancer and 45.4% (range 7.1 to 100) on cancer genetics; results did not differ between three groups for general knowledge but did differ on breast cancer genetics, with genetic counselling group having least knowledge, and genetic counselling and genetic testing having the most; group 1 refused genetic counselling to discuss *BRCA* genes; group 2 participated in genetic counselling but refused *BRCA* testing; and group 3 participated in genetic counselling and *BRCA* testing
Valdimarsdottir *et al.*[230]	105	Mean perceived risk 59.2% (SD=26.5); mean objective risk 28.5% (SD=13.3)	NR
Velicer *et al.*[204]	276	NR	Mean number of correct answers was 2 out of the 7 true or false questions; most women had positive attitudes toward benefits of *BRCA* testing; >60% agreed or strongly agreed with all 7 positive attitude statements; less than half agreed or strongly agreed with 7 negative attitude statements; women with >50% of knowledge questions correctly answered 47% less likely to have positive attitude toward testing compared to lower knowledge levels
Worringen *et al.*[284]	94	88% of participants overestimated risk of developing breast or ovarian cancer	NR

Table 5: Interest in testing

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Biesecker *et al.*[281]	172	After genetic counselling sessions, 135 (78%) chose to undergo testing and 37 (22%) chose not to be tested; 78% of participants who chose to be tested chose to receive results; actual uptake is lower than might be predicted by previous interest survey; intentions or attitudes do not predict behaviour; interest in testing often exceeds uptake	Although participants were randomized to 1 of 2 counselling approaches, no differences in genetic testing uptake were noted between two groups and data were combined for analyses; traveling to National Institute of Health (NIH) or field clinic to participate may have deterred those who were ambivalent about testing; age and marital status significantly associated with decision to test; women aged ≥40 years of age and married participants more interested in undergoing testing. Those tested did not significantly differ from those who chose not to test by gender, cancer status, or by presence of FDRs affected with cancer, nor by type of counselling received, number of years of prior research participation, or research site (NIH versus field clinic); greater family cohesion (measured by Family Environment Scale) and dispositional optimism statistically significant predictors in decision to undergo testing; family conflict, family expressivity, depression, spirituality, and self-esteem levels were not associated with genetic testing decision; family cohesion, optimism, and age were independent predictors of testing [OR 1.05 (95% CI:1.01;1.08); OR 0.87 (95% CI: 0.79;0.95); and OR 3.12 (95% CI:1.32;7.36)]. Greater family cohesion (measured by Family Environment Scale) and dispositional optimism statistically significant predictors in decision to undergo testing. Participants from cohesive families more likely to choose genetic testing
Bluman *et al.*[209]	200	45% of women indicated that their doctors advised them to be tested for *BRCA1* and *BRCA2* mutations; women with higher perceived risk quartile more likely to express definite interest in testing (OR 1.5, 95% CI: 1.1; 2.0); all participants offered testing free of charge; of 142 women in this sample who attended pretest counselling, 134 (94%) sought testing; 84% of women said they would probably be tested.	To provide advantages for their families (92%), to help their children (83%), to be reassured if results were negative (73%), to take steps to prevent cancer (73%) , to plan better for the future (63%), and to decrease anxiety (58%); most important potential disadvantage was worry or uncertainty about effect of testing on insurance
Bluman *et al.*[207]	40	90% of wives underwent testing	Advantages of testing (% of spouses): reduce anxiety (56%), help children (78%), provide advantages for family (85%), help in planning (44%), plan preventive measures (82%), negative results would be reassuring (74%), assist research (90%); disadvantages of testing (% spouses): testing would negatively affect family (8%), only useful if it provides information about cancer risk with certainty (8%), test might be inaccurate (15%), worried about health insurance for family (64%), could not handle emotionally (3%), too much time and effort (0%), better left unknown (3%)

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Brandt *et al.*[203]	400	Program was free and anonymous; any woman, affected or at risk, interested in learning about breast cancer risk could enrol; 400 women enrolled over 40-month period	Women previously affected and unaffected felt that preventive surgery decisions (69% and 52%, p>0.10), surveillance practices (86% and 90%, p>0.25), assessment of children's risks (83% and 68%, p>0.10), and increased breast cancer anxiety (53% and 52%, p>0.25) were “more” or very important issues regarding thoughts about genetic testing
Cappelli *et al.*[216]	110	60% of participants indicated they would like to be tested, 11% did not want to test, and 29% reported needing more time to think about it; at follow-up, 49% (n=23) women had decided to undergo genetic counselling while 51% (n=24) had not yet contacted the counsellor; 5 (22%) had gone for counselling but did not meet criteria for genetic testing; 9 (50%) women proceeded to have *BRCA1* test, 3 (17%) received genetic counselling and opted not to be tested at that time; 6 (33%) were still in counselling and had not reached final decision	More women in breast cancer group (n=43; 72%) wanted testing than did members of general population (23%; n=46) p<0.01; at follow-up, none of demographic variables significantly differed between those who wanted the test and those who did not; women with breast cancer more likely to want test than women in general population (OR=5.87, p<0.01); women who perceived fewer personal costs of having test more likely to want it than were women who perceived such costs to be great (OR=4.39, p<0.01); education level approached significance as predictor of intent with more education being associated with greater likelihood to want testing (OR=1.37, p=0.067); total perceived benefits scores significantly and positively associated with intent to be tested; individual benefits with significant effects on intent providing information to relatives who want to know risk of getting breast cancer, helping make decisions about life and disability insurance, helping make decisions about treatment for breast cancer; and helping decide lifestyle changes to prevent cancer; cost items producing significant results were “the gene test was too much trouble”; “it's better not to know”; “it’s better to let nature take its course.”
Cappelli *et al.*[217]	108	58% (n=61) of participants indicated that they would like to be tested, 8% (n=9) did not want test and remaining 34% (n=36) reported needing more time to think about it; of 108 participants surveyed, 1.9% (n=2) did not answer question on intent to be tested	Intent to be tested differed significantly by group, with more high risk women (68%, n=39) wanting test than general population (45%, n=22, p<0.05); demographic variables and women’s knowledge of breast cancer and genetic testing not found to be associated with intent to be tested; higher levels of perceived risk for getting ovarian cancer associated with wanting gene testing for *BRCA1/2* mutation in high risk group (p<0.05); those who perceived fewer costs associated with testing more likely to want test; total perceived benefits of gene testing scores produced main effect on intent to be tested; women who felt they would be too distressed less likely to want gene testing; survey focused on anticipated distress after testing rather than current psychological distress as predictor of testing decisions; possible that anticipated distress deters testing in otherwise non-distressed women, whereas currently experienced psychological distress precipitates testing; women with experience with breast cancer more likely to express interest in genetic testing for breast cancer susceptibility than women from general population

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Claes *et al.*[222]	64	No significant effects in informing relatives found by those who initiated genetic testing; of 83 eligible participants, 63 participated in study and 20 others declined; all but 1 participant was female; except for 1 participant, all received test result at least 6 months before study interview: 1 participant did not want to know her test result and 2 did not have contact with physician who sent blood sample for DNA analysis; another 3 stated they did not receive any information and that they did not ask for information about result; 1 participant excluded from analysis because she received her test result after testing was offered to unaffected relatives	NR
Claes *et al.*[239]	62	All participants underwent genetic testing	Cancer patients had a genetic test mainly for other persons, especially relatives in descendant line; some misinterpreted genetic test result as revealing absence of genetic predisposition
Clark *et al.*[234]	159	398 probands invited to participate in study; of those invited, 250 completed and returned baseline questionnaires (63% response rate); at time of analysis, 218 participants completed both parts of follow-up; 181 had counselling and made a decision; 13 had counselling but did not decide; 20 scheduled for counselling; 4 declined counselling; only those who completed counselling were offered genetic testing; 159 women completed post-counselling survey; 152 (96%) chose to have genetic testing and 7 (4%) declined; of 152 participants who elected to test, 6 (4%) opted to wait before drawing blood	Advantages of testing listed as important by most participants: desire to aid cancer research (98%), gain information for children (86%) and family members (92%), protect health through screening and prevention (85%), and free testing (91%); among 4% choosing not to be tested, concerns about confidentiality (37%) and worry about being denied medical or life insurance or having premiums raised (58%), anxiety associated with knowing mutation status, sadness, anger, depression, guilt, and fear were reasons most commonly cited as important; among 96% of those choosing testing, offer of free testing, obtaining information for children or family members, and protecting health through screening or prevention were reasons most commonly cited as important; 58% of women indicated they made a decision about genetic testing on their own, rest reported decisions were made with at least some help from others; spouses, sisters, and children were most frequently cited as those who helped in decision process; 57% reported the counselling session helped make decision about testing; 87% felt more confident after counselling; among 42% who did not decide to test on their own, most cited persons giving input to decision were spouses (51%), sisters (28%), children (24%), and parents (15%); although study relied partly on physician referral, 15% of this group said their physician was involved in making decision; 21% satisfied with their decision regarding testing, 74% very satisfied, 1% dissatisfied, 4% unsure; 1 of 6 who were tested unsure whether they wanted to receive results
Croyle *et al.*[235]	60	Among 213 participants who first responded, 84% (n=179) expressed interest in participating, 5.6% (n=12) requested more information, and 8.5% (n=18) requested no further contact; 97.4% of participants completed and returned consent forms; 60 women participated in all, 25 were determined to be carriers, 35 to be non-carriers	NR

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Di Prospero *et al.*[220]	27	All respondents indicated they would undergo genetic testing again, knowing everything they do; 22 subjects (92%) assigned a rating of ≥5 for satisfaction with clinical services received; all were dissatisfied with length of wait for test results	NR
Dorval *et al.*[245]	53	8 individuals declined to receive test results; of 45 individuals who received test results, 4 did not provide data on emotional reactions for study; analyses based on 41 of 45 participants who received test results in *BRCA1* testing program	NR
Foster *et al.*[221]	315	6 participants retrospectively identified as decliners of genetic testing (after counselling) and did not receive questionnaire; 9 individuals did not return questionnaire, leaving 298 who completed questionnaires; (227 females; 71 males); participants asked whose idea it was to attend genetics clinic: 63% reported self-referral, 14% their family's idea, 4% GP recommendation, and 11% referrals from genetics clinic	80% women and 91% men wanted genetic testing for sake of children; mothers of daughters and older women more likely to endorse this than mothers of sons only (daughters 84%; sons 66%) or younger women; in younger groups, women under 35 years of age more likely to endorse making decisions to have children; older women less likely to endorse preparing for future; women more likely than men to want test to prepare for future and to relieve uncertainty; childless men and women more likely to give decisions about having children as reason for wanting test than those who were parents
Hallowell *et al.*[246]	30	100% tested; most women discussed mutation searching with other family members before testing; decision to test was perceived as straightforward with little deliberation; of 20 women for whom data were available, 11 had given blood sample during initial visits to genetics clinic; 2 women reported needing time to deliberate; most participants did not report experiencing emotional difficulties while undergoing mutation testing; waiting for results was not perceived as anxiety provoking; some reported feeling anxious on their relatives' behalf; many reported forgetting about testing until results were received; some who waited over a year said annual update would have been helpful	Reasons for testing included other family members' need for genetic information, curiosity about etiology of cancer in family, desire to benefit future generations of women, and their risk status so they could make decisions about prophylactic surgery
Hamann *et al.*[232]	218	All participants underwent genetic testing and responded to interview; among 218 participants, 104 reported having children <18 years of age; of these, 17.3% noted they would want their children tested for a *BRCA1* mutation, 82.7% did not endorse this; no significant differences noted between carriers and non-carriers in their support of testing for their children; among participants with minor children, no significant difference in support for testing children in general as compared with support for testing children (p=0.58); among those with minor children, 7.7% of individuals who permitted testing for minors did not endorse it for their children; lower levels of test-related distress at follow-up associated with supporting testing of minors; about 5% of individuals did not support testing for minors, but wanted their children tested	NR

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Hughes *et al.*[226]	163	43% of eligible family members elected to receive test results; compared to those who declined genetic counselling, acceptors more likely to be female and had higher education	NR
Hughes *et al.*[247]	43	All study participants (n=43) received *BRCA1/2* rest results	NR
Jacobsen *et al.*[231]	74	While no testing was conducted, interest in testing evaluated; 46% of participants would seek testing as soon as possible, 30% would seek testing in near future, 5% would seek testing in distant future, 16% would not seek testing but thought they might change their minds, 3% would not seek testing and did not think they would change their minds	Readiness to test related to perceived risk; women who planned to test as soon as possible perceived themselves to be at greater risk for breast cancer than women who planned to be tested in future and women who did not plan to test; readiness related to perceived pros and cons of genetic testing; subgroup analysis of con scores indicated that women who did not plan to test perceived more disadvantages than women who planned to be tested in future and women who planned to be tested as soon as possible; among women who planned to test as soon as possible, mean pro scores significantly greater than mean con scores, $t(33)=4.57$, $p<0.001$; among women who planned to be tested in future, mean pro and con not significantly different, $t(25)=–5.40$, $p<0.0001$; women who planned to be tested as soon as possible older than women who planned to be tested in future and women who did not plan to be tested; most women thought that knowing they carried gene would help female relatives decide whether to test, motivate them to perform breast self-examination more frequently, help them decide whether to go for more frequent mammograms, and help them decide whether to undergo preventive surgery; most women thought that knowing they were carriers would increase concerns about developing breast cancer and would cause them to worry about female relatives who may be carriers
Julian-Reynier *et al.*[283]	506	Among FDRs, 79% of women with cancer had obtained their test results or were in process of testing compared to 51% of healthy women and 25% of healthy men; uptake among SDRs and uptake among men lower but followed similar trends; rate of genetic testing uptake among those who attended cancer genetic clinic, including those in process of testing, was 84% (95% CI: 78;l90.4) and did not vary significantly depending on degree of relationship, health status, or gender; among 37 family records, 3 families had no living FDRs or SDRs; in remaining 34 families, nobody attended cancer genetic counselling after index case; among FDRs, 96% women with cancer attended genetic clinics after index case received results compared to 60% healthy women and 25% healthy men; among SDRs, 58% women with cancer attended genetic clinics after index case received results compared with 21% healthy women and 10% healthy men	NR

