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Drugs and Technologies
in Health

RAPID RESPONSE REPORT: SUMMARY WITH CRITICAL APPRAISAL



TITLE: Next Generation DNA Sequencing: A Review of the Cost Effectiveness and Guidelines

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CONTEXT AND POLICY ISSUES

The use of genetic methodologies in health-care is not a novel concept. Since the 1970's when the initial process of genetic sequencing was introduced it has been widely accepted as an extremely valuable investigative process in both research and, more recently, in clinical settings.^{1,2} What is novel about these processes is the scale and scope of screening that is now possible.

Sanger sequencing, or first generation sequencing, is currently considered the gold standard of sequencing methodologies.³⁻⁵ This process works in a liquid phase where only one predefined gene can be targeted in a single run.⁴ This allows for extremely low rates of false-positive and negative errors and has a very high degree of specificity.^{3,6,7} Unfortunately this low level of throughput make the process extremely expensive and time consuming, especially when the disease under examination contains a heterogeneous mutation condition and multiple genes must be screened before significant variants are detected.^{4,7} For example, the current price of sequencing 1 Mb of DNA using the Sanger method costs approximately \$500 USD.¹ As the human genome is approximately 3000 Mb in length, this will mean that a full genomic investigation using Sanger sequencing will cost 1.5 million USD for each individual requiring the process.¹

This cost has led research and health-care communities to seek out novel methods for genetic investigation.⁸ It has also created the optimistic search for the fabled "\$1000 genome test" where an entire genome could be sequenced for \$1000 or less.^{1,9} The first breakthrough towards second generation sequencing, also called next generation sequencing (NGS) or massively parallel sequencing, was published in 2005 allowing, for great increases in throughput and potential cost reduction.^{9,10} Studies have shown that it is now possible to concurrently sequence multiple genomes in under two weeks all in one run.⁹ There are three overall processes that have been developed in NGS: whole genome sequencing (WGS), whole exome sequencing (WES) and targeted gene sequencing (TGS).¹ WGS is capable of sequencing the entire genome in a single run while WES and TGS are more focused. WES

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focuses on only the protein coding regions which make up approximately 1%, or roughly 30 Mb, of the human genome.¹ TGS is the most focused and examines specific genes or regions of interest making it of key interest to clinical investigations.^{1,3,9}

NGS approaches have been used over the past decade in research settings and now are the focus of concerted effort to make them standard practice in clinical settings.² Focus has been given to their use in various medical diagnoses such as cardiomyopathy, noninvasive prenatal diagnosis, inherited cancers, and various inherited Mendelian diseases.^{2,4,9,11} This movement into a clinical setting has created many questions and concerns in the medical community and with interest groups. Chief among these concerns are: what is the proven cost effectiveness of this type of approach, and what procedural guidelines should be followed in regard to experimental methodology, data storage, report generation, and communication of results.

The purpose of this report is to examine the cost-effectiveness and evidence-based guidelines for the use of NGS in clinical settings.

RESEARCH QUESTIONS

1. What is the cost effectiveness of next generation sequencing?
2. What are the evidence-based guidelines regarding the use of next generation sequencing for the diagnosis of genetic disorders?

KEY FINDINGS

Limited evidence was found to establish the cost-effectiveness of these approaches. In the scope of this investigation no established standardized guidelines were identified. The guidelines described are the results of evidence based review and expert opinion, and provide recommendations on implementation of next generation sequencing programs. No recommendations regarding specific clinical applications of the technology were identified.

METHODS

Literature Search Strategy

A limited literature search was conducted on key resources including Medline via OVID, PubMed, The Cochrane Library (2014, Issue 1), University of York Centre for Reviews and Dissemination (CRD) databases, Canadian and major international health technology agencies, as well as a focused Internet search. Methodological filters were applied to limit retrieval to health technology assessments, systematic reviews, meta-analyses, guidelines, and economic studies. Where possible, retrieval was limited to the human population. The search was also limited to English language documents published between January 1, 2009 and January 9, 2014.

Rapid Response reports are organized so that the evidence for each research question is presented separately.

Selection Criteria and Methods

One reviewer screened the titles and abstracts of the retrieved publications and evaluated the full-text publications for final article selection. The final selection of full-text articles was based on the inclusion criteria presented in Table 1.

Table 1: Selection Criteria

Population	Any patient – with focus on patients with cardiomyopathy and inherited cancers
Intervention	Next generation sequencing (any platform)
Comparator	Other sequencing procedures No comparator
Outcomes	Guidelines and recommendations (performing the sequencing, analyzing the data) Cost effectiveness
Study Designs	Health technology assessments, systemic reviews, meta-analysis, economic evaluations, and evidence-based guidelines

Exclusion Criteria

Articles were excluded if they did not meet the refinements outlined in Table 1. Studies must also have been published between January 1, 2009 and January 9, 2014 and be in English. Finally articles were excluded if they were duplications of the same study.

