Protein C Gene Sequencing (Code 20070)
Notice of Assessment

June 2013
1 GENERAL INFORMATION
1.1 Requestor: CHU Sainte-Justine
1.2 Application Submitted: January 1, 2011
1.3 Notice Issued: April 12, 2013

Note:
This notice is based on scientific and commercial information (submitted by the requestor[s]) and on a complementary review of the literature according to the data available at the time that this test was assessed by INESSS.

2 TECHNOLOGY, COMPANY, AND LICENCES
2.1 Name of the Technology
Sequencing for variants in the gene for human protein C (PROC)

2.2 Brief Description of the Technology
The conventional sequencing method (also referred to as the dideoxy method) has six steps: (1) select a specific primer complementary to a single strand of amplified template DNA (PCR or RT-PCR), (2) incubate the template, primer and DNA polymerase along with unlabelled deoxynucleotides (dNTP) and dideoxynucleotides (ddNTP) labelled with a single fluorophore, (3) polymerize until random incorporation of a ddNTP terminates the polymerization of each molecule, (4) separate variously sized polymers according to molecular weight using capillary electrophoresis, (5) excite the terminal fluorophore of each fragment using a laser that traverses the full length of the capillary, (6) read and interpret the sequence based on the fluorescence emission signals (basecalling) (Wilson et al., 1990).

Figure 1: Schematic Representation of Sequencing Using the Dideoxy Method

Image courtesy of the National Forensic Science Technology Center, from NIJ’s DNA analyst training program (Figure from the NFSTC website, http://projects.nfstc.org/gallery/main.php?g2_itemId=3566).
2.3 Company or Developer

In-house protocol using the following analyzer and test kits:\footnote{Based on requestor-supplied data.}
- QuickGene-Mini80 (Autogen)
- AmpliTaq Gold® (Invitrogen)
- BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies)
- ABI 3100-Avant Genetic Analyzer (Applied Biosystems)

2.4 Licence(s): Not applicable

2.5 Patent, If Any: Not applicable

2.6 Approval Status (Health Canada, FDA)

The above-mentioned test kits and systems are not found in Health Canada’s Medical Devices Active Licence Listing (MDALL).

2.7 Weighted Value: 563.0.

3 CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES

3.1 Targeted Patients

Cascade screening for patients with a family history of thrombophilia. The test is particularly useful for prenatal diagnosis because severely affected individuals have a condition largely incompatible with life and the replacement therapy requires an almost daily injection of protein C concentrate, the annual cost of which would be $1,000,000 for an adult (according to information from the requestor).

3.2 Targeted Disease(s)

Protein C is a vitamin K-dependent glycoprotein that plays an important regulatory role in blood clotting through proteolytic inactivation of activated clotting factors (Va and VIIIa). The protein C gene, localized on chromosome 2q13-q14, comprises nine exons spanning 11 kb of DNA. A constitutional protein C deficiency, inherited as an autosomal dominant trait, is associated with various mutations in the protein C gene. This deficiency is associated with an increased risk of venous thrombosis (thrombophilia) (Millar et al., 2000). Thrombophilia is linked to hemostatic disorders resulting clinically in deep vein thrombosis and pulmonary embolisms. There are also more frequent or acquired constitutional forms (HAS, 2006). Diagnosis is usually based on determining protein C activity in plasma using clotting or chromogenic assay plus protein C antigen assay (normal range from 70% to 140%).

Impaired protein C activity can also be found in association with other genetic disorders predisposing for thrombosis, including activated protein C resistance due to the factor V G1691A (or factor V Leiden) gene mutation, and the prothrombin G20210A gene mutation (Tjeldhorn et al., 2010). In a multicultural population such as that of Metropolitan Montreal, the prevalence of thrombophilia in association with impaired protein C activity is unknown and varies with genetic composition (according to information from the requestor).
3.3 
**Number of Patients Targeted**

According to the requestor’s forecasts, one or two tests are expected per month; that is, about ten annually.