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Kinney *et al.*[211]	95	Most participants (82%) indicated they would like to have genetic test if it was available	Significant predictors of intent to test having at least 1 FDR with breast or ovarian cancer (OR=5.1; 95% C I:1.2;20.9) and >50% perceived risk of being gene carrier (OR=64.3; 95% CI:5.1;803.9) or reporting that they did not know risk of being carrier (OR=10.9; 95% CI:2.1;57.7); barriers to testing included cost and availability
Lee *et al.*[205]	258	68 patients (26%) elected to undergo testing, whereas remaining 190 patients declined testing or expressed interest at time of consult, but had not been tested at time of analysis; 33 of 68 individuals who were tested were patients with cancer; more than half of patients underwent full gene sequencing; almost half the patients were tested on same day of consult and 96% within year of consul; 18 of 68 patients had free testing; of remaining 50 patients who had to bear cost of test, 0 of 28 who had the Ashkenazi Jewish screening panel and 13 of 22 who had full sequencing sought insurance reimbursement, of which 7 had prior diagnosis of breast or ovarian cancer	Access to free testing, prior diagnosis of breast or ovarian cancer, and Ashkenazi Jewish heritage only factors associated with genetic testing on univariate and multivariate analysis
Lerman *et al.*[228]	121	75% definitely wanted testing, 20% probably did, 2% did not want testing, 5% uncertain	Reasons for wanting testing: to learn about children's risk (76%), take better care of self (52%), increase screening (71%), to be reassured (70%), plan for future (~45%), childbearing decisions (48%), marital decisions (20%); "expected" impact of test results positive: become depressed (80%), become anxious (77%), feel more in control (68%), impaired quality of life (QoL;32%), consider suicide (1%); negative: less anxious (83%), improve QoL (83%), feel more in control (82%), less depressed (68%), still worry (42%), feel guilty (25%); interest in testing associated with higher education, younger age, likelihood of being carrier, perceived ovarian cancer risk, ovarian cancer worry, and degree of overall mood disturbance
Lerman *et al.*[227]	105	91%wanted genetic testing for breast cancer susceptibility, 4% did not, and 5% were uncertain.	Reasons for wanting testing: to learn about children's risk (~90%), take better care of self (~85%), increase screening (~80%), plan for future (~65%), childbearing decisions (~45%), marital decisions (~30%); reasons for not wanting testing: test accuracy (~25%), worry about insurance (~15%), emotional reactions (~15%), partner's reactions (~8%), family's reactions (~7%); "expected" impact of test results positive: become anxious (83%), become depressed (80%), feel more in control (80%), impaired QoL (46%), negative effect on marriage (16%), consider suicide (2%); negative: less anxious (76%), improve QoL (76%), still worry (72%), less depressed (64%), positive effect on marriage (52%), feel guilty (32%); women with less formal education more motivated by childbearing decisions and future planning than those with higher education; married women more motivated by wanting to take better care of self; women who perceived their risk to be high and more depressed were more likely to be motivated by childbearing decisions

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Lerman *et al.*[132]	192	Of 192 subjects who completed baseline interview, 60% requested BCRA1 test results	Perceived benefits of testing: to learn about children's risk (78% very, 18% somewhat), indicator of need for increased screening (70% very, 23% somewhat), to plan future (67% very, 24% somewhat), to make surgery decisions (63% very, 22% somewhat), to be reassured (61% very, 30% somewhat), to make childbearing decisions (41% very, 20% somewhat); perceived limitations or risks of testing: worried about losing insurance (16% very, 18% somewhat), concerns about effect on family (15% very, 22% somewhat), do not believe it can prevent cancer (9% very, 23% somewhat), could not handle it emotionally (16% very, 44% somewhat), inaccurate test results (7% very, 33% somewhat), mistrust of modern medicine (4% very, 12% somewhat); among unaffected carriers, 17% decided to obtain prophylactic mastectomy and 17% were undecided; for oophorectomy, 33% and 17% respectively; of 192 participants, 115 (60%) requested test results, and 77 (40%) declined; among 155 participants, 53 (46%) mutation carriers and 62 (54%) were not; *BRCA1* testing statistically significantly associated with female sex, having high school education and beyond, having health insurance, having greater number of FDRs with breast (but not ovarian) cancer, baseline knowledge about *BRCA1* testing and perceived importance of benefits of *BRCA1* testing; logistic regression model including sex, health insurance, education, clinical status, number of FDR, knowledge of susceptibility and perceived benefits of testing, significant variables were having health insurance (OR=3.74, CI:2.06;6.8), number of FDR with breast cancer (OR=1.59, CI:1.16;2.16), knowledge (OR=1.85, CI:1.36;2.5), and perceived benefits (OR=1.45, CI:1.13;1.86)
Lerman *et al.*[206]	149	58% (86 of 149) participants requested test results	Psychological distress was statistically significantly associated with test use whereas global mood was marginally positively related to test use; hierarchical logistic regression model revealed that younger age (less than 50, OR=2.5, CI:1.1;10), being female (OR=2.7, CI:1.2;6.1), higher objective risk (OR=5.5, CI:2.5;11.9), and psychological distress (OR=2.9, CI:1.3;6.5) significantly associated with test use
Lerman *et al.*[237]	327	63% underwent testing	NR
Lerman *et al.*[236]	298	In African American women, expanded counselling led to significantly greater increases than education only in intentions to be tested and provision of blood sample	Provision of blood sample: only referral type had significant association with provision of blood sample; 44% of self-referred versus 30% of patient-referred participants gave blood samples; at baseline, on scale of 1 to 4 (1=no interest, 2=considering, 3=probably will, and 4=definitely will have genetic testing), Caucasians had mean score of 2.68 (SD=1.1) and African Americans scored 2.47 (SD=1.0); family history, baseline intentions, treatment group were significantly associated with 1 month testing intentions, as was race by treatment interaction

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Liede *et al.*[225]	59	All participants had genetic counselling and testing, and received positive results; 3 men had previously diagnosed breast cancer, of whom 2 performed self-examination on regular basis; 7 men (15%) with no previous diagnosis of breast cancer performed self-examinations, including 4 (25%) *BRCA2* carriers; all but 2 were at least "satisfied" with genetic counselling; mean response on 5-point Likert scale was 4.2; 4 men (7%) indicated some missing information such as risk of colorectal cancer risk and health care providers' limited knowledge of male breast cancer; 2 men uncertain about recommending genetic testing and 2 men would not recommend testing	Reasons for seeking genetic counselling included: for families (14 of 59) or children (16 of 59), to learn about personal risk for cancer (26 of 59), and family's recommendation (4 of 59); motivation for testing not associated with cancer status, age, education, or daughter's cancer status
Lodder *et al.*[244]	28	All men underwent genetic testing	All 25 men with children wanted to obtain certainty about whether they could have transmitted mutation to offspring; 24 of 28 participants had a 50% risk of inheriting a mutation, 4 were identified as mutation carriers; of 24 non-carriers, 7 did not return post-test questionnaires, 3 declined answering questions on psychological functioning having received a favourable test outcome, 4 did not specify reasons for declining further participation; results available for 4 mutation carriers and partners, and 17 non-mutation carriers and partners; 1 of 3 men without children and his partner wished to include test outcome in decision whether to have children
Lodder *et al.*[242]	63	All participants tested for mutations; 26 women carriers and 37 non carriers	14 mutation carriers underwent prophylactic mastectomy within 1 year after disclosure of test result, 8 of whom underwent prophylactic oophorectomy; 12 carriers opted for breast surveillance, 5 underwent prophylactic oophorectomy
Lynch *et al.*[201]	181	All 181 had genetic testing (part of inclusion criteria); 75% of those coming for genetic services were women	Reasons for seeking risk assessment: concern for family (56%), surveillance (30%), curiosity (17%), consideration of prophylactic surgery (7%), relieve anxiety (5%), research purposes (5%); among women who tested positive, 35% considered prophylactic mastectomy, 76% considered prophylactic oophorectomy; 25% of all respondents worried about discrimination by insurance companies
Mehnert *et al.*[218]	100	Of women who had taken advantage of genetic counselling, 10 were eligible for testing based on indication criteria. 8 women decided to undergo testing of women without genetic counselling (n=77), 40 (52%) were in favour of *BRCA* testing, 19 (25%) were against it, and 17 (22%) were undecided	No correlation found between desire to use genetic services and sociodemographic variables, subjectively experienced threat of cancer, or subjective perception of risk; arguments against testing included fear of psychological burden that a positive result might place on them, limited explanatory power of test results, risky procedures of tests, non-existence of therapeutic follow-up, worry about relatives, missing indications, and fears of misuse of data; ill women more critical of positive aspects of testing than healthy women; mean values on QoL Scale=3.4 versus 3.7 ($p<0.05$)
Meiser *et al.*[233]	143	At 12-month follow-up, 90% of those having undergone testing pleased that they had, 8% unsure (half carrier, half non-carrier), 1% regretted it (one carrier woman)	NR

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Patenaude *et al.*[202]	36	80% (29 of 36) accepted and enrolled in program, 17% (n=6) declined, 3% (1 person) postponed *BRCA1* testing	Reasons for accepting included wish to end uncertainty about cancer risk, want to set up appropriate risk management strategies (prophylactic surgery), knowledge about children's risk; reasons for declining include fear of emotional impact if positive, uncertainty about medical benefits of knowing, and pressure of other medical problems; after first counselling session, all subjects requested to have blood drawn for testing; not yet known if they will want to know results when available
Phillips *et al.*[229]	102	Everyone interested in testing (part of inclusion criteria)	Factors influencing testing: potential benefit to other family members (96%), desire to contribute to research (96%), curiosity (92%), potential relief if negative (74%), need to know (71%), knowledge may help prevent cancer occurrence (52%), may prevent cancer death (49%), potential impact on ovarian (52%) and breast (28%) screening, potential impact on attitude towards prophylactic oophorectomy (32%) and mastectomy (24%); concerns were potential insurance discrimination (28%), worries regarding confidentiality (24%), test accuracy (30%), potential for negative impact on individual family members (26%), potential employer discrimination (8%), potential guilt (12%), negative impact on family as a whole if positive (7%), impact on life planning (18%), and childbearing decisions (4%); factors associated with specific reasons for testing: younger women more likely to be influenced by screening and surgery reasons, and confidentiality and insurance discrimination concerns; no associations found between education level or degree of family history, and factors influencing decision making; cultural determinants of decision making: 47% definitely and 27% somewhat influenced by potential to improve health of Jewish community; 17% concerned that being gene carrier might alter marriage prospects for self or family members; 15% concerned that genetic information might be used to single out individuals of particular ethnic group
Press *et al.*[213]	246	Hypothetical situation: 70% interested, 9% uncertain, 20% not interested	Reasons for interest: knowledge, such as prevention or risk reduction, reduction of uncertainty (72=39%), reassurance (13= 7.7%), no reason not to (12=6.5%), fear of breast cancer (10=5.4%), benefit to science (5=2.4%), benefit to future generations and increased reproductive options (both 4=2%); interest consistent with negative and positive predictive values, interest greater with short-term increased risk of breast cancer, interest greater if positive result led to gene therapy, and lower if it led to prophylactic mastectomy
Randall *et al.*[197]	60	NR	At baseline, cases cited the following reasons for testing: research contribution (93%), determine risk for children (84%), learn about risk status (74%), future planning (66%); no data available for controls; cases significantly more concerned about testing than controls, with most common concerns including not trusting accuracy of results, and potential emotional impact of results on family
Reichelt *et al.*[238]	232	78% (180 of 232) received results, 6% (14 of 232) undecided, 16% (38 of 232) declined	

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Richards *et al.*[214]	309	289 (94% ; 253 females, 36 males) requested DNA test, decliners all females; six participants tested positive for 165delAG	Reasons for testing: concern for risk (80% F, 56% M), learn about potential precautions if at increased risk (80% F, 47% M), concern for risk for children (72% F, 86% M), hope for negative results (66% F, 42% M), wish for better self-care (59% F, 42% M), concern for risk for other family members (53% F and M), feel in more control of life (47% F, 28% M), future planning (41% F, 33% M), recommended by spouse (23% F, 42% M); reasons for not testing: concern over health insurance (79%), family members' and spouse's worry if result is positive (58%, 32%, 21% respectively), already careful about routine surveillance (32%), does nothing to avoid cancer (32%), would worry even if result is negative (16%), discouraged by doctor (10%), risk is low (10%), concern over test accuracy (5%); approximately 85% women had annual breast and pelvic examinations by physician during study period, regardless of family history; younger women (<40) less likely to have had annual mammograms (RR=0.36, CI:0.25;0.54) and clinical breast examination (RR=0.87, CI:–0.76; 0.99) but no age difference for annual pelvic examination; significant overall improvement in knowledge after education; before and after education, no apparent difference between requesters and decliners of genetic testing
Schwartz *et al.*[223]	279	100% part of inclusion criteria	NR
Schwartz *et al.*[224]	290	238 (82%) tested and received test results; 18% declined	Predictors of uptake: spiritual faith and perceived ovarian cancer risk significantly associated with uptake, whereas perceived breast cancer risk marginally associated with uptake; final multivariate model included spirituality, perceived ovarian cancer risk, perceived breast cancer risk, and perceived breast cancer risk by spirituality interaction; model showed spiritual women 80% less likely to receive test results than non-spiritual women (OR=0.2, CI:0.1;0.5); women with high perceived ovarian cancer risk twice as likely to get results (OR=2.4, CI:1.3;4.7); among women with high perceived breast cancer risk, spirituality unrelated to uptake; among women with low perceived risk, high spirituality 80% less likely to take test (OR=0.2, CI:0.1;0.5)
Tercyak *et al.*[200]	133	100%	NR
Tercyak *et al.*[198]	42	100%	NR