Critical Appraisal of Individual Studies

Systemic reviews were assessed using the Assessment of Multiple Systemic Reviews (AMSTAR) tool.¹² Guidelines were assessed using the Appraisal of Guidelines for Research and Evaluation (AGREE) tool.¹³ A numeric score was not calculated, instead the strengths and limitations of included studies were described narratively.

SUMMARY OF EVIDENCE

Quantity of Research Available

The initial literature search identified 383 publications for investigation. After review of titles and abstracts 344 articles were rejected as not meeting the selection criteria. A total of 39 publications were requested for full-text review, and of these two were found to fulfill all of the required conditions. There were also three publications found to be relevant in the grey literature. A PRISMA flowchart outlining the study selection process is provided in Appendix 1.

Summary of Study Characteristics

Cost effectiveness of next generation sequencing

There was one systematic review identified for the analysis of the cost effectiveness of an NGS process in the literature search. In addition, one health technology assessment and one systematic review were identified from the grey literature for this question. Details of individual study characteristics are provided in Appendix 2.

The health technology assessment was produced in the United Kingdom.⁴ This review was carried out in 2010 and 2011 by an expert steering group and focused on all approaches of NGS. One of the systematic reviews that was found was conducted in Germany¹ and focused on the costs associated with WGS though no exclusion was made for other NGS approaches. The final systematic review⁵ analyzed economic studies conducted on hereditary breast cancer in England and Wales including studies published between 1996 and 2012. NGS approaches were compared against other standardized methods, typically Sanger sequencing followed by multiplex ligation-dependent probe amplification.

In the three systematic reviews of cost effectiveness in this report there was no limitation placed on the type of economic evaluation methodology that was included.

Evidence-based guidelines regarding the use of next generation sequencing for the diagnosis of genetic disorders

There was one procedural guideline³ identified in the literature regarding the use of next generation sequencing for the diagnosis of genetic disorders. It was produced in the United States of America in 2013 with an aim of developing a standardized protocol for NGS approaches for clinical geneticists. In addition, an examination of the grey literature identified another evidence based guideline.⁷ This publication was produced in the United Kingdom in 2012. It is a summary of the efforts of the attendees of the next generation sequencing good practice meeting. This meeting was attended by delegates from the UK Clinical Molecular Genetics Society.

Individual guideline characteristics are provided in Appendix 3.

Summary of Critical Appraisal

Details of the critical appraisal of individual studies are provided in Appendix 4.

Cost effectiveness of next generation sequencing

The included health technology assessment⁴ provided an extensive review of many aspects of NGS, including health economics. However, it was unclear whether grey literature was searched for inclusion in the review, criteria for study inclusion was not provided, and characteristics of included and excluded studies were not described. When considering the use of NGS, Sanger sequencing was used as a reference.

Both included systematic reviews^{1,5} were based on comprehensive literature searches conducted in multiple databases. The study selection criteria were well described in both

reviews. In one review⁵ article selection was performed in duplicate by independent reviewers, however in the second review¹ it was unclear whether duplicate selection took place.

Evidence-based guidelines regarding the use of next generation sequencing for the diagnosis of genetic disorders

There were two guidelines identified regarding NGS implementation.^{3,7} Both were based on reviews of the relevant literature. The first guideline⁷ contained the most concise procedures for the use of NGS technology in clinical practice. A summary of the findings of an expert panel from the UK Clinical Molecular Genetics Society was given. Detailed in the guidelines are specific requirements for investigative laboratories to follow ISO practices. The authors made assumptions that laboratories will follow “good-practice” methods but no formal criteria for this was given.

The second guideline³ contained an extensive literature analysis and gave recommendations on the entire scope of NGS usage. Clear details were given for the incorporation of quality control guidelines and the use of proficiency testing for involved laboratories. Unfortunately the criteria for literature inclusion/exclusion were not detailed and the specific research question was not stated.

Summary of Findings

A summary of individual study findings is provided in Appendix 5.

Cost effectiveness of next generation sequencing

The health technology assessment⁴ reviewed in this investigation compared the use of NGS against Sanger sequencing. They found that Sanger sequencing results in a low error rate when mutation occurs in a single gene, but when the mutation is heterozygously expressed these error rates dramatically increase. Costs associated with Sanger sequencing were reported to be approximately \$500/Mb (USD) compared to \$0.50/Mb using NGS. The costs associated with an NGS investigation vary greatly depending on the methodology used, the gene or genes being investigated, and the laboratory that is conducting the analysis. The authors stated that there is a distinct lack of randomized controlled trials investigating the cost effectiveness of NGS approaches.