3.4 
**Medical Specialties Involved (and Other Professions, If Any)**

Hematology, pediatrics and obstetrics–gynecology

3.5 
**Testing Procedure**

The test is performed on genomic DNA extracted from peripheral blood leukocytes.

4 
**TECHNOLOGICAL BACKGROUND**

4.1 
**Nature of the Diagnostic Technology**

According to information from the requestor, this test would replace testing for the 3363 inserted (Ins) C mutation (code 20986) of the PROC gene. Tests for other mutations of the PROC gene are listed in the Index; namely, mutation R178Q (code 20987), Splice 3222 (code 20988) and mutation T298M (code 20989). CHUM² is conducting research on three mutations (3363 Ins C, R178Q and T298M) by PCR.

4.2 
**Brief Description of the Current Technological Context**

3363 Ins C mutations and the two missense mutations (R178Q and T298M) have been identified in seven French-Canadian families affected by a type I protein C deficiency. The 3363 Ins C mutation was probably introduced in North America by a French colonist couple who settled in Quebec and Vermont in 1669 (Couture et al., 1998). In 2001, Couture et al. showed that all patients heterozygous for this mutation living in North America are related within 10 generations to this French couple.

4.3 
**Brief Description of the Advantages Cited for the New Technology**

Sequencing allows for identifying any known or unknown mutation, in contrast to testing one by one for the mutation in question (according to information from the requestor).

4.4 
**Cost of Technology and Options**: Not Analyzed

5 
**EVIDENCE**

5.1 Clinical Relevance

5.1.1 Other Tests Replaced

According to information from the requestor, this test would replace testing for the 3363 Ins C mutation of the protein C gene. Because sequencing allows for identifying any known or unknown mutation, this test would replace testing for mutations one by one.

5.1.2 Clinical Relevance Validity

According to the literature, PCR followed by DNA sequencing allows the identification of many mutations, whether new or previously known and registered in a continually updated mutations database (Reitsma, 1996). About 200 different mutations of the protein C gene have been registered in the PROC Database (Reitsma et al., 1995, cited in Tjeldhorn et al., 2010). Missense mutations and nonsense mutations account for about two thirds of the PROC gene mutations. The other mutations include splice site mutations, small insertion mutations and deletion mutations. Tables 1 and 2 summarize the selected studies.

Cascade screening

The most severe protein C deficiencies are generally homozygous and manifest clinically as severe thrombotic events occurring at an early age. In the six selected studies that reported cascade screening results, the majority of reported probands were homozygous, with a severe protein C deficiency (< 10%). According to Millar et al. (2000), neither protein C activity level nor the nature of the PROC gene lesions detected are good prognostic indicators of the age of onset or clinical severity of thrombotic symptoms. Other factors may complicate the relationship between the genotype and clinical phenotype. For example, in two patients, the presence of factor V Leiden in addition to protein C gene lesions may have precipitated the thrombotic events.

Non-family screening

The studies reviewed have identified one or more mutations in different patient populations with a protein C deficiency. These mutations lead to variability in clinical expression. The mutation detection rate depends on the percentage of protein C activity. As reported by Caspers et al. (2012), a mutation detection rate of 100% was found at protein C activity levels ranging from 10% to 25%. The mutation detection rate was higher than 60% when the protein C activity levels were 50% or greater, and was absent for activity higher than 70%; the authors do not advise genotyping for the latter range. Briefly stated, the lower the level of protein C activity, the greater the mutation detection rate. In another study involving the screening of mutations by denaturing gradient gel electrophoresis (DGGE) prior to sequencing, a diagnosis of germline protein C deficiency was confirmed in 39% of the patients with borderline plasma protein C antigen levels, showing that PROC gene analysis improves the diagnosis of protein C deficiency in such patients (Alhenc-Gelas et al., 2000).