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Tessaro *et al.*[248]	66	NR	Strong sense of altruism among women (help other women by contributing to scientific knowledge); some women not interested in knowing results of test if they got tested; confidentiality and fear of discrimination were concerns; women also concerned about insurance companies finding that they declined testing; affected women more aware of potential impact on insurance given experience with cancer; lack of proven options if test was positive regarded as disadvantage for testing; one advantage identified by women was to be in better control of situation relative to breast cancer, especially for unaffected women; preventive prophylactic surgeries mentioned; affected women mentioned that mutation status would help them make decision regarding treatment for breast cancer and in considering risk for second primary cancer and they would have made different health behaviour decisions if they had known
Thompson *et al.*[212]	76	17 (22.4%) declined genetic counselling (GC-), 19 (25%) underwent counselling but not testing (GC+GT-), 40 women (52.6%) underwent genetic counselling and testing (GC+GT+)	Perceived benefits and barriers to testing: 6 of 7 benefits endorsed by >70% of women with majority indicating that positive result would motivate more frequent breast self examinations and help family members decide about testing 7 of 14 barriers endorsed by >50% of women with most commonly cited being worry about passing gene to children and worry about family members who might be carriers; no differences in perceived benefits between groups, but there was a trend for barriers; women in GC- group reported greater concerns about stigmatization than other two groups; anticipated higher levels of negative reactions to positive results than GC+GT+ group; women in GC- and GC+GT- groups showed stronger anticipation of guilt about family members if they were positive than GC+GT+ group; no significant differences in sociodemographic variables among groups but there was trend for women in GC+GT- group to be younger and for GC+GT+ women to have higher income
Valdimarsdottir *et al.*[230]	105	55% provided a blood sample.	Correlates of provision: older women (50+) tended to be more likely to provide blood samples (69% versus 49%) and women with higher levels of perceived (71% versus 43%) and objective (68% versus 48%) risk significantly more likely to provide blood samples; cancer-specific distress also significantly associated with moderate level of distress leading to higher percentage of women providing blood samples (77%) than women with low (52%) or high (38%) level of distress; in logistic regression model, age, perceived risk, objective risk, cancer-specific distress were all significantly associated with blood sample provision; relative to women with moderate distress level, low distress had OR=0.24, 95% CI:0.1;0.5, high distress had OR=0.11, 95% CI:0.03;0.4); objective and perceived risk also associated (OR=4.4, 95% CI:2.7;18.5; OR=2.5, 95% CI:2.7;6.7 respectively)

Author	Sample Size	Uptake of Testing	Reasons forInterest or No Interest in Testing
Velicer *et al.*[204]	276	NR	Intent for testing differed according to who pays for testing (26% if self versus 67% if insurance); among 26% who would get tested by paying themselves, 93% would not pay >$200; for those willing to pay, intent for testing was associated with positive attitude towards testing and having discussions about testing with relatives; if covered by insurance, intent to obtain testing associated with marital status (OR=3.9, 1.6 to 9.8) , positive attitude toward testing (OR=3.7, 1.6-8.7), and having daughters (OR=2.3, 1.1 to 4.8) in multivariate logistic regression
Wood *et al.*[199]	35	3 women (8.6%) decided not to receive test results; 91% completed entire counselling process	NR
Worringen *et al.*[284]	94	77% very certain they wanted to find out mutation status; after counselling, testing done in 48% respondents' families; 43% participants decided not to undergo testing; 9% undecided at time of data analysis	Most did not test because probability of having mutation was very small, or no tissue or blood sample available from ill family member to test; 92% of those who underwent testing certain they wanted to know whether they carried mutation before counselling; this was true of 64% of those who did not undergo testing; undertaking testing dependendent on meeting inclusion criteria for testing; statistically significant relationship between implementation of testing and certainty expressed before counselling that there was desire to know about possibly having mutation; no significant differences between those wanting and not wanting testing in terms of sociodemographic characteristics; among sociodemographic, risk-related, and psychology-related data, expectations from testing, early detection measures, perceived benefits of early detection, and variables that depict access to counselling; factors associated with test intention were recommendation by others, earlier participation in study, stress by illness of relatives, desire for certainty, and ability to cope with unfavourable test outcomes

Table 6: Psychological impact

Author	Sample Size	Distress or Depression
Audrain *et al.*[219]	256	Mean general distress score based on Hopkins symptom Checklist (HSCL-25) 38.07; mean cancer specific distress score based on Impact of Events Scale (EIS) 7.71; average score for monitoring 9.95; average score for optimism 16.72; responses to Likert-style appraisal items not normally distributed; marital status and total number of FDRs with breast or ovarian cancer associated with general distress; unmarried subjects had higher general distress scores than married subjects (40.66 versus 36.81; t(245)=-2.63), p=0.01); subjects with ≥2 FDRs with breast or ovarian cancer scored lower on general distress than subjects who had 1 affected FDR (34.98 versus 38.29; t(252)=2.34, p=0.03); cancer-specific distress marginally associated with age and race; participants aged 18 to 30 years (mean 8.96) and those aged 31 to 50 years (mean 8.24) had higher cancer-specific distress scores than subjects >50 years of age [mean=6.10; F(2.243); p=0.10]; non-Caucasians had higher cancer-specific distress scores than Caucasian subjects [10.92 versus 7.42; t(245)=1.68, p=0.10]; monitoring associated with higher levels of general distress (r=0.14, p=0.02); optimism predicted lower levels of general distress (r=-0.62, p=0.0001) and cancer-specific distress (r=-0.24, p=0.0002); perceived control over developing breast cancer exhibited statistically significant associations with general distress [t(243)=2.66, p=0.008] or cancer-specific distress [t(245)=245, p=0.04]; among participants who perceived risk as higher, perceived control over developing breast cancer associated with lower levels of general distress [t(103)=3.2, p=0.002]; among participants with lower perceived risk, perceived control was unrelated to distress [t(138)=0.59, p=0.56)]; among women with heightened risk perceptions, those who perceived themselves as having control had mean general distress score of 34.73 (SD=8.38) compared to score of 40.98 (SD=10.93) among those who did not perceive themselves as having control
Cappelli *et al.*[216]	110	Women in breast cancer group showed higher overall degree of concern than members in general population; higher degree of concern noted in breast cancer group regarding children's risk of developing breast cancer
Claes *et al.* [222]	63	Coping skills assessed by Utrecht Coping List, Dutch adaptation of Westbrook Coping Scale; participants inclined to use coping strategies more often than normal population: active coping (20>17.7, t=4.21, p<0.001); palliative coping (18.2>16.3, t=2.86, p<0.01); social support seeking (14.8>12.9, t=2.84, p<0.01), comfort ideas (14.4>12.4, t=4.24, p<0.001); patients less inclined than normal population to express emotions as coping strategy (5.8<7.1, t=-3.61, p<0.001); mean scores on avoidance and depressive coping not significantly different from general population
Claes *et al.* [239]	62	All affected non-carriers expressed relief upon receiving test results; 83% carriers reported advantages of knowing test results and expressed them in terms of ability to take action for themselves and relatives; differences in distress about breast cancer significantly higher than that over ovarian cancer (Wilcoxen signed ranks test, p=0.01 to 0.03); avoidance about breast cancer higher than avoidance for ovarian cancer but only significant in carriers (Wilcoxen signed ranks test, p<0.005); self-report questionnaires did not reveal differences in general and cancer-specific distress as function of genetic test result
Clark *et al.*[234]	159	Prayer most cited coping technique (57%); talking to a friend reported by 45%; 13% spoke to physicians, 20% used relaxation or other techniques to reduce tension; 19% exercised more or less than usual; and 12% ate more or less than usual; women <50 years of age and those who received college degree more likely to use relaxation or other techniques to reduce tension; women with less than college education more likely to pray to deal with thoughts and feelings about cancer or genetic testing

Author	Sample Size	Distress or Depression
Croyle *et al.*[235]	60	Mean State Anxiety scale score 34.87 (SD=11.91) at baseline; average level nearly identical to that observed in normative sample of women; State Anxiety scale scores at baseline significantly correlated with State Anxiety scores and IES total scores at follow-up; State Anxiety scale baseline scores significantly predicted IES scores at follow-up; participants who were more anxious at baseline manifested more test-related distress in post-test interview; significant effect of test results on test-related distress, with mutation carriers experiencing greater distress than non-carriers; mean adjusted IES scores for non-carriers at follow-up 9.16 (SD=6.39) for those with no cancer or cancer-related surgery; 9.56 (SD=5.9) for those with history; for carriers, means 22.38 (SD=5.02) for those without cancer or cancer-related surgery; 11.58 (SD=5.48) for those with history; Age and State Anxiety scale score at baseline significantly predicted post-test State Anxiety scale scores; baseline anxiety scores predicted scores approximately 20% lower at follow-up, reflecting decline in general distress between baseline interview and 1 to 2-week follow-up interview; only mutation status significant, reflecting that carriers reported more general distress than non-carriers
Di Prospero *et al.*[220]	27	Of 24 respondents, 58% (n=14) not or a little worried about developing cancer; 42% (n=10) moderately or very worried before receiving test results; 18 subjects (75%) indicated distress level increased after receiving results
Dicastro *et al.*[282]	155	41% non-carriers reported high grade of cancer fear before counselling, compared with 17% of carriers; cancer fears less pronounced in 60% non-carriers after counselling and 14% carriers. 43% carriers and 3% non-carriers reported worsening of cancer fears after genetic counselling (p=0.001); as result of counselling and testing, levels of sleeplessness (increase from 1.46 to 1.8, p=0.01), feeling tense and moody (increase from 1.65 to 1.76, p not significant), or anxiety attacks (increase from 1.69 to 2.0, p=0.01), increased in carriers, whereas they decreased in non-carriers: 1.7 to 1.34, 2.0 to 1.42, 1.74 to 1.43 respectively (all significant at p=0.01); no significant differences found between carriers and non-carriers before and after genetic counselling in perception of general health, change of workplace or profession, patterns and frequency of leisure time habits, levels and frequency of physical activity, seeking methods of alternative medicine, or frequency of sexual intercourse; mutation carriers reported worsening of anxiety-related symptoms after notification, whereas non-carriers reported relief and less anxiety-related symptoms; both groups' pre-and post-counselling and testing levels of distress and anxiety did not interfere with everyday life activities even upon worsening; results support notion that oncogenetic counselling and testing has negligible effect on long-term psychosocial parameters; mutation carriers had better recollection of specific surveillance and prevention schemes than non-carrier counterparts; up to 63% of carriers and non-carriers could answer 1 of 4 questions correctly 1 to 3 years after initial counselling; questions aimed at assessing knowledge of participants regarding basic genetics of cancer inheritance
Dorval *et al.*[245]	53	Subjects anticipated they would have different emotional reactions upon disclosure that they carry mutation than upon disclosure they did not carry a mutation; hypothetical disclosure of negative test results associated with higher levels of anticipated favourable emotions, relief and happiness) than was positive test results ($p<0.0001$); mean levels of anticipated sadness, anger, and worry upon disclosure of positive test results significantly higher than mean levels of same anticipated emotions upon disclosure of negative test results ($p<0.002$); anticipated levels of guilt low and not statistically different when participants anticipated disclosure that they were carriers and when they anticipated negative test results; post disclosure guilt ratings low, suggesting participants did not experience strong guilt after disclosure of a positive or negative test result; non-carriers and carriers accurate in anticipating emotional reactions to test results; *BRCA1* mutation carriers overestimated feelings of sadness, anger, and worry, whereas ≤14% underestimated these feelings; affected *BRCA1* mutation carriers tended to underestimate these reactions more frequently; 43% more sad, 57% more angry, 57% more worried after result disclosure than they had anticipated; 86% affected *BRCA1* carriers reported more intense feelings of sadness, anger, or worry than they had anticipated in response to a positive result; of 10 unaffected (cancer free) *BRCA1* carriers, 30% underestimated ≥1 of these feelings
Foster *et al.*[221]	315	Younger women expressed higher cancer worry (<50 years of age, median 12 cancer specific worry score) than older women (>50 years of age, median 10 cancer specific worry score) ($p<0.001$; MW); 48 (21%) women stated they worried about developing cancer frequently or constantly; 38 (17%) felt cancer-related worry was definite or severe problem; compared with older women, younger women worried more often, and found it more of a problem; 50 women (24%) reported absence of intrusive thoughts; 36 women (17%) recorded avoidance score of 0; cancer-related worry was not associated with higher level of risk management activity