In the first systematic review,¹ the use of Sanger sequencing was compared to NGS methodologies. It was found that to sequence the entire genome of a patient using Sanger sequencing would cost approximately \$1.5 million USD. When analyzing the costs of NGS they found that the prices varied greatly from laboratory to laboratory and that the costs associated with variant filtering/data analysis were much higher than the actual experimentation. There was also a higher risk of false positive results when using NGS which requires that confirmatory testing be completed when variants are found. The authors stated that it is very difficult to determine cost effectiveness of NGS due to the multifaceted nature of the interpretation of results. In many circumstances this requires experts from various different backgrounds such as molecular, clinical and genetic counselling.

The final systematic review⁵ compared the use of Sanger sequencing along with multiplex ligation-dependent probe amplification to NGS in the investigation of hereditary breast cancer.

The authors concluded that there is not sufficient information to make an informed analysis of the cost effectiveness of NGS.

Evidence-based guidelines regarding the use of next generation sequencing for the diagnosis of genetic disorders

Ellard et al.⁷ focused on the use of TGS to make a guideline standard for variant detection and reporting. They provided guidelines based on those that have been used for Sanger sequencing. Guidelines focused on the TGS process. Specific guidelines for use of NGS for specific clinical conditions were not provided. Recommendations included:

- Sample Collection
 - all harvested/treated/stored in quality controlled environment and methodology
- Amplification / Hybridization / Multiplexing
 - check for primer binding site SNPs
 - avoid GC rich areas
 - use of index tagging or molecular barcodes is preferential
 - use appropriate negative controls in PCR step
- Library Preparation / Region of Interest
 - Depth of coverage determined by sensitivity of assay used, the targeting or sequence method and the type of mutation detected
 - Include coding regions and conserved splice sites
- Data Analysis
 - Software for analysis of the obtained sequences can be obtained commercially or through open-source formats
 - validation of software and software upgrader may be done using existing data
- Annotation
 - Done according to Human Genome Variation Society
 - include reference sequence on reports, recommend to include genomic coordinates with hg build number
- Amplification / Hybridization / Multiplexing
 - check for primer binding site SNPs
 - avoid GC rich areas
 - use of index tagging or molecular barcodes is preferential
 - use appropriate negative controls in PCR step
- Filtering
 - The filtering variants for insignificant polymorphisms depends upon the likely mode of inheritance (for example an unaffected adult with a heterozygous variant is unlikely to be the cause of a dominant congenital disorder)

- Data Storage
 - Is not essential to keep all data generated on file as is extremely large and platforms are always changing, in addition the DNA should be stored for future use
 - is essential to store the output file from the variant annotation step and the laboratory may or may not store the FastQ, SAM or BAM files for further use

- Reporting
 - Reporting should follow the principles of the Clinical Molecular Genetics Society best practice guidelines
 - positive variant result
 - must confirm results using a fresh DNA dilution in situations where there is no robust sample identification in place
 - negative variant result
 - should report expected diagnostic yield (proportion of cases with phenotype in which a variant is detected using the platform utilized min the current study)
 - include limitations of assay with an explanation of false positives due to:
 - PCR primer binding site polymorphism causing allelic dropout
 - Tissue mosaicism
 - Deleted/duplicated region in the absence of copy number analysis
 - variants of unknown result
 - May be reported but should be done in a separate report and is not necessary to confirm using a second method
 - Submission to an online database is recommended so that findings may be utilized in an open-source format

It was also recommended that any laboratory conducting NGS examinations should have ISO 15189 and/or ISO 17025 in place.

Rehm et al.³ prepared a review that examined WGS, WES and TGS with a focus on guideline development. All approaches involve three general steps; preparation of sample, sequencing of sample, and data analysis. In the preparation stages laboratories are recommended to have in place established protocols to avoid sample mix-up/mislabeling. The following recommendations were made:

WGS:

- When this method is used it is typically best to investigate coding regions for mutation first and if none are found then the focus will shift to noncoding regions
- Is ideal for the examination of copy number or structural variants

WES:

- Is best suited for the identification/detection of genetic variants in genes already established to be disease causing. Additionally it may be used for the identification of novel gene-disease relationships.
- In a clinical setting WES is recommended for use on known genes and if no variation is found then may reanalyze the remaining exome and identify novel mutations

- Sensitivity is lower than TGS as the depth of coverage for an exon is not uniform. As a result of the exons of known trait causing gene variations being highly numerous this lack of depth has made the use of this method rare. Additionally this lack of uniformity requires that gaps be filled in using Sanger sequencing making it impractical

TGS:

- Best suited to disease investigation as the limited interest region makes coverage depth greater
- Makes for easier data analysis as not as much data to sort through
- Study region should always be limited to genes with appropriate level of data proving their role in trait development
- If the gene panel being examined includes genes with overlapping phenotypes the clinician should be approached to restrict the panel to specific genes
- The region of interest should be analyzed for the expected number of variants as the variant number with clinical relevance will be approximately proportional to the size of the area of interest
- Care must be taken on which databases reference sequences are obtained from as few, if any, are curated to clinical/medical grade applications. Since virtually all are created for research purposes they will contain misclassified variants, benign variation misclassified as trait causing and may not include variants specific to family inherited conditions. Recommended databases are: dbSNP from NCBI, NHLBI Exome Sequencing Project and the 1000 Genomes Project.

