Thyroid-Stimulating Hormone Receptor (TSHR) Gene Mutation Test by Sequencing and MLPA (Reference 2014.01.008)

Notice of Assessment

June 2014
1 GENERAL INFORMATION

1.1 Requestor: CHU Sainte-Justine

1.2 Application for Review Submitted to MSSS: January 14, 2014

1.3 Application Received by INESSS: March 1, 2014

1.4 Notice Issued: June 30, 2014

Note
This notice is based on the scientific and commercial information submitted by the requestor 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 LICENCE(S)

2.1 Name of the Technology
Thyroid-stimulating hormone receptor (TSHR) gene mutation test by sequencing and multiplex ligation-dependent probe amplification (MLPA).

2.2 Brief Description of the Technology, and Clinical and Technical Specifications
Sanger sequencing is a basic technique [Kircher and Kelso, 2010].

MLPA is a technique used for detecting locus copy number changes [Schouten et al., 2002]. It is a targeted detection method offering the advantage of studying several loci simultaneously. The principle is to obtain, for each locus, an amplified fragment of a different length in order to distinguish and quantify the loci (Figure 1). Two differently sized probes are matched to each locus. Each probe has two parts: one complementary to the target sequence (specific hybridization) and the other needed for the polymerase chain reaction (PCR) amplification of all the loci studied. Each fragment can thus be quantified and compared to a control, thereby enabling the detection of the locus copy number changes [Keren and Sanlaville, 2008].
2.3 **Company or Developer:** Protocol provided by the requestor.

2.4 **Licence(s):** Not applicable.

2.5 **Patent, If Any:** Not applicable.

2.6 **Approval Status (Health Canada, FDA):** Not applicable.

2.7 **Weighted Value:** 743.89.

3 **CLINICAL INDICATIONS, PRACTICE SETTINGS, AND TESTING PROCEDURES**

3.1 **Targeted Patient Group**

The test targets Quebec neonates with positive newborn blood screening for congenital hypothyroidism (CH), normal thyroid anatomy and no autoimmunity.

3.2 **Targeted Disease(s)**

The incidence of CH is about 1 in 3,000 to 4,000 live births [Toublanc, 1992]. Without early treatment, irreversibly impaired growth and cognitive development, as well as neuromotor damage, will appear gradually in most CH-positive newborns [Laflamme et al., 2006]. Thyroid dysgenesis, defined as an abnormal gland development or migration, is responsible for 85% of CH cases. The remaining 15% are associated with defective thyroid hormone synthesis (dyshormonogenesis). Thyroid dysgenesis may be caused by thyroid agenesis, hypoplasia or ectopy [Park and Chatterjee, 2005]. The CH cases targeted by the requestor are the subclinical cases, i.e., the clinical presentation is consistent with thyroid stimulating hormone (TSH) resistance, a genetic disease (OMIM #275200) characterized by [Beck-Pecco et al., 2006; Refetoff, 2003]:

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![Figure 1: Principle underlying MLPA](image URL)
- Serum TSH > normal;
- Serum thyroxine (T4) and serum triiodothyronine (T3) ≤ normal;
- Thyroid size ≤ normal;
- Absence of thyroid autoantibodies: antithyperoxidase, antithyroglobulin, anti-T3, anti-T4, anti-TSH.

Subclinical CH is common in the adult population. The prevalence of this condition is 9%, according to the results of a population survey conducted in Colorado (United States) in 1995, with 25,862 participants [Canaris et al., 2000]. Pediatric population data suggest a prevalence below 2% [Wu et al., 2006]. Mutations of the TSH receptor (TSHR) gene lead to a variable loss in its function and are responsible for TSH resistance. When the sensitivity of thyroid tissue to TSH stimulation (by TSHR) is partially conserved, an elevation in serum TSH can compensate for the receptor defect (partial TSH resistance). A major defect in TSHR function, resulting in a complete loss of sensitivity to TSH, leads to impaired gland growth (hypoplasia) and thyroid hormone production (complete TSH resistance) [Persani et al., 2010].

3.3 Number of Patients Targeted
The requestor estimates the number of newborns to be tested at approximately 50 cases annually. According to Deladoëy et al., out of the 620 babies born in Quebec between 1990 and 2009 who screened positive for CH, 179 would have been candidates for molecular analysis of the TSHR gene, i.e., 1 case in 9,278 live births, thus about 10 new cases annually (~ 90,000 live births annually in Quebec) [Deladoëy et al., 2011].