Author	Sample Size	Distress or Depression
Foster *et al.*[249]	34	90% test decliners felt at least somewhat likely to have mutation; 11% decliners said they worried about developing cancer "frequently" or "constantly" 4% felt their cancer worry was "definite or severe" problem; apprehension of result was recorded as factor causing most difficulty (74% of individuals); taking time from work, family, social obligations (30%) and travelling to clinic (33%) rated as difficult by approximately a third of decliners
Hagoel *et al.*[243]	165	Probands or nonprobands and carriers or noncarriers did not differ regarding demographic characteristics, health behaviours, distress, resources, or social integration; affected individuals evaluated themselves as less healthy than those not affected by cancer (OR 4.2, 95% CI:1.8;9.5, p<1) with marginal trend to chronic stress; affected individuals had lower sense of coherence than nonaffected individuals[243]
Hallowell *et al.*[246]	30	Emotional response to results: affected women (women with cancer) regarded testing as incurring costs and benefits; few carriers reported they were surprised to receive a confirmatory result; many carriers said they were pleased to have had etiology of their family history confirmed and to gain knowledge that ended their own and others' uncertainty about status; many women in waiting and inconclusive groups hypothesized that they would feel this way if they were confirmed as carriers; women who received inconclusive results are under-researched group; women in this group reported range of emotions varying from relief or elation to disbelief, acceptance, to disappointment and anger or frustration; some women with inconclusive results misinterpreted result as meaning that cancers in family not caused by genetic mutation; others who misinterpreted spoke of puzzlement or disbelief that mutation was not detected; some women expressed disappointment that technology could not identify mutation in sample, primarily because it meant that relatives could not obtain testing and were left in uncertain position regarding magnitude of risks of developing cancer and risk management
Hamann *et al.*[232]	218	Chronbach's alpha for STAI measure 0.92 at baseline and 0.93 at 4 to 7 month follow-up; IES at follow-up had Chronbach's alpha of 0.88
Kinney *et al.*[211]	95	45.55% of those intending to undergo testing had depressive symptoms; 23.5% of those of those who did not intend to test had depressive symptoms; psychological distress relatively high; mean CES-D score 15.0 (SD=12.4); scores did not significantly differ by gender or cancer status; prevalence of depressive symptoms 41%, median score on Intrusion subscale of Impact of Event Scale 9.0
Lerman *et al.*[227]	105	Average level of depression comparable to that of general population (10.0 versus 8.1 on MHI respectively), while scores on Intrusion subscale comparable to those in clinical population that included people under treatment for traumatic stress syndromes (14.3 versus 12.9); over half of subjects reported having feelings and thoughts about breast cancer, and 1 in 5 had trouble falling asleep because of thoughts and feelings
Lerman *et al.*[132]	192	Although baseline level of depression not significantly different at baseline between non-carriers, carriers, and non-decliners, there was a statistically significant difference at 1-month follow-up
Lerman *et al.*[206]	149	Mean psychological distress measure (IES) 6.2 (SD=6.7), that of global mood distress (CES-D) 7.4 (SD=8.7); both positively skewed
Lerman *et al.*[237]	327	Mean baseline stress measure 7.8±0.4 (range 0 to 33); mean score for baseline depression measure 8.6±0.5; among those with high baseline stress levels, depression increased among test decliners, decreased among non-carriers, and remained same among carriers at 1 month and 6 month follow-up
Lerman *et al.*[236]	298	Mean baseline distress scores 6.44 (SD=7.0) for Caucasian and 5.09 (SD=7.4) for African American (p=0.17); multivariate, only baseline distress had significant association with 1-month distress levels

Author	Sample Size	Distress or Depression
Lodder *et al.*[242]	63	Mean levels of general and cancer-related distress levels before and after disclosure in mutation carriers not significantly different from non-mutation carriers with prior risk of 25%; mean levels before result higher than those of normal female population in women who were later found to be carriers who opted for mastectomy and in non carriers, and similar to that of normal female population in women who were later found to be mutation carriers who opted for intensive surveillance; at 1 year follow up, mean levels of anxiety of 3 groups similar to or lower than those of normal female population; at pre-test, mutation carriers opting for prophylactic mastectomy had similar estimation of importance of physical appearance and sexual relationship as those undergoing surveillance; higher levels of anxiety and cancer-related distress found in mutation carriers opting for mastectomy than in other groups; difference greatest after disclosure of test result and smallest at 1-year follow-up; mutation carriers opting for regular surveillance had lower anxiety levels than other two groups, except for post test assessment; levels of cancer-related distress at post-test and follow-up similar to those of non mutation carriers; non mutation carriers reported lower levels of general and cancer-related distress and post-test and follow-up than they had reported at pre-test; proportion of women with high levels of anxiety 1 year after test outcome 29% for mutation carriers opting for prophylactic mastectomy, 16% for non-mutation carriers; all mutation carriers with high anxiety at 1 year follow-up reported anxiety on previous two post-test assessments
Lynch *et al.*[201]	181	Emotional response among carriers: appeared sad (36%), appeared surprised (27%), reported guilt (8%), appeared angry (6%), reported relief (4%), no apparent reaction (19%); emotional response among non-carriers: appeared happy or relieved (80%), appeared surprised (8%), reported survival guilt (4%), no apparent reaction (10%)
Mehnert *et al.*[218]	100	Total mean psychological health score based on Quality of Life Scales 3.8; third of all women wanted psychological support during decision phase before genetic testing; in event of positive result, 54% of all women wanted psychological support
Meiser *et al.*[233]	143	Carriers had significantly greater *BRCA* distress 7 to 10 days and 12 months post-notification than untested women and trend for higher *BRCA* distress 4 months post-notification; carriers showed significant decrease in state anxiety 12 months post-notification, as did non-carriers at 7 to 10 days post notification; non-carriers showed trend for lower state anxiety at 4 months post-notification relative to untested women and significant decrease in depression score at 4 months post-notification; findings persisted even after adjusting for type of family history and before or after counselling variable
Randall *et al.*[197]	60	At baseline, no difference found in psychological adjustment; 8 cases and 7 controls mildly and 2 cases and 3 controls moderately depressed while anxiety scores comparable to general population; follow up, no associations found with any of psychological variables
Reichelt *et al.*[238]	232	For women without history of cancer, clinical levels of mental distress varied from 6 of 142 (4.3%) on HADS-Depression scale to 26 of 142 (18.0%) on HADS-Anxiety scale; all results from other questionnaires within this range; for women with history of cancer, proportion of mental distress varied from 3 of 25 (12.5%) on HADS-Depression to 10 of 25 (41.7%) on IES-Intrusion subscale; statistically significantly higher number of cases among those with history of cancer (41% versus 10.7%) and (37.5% versus 12.7%) according to IES-I and GHQ respectively; no differences found between men and women
Schwartz *et al.*[223]	279	Groups did not differ at baseline or on change in cancer-specific or general distress; multivariate analysis showed no impact of test results on change in cancer-specific distress or general distress; among relatives, no baseline differences found between positives and negatives on cancer-specific or general distress; women with negative results exhibited significantly decreased cancer-specific distress than those with positive results; test result unrelated to change from baseline to follow-up on general distress; among relatives, at risk women with positive test results had significantly higher breast and ovarian cancer perceived risks; after adjusting for baseline scores and employment status, those with negative results showed significantly more decreased cancer-specific and general distress; accounting for familial clustering (via GEE modeling) confirmed that negative results significantly associated with cancer-specific distress and revealed reduced general distress
Sheridan *et al.*[240]	102	Carriers reported they were angry (17.9%), anxious (35.7%), worried (50%), and depressed (16.1%) after they received results; at time of test results, carriers reported feeling worried (41.1%) and relieved (25%); non-carriers reported feeling relieved (84.8%) after receiving results

Author	Sample Size	Distress or Depression
Tercyak *et al.*[200]	133	Baseline levels of psychological distress variables in sub-clinical ranges: CES-D M=9.4 (SD=10.1), IES=10.1 (SD=8.1) compared to normative data; no differences found between mothers and fathers in demographic variables and in baseline CES-D or IES scores; model for cancer-specific distress included baseline IES score, mutation status, cope scores, and communication status; no relationship found between cancer history and distress
Tessaro *et al.*[248]	66	Theme of concern for family members, with hopes of it better equipping them in making health care decisions; some concerns arose for potential stress resulting from positive result of letting go of healthy behaviours that could result from negative test result; concerns about stress involved in knowing or not knowing; testing and knowing results could lead to anxiety from being at increased risk or to relief from uncertainty and sense of empowerment; affected women reported less stress related to testing because they had been through more stressful experience of having breast cancer
Thompson *et al.*[212]	76	Distress: mean IES Score 9.9 (SE=1) for everyone; 14.5% had score >19, which is in the range for warranting clinical concern; 18% of GC-, 73% of GC+GT, and 58% of GC+GT+ women were about median in intrusive thoughts; using multivariate model including income, age, knowledge about genetics, perceived barriers of testing, and intrusive thoughts, significant associations were found between group membership and perceived barriers, and intrusive thoughts about breast cancer and trend with knowledge about breast cancer genetics
Valdimarsdottir *et al.*[230]	105	Mean IES intrusion subscale 6.3% (SD=7.5)
Wood *et al.*[199]	35	Statistically significant reduction in anxiety level seen between pre- and post-test results notification, irrespective of test results. Significant decreases in intrusive thoughts related to testing from pre to post test counselling was found among women who tested negative, with similar decrease in avoidance related to testing approaching significance (p=0.07); increasing trend in distress level among women who tested positive; women with more recent diagnoses (<1 year) reported higher cancer-specific and testing-specific distress than those diagnosed >1 year ago
Wylie *et al.*[241]	57	Distress levels reached clinically significant levels 1 week after results received and remained above clinical thresholds when measured 4 months, 1 year, and 2 years after testing for those tested who perceived their spouse to be anxious and non-supportive at time of testing

Table 7: Social issues

Author	Sample Size	Social Issues
Armstrong *et al.*[208]	305	Use of *BRCA1/2* counselling between 1996 and 1997 positively associated with being Caucasian and non-Jewish (OR 4.1, 95% CI:1.3;13.5) and being Caucasian and Jewish (OR 8.8; 95%CI:2.2;35.5)
Audrain *et al.*[219]	256	Being married associated with lower levels of general distress, which may reflect availability of social and emotional support; women with 1 FDR more distressed than women with ≥2 FDRs
Brandt *et al.*[203]	400	Although opinions regarding insurance and employment discrimination did not vary significantly between groups (p=0.09 and p=0.25 respectively), over half of affected women felt these issues were important, more important, or very important with respect to genetic testing, whereas over half of at-risk women ranked these issues with ≤2
Blumen *et al.*[207]	40	Nearly all couples discussed testing, 30% reporting they discussed the topic a lot; one-third of spouses read wives' printed material, 25% looked elsewhere for more information
Cappelli *et al.*[216]	110	Intent did not predict actual genetic follow-up; perceptions of costs of testing may induce women to consider prospect of learning *BRCA1/2* status more carefully before acting
Cappelli *et al.*[217]	108	Individuals perceived benefits in providing relative information regarding risk, helping make career decisions, helping plan retirement, helping make decisions about life and disability insurance, helping make marriage and relationship decisions, helping make decisions about treatments for breast cancer
Claes *et al.*[222]	64	1 non-carrier of familial mutation informed parent and siblings that test result inconclusive, in concordance with professionals' interpretation; 8 participants from inconclusive group understood that test result revealed absence of genetic predisposition; 5 informed all close relatives about result, other 3 informed some close relatives; noneinformed distant relatives about result; 21% of conclusive group and 7% of inconclusive group tried to systematically inform distant relatives, while others did not apply systematic approach; about 40% of participants of conclusive group and half of participants of inconclusive group informed close relatives because they had the opinion that this information was important to them; preference to inform distant relatives who also obtained diagnostic genetic testing found in 47% of participants in conclusive group and 40% thought that information important for distant relatives; close relatives like children, siblings, and parents usually informed about diagnosis; distant relatives rarely informed about diagnosis; of 19 participants who provided argument against informing close relatives, 68% assumed that other relatives would pass on information; 57% participants said there was lack of contact with relatives or participant assumed other relatives had informed them; close relatives more likely to be informed about blood sampling than distant relatives; significant differences in informing male versus female relatives found for siblings, SDRs, and third degree relatives; data could be presented for 56 participants (24 conclusive and 32 inconclusive); when distant relatives not informed, >40% of participants in conclusive group assumed that other relatives would pass on information; participants of inconclusive group found information less important for distant relatives and <20% assumed that information passed on by other relatives; little or superficial contact impeded dissemination of information to distant relatives in both groups; participants who informed distant relatives had lower scores on UCL sub-scale palliative coping (behaviour aimed at reducing tension rather than trying to change problematic situation) than those who did not inform distant relatives (16.8<19.2 t=-2.13, p<0.05); participants who informed distant relatives also had lower scores on UCL sub-scale comforting ideas (behaviour aimed at setting mind at rest (13.3<14.9, Wolcoxon test=-2.08, p<0.05)
Claes *et al.*[239]	62	56% (10 of 18) carriers reported changes in relationship with children as consequence of genetic test result, as opposed to 33.3% (2 of 6) non-carriers; 28% (5 of 18) carriers reported changes in relationships with relatives compared to 1 of 6 non-carriers