Both WGS and WES allow for investigation without any bias from the assumption that a mutation will be found in a specific area. They typically require much more communication between experts and clinicians and will potentially give a host of false-positive results due to variation in genes unrelated to the condition being detected. It was therefore recommended that these methods only be utilized if targeted approaches have not yet been created. It was stated that in this type of situation it is the responsibility of the laboratory manager to ensure that all involved parties understand the significance and limitations of the test results.

Data analysis is divided into four stages: base calling, read alignment, variant calling and variant annotation. Regarding these stages, the guideline stated the following:

Base calling:

- Typically completed by software provided with purchase of sequencing machine
- Must ensure that provided software is efficient at identification bias that is inherent to each machine

Read alignment:

- Read length will vary depending on platform used
- Recommended that alignment be completed using full reference gene (this is true even for WES or TGS to ensure that mapping errors are decreased as much as possible)

Variant calling:

- Recommended that alignment be completed using full reference gene (this is true even for WES or TGS to ensure that mapping errors are decreased as much as possible)

Variant calling:

- Depth of coverage is critical here and a higher depth degree will equal higher accuracy

Variant annotation:

- Must contain information such as mutation location (intron, exon etc.), if variation causes amino acid change and whether it causes the development of a trait must be included.

When WGS and WES platforms are used variant detection can result in millions of occurrences. This requires that the data be filtered in order to separate the relevant results from those that are insignificant. The overall goal of variant filtering is to reduce the numbers of variants that require professional analysis. According to the guideline, factors such as mutation in disease relevant gene, inheritance pattern in family, variant type, existence in control population, identification of *de novo* occurrence, pattern of gene expression, scores for *in silico* analysis of protein function, and biological pathway analysis must all be considered when this is completed. It was recommended to use a defined stepwise process whereby an initial analysis for obvious changes followed by subsequent passes of greater depth are carried out.

Once all of the criteria for a test have been initialized, laboratories can optimize the processes as required. This is typically where questions such as; “How many samples can be pooled in a single run?” are answered. It is imperative to investigate all sample types/DNA sources used in the clinical diagnostics. Once this is completed an optimized protocol can be developed and validated. It was recommended validation use reputable reference samples that have undergone widespread Sanger sequencing in the past. It was further stated that it is best to use samples which are renewable which will make it possible to use them as a future source for quality control. According to the guideline, WGS and WES validation must be more focused on areas such as average coverage distance and the percent of bases that meet a set standard for coverage threshold. A comparison to Sanger sequencing methods that cover a similar area of interest should be used, and typically laboratories use 95-98% concordance as the minimum acceptable level.

Regarding data storage, the guideline suggested that it may be either housed on site or offsite as desired. However, it stated that in instances where cloud computing is used it must be ensured that the environment is compliant with laws governing data traceability. It was recommended that the laws outlined in the Health Insurance Portability and Accountability Act be followed. It was advised to store data and reports for at least two years post investigation, though the large imaging files direct from the sequencing run were not deemed to be required.

The guideline stated that reporting must list the variants that are found in standardized formats. In the United States these formats follow Human Genome Variation Society nomenclature and are classified according to American College of Medical Genetics and Genomics guidelines. In TGS examinations results were recommended to be presented with the trait causing variants first followed by negative results. In addition it was recommended that all investigated genes should be documented. It was deemed the responsibility of the laboratory in question to decide if they wish to report benign results, though if reported it was stated that they must be clearly marked as being benign. Regarding reporting for WGS and WES, it was stated that examinations must contain information on the data analysis process and parameters such as coverage level and gene coverage value along with any limitations. The guidelines further stated that if a proband is utilized, then only minimal information needs to be included in order to protect privacy and names/relationship to patient should never be included.

It was recommended that for every platform used, an established method for quality analysis and quality control needs to be developed. Key focus was recommended to be given to sample identity. It was stated this process should have defined checkpoints where samples are investigated for contamination or high error rates at various stages of progression.

Recommended stop sites were during DNA fragmentation, during sequencing run and post-run/pre analysis of read quality. It was also highly recommended that a process for proficiency testing be instituted, typically this is done twice yearly.