3.4 Medical Specialities and Other Professions Involved
Medical biochemistry and pediatric endocrinology.

3.5 Testing Procedure
Direct sequencing of purified DNA samples from blood or saliva.

4 TECHNOLOGY BACKGROUND
4.2 Brief Description of the Current Technological Context
CH screening has been included in Quebec’s newborn blood screening program since April 1974 [Laflamme et al., 2006].
At present, CH cases identified by newborn screening and considered eligible for molecular analysis of the TSHR gene are sent outside Quebec. In 2012–2013, MSSS data on CH screening make no mention of a referral outside Quebec. A diagnostic algorithm has been prepared with input from endocrinologists at CHU Sainte-Justine (see Appendix A).

4.3 Brief Description of the Advantages Cited for the New Technology

The TSHR gene mutation test has a three-pronged objective:

1. Corroborate molecular analysis results for patients with slightly increased blood TSH levels.
2. Identify cases with fully or partially compensated resistance in order to stop treatment of hypothyroidism and monitoring of TSH levels.
3. Determine which relatives are carriers of a heterozygous mutation associated with a loss of function of the TSHR gene.

4.4 Cost of Technology and Options: Not assessed.

5 EVIDENCE

5.1 Relevance and Clinical Validity

5.1.1 Other Tests Replaced: This test is unique.

5.1.2 Diagnostic or Prognostic Value

The thyroid-stimulating hormone receptor gene contains 10 exons and codes for an imposing 764-amino-acid protein comprising an N-terminal extracellular TSH-binding domain, a 7-alpha-helix transmembrane portion, and an intracellular domain consisting of 3 loops and a C-terminal tail (Figure 2).

“Resistance” refers to loss of function (LoF) of the receptor caused by mutations affecting the TSHR gene. LoF mutations are acquired as monoallelic (heterozygous) or biallelic (homozygous or compound heterozygous) mutations. They are associated with a wide spectrum of phenotypes, ranging from simple hyperTSHemia to severe congenital hypothyroidism without structural thyroid abnormalities (euthyroidism) or autoimmunity (Table 1) [Park et al., 2004].

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1 Personal telephone conversation with Dr. Jean-François Soucy at CHU Sainte-Justine (April 29, 2014).
2 Test request form and personal telephone conversation with Dr. Jean-François Soucy at CHU Sainte-Justine (April 29, 2014).
Figure 2: Schematic representation of TSHR gene structure showing mutations identified as causing loss of function as of 2010

Table 1: Diagnostic criteria for TSH resistance syndrome (adapted from Cassio et al., 2013)

<table>
<thead>
<tr>
<th>TSH RESISTANCE</th>
<th>AGE AT DIAGNOSIS</th>
<th>CH NBS</th>
<th>TSH</th>
<th>T4L</th>
<th>THYROID SIZE</th>
<th>DIFFERENTIAL DIAGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncompensated</td>
<td>Newborn</td>
<td>+</td>
<td>↑↑</td>
<td>↓</td>
<td>Severe hypoplasia</td>
<td>Biallelic TSHR LoF Mut; Thyroid TF Mut; Inactive TSH</td>
</tr>
<tr>
<td>Partially compensated</td>
<td>Newborn Infant</td>
<td>+/-</td>
<td>↑↑</td>
<td>↓/=</td>
<td>Hypoplasia or normal</td>
<td>Biallelic/monoallelic TSHR LoF Mut</td>
</tr>
<tr>
<td>Fully compensated</td>
<td>Infant</td>
<td>-</td>
<td>=</td>
<td>=</td>
<td>Normal or slight hypoplasia</td>
<td>Thyroid TF Mut; Inactive TSH; PHP1a; Autoimmunity</td>
</tr>
</tbody>
</table>

Abbreviations: CH = congenital hypothyroidism; LoF = loss of function; Mut = mutation; NBS = newborn blood screening; PHP1a = pseudo-hypoparathyroidism type 1a; T4L = free thyroxine; TF = transcription factor; TSH = thyroid-stimulating hormone; TSHR = TSH receptor.

The degree of resistance of the TSH receptor to the action of its ligand, TSH, varies with the type and position of the mutation. To date, more than 60 different mutations in the TSHR gene have been described in association with different degrees of TSH resistance [Cassio et al., 2013]. The reported sequence variations are mainly point variations or small insertions/deletions (indels) that frequently cause missense mutations, as well as nonsense mutations or frameshift mutations [Cassio et al., 2013]. The mutations are distributed all along the gene sequence. No full-gene deletion has been reported, but deletion of a complete exon has been described [Cangul et al., 2010].
A summary search of the scientific literature identified a few clinical studies suitable for comparing the evidence supporting the use of the proposed test. Table 2 presents these case-series studies of newborn blood screening (NBS) for CH. These studies corroborate the clinical phenotype of compensated TSH resistance with molecular alteration of the TSHR gene.