Author	Sample Size	Social Issues
Di Prospero *et al.*[220]	27	1 focus group participant, with previous diagnosis of cancer, stated she had told no one in her family of her results because she was unsure they would want to know; another did not have immediate living relatives to tell; other 6 participants and 12 (75%) respondents reported telling all their immediate family members of test results; remaining 4 respondents stated they felt some relatives too young to receive sensitive information or they did not feel close enough to certain relatives to share results. Reported reactions of family members were distributed evenly as "a little" (10 of 24, 42%) or moderately (9 of 24, 38%) worried about proband and risk; 62% (n=5) focus group participants favoured regular support group, meeting monthly or semiannually; most indicated they would be happy with peer-led group or professionally led group; 25% (n=4) respondents indicated interest in support group; others felt supported by family and friends; 92% subjects indicated interest in follow-up with genetic counselling team for reasons including updates on new research studies or treatments, and opportunity to have psychological well-being assessed
Dicastro *et al.*[282]	155	30 women involved their partner in counselling, of whom 13.5% non-carriers and 44.8% mutation carriers (p=0.0001); educational level of women attending oncogenetics service higher than that of general population; majority have academic degree
Foster *et al.*[249]	34	Barriers to testing include travelling to genetics clinic (33%) and taking time away from work or family (30%)
Hallowell *et al.*[246]	30	Women in all groups talked of anxiety about risk of developing another form of cancer and anxiety about other relatives' risks, particularly sisters' and daughters' risks; women in carrier group reported that they had found disclosure of this information to their kin particularly burdensome and ethically contentious; in some cases, they had not reflected on which of their relatives, in addition to sisters and offspring, would be implicated by the outcome of test until after they received results; most women in inconclusive group did not report experiencing any problems disclosing inconclusive test results to their kin
Hamann *et al.*[232]	218	Men more likely to support *BRCA1* testing in children; *BRCA1* mutations confer greater risks of cancer on women than men, men may not have perceived the information gained from testing to be personally threatening; differences in demographic factors may contribute to divergent findings of studies; sample included only individuals of northern European descent
Hughes *et al.*[210]	407	Significantly more Caucasian women compared to African American women recruited through self referrals (88% versus 12%); Caucasian women had significantly higher education levels and household incomes than African American women; Caucasian women more likely to be married and have health insurance than were African American women; Caucasian women reported significantly greater exposure to genetic testing through increased use of genetic testing services and more exposure to written and verbal information about testing
Hughes *et al.*[226]	163	Majority of carriers and non-carriers communicated test results to sibling or to offspring age >18; 81% carriers and 87% non-carriers communicated their results to sister and 61% carriers and 68% non-carriers communicated results to brother; carriers previously affected with cancer and those who were older more likely to communicate their result to adult child; 72% carriers and 70% non-carriers communicated results to children age >18; carriers and non-carriers less likely to communicate results to offspring ≤18 years old (46% of carriers and 46% of non-carriers); females significantly more likely than males to communicate test results to sister and to young child 18 or younger (89% versus 69%, chi square 7.47, p=0.006 and 54% versus 12%, chi square=8.85, p=0.003 respectively); heightened perceived risk of having *BRCA1/2* mutation associated with communication to brother only in non-carriers; gender had significant positive association with communication of test results to offspring age ≤18 (OR=8.6, CI:1.4;32.9, p=0.02) and perceived risk of having *BRCA1/2* mutation had a marginally significant association with this outcome (OR=2.4, CI:9.4;6.4, p=0.07); among non-carriers, perceived risk of having mutation associated significantly with communication of test results to brother and to offspring age ≥18 years; non-carriers with higher risk perceptions significantly more likely to communicate test result to brother and child age ≤18 years than non-carriers with lower risk perceptions; older respondents significantly more likely than younger respondents to communicate results to adult child age ≥18 years.

Author	Sample Size	Social Issues
Hughes *et al.*[247]	43	*BRCA1/2* test results communicated to 85% of sisters, and carriers communicated results to significantly more sisters compared to those whose results were uninformative (96% versus 76%); Fishers exact test (FET)=0.02); probands communicated test results to 85% of sisters and *BRCA1/2* mutation carriers communicated results to significantly more sisters than probands whose results were uninformative; carriers communicated results to 96% of their sisters whereas uninformatives communicated test results to 76% of their sisters (FET=0.02); test results communicated to 25% of sisters on same day as disclosure and results were communicated to 70% sisters within 1 week of receiving test results; most important reason for communicating results to provide genetic risk information; compared to uninformatives, carriers communicated results to significantly more sisters to obtain emotional support (74%) and to get advice about medical decisions (42%) (FET=0.001); carriers also discussed possibility of discrimination and recommendations for cancer management with more sisters; most important reason for not sharing results was because probands not close to relative and least important reason was because of guilt or anxiety; not being close to relative was important reason for not communicating results to 45% of sisters, whereas experiencing guilt or anxiety was important reason for not communicating results to 8% of sisters
Hughes *et al.*[250]	28	Rates of test acceptance lower among women with greater perceptions of familial interdependence (41% versus 91%, p=0.02)
Julian-Reynier *et al.*[283]	506	Attendance rate higher among FDRs (34%, 89 of 173) than SDRs (18%, 44 of 246) (OR=4.86; 95% CI:3.06;7.76, p<0.001); also higher among women with cancer (83%, 30 of 36) than among healthy women (36%, 75 of 208) (OR=8.86; 95% CI:3.53;22.27; p<0.001) and among women (43%, 105 of 244) compared with men (16%, 28 of 175)(OR=3.97, 95% CI:2.46;6.39; p<0.001); difference also significant (p<0.01) for gender inside first and second degree groups
Kinney *et al.*[211]	95	Most participants reported attending specific clinic where communication with health professionals high; younger age associated with interest in testing, as were history of breast or ovarian cancer, and ≥1 FDRs with breast or ovarian cancer; no significant associations between those who intended to and those who did not intend to test regarding gender, income, religion, health insurance, primary care provider, or communication
Lee *et al.*[205]	258	Eligibility for free testing, history of breast or ovarian carcinoma, Ashkenazi Jewish versus non-Ashkenazi Jewish heritage, genetic risk category, and age category associated with test utilization and in multivariate analysis, the first 3 remained statistically significant factors associated with testing; 26% of 50 patients who did not have access to free testing sought insurance reimbursement of which >50% had prior diagnosis of breast or ovarian cancer
Liede *et al.*[225]	59	Majority of men discussed result with family member; 88% men participated in family conversations about breast and ovarian cancer; 10 men said family relationships had changed since they received results, most said family relationships had been strengthened
Lodder *et al.*[244]	28	If men became identified as carriers, all intended to postpone informing children about possible risks for several years; 2 of 14 men with adult daughters opt to inform daughters about testing after receiving results; increase in problems with children was expected by 19 of 25 men with children and half of their partners
Lodder *et al.*[242]	63	Increase in problems regarding breast-related body image at follow-up reported by mutation carriers who underwent prophylactic mastectomy, unlike women of other groups; increase in problems in intimate relationship found in carriers undergoing mastectomy and those opting for surveillance; first group reported more problems than the latter at pre-test and follow-up; breast-related body image and intimate relationship of non-mutation carriers improved at follow-up
Mehnert *et al.*[218]	100	54% of women described problematic communication regarding cancer specific communication in family; 20% said that cancer was unspoken subject; in 18% of cases, communication about cancer restricted to factual information; for 16% of women, conversations about cancer and burdens connected with it only possible with few relatives; women participated in study on recommendation of medical facility or attending physician and those with higher level of education had a higher level of knowledge about counselling; women who received genetic counselling had higher educational level than women who had not been counselled, in ratio of 61% to 29% (p<0.01)
Schwartz *et al.*[224]	290	25% Catholic, 33% Jewish, 31% Protestant, 10% other; spiritual faith: 42% very strong, 58% not strong moderately strong

Author	Sample Size	Social Issues
Sheridan *et al.*[240]	102	88.2% participants shared results with ≥1 immediate family members; 72% shared results with ≥1 members of extended family; 37% shared results with family member under age 18; individuals reported that family members' reactions to results varied; carriers reported that family members experienced guilt, anger, anxiety, and fear; non-carriers reported that family members experienced relief, non-concern, and guilt
Tercyak *et al.*[200]	133	63 parents (47%) shared news of mutation status with children; among carriers, about same numbers disclosed as non-disclosed (53% versus 47% respectively); among non-carriers, rates were 43% versus 57% respectively; disclosure rate 49% and among parents with older children (14 to 18 years of age) and 37% with younger children (<14 years of age); mothers more likely to disclose than fathers (51% versus 29%, p=0.05); women who reported using more active coping, higher levels of baseline general distress, and those who reported higher levels of post-counselling general distress also found to be more likely to communicate; multivariate model: after controlling for gender, mutation status, and cancer history, baseline general distress significantly associated with communication (OR=3.45, CI:1.32;8.96); as in general distress model, communication of *BRCA1/2* genetic test results to children not significant predictor for post-counselling IES scores beyond that accounted for by active and avoidance coping
Tercyak *et al.*[198]	42	Rate of disclosure 53%; factors associated with disclosure were child age (mean age 13.5 versus 11.6 for disclosed and undisclosed respectively), having had more conversations about maternal health with children, more interested in pediatric genetic testing, and stronger intentions to share results; mothers who disclosed test results reported better dyadic adjustment in relationships with children and more open parent-child communication styles; reasons for disclosure included child's right to know (50%), result was good news negative result (23%), prevent child's worries or promote greater trust and open communication (17%), and other (10%); reasons for non-disclosure included child being too young to understand (47%), child would become too worried or anxious if he or she knew (22%), lack of apparent interest by child (19%), test result not important enough to warrant discussion (12%). Multivariate model using GEE, which adjusts for potential clustering of children from same families, after accounting for significant effects of child age and maternal communication history, mothers reporting more open communication styles nearly 6 times more likely to disclose results to children (OR=5.88, CI:1.63;21.22)
Tessaro *et al.*[248]	66	Women expressed support needs to help make decision about testing, with testing experience, and with receiving of results; decisive about who they would divulge the results to, especially among affected women; ranged from not telling anyone to telling everyone; support groups and religious faith experiences reported as potential sources of support; whereas affected women considered genetic testing to be more of a personal decision, relatives appeared to be more of a family issue; many wanted physician to play active role in decision; women consistently expressed need regarding guidelines for genetic testing based on current professional knowledge (partly to form unified front among doctors)
Velicer *et al.*[204]	276	Respondents discussed breast cancer genetics testing with relatives more (53%) than with health care providers (8.3%); most common initiators of conversations were survivor (70.5%), daughter of survivor (26.8%), sister (21.4%)
Wood *et al.*[199]	35	64% found counselling process extremely helpful in future medical decision making; most useful aspect was multidisciplinary counselling effort by genetic counsellor and oncologist; suggested areas for improvement: assistance communicating with family (54%, family session or brochure); barriers to communication: knowing when to tell at-risk family members, geographic distance, family member's denial
Wylie *et al.*[241]	203	Effect of having burdensome spouse continues to elevate distress level of tested person

Table 8: Conclusions, study limitations, potential impact of bias

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Armstrong *et al.*[208]	305	Even after adjusting for breast cancer risk, substantial racial disparity in use of *BRCA1/2* testing	Substantial difference in response rates between cases and controls (response bias); single academic health system studied with single testing site	Response bias
Audrain *et al.*[219]	256	Women who self-referred for genetic counselling and possible testing scored in moderate range on measures of general and cancer specific distress; women with higher levels of general distress less optimistic and had heightened breast cancer risk perceptions accompanied by feelings of low perceptions of control over development of breast cancer; women with higher levels of cancer specific distress had low perceptions of control over developing breast cancer; consideration of personality for women who self-refer for genetic counselling may help identify groups who may be vulnerable to distress and who may benefit from psychosocial interventions	Cross sectional study design does not permit determining whether psychological distress promotes certain appraisals of perceived cancer risk or whether certain appraisals generate psychological distress; following women over time and determining whether changes in appraisals are followed by changes in distress could address issue; participants in study all self-referred; no population-based comparison group; women who self-refer may be more distressed than other high risk women; most participants Caucasian with at least high school education; factors may limit generalizability of results to all high risk women	Among all eligible subjects, 301 completed baseline interview; among 301 who completed interview, 256 completed counselling visit; of remaining 45 women, 30 declined participation or withdrew from study before counselling visit; 15 could not be reached to schedule visit; compared to non-participants, participants significantly more likely to be Caucasian and have higher level of education
Biesecker *et al.*[281]	172	Age is strongest predictor of decision to undergo testing; 1 of strongest motivations to test is to learn about risks to one's children; participants with dispositional optimism less likely to choose testing	Potential criticism of studying families who have participated in NCI research is potential lack of ability to generalize results, even to other hereditary breast or ovarian cancer families; some members of families had not participated in first epidemiological investigation so we could compare those who had participated in research, some for 20 to 25 years to those who had not participated in research; previous participation did not predict decision to undergo testing	None
Blandy *et al.*[215]	30	General lack of knowledge despite a high level of satisfaction regarding information given by geneticist; challenge for genetic counselling is to ensure that consulting patients not only receive complete information but also understand information and anticipate impact of test result before deciding to take test	Study's small sample size limited ability to interpret negative results	Further investigation with larger samples and longer follow-up will allow information to circulate in study families beyond first-degree relatives

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Bluman *et al.*[209]	200	Study results add to evidence indicating that women overestimate chances of having *BRCA1* and *BRCA2* mutations and lack basic knowledge about cancer genetics; informed decision should reflect risks and benefits of testing, and realistic appraisal of individual probability of having mutation	Participation voluntary and testing was free so may have resulted in selection biases; all patients included in analysis affected with breast or ovarian cancer; personal history affects risk perceptions, and may have impact on intentions to be tested; some women may have faced problems with insurance as a result of cancer diagnoses and may have less concern about insurance discrimination as result of testing; homogeneous nature of the population with respect to ethnicity limits generalizability of results to more ethnically diverse population; study methods not completely representative of clinical practice; women could self-refer to project; majority recruited through proactive methods	331 probands with history of breast or ovarian cancer and family history of breast or ovarian cancer invited to participate in study in January 1998; of those invited, 208 completed and returned baseline questionnaires by February 1998; mean time for survey mailing to survey receipt 25 days, maximum 231 days; some non-responders at time of analysis eventually responded
Bluman *et al.*[207]	40	Knowledge of cancer genetics and genetic testing for *BRCA1* and *BRCA2* limited among women and spouses; one-third of spouses indicated they would like additional information about testing; most spouses indicated they thought wives had mutation and breast cancers would recur; spouses satisfied with role in decision making process; future interventions to improve decision making should be undertaken	Participation in overall randomized trial and study involving spouses voluntary, and genetic testing was free, so may have led to selection bias regarding women who decided to participate; all wives had personal history of breast or ovarian cancer	Personal history of breast or ovarian cancer may have led to overestimation of risk of having mutation in *BRCA1* or *BRCA2*, and risk of recurrence by wives and spouses; population studied homogeneous, making it difficult to generalize results of study to more educationally and ethnically diverse population
Brandt *et al.*[203]	400	Women with previous diagnosis of breast cancer may have different primary motivators and degrees of concern regarding genetic testing than unaffected counterparts	Study contains limitations, such as small size and homogeneity; views reflected are of self-selected, educated, suburban, primarily Caucasian women; extrapolation of opinions may be limited; issues regarding gene testing addressed in the questionnaire subjective, individual answers not compared with history of surgery, number or sex of children, familial mutation status, or personal factors that may influence responses in both groups	None