Regarding choosing a sequencing platform for laboratory use, several aspects were recommended for consideration. Firstly proper selection was dependant on the use that it will get. Therefore, conditions such as region of interest size, cost, turn-around time, coverage depth, and sample volume must be answered. High throughput platforms have the capability to reduce costs per run but only if there are an adequate number of samples to make it efficient. It was recommended that if the platform will be used for prenatal therapeutic processes that a platform able to produce results in as short a time as possible be considered. Likewise if the typical analysis is expected to be on mutations at levels lower than germline heterozygosity a platform with a high depth of coverage was recommended.

Limitations

The analysis of cost-effectiveness in this report was limited by a distinct lack of robust published data. In the publications that met the screening criteria, statements were made in each that definite insight into this question cannot be given and more investigative assessment is needed. The publications that were included suffered from a lack of description of included study characteristics and provided insufficient methodological detail and inclusion/exclusion criteria.

The guidelines analyzed for this report contained recommendations that were of an unfocused nature and would leave actual hands-on techniques open to interpretation. There were also areas where more detail may be needed on how specific methods improve results over other sequencing approaches. These guidelines were focused on implementation of the sequencing program as opposed to specific clinical methodologies. In addition all authors have indicated that guidelines will deviate depending on the laboratory that is examined which makes it difficult to give recommendations for policy development. Some of the included reports were missing clear methodology and in one instance no clinical question was clearly stated.

CONCLUSIONS AND IMPLICATIONS FOR DECISION OR POLICY MAKING

The question of the cost-effectiveness of next generation sequencing remains unclear. All studies identified in this report have concluded that there is a lack of powerful economic investigations published to date. This is a result of the complexity of analyzing the costs associated with all processes subsequent to the sequencing run itself. In addition health-care costs related to the high proportion of ambiguous variants detected using NGS techniques remain unknown.

The evidence based guidelines included in this report give high-level recommendations on implementation of the technology, but recommendations for specific applications are lacking. The studies do provide some tools for specific platform use and may be of interest to decision makers. It has been found that the guidelines included in this report are not standardized and may not be widely accepted in current clinical practice. This likely is a result of the fact that NGS approaches have only been examined recently in health-care situations. As time goes on and

further advances are made with regard to all aspects of NGS development and data analysis, robust protocols will be needed so that clinical methodologies may be established.

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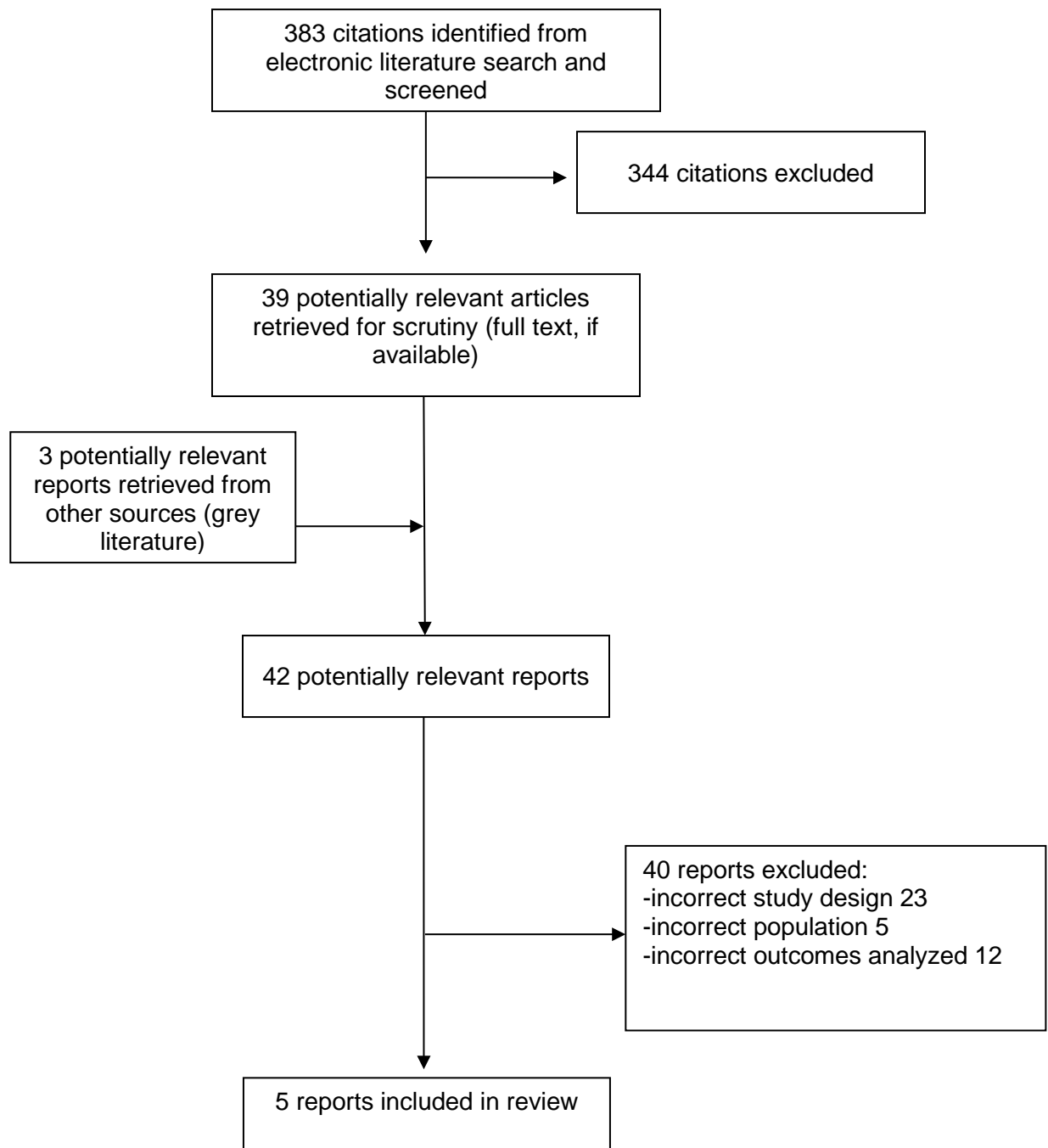
REFERENCES