Table 2: Case-series corroborating compensated TSH resistance (partial or complete) with a mutation of the TSHR gene

<table>
<thead>
<tr>
<th>AUTHOR, YEAR</th>
<th>CASES (N)</th>
<th>METHOD</th>
<th>VARIATION (N)</th>
<th>GENOTYPES</th>
<th>L-T4</th>
<th>FAMILY FORM</th>
<th>FUNCTIONAL STUDIES (MUTATIONS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calebiro et al., 2012</td>
<td>NBS CH+ (62)</td>
<td>dHPLC Sanger</td>
<td>7 new ones</td>
<td>All HTZ: 5 MM, 1 ARF, 1 intron</td>
<td>4 treated</td>
<td>All cases have a tested parent: 3 with proven co-segregation and TSH ↑, 2 carriers without CH and 2 non-carriers</td>
<td>R609Q, T607I, P162L, I583T and Y466C TSH binding ↓. P162L and Y466C surface expression and biological activity ↓. Q33PfsX46: protein truncation IVS4 + 2A &gt; G: splicing error</td>
</tr>
<tr>
<td>Ma et al., 2010</td>
<td>NBS CH+ (18)</td>
<td>SSCP Sanger</td>
<td>1 known</td>
<td>HMZ R450H</td>
<td>?</td>
<td>6 parents R450H/wt, TSH↑/T4 normal</td>
<td>No details</td>
</tr>
<tr>
<td>Nicoletti et al., 2009</td>
<td>NBS CH- (38)</td>
<td>PCR Sanger</td>
<td>9 known 2 new ones</td>
<td>All HTZ: 1 NM, 1 ARF, 9 MM</td>
<td>5 treated</td>
<td>All cases have a carrier parent: no CH feature (6), CH only (3) or with AA+ (2)</td>
<td>P68S shows loss of TSH binding without loss of biological activity</td>
</tr>
</tbody>
</table>

Abbreviations: AA = antithyroid antibodies; ARF = altered reading frame; CH = congenital hypothyroidism; dHPLC = denaturing high-performance liquid chromatography; HMZ = homozygous; HTZ = heterozygous; L-T4 = levothyroxine treatment; MM = missense mutation; N = number; NBS = newborn blood screening; NM = nonsense mutation; PCR = polymerase chain reaction; SSCP = single-strand conformation polymorphism; TSH = thyroid-stimulating hormone; wt = wild type.

The considerable clinical heterogeneity of TSH resistance shows that factors other than the function of the TSHR gene are involved. In fact, the transmission of bigenic lesions [Lado-Abeal et al., 2011; Sripiprapradang et al., 2011; Lado-Abeal et al., 2010], the non-coverage of certain gene regions (5’UTR, 3’UTR, certain promoter regions in cis) by sequencing, and the complex rearrangements limit the efficacy of genetic testing. To date, large deletions involving the entire gene have not yet been identified. However, in 2010 Cangul et al. published a study describing a case with complete deletion of exon 2. The homozygous carrier of this mutation was from a consanguineous family and presented severe thyroid hypoplasia [Cangul et al., 2010]. No study using MLPA as a technique for detecting mutations was identified.
5.1.3 **Therapeutic Value**

The primary purpose of the proposed test is to reduce the number of hormonal assays and thyroid ultrasounds in confirmed cases of compensated TSH resistance. The molecular test will serve to establish the patient’s genotype and, following a temporary suspension of treatment (L-T4) and a complete clinical assessment, evaluate the relevance of stopping treatment. In several cases presenting a monoallelic or biallelic TSHR mutation, L-T4 treatment was withdrawn during childhood on the basis of a clear demonstration that persistent hyperTSHemia does not cause hypothyroidism, a condition that affects growth and cognitive development. Table 3 presents the clinical description of a few cases detected by NBS and reported in the literature.
Table 3: Clinical characteristics of congenital hypothyroidism cases with normal-sized or mildly hypoplastic thyroid in normal position, hyperTSHemia and normal serum T4 hormone