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Cappelli *et al.*[216]	110	60% of women with and or breast cancer indicated they would like breast cancer gene testing; women with breast cancer almost 6 times more likely to want test than general population; 49% of breast cancer participants who intended to have test sought advice from genetic counsellor; at follow-up no significant relationship between intent to be tested and genetic follow-up; actual use of genetic testing lower than anticipated use of test	Total cancer-related concerns variable did not independently predict intentions or actions regarding testing; caution should be taken in interpreting results as this represents sum of responses to 2 survey variables; results cannot be generalized to unaffected family members of breast cancer patients; women opposed to health intervention may be less inclined to disseminate information about it to relatives; if widespread testing is implemented, genetic counselling protocols followed by many institutions in Canadian health care system will stipulate that affected family member must be tested, and mutations subsequently identified before unaffected relatives proceed to test; actual genetic follow-up variable may limit comparison of results with those from recent studies examining uptake rates for testing	Results not generalizable
Cappelli *et al.*[217]	108	Personal risk appraisal is predictor of health-related attitudes; over half of women in general population and high risk groups would be interested in gene testing to identify breast cancer or ovarian cancer susceptibility if it were offered; women considered at higher risk for developing disease more likely to be interested in testing, and certain aspects of personal risk appraisal associated with interest in testing; women who perceive more benefits and fewer costs inherent in testing more likely to be interested in testing	High risk women who participated in study recruited from sample of affected relatives who had participated in previous study; possible that both groups of women held favourable attitudes towards genetic testing, which influenced decision to participate in study; participants predominantly Caucasian, middle to upper class and well-educated; results not necessarily generalizable to all Canadians	Results not generalizable
Claes *et al.*[222]	64	Conclusive group thought that information important enough to be disseminated in family; differences in personal evaluation of importance of test result may be significant factor in informing distant relatives; a part from genetic test result (conclusive versus inconclusive), age also significant factor in informing distant relatives; the younger the patient, the more willingness to communicate genetic test result; at least part of absence of requests for testing in distant relatives explained by fact that they were	Less than one fourth of contacted eligible patients did not want to participate in study; because of study procedure, no additional information on these patients retrieved; maybe these patients less willing to talk about cancer and testing, and may be less willing to share information with relatives; if this is true, findings about communication may be overoptimistic; of participants, all patients and physicians informed about test result by genetic centre before middle of 2000; interval between interview, blood sampling, notification, and	None

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
		not informed about result of affected relative or test availability	dissemination of information to family especially after receiving test result large; regarding dissemination of information to family, especially after receiving test result, unable to obtain detailed data about the content of the information provided to relatives, except some cases; future studies with shorter follow-up times needed	
Claes *et al.*[239]	62	Not to take for granted that genetic testing in cancer patients will not have negative impact on emotional well-being because they had to cope with cancer diagnosis; apart from non-carriers, carriers and patients with conclusive results experience negative consequences and can benefit from counselling	Retrospective study has limitations; group of participants self-selected; less resourceful patients less likely to participate in study; patients studied may differ from those who will be studied when genetic testing is clinical service or more common practice	Results not generalizable
Clark *et al.*[234]	159	Little variation in testing decision; 96% of respondents elected to proceed with testing; most participants focused on potential for beneficial effect rather than potential negative effects; most felt counselling necessary and more than half said they made a decision about testing on their own; satisfied with decisions but said they used or changed coping strategies to deal with anxiety related to cancer or genetic testing after decision	Offer of free testing may have encouraged participation among women unwilling or unable to pay for testing otherwise; possible that this study surveyed a group more representative of general population of women with cancer than those with access to testing; all but 4% of sample elected to test, so cannot determine whether concentrations on pro versus con of testing differ significantly between those proceeding with and those declining testing; 70% participants thought researchers wanted them to have testing; perception persisted despite messages emphasizing that women should make autonomous decisions and in spite of fact that physicians on team did not recommend testing as right decision; women referred by physicians may have felt that going through with testing more "full" participation, more pleasing to researchers or helpful; 1% felt pressured into making decision about genetic testing	Potential referral bias

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Croyle *et al.*[235]	60	Genetic test results can have short-term impact on psychological functioning even when in-person counselling is provided; IES scale identified group of participants with high levels of distress after testing and more sensitive to interactive effect of cancer or cancer-related surgery history and test result; findings suggest that medical experiences of women before testing and expectations they have regarding genetic status warrant more thorough investigation; because most women in population-based screening programs would not have cancer or cancer-related operations, findings that distress is highest among carriers with these characteristics has significant public health implications	Sample size limits ability to test more comprehensive models of role of experience and expectations in adjustment to genetic testing; study included measures of general distress (the State Anxiety scale) and test-specific distress (the IES); results of this study might have been interpreted differently had only 1 of the 2 measures been included	58 of 60 participants included in analysis for general distress; all participants in study members of predominantly Mormon kindred of northern European descent; many had participated in genetics research by providing blood samples and family history information; because of this, level of health knowledge and cancer risk awareness may have been higher in this sample than general population; adverse psychological effects of genetic testing might be more likely among individuals with less health knowledge and risk awareness
Di Prospero *et al.* [220]	27	While participants indicated perception of cancer risk and worry about cancer increased after learning of mutation status, none regretted decision to undergo testing; almost 40% indicated interest in attending ongoing support groups and regular follow-up with genetic counselling team; perceived benefit of testing was increased surveillance offered to those found to have mutation; participants reported wait for results too long; time from blood sampling to receiving results up to three years	Results based on small sample and should be interpreted with caution; subjects mainly middle-aged Caucasian women with at least 1 child; lower perception of risk and lower distress levels reported compared to review of US studies; discrepancy may be due to fact that most participants had defined support system and were beyond age of having to make life decisions based on genetic test results	8 (30%) of 27 people invited to focus group attended; 16 (62%) of 26 mailed questionnaires completed and returned
Dicastro *et al.*[282]	155	Oncogenetic counselling and testing not associated with adverse psychological sequelae, impact that it has on mutation carriers in increasing anxiety-related symptoms minimal, and retention of cancer risk related information 1 and 3 years after counselling sessions is disappointing	Study is selected, retrospective analysis <40% of women counselled in 1 facility in Israel; no apparent differences in measurable parameters between responders and decliners; more comprehensive study is indicated before reaching conclusions; questionnaire used for assessing anxiety and distress levels is not the 1most commonly used in similar studies	Reduced generalizability

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Dorval *et al.*[245]	53	Majority of participants in initial programs accurate in predicting emotional reactions to disclosure of test result and accuracy of anticipation of distress emotions associated with post-disclosure psychologic adjustment; affected *BRCA1* carriers felt more distressed than they had anticipated after learning carrier status; most *BRCA1* carriers who had cancer underestimated distress reactions to disclosure; after disclosure, cancer patients may have greater awareness of increased risk of second cancer and may be more conscious of genetic contribution to increased risk of cancer in offspring; modest levels of guilt reported in anticipation of positive results and in anticipation of negative results; post-disclosure guilt ratings remained low, suggesting participants did not experience strong guilt after disclosure of positive or negative test result	Act of asking about anticipated reaction may be viewed as intervention that may have enhanced participants' awareness of potential emotional impact of test results; accuracy of anticipated versus actual emotional reactions may be overestimated in this study compared with settings in which anticipated reactions not explicitly addressed; despite this difficulty, likelihood of recall bias limited by prospective design of study; anticipated reactions assessed months before result disclosure; differences in periods of data collection for testing programs might have reduced comparability of information, although extent and direction of biases difficult to assess; application of these findings is limited by small number of participants	Reduced generalizability
Foster *et al.*[221]	315	Women more likely to report cancer-specific distress than general psychological distress; younger women report more cancer-specific worry than older women; more younger women worry more often about developing cancer and find it more of a problem than older women; men and women do not report unusually high levels of general distress, although this may be conservative; most women reported intrusive thoughts about developing cancer; significant proportion of women overestimate population risk of breast and ovarian cancer; younger women more likely to provide accurate figure for breast but not ovarian cancer risk; most women think it is likely they will develop breast or ovarian cancer and that risk is higher than that of average woman; data illustrate that while general mental health is not adversely affected by prospect of genetic testing, cancer-related worry is prevalent among premenopausal women, many of whom self-refer to genetics services	Self-referred women more likely to think of themselves as higher risk than those in other referral groups; cancer-related worry not associated with risk perception; may be because women in study eligible for genetic testing; most consider themselves to be at increased risk; given low variation in risk perception, difficult to assess relation between cancer worry and perception of risk at baseline	

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Foster *et al.*[249]	34	Barriers to testing need to be discussed in genetic counselling sessions	Small sample size	Reduced generalizability
Hagoel *et al.*[243]	165	Being carrier could not be considered psychosocial risk factor, nor does it affect carriers' resources and lifestyle	Findings limited by study design and time frame; cross-sectional design does not allow conclusion that predictive genetic testing has no adverse effect, as there was no pretest comparison; no data on long term effect of carrier status	Reduced generalizability, study participants self selected
Hallowell *et al.*[246]	30	Similar reasons for undergoing testing reported in studies of unaffected high risk women; motivation for undergoing testing to help family members; study suggests that women previously affected with breast or ovarian cancer do not experience genetic testing process, or waiting for result, as anxiety provoking, in contrast to findings of Canadian study by Di Prospero *et al.;* few participants expressed dissatisfaction about delay between blood being drawn and receiving result; benefit was seen as gaining genetic information to enable family members to establish mutation status, gain access to services, and potentially end uncertainty about risks; burdens of testing identified as increased anxiety about risks and others' risks of cancer and disclosure of results to kin	Retrospective study at one centre; no indication of ethnic background of participants	
Hamann *et al.*[232]	218	Majority of tested individuals with minor children did not want own children to be tested; among respondents with children <18 years of age, less than one-fifth supported testing for children; results indicate that majority of tested individuals did not support practice or desire testing for own children	Did not include measures of attitudes toward testing children before participants counselled and tested; although effects of counselling and testing on attitudes toward testing of minors can be speculated, cannot be demonstrated that attitudes changed from pre- to post-testing; limited generalizability of sample to others receiving *BRCA1/2* results; participants members of large kindred of northern European descent, majority identified as members of Church of Jesus Christ of Latter Day Saints; participants not representative of the US population in terms of race or religion; Mormons more likely to be married, have larger families and social networks than non-Mormons	Reduced generalizability

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Hughes *et al.*[210]	407	Women with family history of breast and ovarian cancer understand facts about inheritance of breast cancer and *BRCA1* testing, but lack knowledge in some areas; women at increased risk for breast or ovarian cancer generally have positive attitudes about genetic testing for breast ovarian cancer risk; most important benefits of testing were to know if additional measures are needed to prevent cancer and to know if cancer screening tests needed more often; Caucasian women had higher levels of knowledge than African American women; most African American and Caucasian women rated benefit items dealing with cancer prevention and detection as being of importance; ethnic differences in knowledge and perceptions of benefits of genetic testing	Participants self-referred or patient-referred	82% of eligible women contacted completed baseline interview; 7% refused survey; 11% could not be reached; final sample included 407 women
Hughes *et al.*[226]	163	Results of study demonstrate that recipients of *BRCA1/2* genetic test results communicate with at-risk family members; recipients most likely to have communicated with sister	Did not address content of communication beyond disclosure of test results; no examination of whether testing encouraged or discouraged or whether individuals experienced difficulties in communicating with family members; accuracy or consequences of communicating results not assessed; sample composed of only Caucasian subjects identified from hereditary cancer registry in which cascade testing throughout family not used; likelihood and determinants of communication to at-risk relatives may differ among subjects of different ethnic backgrounds; communication with relatives may differ in families who receive test results at different times	
Hughes *et al.*[247]	43	Study suggests that probands likely to quickly communicate results to relatives; although needs for social support may motivate family communication, emotionally distant relationships may be barrier to communication; test results communicated to 85% of sisters; 95% of discussions took place within week of receiving results; most important reason for communicating results among probands to provide sisters with information about risk of	Study based on small sample of *BRCA1/2* probands who received test results and analysis limited to communications with sisters; only communication process and content from perspective of proband evaluated and relatives' reactions to receiving information not evaluated; possible that communication with relatives facilitated by the counselling process in which probands provided with written information that could be shared with family members; having	