1. Frank M, Prenzler A, Eils R, Graf von der Schulenburg JM. Genome sequencing: a systematic review of health economic evidence. *Health Econ Rev* [Internet]. 2013 [cited 2014 Feb 6];3(1):29. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3874598>
2. Bombard Y, Bach PB, Offit K. Translating genomics in cancer care. *J Natl Compr Cancer Netw*. 2013 Nov;(11):1343-53.
3. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, et al. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med* [Internet]. 2013 Sep [cited 2014 Feb 6];15(9):733-47. Available from: https://www.acmg.net/docs/ACMG_Lab_Standards_Next_Generation_Sequencing_Sept2013.pdf
4. Next steps in the sequence. The implications of whole genome sequencing for health in the UK [Internet]. Cambridge (UK): PHG Foundation; 2011 Sep. [cited 2014 Feb 6]. Available from: <http://www.phgfoundation.org/file/10363/>
5. Sullivan W, Evans DG, Newman WG, Ramsden SC, Scheffer H, Payne K. Developing national guidance on genetic testing for breast cancer predisposition: the role of economic evidence? *Genet Test Mol Biomarkers*. 2012 Jun;16(6):580-91.
6. Cordero P, Ashley EA. Whole-genome sequencing in personalized therapeutics. *Clin Pharmacol Ther*. 2012 Jun;91(6):1001-9.
7. Ellard S, Charlton R, Lindsay H, Camm N, Watson C, Abbs S, et al. Practice guidelines for targeted next generation sequencing analysis and interpretation [Internet]. London: Clinical Molecular Genetics Society; 2012. [cited 2014 Feb 6]. Available from: <http://www.cmgs.org/BPGs/BPG%20for%20targeted%20next%20generation%20sequencing%20final.pdf>
8. Ku CS, Cooper DN, Iacopetta B, Roukos DH. Integrating next-generation sequencing into the diagnostic testing of inherited cancer predisposition. *Clin Genet*. 2013 Jan;83(1):2-6.
9. Meldrum C, Doyle MA, Tothill RW. Next-generation sequencing for cancer diagnostics: a practical perspective. *Clin Biochem Rev* [Internet]. 2011 Nov [cited 2014 Jan 13];32(4):177-95. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3219767>
10. Norton N, Li D, Hershberger RE. Next-generation sequencing to identify genetic causes of cardiomyopathies. *Curr Opin Cardiol*. 2012 May;27(3):214-20.
11. Marquardt JU, Andersen JB. Next-generation sequencing: application in liver cancer-past, present and future? *Biology (Basel)* [Internet]. 2012 Aug 31 [cited 2014 Jan 13];1(2):383-94. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3674503>
12. Shea BJ, Grimshaw JM, Wells GA, Boers M, Andersson N, Hamel C, et al. Development of AMSTAR: a measurement tool to assess the methodological quality of systematic

reviews. BMC Med Res Methodol [Internet]. 2007 [cited 2014 Feb 4];7:10. Available from: <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1810543/pdf/1471-2288-7-10.pdf>

13. The AGREE Collaboration. Appraisal of guidelines for research and evaluation (AGREE) instrument [Internet]. London: The AGREE Research Trust; 2001 Sep. [cited 2014 Jan 24]. Available from: <http://www.agreetrust.org/?o=1085>