<table>
<thead>
<tr>
<th>AUTHOR, YEAR</th>
<th>CASE (SEX, AGE)</th>
<th>NBS CH</th>
<th>LEVOthyroxine (l-T4)</th>
<th>MOLECULAR ANALYSIS</th>
<th>CHARACTERISTICS OF PROBAND</th>
<th>CHARACTERISTICS OF RELATIVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucas-Herald et al., 2013</td>
<td>M, 11 d</td>
<td>+</td>
<td>94 d</td>
<td>3.1 y</td>
<td>At 3 y: normal growth (size and weight). L-T4 treatment stopped. After 6 weeks, child is doing well and is active: TSH ↑ and T4 normal</td>
<td>Mother, C390F/wt, L-T4 for 6 y. L-T4 stopped: TSH ↑, T4 normal. Resumed after 6 weeks because of tiredness.</td>
</tr>
<tr>
<td>Moia et al., 2013</td>
<td>F, 6 y</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>At 6 y: normal neuromotor growth and development</td>
<td>Mother, W520X/wt, TSH?, T4?</td>
</tr>
<tr>
<td>Bas et al., 2012</td>
<td>M, 3 d</td>
<td>+</td>
<td>30 d</td>
<td>No</td>
<td>P162A/P162A</td>
<td>Parents consanguineous P162A/wt, 1 case with goiter in the family</td>
</tr>
<tr>
<td>Narumi et al., 2009</td>
<td>M, 5 d</td>
<td>+</td>
<td>54 d</td>
<td>2 y</td>
<td>R450H/R450H</td>
<td>At 17 y, TSH ↑↑ and T4 normal</td>
</tr>
<tr>
<td></td>
<td>F, 6 d</td>
<td>+</td>
<td>14 d</td>
<td>12 y</td>
<td>G132R/R450H</td>
<td>At 17 y, TSH ↑↑ and T4 normal</td>
</tr>
<tr>
<td></td>
<td>M, 5 d</td>
<td>+</td>
<td>23 d</td>
<td>3 y</td>
<td>D403N/R450H</td>
<td>At 12 y, TSH ↑↑ and T4 normal</td>
</tr>
<tr>
<td></td>
<td>M, 4 d</td>
<td>+</td>
<td>5 d</td>
<td>5 y</td>
<td>R450H/wt</td>
<td>At 5 y, TSH ↑ and T4 normal</td>
</tr>
<tr>
<td></td>
<td>M, 5 d</td>
<td>+</td>
<td>1 d</td>
<td>1 year</td>
<td>R450H/wt</td>
<td>At 13 y, TSH ↑ and T4 normal</td>
</tr>
<tr>
<td></td>
<td>F, 5 d</td>
<td>+</td>
<td>Untreated</td>
<td></td>
<td>A204V/wt</td>
<td>At 12 y, TSH ↑↑ and T4 normal</td>
</tr>
<tr>
<td>Mizuno et al., 2009</td>
<td>M, 33 d</td>
<td>+</td>
<td>20 mo</td>
<td>15 y</td>
<td>R450H/R450H</td>
<td>No CH features at birth. After 1 m suspension of treatment for cases 1, 2 and 5: TSH ↑, T4 normal for cases 2 and 5, but T4 ↓ for case 1. Cases 3 and 4 tested prior to start of treatment. 4 of 5 cases IQ tested at 6 y with average or above average results (101 to 121).</td>
</tr>
<tr>
<td></td>
<td>M, 27 d</td>
<td>+</td>
<td>4 mo</td>
<td>13 y</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M, 35 d</td>
<td>+</td>
<td>44 mo</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M, 61 d</td>
<td>+</td>
<td>32 mo</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F, 17 d</td>
<td>+</td>
<td>17 d</td>
<td>8 y</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3 The Denver Developmental Screening Test is used to assess the developmental progress of children in various areas (gross motor function, language, fine motor function and social contact). Test du développement de l’enfant (in French) [website], available at: http://protectnet.wordpress.com/2011/11/13/test-de-developpement-de-denver/.
<table>
<thead>
<tr>
<th>AUTHOR, YEAR</th>
<th>CASE (SEX, AGE)</th>
<th>NBS CH</th>
<th>LEVOTHYROXINE (L-T4)</th>
<th>MOLECULAR ANALYSIS</th>
<th>CHARACTERISTICS OF PROBAND</th>
<th>CHARACTERISTICS OF RELATIVES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yuan et al., 2008</td>
<td>M, 30 d</td>
<td>+</td>
<td>60 d</td>
<td>No</td>
<td>PCR + Sanger</td>
<td>G245S/wt</td>
</tr>
<tr>
<td>Tonacchera et al., 2007</td>
<td>M, 13 y</td>
<td>-</td>
<td>13 y</td>
<td>14 y</td>
<td>PCR + Sanger</td>
<td>Q8fsX62/wt</td>
</tr>
<tr>
<td></td>
<td>F, 31 y</td>
<td>?</td>
<td>21 y</td>
<td>31.3 y</td>
<td></td>
<td>D410N/wt</td>
</tr>
<tr>
<td></td>
<td>M, 38 y</td>
<td>?</td>
<td>Untreated</td>
<td></td>
<td></td>
<td>P162A/wt</td>
</tr>
</tbody>
</table>