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
		having mutation; no differences between mutation carriers and uninformatives in terms of motivation for providing risk information to sisters; concern about upsetting relative an important reason for not communicating test results	evaluated communication to sisters only, communication to males not considered	
Hughes *et al.*[250]	28	Cultural beliefs and values may influence genetic testing decisions among African-American women	Small study sample	Generalizability to other ethnic populations
Jacobsen *et al.*[231]	74	Results of study indicate that decision of many women at familial risk to seek genetic testing related to perceptions that advantages of learning carrier status outweigh disadvantages; results are consistent with transtheoretical model of behaviour change and demonstrate usefulness in understanding decision-making about genetic testing; responses suggest that notification of genetic carrier status likely to have significant impact on women's psychological well-being and on breast cancer surveillance and prevention behaviours	Outcome in study women's readiness to undergo hypothetical genetic testing for breast cancer susceptibility; whether future genetic testing will possess same characteristics as this hypothetical test unknown; relation of readiness to undergo testing to decisions about testing unknown; sample in study predominantly Caucasian and well-educated; results may not be generalizable to women at familial risk with different demographic characteristics; study limited to women with ≥ FDR diagnosed with breast cancer; possible interest in testing among those not at familial risk or at lesser familial risk not assessed	94 women meeting criteria invited to participate in study; 82 agreed; among those who declined, 12 reported lack of time; 8 women provided incomplete data
Julian-Reynier *et al.*[283]	506	On average, for every *BRCA1* mutation detected, there are 2 at-risk unaffected female FDRs to be tested; 8 months elapsed before ≥1 close relative informed after index case had been given results in 75% of families; high rate of interest in testing shown by female FDRs; in 85% of families, ≥1 relative attended cancer genetic clinic after index case informed	Families selected from available French cancer genetic clinics to prevent bias towards families involved in research studies, yet sample not representative of national family profile; having asked for first cases obtained at every centre, more families may have been selected with large numbers of people involved, leading to an overestimate; in most analyses, records selected were those where information was divulged to first case family members at least 8 months previously; arbitrary threshold may have underestimated time required for information to spread	3 families excluded: 1 *BRCA2* mutated family and in the other 2, information on FDRs and SDRs incomplete

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Kinney *et al.*[211]	95	Study provides information about knowledge deficits, attitudes, and beliefs to consider when designing genetic education and counselling intervention for this and similar high risk families; high interest level in genetic testing despite limited knowledge about cancer genetics among at risk African Americans; negative association between beliefs about God as controlling force over health and adherence to breast cancer screening guidelines observed in K2099 cohort	Study measured intention to undergo genetic testing rather than behaviour; interest in *BRCA1/2* testing overestimates uptake among Caucasians; results of study should be interpreted with caution; participants had favourable attitudes toward health care providers in terms of communication and rapport; many African Americans have distrust of medical system, which may inhibit them from using services; findings may not reflect beliefs, attitudes, and knowledge among other African Americans who carry gene mutation associated with hereditary breast cancer; odds ratios have wide confidence intervals and are imprecise, possibly because of small sample size; choice of in-person or telephone interviews may be suboptimal; design established in partnership with key informants of K2099 as strategy to enhance recruitment	Of 121 eligible K2099 members, 79% participated in study; compared to non-participants, participants more likely to be female and participated in prior linkage study; respondents younger than non-respondents
Lee *et al.*[205]	258	Actual utilization of *BRCA1/2* genetic testing in clinical setting lower than in research and hypothetical settings; potential obstacles include cost, fear of insurance discrimination, and need to involve affected family member in testing process; one-quarter of patients eligible to consider testing in this population eventually tested or arranged to have affected family member tested; only factors associated with genetic test use access to free testing, personal history of breast or ovarian cancer, and Ashkenazi Jewish heritage; approximately one-third of entire patient population tested found to carry mutation; factors previously reported to be important in hypothetical and research situations, such as education level, reproductive status, involvement in screening, healthy lifestyle, and high perceived risk of breast cancer, did not have impact on test utilization in study	Genetic risk estimates provided to patients not based on single model or delivered in standardized manner; significant limitations to any risk assessment model; risk estimates derived from Couch model empirically doubled, resulting in possible overestimation of risk because *BRCA2* mutations less prevalent than *BRCA1* mutations; prospective study with systematic documentation of genetic risk categories would have provided more valuable and relevant information regarding genetic risk categories and how they may affect genetic test utilization; passage of legislation to protect against genetic discrimination, increasing willingness of insurance companies to reimburse for genetic testing, and emerging data on cancer preventive measures may affect testing uptake	

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Lerman *et al.*[228]	121	Demand for genetic testing may be great, even among low risk women; adequate resources for psychological counselling essential before initiating widespread genetic testing; informed consent protocols to educate about benefits and limitations of predictive testing are critical	Subjects responded to hypothetical scenario in defined population of women at increased risk; therefore may not be generalizable to actual genetic testing situations or to other subject populations; small sample size; study focused on FDRs of ovarian cancer patients at tertiary care centre who were predominantly Caucasian and middle class, thus not necessarily generalizable to high risk women in local community hospitals; subjects at low risk (1 FDR with cancer)	No comparison between groups being made; descriptive study, therefore bias not an issue; participation rate not reported
Lerman *et al.*[227]	105	Demand for genetic testing is high, even among those not likely to have predisposing mutations; informed consent protocols to educate about the benefits and limitations of predictive testing are critical	Subjects responded to hypothetical scenario in defined population of women at increased risk and therefore may not be generalizable to actual genetic testing situations or to other subject populations; reason for 1 year gap between first and second interview not clear; no information given about rate of participation, no indication of how many people refused	No comparison between groups being made; descriptive study, therefore bias not an issue; participation rate not reported
Lerman *et al.*[132]	192	Some HBOC families will want *BRCA1* testing; highest use of testing may be found among those of higher socioeconomic status and greater number of affected FDRs; psychosocial impact of testing promising but more research necessary before recommending counselling	Study population consisted of individuals in hereditary breast and ovarian cancer registry who were Caucasian and highly educated; many involved in prior genetics studies	N/A
Lerman *et al.*[206]	149	58% participants requested genetic test results; after controlling for demogarphic factors and risk status, cancer-specific distress significantly and positively associated with *BRCA1* test use, whereas global distress was not	Study design prospective observational, rather than RCT for providing education thus could not assess impact of education on distress levels; 24% eligible individuals did not participate; less likely to have provided a blood sample and to request test results; no way of knowing if they differed by distress levels; participants from HBOC registry, all were Caucasian, highly educated, and had health insurance	Participants more likely to have given blood sample before and to request testing; not having to get blood test again may influence test use; generalizability of results may be limited; potential attrition bias
Lerman *et al.*[237]	327	Genetic testing decliners from *BRCA1/2*-linked families with high levels of cancer-related stress may be at increased risk of depression and therefore may benefit from education and counselling; should be monitored for potential adverse effects	Study population consisted of individuals in hereditary breast and ovarian cancer registry; Caucasian and highly educated; received general information about hereditary cancer as a result, and test results more readily available; may not be representative of clinical setting	Authors report having tested for attrition bias; no significant difference on demographic variables or baseline depression level; those lost to follow-up significantly more likely to have had cancer

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Lerman *et al.*[236]	298	Among low- to moderate-risk African American women who are counselled in research setting by African American counsellor, pretest education and counselling may motivate rather than deter *BRCA1* testing; among Caucasian women, alternative counselling approaches may not influence testing intentions	Almost all counselling for African American participants provided by African American nurse educators, thereby potentially making results less generalizable to African Americans counselled by Caucasian educators; aspects of training or background of counsellors may influence results; some differences in interventions may exist between 2 sites that may have contributed to racial differences; differences between 2 study arms may be due to differences in time spent with counsellors; provision of blood sample proxy for intention for testing; family history information self-reported so it could be less accurate than medical records; fewer African Americans relative to Caucasians; potential selection bias due to different participation rates; African Americans recruited from different hospital settings than Caucasians; African Americans had significantly lower participation rate; enrolled African Americans older, had higher incomes and lower baseline distress than non-participant African Americans; enrolled Caucasians had higher baseline distress than non-participant Caucasians; although authors indicate no significant difference between participants and those lost to follow-up, no numbers are given; appear not to have assessed losses by intervention groups	Some significant differences in participants and non-participants, such as education, marital status, ethnicity, income, and family history; may have impact on results
Liede *et al.*[225]	59	Primary reason for seeking genetic counselling concern for their daughters; 88% men participated in family conversations about breast and ovarian cancer; 47% participated in conversations about prophylactic surgery; most men believed they were at increased risk of developing cancer of all kinds; fewer than half of men with no previous diagnosis stated that prostate cancer surveillance practices had changed after receiving results. More than half of men had intrusive thoughts of cancer risk. Satisfaction with genetic counselling generally high; issues needing greater awareness by practitioners, as identified by respondents: pressures influencing men to request testing,	Some results unclear as to whether they referred to breast cancer or prostate or colorectal cancer	15 respondents participated by telephone; 4 men did not return questionnaire, 3 men lost to follow-up, and 1 refused participation

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		difficulties in setting up surveillance regimens for breast and prostate cancer, lack of information about men's experiences medical community; increased focus on men necessary		
Lodder *et al.*[244]	28	Distress levels before result in tested men and partners low; many men and partners expected test result to affect children's but not their level of problems; men without daughters and those with optimistic personality had especially low distress before disclosure; most men reported they did not avoid the issue; 4 of 28 men identified as mutation carriers; high distress after disclosure of result reported by 1 mutation carrier and by 3 non-mutation carriers; transcripts from interviews showed large variation of psychological reactions in male mutation carriers; low pre-test distress in males does not necessarily indicate avoidance of issue; interviews gave impression that many men perceived implications from unfavourable test outcome as distant, and that they are not to be blamed for possibility of passing mutation to offspring	30% men resigned from participation in study; study sample small and not randomly selected; approximately half participating men had ≥1 male relatives participating in study; implies that sample not statistically independent; because of sample-related restrictions, cautious interpretations possible	
Lodder *et al.*[242]	63	Women opting for prophylactic mastectomy had significantly higher distress levels than mutation carriers who opted for surveillance and non mutation carriers; difference in distress highest at pre- and post-test had almost disappeared at 1 year follow up; mutation carriers opting for prophylactic mastectomy more often in 30s, more often had young children, and had longer awareness of genetic nature of cancer in family than those opting for regular surveillance; adverse effects observed in women who underwent prophylactic mastectomy regarding perception of how breast region looked and felt, and intimate relationship and physical well being; women opting for prophylactic mastectomy reported more distress than other women in study, distress levels significantly decreased ≥6 months after surgery, possibly because of significant risk reduction of developing breast cancer	Limitation of study observations is that validation study on Body Image/Sexuality study has not been applied; might exclude definite conclusions about level of problems with breast-related body image and sexuality in women undergoing prophylactic mastectomy; because small number of partners participated in study, perceptions of implications of prophylactic mastectomy sparsely represented; whereas partners of women having undergone prophylactic mastectomy did not report having more problems with wife's appearance than partners of other women, surgery did seemed to have negative effect on frequency of intimate contact with spouses ≤8 months after surgery	

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Lynch *et al.*[201]	181	DNA testing must be performed in context of genetic counselling and many complex clinical and non-clinical issues important in process	DNA from peripheral blood lymphocytes taken from certain members of these families collected and stored for DNA testing purposes before discovery of *BRCA1* gene; some had died, some had been lost to follow-up, and unknown subset of family members elected not to participate in studies; limits ability to assess proportion of individuals who elect to seek testing and receive test results; they (as opposed to public) would not likely become aware of potential risks and benefits of genetic testing; some issues mentioned in discussion; unclear whether these issues were "measured" or interpreted based on available data; given lack of information about how subjects selected, unclear to whom these results apply	Study is descriptive so there is no issue of bias but external validity; study results are inadequate and unclear; no indication as to how 14 families chosen from registry of 150; no comparison of 181 individuals who completed testing and those who did not; unclear as to which results reported by participants versus those interpreted by counsellor
Mehnert *et al.*[218]	100	Results show that women little informed and mainly informed by media; women overestimated risk of falling ill with cancer; advice-seeking women positively predisposed to BRCA testing whereby those who had cancer more critical of diagnosis than healthy women; random sample studied felt emotional burden of fear of occurrence or reoccurrence of cancer; overestimated risk of falling ill with cancer by an average of 45%; advice-seeking women positively disposed to *BRCA* testing whereby those who had cancer more critical of test than healthy women	Women responding to local request for volunteers may be bias toward testing and not reflective of population as a whole	Reduced generalizability
Meiser *et al.*[233]	143	Non-carriers derive psychological benefits from genetic testing; carriers anticipate sustained increase in breast cancer distress after disclosure (although no other adverse psychological outcomes observed in group)	Sample size limitations	Unclear how controls were selected
Patenaude *et al.*[202]	36	Factors that may influence utilization of cancer genetic testing programs include programmatic demands, nature and immediacy of cancer risk, demographic factors, perceived lethality of cancers involved, clarity of surveillance recommendations, and perceived efficacy of screening, ego strength and family experience with cancer	Data are preliminary; sample size of 2 families, not enough information about study participants to determine to whom it is applicable; data may not support author's conclusions	Insufficient information to determine appropriateness of study design; sampling method not reported, no comparison of acceptors versus decliners, final results not yet available

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Phillips *et al.*[229]	102	Most important factors influencing decision making: research contributions, potential benefit to family members, curiosity and potential relief (if negative); perceived risks of testing: insurance discrimination, confidentiality, accuracy and interpretability of results, potential impact on marriage prospects for family members, and focus on Jewish community	Small sample size, and opinions of those not undergoing testing not considered; all subjects had breast cancer, so results may not apply to unaffected women, test decliners should have been included as comparison group; no information provided regarding non-responders	Descriptive study, bias not an issue; no information given about non-responders (32 of 134); may influence external validity
Press *et al.*[213]	246	Education about breast cancer gene and breast cancer risk very important	Study is limited by use of "hypothetical" situations as opposed to actual uptake of testing, unequal educational level by ethnic background, potential confusion among participants in meaning of having breast cancer gene and breast cancer; hypothetical situation used may have affected results; given disparities by ethnicity, stratified or multivariate analyses may have been more appropriate	Interest for testing assessed by family history, ethnicity, test performance
Randall *et al.*[197]	60	Women in study not found to have adverse psychological effects from genetic testing and counselling process; no assumptions can be made about post-test result disclosure psychological adjustment	Omission of measurement of subjective risk perception, small sample size, affected women may be more anxious about genetic testing then found in study; investigators only sampled women who sought testing, those who were too anxious to seek testing or counselling not included; controls chosen after physicians approved them such that physicians may have imposed selection criteria unintentionally that may have influenced results; none of women received test results; post-notification impact could not be examined; inadequate follow-up, as there was no information about impact of receiving test results	Differences noted between cases and controls in terms of education and marital status; sample selection process not identical for two groups
Reichelt *et al.*[238]	232	Results show apparent lack of adverse psychological reactions to offer of predictive genetic testing in men and women without history of cancer, but show raised levels of mental distress and number of cases among women with history of cancer; uptake of testing higher than in other studies; activity of predictive testing may continue without undue fear of adverse psychological effects in those offering testing	Results may only apply to self-referred subjects	Women with versus without a history of cancer compared; may not be most appropriate comparison