APPENDIX 1: Selection of Included Studies



Appendix 2: Study characteristics – cost-effectiveness of next generation sequencing

First Author, Publication Year, Country	Eligibility Criteria	Literature Search Strategy	Number of Included Studies
Health Technology Assessment			
Foundation for Genomics, and Population Health, ⁴ 2011, United Kingdom	Examined studies evaluating the use of 2 nd and 3 rd generation sequencing strategies.	<ul style="list-style-type: none"> • Focus group on genomic service within the NHS* United Kingdom 	Report utilized 367 articles (no breakdown of specific types) Included information from workshop panels of 85 experts
Systematic Reviews			
Frank et al., ¹ 2013, Germany	Included studies from 2002 to 2012 that were completed in either English or German and examined economic evaluation of genome sequencing. Conducted using the criteria from the German Institute for Medical Documentation and Information.	<ul style="list-style-type: none"> • Studies extracted from Deutsches Ärzteblatt, BIOSIS Previews, Cochrane Database of Systematic Reviews, DAHTA-Datenbank, EMBASE Alert, EMBASE, GMS, GMS Meetings, Social SciSearch, Health Technology Assessment Database, SciSearch, Krause & Pacherneegg Verlagsdatenbank, MEDLINE, NHS Economic Evaluation Database, Thieme 	5 studies included (articles not classified according to study design)
Sullivan et al., ⁵ 2012, United Kingdom	Included studies from 1996 to present. Studies included must be economic evaluations on hereditary breast cancer using. Conducted using Centre for Reviews and Dissemination systematic review methods.	<ul style="list-style-type: none"> • Grey literature and hand search included • Economic evaluations only found using MEDLINE, EMBASE, PsychINFO and NHS Economic Evaluation Database • Conducted phone interviews with 14 laboratories to determine platform being used 	15 systematic evaluations were included

*NHS – National Health Service

Appendix 3: Study characteristics – guidelines for next generation sequencing

First Author, Publication Year, Country	Eligibility Criteria	Included Study Designs	Number of Included Studies
Evidence Based Guidelines			
Ellard et al., ⁷ 2012, United Kingdom	Not clearly defined. Authors give inconclusive statement about publication selection requiring them to examine the quality of the entire next generation sequencing process.	<ul style="list-style-type: none"> • Not well defined. Authors state are the combined efforts of attendees of the next generation sequencing good practice meeting in 2012 and experts from the UK Clinical Molecular Genetics Society 	4 studies included (articles not classified according to study design)
Rehm et al., ³ 2013, United States	Focus is on clinical guidelines developed by American College of Medical Genetics and Genomics. Included publication criteria not clearly defined.	<ul style="list-style-type: none"> • Not defined 	19 publications included (articles not classified according to study design)

Appendix 4: Summary of critical appraisal

First Author, Publication Year	Strengths	Limitations
Health Technology Assessment		
Foundation for Genomics, and Population Health, ⁴ 2011	<ul style="list-style-type: none"> • Extensive review of all aspects of NGS including sequencing platforms, current applications in heritable disease, informatics, and health economics • Use comparator of Sanger sequencing for analysis of NGS techniques 	<ul style="list-style-type: none"> • Lack of description of study characteristics and excluded studies. • Unclear whether grey literature was searched. • Criteria for study inclusion not given and no detail of single or duplicate selection is given • Expert advisory panel inclusion criteria not given
Systematic Reviews		
Frank et al., ¹ 2013	<ul style="list-style-type: none"> • Clearly defined the study selection criteria and included PRISMA chart • Extensive literature search conducted on multiple databases • Critical appraisal is included and provides basis for logical conclusions 	<ul style="list-style-type: none"> • Included economic evaluations not specified as to study methodology • No apparent examination of grey literature • Unclear on who was involved in the study selection (single or multiple participants) • Outcome values not given, only briefly discussed
Sullivan et al., ⁵ 2012	<ul style="list-style-type: none"> • Extensive literature search conducted on multiple databases • Clearly defined the study selection criteria (methodology well documented) • Critical review of literature follows a defined process and appropriate conclusions are made 	<ul style="list-style-type: none"> • Several of the included studies utilized expert opinion when there was insufficient defined data contained in the reports but did not discuss how the expert opinion was obtained • No clarification as to singular or duplicate study selection given
Evidence Based Guidelines		
Ellard et al., ⁷ 2012	<ul style="list-style-type: none"> • Broken down into easy to follow sections covering whole range of process • Gives most concise guidelines of all reports examined. • Contains details for ISO regulation • Utilized panel of experts from UK Clinical Molecular Genetics Society for guideline development 	<ul style="list-style-type: none"> • Authors state that laboratories included in review are assumed to follow good practice standards but no hard evidence is given to prove this • Limited description has been included, are instances where more detail could have been given • Specific quality control recommendations are not included • There is no detailed examination of associated costs for the implementation of these guidelines
Rehm et al., ³ 2013	<ul style="list-style-type: none"> • Extensive literature review • Each stage of process is broken down and examined • The guidelines are readily supported with tools for application • Contains examples of quality control/quality assessment and proposes use of proficiency testing program 	<ul style="list-style-type: none"> • Specific clinical questions were not clarified • The inclusion or exclusion criteria for literature was not clearly defined • There is no detailed examination of associated costs for the implementation of these guidelines