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3 A missense mutation is a mutation that replaces a codon specifying one amino acid with a codon that specifies another.
4 A nonsense mutation is a mutation that replaces a codon specifying one amino acid with a stop codon (effectively truncating the protein).
5 Splicing is a process involving the excision of introns and splicing of exons in RNA (Chartier, 1991).
6 A person who draws attention to a genetic problem in a family; also referred to as an index case.
The risk of thrombosis is higher among patients carrying mutations than among patients not carrying any, but the risk of developing venous thrombosis varies, according to Tjeldhorn et al. (2010). In that study, although four of the six siblings were homozygous for the A267T mutation in the PROC gene, with protein C activity levels ranging from 8% to 41%, only one developed venous thrombosis. These results indicate that the genotype-phenotype relation in this protein C deficient family is very complex.

To summarize, sequencing is widely used and its value proven for cascade screening of severe protein C deficiency and for determination of the specific mutation affecting members of the same family in order to confirm the constitutional nature of the deficiency. Given the number of mutations that can be detected, it is not recommended to search for a specific type of mutation or to conduct genetic screening outside of a familial context.
Table 1: Studies of the Genetic Analysis of Protein C Deficiency (Cascade Screening)

<table>
<thead>
<tr>
<th>STUDY (ORIGIN OF POPULATION)</th>
<th>N</th>
<th>PROTEIN C ACTIVITY</th>
<th>GENETIC FORM</th>
<th>MUTATION (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cafolla, 2012 (Italian)</td>
<td>15</td>
<td>Proband 1: 5% (TE)</td>
<td>Homozygous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proband 2: 9% (TE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tang, 2012 (Chinese)</td>
<td>34*</td>
<td>34 probands:</td>
<td></td>
<td>18, of which 12 are new</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from 6.3% to 62.3% (TE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yu, 2012 (Chinese)</td>
<td>11</td>
<td>1 proband: 5% (T)†</td>
<td>Homozygous</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Park, 2011 (Korean)</td>
<td>5</td>
<td>1 proband: 30% (PE)</td>
<td>Heterozygous</td>
<td>1 new</td>
</tr>
<tr>
<td>Tjeldhorn, 2010 (Lebanese)</td>
<td>9</td>
<td>1 proband: 8% (RVT)</td>
<td>Homozygous</td>
<td>1 new</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daughters of proband: 3% and ND</td>
<td>Parents heterozygous for the mutation in question, one brother, two sisters and the daughter of the proband are homozygous</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other members: from 33% to 63%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millar, 2000‡</td>
<td>9</td>
<td>9 probands: between 1% and 8% (SPCD)</td>
<td>5 homozygous 4 heterozygous</td>
<td>7, of which 3 are new</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from 30 to 100%§ from 32 to 65%∥</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: SPCD = severe protein C deficiency; PE = pulmonary embolism; N = number of patients tested by means of protein C gene sequencing; ND = not determined; PC = protein C; T = thrombosis; TE = thromboembolism; RVT = recurrent venous thrombosis.
* 34 probands with a congenital protein C deficiency and a personal and family history of thromboembolic disease.
† Right iliofemoral thrombosis and partial vaso-occlusion.
‡ Indian, Finnish, British, Swedish, Venezuelan.
§ Fathers of the probands.
∥ Mothers of the probands.
<table>
<thead>
<tr>
<th>STUDY (ORIGIN OF POPULATION)</th>
<th>N (DIAGNOSIS)</th>
<th>PROTEIN C ACTIVITY</th>
<th>NUMBER OF MUTATIONS IDENTIFIED</th>
<th>NUMBER OF DIFFERENT MUTATIONS</th>
<th>NEW MUTATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ding, 2013 (Chinese)</td>
<td>23 (DVT)</td>
<td>from 19% to 69.4%</td>
<td>15</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Lu, 2013 (Chinese)</td>
<td>40 (CI)</td>
<td>NR</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Caspers, 2012 (German)</td>
<td>211 (TE)</td>
<td>from 27.5% to 67%</td>
<td>146</td>
<td>77</td>
<td>31</td>
</tr>
<tr>
<td>Pai, 2012 (Indian)</td>
<td>102 (VT)</td>
<td>from 9% to 63%</td>
<td>8</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Takagi, 2009 (Japanese)</td>
<td>6 (DVT or PF or PE)</td>
<td>One case: &lt; 10% from 10% to 52%</td>
<td>8 (2 mutations in one patient)</td>
<td>6*</td>
<td>NR</td>
</tr>
<tr>
<td>Altinisik, 2008 (Turkish)</td>
<td>50 (VT)</td>
<td>NR†</td>
<td>9 patients (18%) with mutations</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Labrouche, 2003 (Not indicated)</td>
<td>17 (VT or PE)</td>
<td>from 4% to 74%</td>
<td>11</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Alhenc-Gelas, 2000‡</td>
<td>129§</td>
<td>Values differ with the deficiency type</td>
<td></td>
<td>104</td>
<td>78</td>
</tr>
</tbody>
</table>