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Richards *et al.*[214]	309	Genetic testing interest very high among those who attended education session, group education model efficient and effective, carriers of common *BRCA1* and *BRCA2* mutations in the study population identified only among those with history of breast or ovarian cancer	Apparent bias toward inclusion of participants with positive breast or ovarian cancer family histories	No comparison between participants and non-participants made
Schwartz *et al.*[223]	279	Study does not provide evidence for adverse psychologic effects among women participating in clinic-based *BRCA1/2* testing programs, and may even provide benefits for those with negative results	Differential drop out - those with negative or uninformative results more likely to drop out than women with positive results, which may affect results if there were differences in distress levels between them; homogeneity of study sample: results should be validated with more ethnically diverse and other primary care settings in which testing may be offered; settings that fail to provide extensive pretest and post-test genetic counselling may not yield such favourable results; all subjects self-referred; different tests offered by ethnic group; Jewish probands offered testing for 3 founder mutations, whereas non-Jewish probands were offered full *BRCA1/2* testing; among relatives, non-Jewish relatives only tested for presence or absence of deleterious mutation identified in family, whereas Jewish relatives tested for 3 founder mutations; some mutations not tested for may have been missed	Although no differences in baseline sociodemographic or psychological variables between those who completed follow-up versus those who dropped out, difference in test results; probands with positive test results more likely to complete follow-up than those with "uninformative" results
Schwartz *et al.*[224]	290	High levels of spiritual faith may deter genetic testing among some women with familial breast cancer	Sample: all self-referred for genetic counselling and agreed to complete baseline telephone interview, thus 83% uptake may be overestimation of "all eligible women"; all study participants affected with breast cancer and members of high risk families, thus not applicable to unaffected women and women from low-risk families; all testing and counselling free of charge thus may have also overestimated rates for population when cost is incurred on patients; measure of spirituality based on 1 item; better measure of spirituality may have been more appropriate; all participants were self-referred	

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Sheridan *et al.*[240]	102	Carriers reported feeling angry, worried, anxious, depressed after receiving results; non-carriers reported feeling relieved; most shared results with immediate family members; results were in keeping with other studies	Survey based on small population size and limited to 1 geographic location in Ontario	Multi-centre survey to include a province of Ontario may be more generalizable to population
Tercyak *et al.*[200]	133	Persons who experienced greater pre-counselling general distress and those who engaged in more post-counselling coping efforts, more likely to disclose *BRCA1/2* information to children	No detailed information about children, small number of men due to low incidence rate of breast cancer for them, disclosure outcome tested soon after and additional information may have been communicated afterwards, found subclinical (not to be based on clinical criteria) symptoms of general and cancer-specific distress	Participants should be followed up for longer
Tercyak *et al.*[198]	42	Study demonstrated links in communication process among female *BRCA1/2* testing participants and minor children; developmental factors such as child age, maternal communication history variables, open parent-child communication styles at baseline strongly related to increased rate of communication of *BRCA1/2* test results to youngsters	Sample size, leaving open possibility of referral bias in direction of participants with heightened awareness of hereditary cancer risks stepping forward, maternal report on children's behaviour and self-reports on communication may be skewed because of data clustering effects, communication outcome assessed basic and other sources of family discussions were not taken into account, immediate and long-term impact of communication behaviours must be evaluated more thoroughly; women from free comprehensive patient education, genetic counselling and *BRCA1/2* testing clinical research effort; may not be representative of all "eligible people" rationale for choice of 1 month as follow-up not explained; may be more appropriate to use longer follow-up	Participants should be followed up for longer
Tessaro *et al.*[248]	66	Women need to be provided with balanced information about positive and negative aspects of genetic testing, determine how best to include physicians' advice in decision making process, consider effects of testing on family relationships, and provide more public education about what genetic testing is and what it means	Disproportionate number of college-educated women among affected and unaffected women; despite efforts to obtain diversity in race, income and education, 23% minority representation and most women highly educated; 2 groups of affected women recruited from community support groups primarily African American; tended to have less knowledge about genetic testing; study was qualitative in design, thus issues around testing raised but no association between issues and uptake can be made	

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Thompson *et al.*[212]	76	Participants declining counselling had significantly less knowledge about *BRCA* genetics than those who accepted counselling and testing; no differences found between groups on perceived benefits but more barriers reported by those who declined counselling; cancer specific distress positively associated with participation in counselling, regardless of participation in testing	Although pros and cons of testing assessed, pros and cons of counselling were not; low response rate reduce generalizability of results; low participation rate may affect results as it could be related to interest in and attitude towards counselling and testing	Participation may be associated with interest in counselling or testing; 46% did not return questionnaire; no comparisons made of responders and non-responders
Valdimarsdottir *et al.*[230]	105	Cancer-specific distress affects genetic testing decisions for *BRCA1:* genetic counselling needs to address cancer-specific distress because it can affect probability of women making informed decision	Majority of women Caucasian and well educated, thus not generalizable to other ethnic backgrounds and education groups: small sample size: focused on *BRCA1;* response rate not given; authors should acknowledge difference between providing blood sample and wanting test results	How subjects selected not reported; may affect results; participation rate not reported
Velicer *et al.*[204]	276	Health care providers and long-term breast cancer survivors not discussing *BRCA* genetics with each other; strong association between intent to obtain genetic testing and insurance coverage for testing; increasing survivors' knowledge may not result in large influx of testing requests but may provide information necessary to make informed decisions and input by health care providers may be helpful in this process	Cross sectional design cannot establish causality; no information on whether these women got tested, only have "intention"; no information available about source of information about *BRCA* genetics; measure of willingness to pay could have been more refined, outcome variable, hypothetical in that no data available as to whether they subsequently went for testing	
Wood *et al.*[199]	35	Genetic counselling and testing can be done without significant increase in depression and distress; nonetheless, caution necessary for individuals having been diagnosed with breast cancer in preceding year because of higher distress level	Small sample size; 1 month follow-up seems insufficient for purpose of study, even for pilot study	

Author	Sample Size	Author's Conclusions	Limitations of Study	Impact of bias, if any
Worringen *et al.*[284]	94	Persons who contact genetic counselling centres motivated to undergo genetic testin; attending physicians and family members play roles in motivating individuals to seek testing; people who do not feel capable of coping with possibility of having mutation forgo not only predictive testing but genetic counselling also	Gender, education, and age failed to play role in predicting intention to test, level of education in random sample high, proportion of men small; in addition to situational-related or culturally determined random sample effects, methodological limitations of study may account for differences in results compared to other studies; distribution of dependent variable "intention to test" skewed and number of persons who did not want to undergo testing turned out to be small; most independent variables and dependent variables determined by use of 1 item, so they can only be granted limited degree of reliability	
Wylie *et al.*[241]	57	Tested person's perception of his or her spouse's support at time of testing predictive of tested person's psychological distress ≤2 years after testing	Sample largely made up of members of Church of Jesus Christ of Latter-Day Saints (Mormons); effects of cancer family history may limit utility of study	Religious characteristics may make this sample different from other populations, though potential support available to members of church suggests that results are conservative

APPENDIX 8: Summary of Study and Patient Characteristics, and Ethical Considerations

Author (Country)	Study Characteristics		Patient Characteristics		Results of Ethical Considerations		
	Technique	Sample (% females)	Mean Age in Years (range)	Family History (cancer)	Informed Consent Issues	Privacy and Confidentiality	Familial Implications
Armstrong (US)[253]	Mail survey	636 (100)	48 (20 to 80)	Participants in breast cancer risk assessment (27% Ashkenazi Jewish descent; 18% with *BRCA* mutations) (breast or ovarian)	NR	Fear of life insurance discrimination rated moderate or very important factor by 55% of participants (n=574); women who were concerned about life insurance discrimination less likely to undergo genetic testing (RR 0.67, 95% CI:0.52;0.85); fear of discrimination was not associated with breast cancer risk or change in insurance coverage (p>0.24)	NR
Benkendorf (US)[254]	Baseline 20 minute structured phone interview and set of self report questionnaires	238 (100)	44 (22 to 75)	≥1 affected first-degree relative (breast or ovarian)	98% voluntary nature of testing; 95% undergo testing against MD recommendation (higher percentage in blacks or in women with higher coping styles, p<0.05); 88% parents to decide testing on behalf of minor children (higher percent in blacks or in women with higher coping styles, p<0.05)	Health professionals sharing genetic information without patient's consent: to employer, 97% object to insurance, 95% object	Health professionals sharing genetic information without patient's consent: to immediate family, 87% object; to spouse, 84% object; women may be denied access to testing if MD recommends against it; study mentions women often have more information about genetic risks than MD and may warrant testing in spite of MD recommendations

Author (Country)	Study Characteristics		Patient Characteristics		Results of Ethical Considerations		
	Technique	Sample (% females)	Mean age in Years (range)	Family History (cancer)	Informed Consent Issues	Privacy and Confidentiality	Familial Complications
Durfy (US)[252]	Analysis of consent forms and protocol materials	Seven centres	NA	NR (breast)	100% provide implications of positive or negative test results; 71% describe physical risks associated with blood drawing or indicate test limitations of not detecting all possible mutations; 43% state voluntary nature of testing or precise costs for test; 29% state testing has psychological impact; 14% report on time frame for receipt of test results	All centres address confidentiality issues in their forms; 6 centres (86%) require written consent to release test results; 3 centres (43%) provide test results to ordering MD; 29% only to individual tested; 29% did not indicate to whom tests results provided; 86% cite risk of insurers and 43% of employers learning of test results; 29% indicate test results may be used for research	NR
Goelen (Belgium)[257]	Grounded Theory Approach using recordings from genetic counselling sessions	45 (69)	NR	≥2 first-degree relatives (breast or ovarian cancer), or ≥1 (breast cancer) case diagnosed <age 50 years; availability of genetic material from ≥1 affected family member	Individuals in families had and promoted respect for autonomy in decisions of others, including children	Individuals who received test results had concerns about privacy and confidentiality in families; when some family members shared results, others felt expected to do the same; by sharing results with spouses and partners, in-laws found out; raised concerns that community would know; same concern true of siblings knowing test results	Probable carriers affected with cancer valued opportunity to make contribution to others in the family; those with negative test results concerned about helping relatives, including those who were undecided about testing

Author (Country)	Study Characteristics		Patient Characteristics		Results of Ethical Considerations		
	Technique	Sample (% females)	Mean Age in Years (range)	Family History (cancer)	Informed Consent Issues	Privacy and Confidentiality	Familial Implications
Hallowell (UK)[258]	Grounded Theory Approach using 1 or 2 hour open ended interviews (recorded and transcribed)	30 (100)	NR (39 to 71)	40% with no mutation, 33% with mutation, 27% awaiting test results; 60% with maternal history, 23% with paternal history, and remainder with unclear history; 87% with 1 affected first-degree relative (breast or ovarian)	When providing "informed consent" to undergo testing, participants not aware of implications of role in disseminating information to other family members; participants not prepared for potential burdens and responsibilities associated with being first member of family to be tested	NR	If affected individuals prefer not to undergo testing, other family members would be denied access to such testing; participants felt moral duty to undergo testing to benefit family members, but also burdened by dilemma of possibly causing harm by giving "bad news" to those members who may not have wanted to know genetic status
Lehmann (US)[255]	Population-based survey	200	46 (100)	Jewish women selected because of increased frequency of *BRCA1/2* mutations (breast)	NR	NR	97% of respondents believed that patients should inform at risk family members of increased chance of developing breast cancer; 83% of respondents believed that physicians should inform patients of familial implications of genetic information; 22% of respondents believed that physicians should seek out and inform at risk family members against patient's wishes

Author (Country)	Study Characteristics		Patient Characteristics		Results of Ethical Considerations		
	Technique	Sample (% females)	Mean Age in years (range)	Family History (cancer)	Informed Consent Issues	Privacy and Confidentiality	Familial Implications
Peterson (US)[256]	Chart reviews of genetic counselling sessions; telephone surveys	184 (93)	45 (NR)	Suggestive of hereditary disease (breast or ovarian)	NR	Barriers to testing were Cost, fear of insurance discrimination, concerns about loss of confidentiality; no cases of overt discrimination found; having insurance pay for testing not factor in decisions to have test; majority wanted test results kept confidential from insurers	NR
Winter (US)[251]	Telephone interview, open-ended questionnaire	376 (79)	65	21% first-degree; 49% second-degree relatives (breast)	Nearly one-quarter of study cohort unaware of family history of cancer before contact by study; participant perception of cancer family history different from study family history	28% (15 of 53) expressed privacy concerns (sharing personal information over telephone with unknown callers; fear of adverse personal consequences associated with insurance companies; receiving junk mail); most privacy concerns expressed by participants aware of family history	Frequency of privacy concerns by relatives independent of gender or relationship to proband
Phillips (Canada)[229]	Self-administered questionnaire	134 (100)	(32 to 87)	Research-based testing program for Ashkenazi Jewish women; 32% with one or more first-degree relative with cancer; 41% with no family history (breast or ovarian)	NR	Perceived risks of undergoing *BRCA* testing related to insurance discrimination (28%) and confidentiality of test results (24%)	Potential benefit to other family members as factor influencing the decision to undergo testing (78%)