Appendix 5: Summary of findings – cost-effectiveness of next generation sequencing

First Author, Publication Year	Main Study Findings	Authors' Conclusions
Health Technology Assessment		
<p>Foundation for Genomics, and Population Health,⁴ 2011</p>	<p>Sanger sequencing has a low error rate though not at 0 (error rate at 1 in 1000-10000 bases) for individual gene runs but when a condition where variants are found in a heterozygous gene set is discovered analysis is extremely difficult.</p> <p>Sanger sequencing is expensive at ~\$500/Mb compared to less than \$0.50/Mb for NGS platforms.</p> <p>Cost of WES for examination of colorectal cancer in United Kingdom varies greatly from laboratory to laboratory (e.g. Cost for single test ranged from £430 up to £1050). Additionally the time to produce the clinical report varied from 40 days up to 80 days.</p>	<p>-It is not the responsibility of the health-care system to investigate variants detected in genes outside of the initial analysis agreed to by the patient.</p> <p>-Effort should be taken to reduce the amount of incidental variants detected unless they are believed to have the potential to cause dramatic health effects.</p> <p>-There is a lack of randomized controlled trials to analyze the cost and benefits associated with WGS in clinical and research settings. This is a result of all economic evaluations lacking any “real” information on how the increased variant detection in ambiguous genes from NGS runs impacts the health-care system.</p> <p>-Currently there is a lack of economic evaluations on NGS applications that effectively examine association between diagnostic evaluation and endpoint patient diagnosis.</p> <p>- In situations where NGS is not currently being used then the implementation of an NGS pathway is not recommended due to the expense of initial start-up costs.</p>
Systematic Reviews		
<p>Frank et al.,¹ 2013</p>	<p>Sanger sequencing is most expensive process being used (1Mb costs ~\$500 therefore to sequence entire human genome will cost ~\$1.5 million per patient)</p> <p>Prices vary from laboratory to laboratory, closest estimates are:</p> <ul style="list-style-type: none"> - Roche/454 GS FLX Titanium platform costs \$12.40 to \$84.39 per Mb - Illumina HiSeq2000 is least expensive and costs ~0.10/Mb therefore whole genome is estimated at ~\$300 for WGS process <p>Expenses associated with sequence analysis/filtering/data management are typically higher than actual sequencing process. These processes are most expensive for platforms that use short read length.</p>	<p>- It is extremely difficult to estimate costs associated with processes after actual sequencing run as they require multiple different professionals from molecular and computational biologists to genetic counsellors, pathologists and clinicians.</p> <p>- May be more cost effective to reanalyze a patient than to store the data from a previous run due to expense of data storage equipment (stipulated that this is for current dates only as prices for this type of equipment are rapidly dropping).</p> <p>- The need to confirm NGS results using established methods will not be</p>

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	<p>Data storage is major concern as a single WGS run will result in 2.5 terabytes of hard disk space.</p> <p>Currently the risk of false positive results cause a requirement for confirmation testing especially in cases of a recessive disorder (these have a compound heterozygote)</p>	<p>required in near future as protocols for these processes are rapidly becoming more robust and reliable.</p> <ul style="list-style-type: none"> - Results from a WGS run are still quite complex and the transition of the data to a patient is equally as multifaceted since every person has a different view of what will be a concern. - Stipulate that the industry is still far from the \$1000 genome and even if it is reached it will still cost ~\$600 million per year to sequence every child born in Germany.
<p>Sullivan et al.,⁵ 2012</p>	<ul style="list-style-type: none"> -In the United Kingdom it costs ~£600 for proband and £120 for family member of a person already carrying a BRCA1 or BRCA2 mutation using Sanger sequencing -Standard test currently used for analysis of BRCA1 and BRCA2 in United Kingdom is Sanger sequencing followed by MLPA* though two laboratories utilize NGS processes -Authors cannot state NGS is cost effective due to a lack of sufficient robust evaluation 	<ul style="list-style-type: none"> - There is a lack of sufficient economic evidence to support or deny the switch to NGS or keep standard Sanger-MLPA processes. - Currently there are programs in the European Union to establish NGS in clinics, these are; TECHGENE, EURO-GENE-SCAN and NMD-CHIP

*MLPA – multiplex ligation-dependent probe amplification