Abbreviations: PE = pulmonary embolism; CI = cerebral ischemia; N = number of patients tested by means of protein C gene sequencing; NR = not reported; PC = protein C; PF = purpura fulminans; TE = thromboembolism; VT = venous thrombosis; DVT = deep vein thrombosis.

* All mutations previously reported in Japanese patients, except for c.439G > A, which had been already reported in two German families.
† Seven of nine patients with low protein C activity levels (value not reported), two patients with borderline levels: 73% and 80%.
‡ Population: France (n=122); Swiss (n=2); Tunisian (n=1); Canadian (n=1); German (n=1).
§ Patients with personal or family history of thrombosis.
‖ Values expressed as mean: Type I: 0.49 (0.06 to 0.66); Type II-AC (based on anticoagulant activity): 0.45 (0.11 to 0.68); Type II-AM (based on amidolytic activity): 0.47 (0.11 to 0.68); unknown classification: 0.33 (0.06 to 0.58); borderline protein C level: 0.70 (0.53 to 0.90).
5.1.3 **Therapeutic Value:** Not applicable

5.2 **Analytical (or Technical) Validity**

The literature search strategy for the requested analysis and indication did not include locating studies on the analytical validity of sequencing. However, the sequencing technique is the same for many diseases. The requestor is a leader in this area and, in its application, it stated that quality controls are applied at every step. In addition, the laboratory complies with the guidelines issued by the Canadian College of Medical Geneticists (CCMG) and the American College of Medical Genetics and Genomics (ACMG); it also provides for the participation of external controls.

5.3 **Recommendations for Listing in Other Jurisdictions**

The practice guidelines identified do not consider the issue of sequencing.

6 **ANTICIPATED OUTCOMES OF INTRODUCING THE TEST**

6.1 **Impact on Material and Human Resources:** Not assessed

6.2 **Economic Consequences of Introduction into Québec’s Health and Social Services System:** Not assessed

6.3 **Main Organizational, Ethical, or Other (Social, Legal, Political) Issues:** Not assessed
7 INESSS NOTICE IN BRIEF
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<table>
<thead>
<tr>
<th>Status of the Diagnostic Technology</th>
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<tbody>
<tr>
<td>☐ Established</td>
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<tr>
<td>☒ Innovative</td>
</tr>
<tr>
<td>☐ Experimental (for research purposes only)</td>
</tr>
<tr>
<td>☐ Replacement of technology: ___________ , which becomes obsolete</td>
</tr>
</tbody>
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<thead>
<tr>
<th>INESSS Recommendation</th>
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<tbody>
<tr>
<td>☒ Keep test in the Index for the disease and the conditions described in the application</td>
</tr>
<tr>
<td>☐ Remove test from the Index</td>
</tr>
<tr>
<td>☐ Reassess test</td>
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<tr>
<th>Additional Recommendation</th>
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<tbody>
<tr>
<td>☐ Draw connection with listing of drugs, if companion test</td>
</tr>
<tr>
<td>☐ Produce an optimal use guide</td>
</tr>
<tr>
<td>☐ indicators, when monitoring is required</td>
</tr>
</tbody>
</table>
REFERENCES


Chartier A. Glossaire de génétique moléculaire et génie génétique. Paris, France: Institut national de la recherche agronomique (INRA); 1991.