Abbreviations: AA = antithyroid antibodies; F = female; CH = congenital hypothyroidism; d = days; L-T4 = levothyroxine treatment; mo = months; n = number; NBS = newborn blood screening; NM = nonsense mutation; M = male; PCR = polymerase chain reaction amplification; TSH = thyroid-stimulating hormone; wt = wild type (without mutation); y = years.
5.2 Analytical (or Technical) Validity

Sanger sequencing is capable of detecting virtually all nucleotide substitutions and small insertions or deletions (indels) within a PCR amplicon. However, certain larger indels might not be detected. CH often has an autosomal recessive pattern of inheritance, and this peculiarity could prove to be clinically significant. In fact, Cangul et al. report the case of a consanguineous family with several members presenting a severe form of congenital hypothyroidism (CH). Through genetic linkage studies of several known CH loci, these researchers pinpointed the TSHR gene as a possible source of the defect. Exon 2 amplification was attempted several times, unsuccessfully. Subsequently, transcript analyses by RT-PCR revealed complete deletion of exon 2. The affected cases were homozygous for the mutation and both parents were heterozygous carriers. In this case, the molecular analysis of TSHR by Sanger sequencing was insufficient to detect the mutation in question. RT-PCR or MLPA are thus essential techniques for complementing Sanger sequencing [Cangul et al., 2014].

5.3 Recommendations from Other Organizations

No recommendation has been found for sequencing the TSHR gene in relation to diagnosing and managing congenital hypothyroidism.

6 ANTHICIPATED OUTCOMES OF INTRODUCING THE TEST

6.1 Impact on Material and Human Resources

Demonstration of compensated, persistent hyperTSHemia caused by a mutation of the TSHR gene could allow some monitoring and treatments to be withdrawn.

6.2 Economic Consequences of Introducing Test Into Quebec’s Health Care and Social Services System

Not assessed.

6.3 Main Organizational, Ethical, and Other (Social, Legal, Political) Issues

Not assessed.

7 IN BRIEF

7.1 Clinical Relevance

Some cases of congenital hypothyroidism detected at birth present persistent hyperTSHemia with no functional or physical disorder of the thyroid gland. In such cases, the molecular analysis of the TSHR gene, in combination with a complete clinical assessment, may demonstrate that the condition is stable and that levothyroxine treatment (L-T4) to normalize TSH serum concentrations is unnecessary.
7.2 Clinical Validity

Several case-series studies of congenital hypothyroidism cases that were detected at birth and enrolled in clinical tests and complementary molecular analyses have been identified. The evaluation of the mutational status of the TSHR gene using classical sequencing has highlighted several cases of compensated TSH resistance. To date, only one mutation easily identified using MLPA has been detected in a non-classical homozygous case from consanguineous parents.

7.3 Analytical Validity: No data available.

7.4 Recommendations from Other Organizations: No data.


## 8 INESSS NOTICE IN BRIEF

Thyroid stimulating hormone receptor (TSHR) gene mutation test by sequencing and MLPA

<table>
<thead>
<tr>
<th>Status of the Diagnostic Technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ Established</td>
</tr>
<tr>
<td>☐ Innovative</td>
</tr>
<tr>
<td>☒ Experimental (for this use)</td>
</tr>
<tr>
<td>☐ Replacement for technology________, which becomes obsolete</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INESSS Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ Include test in the Index</td>
</tr>
<tr>
<td>☐ Do not include test in the Index</td>
</tr>
<tr>
<td>☒ Reassess test once supplementary data on clinical and technical validity become available</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ Draw connection with listing of drugs, if companion test</td>
</tr>
<tr>
<td>☐ Produce an optimal use manual</td>
</tr>
<tr>
<td>☐ Identify indicators, when monitoring is required</td>
</tr>
</tbody>
</table>
REFERENCES


Keren B and Sanlaville D. Nouveaux outils diagnostiques du retard mental. Médecine Thérapeutique Pédiatrie 2008;11(4);230-41.


Park SM, Clifton-Bligh RJ, Betts P, Chatterjee VK. Congenital hypothyroidism and apparent athyreosis with compound heterozygosity or compensated hypothyroidism with probable hemizygosity for inactivating mutations of the TSH receptor. Clin Endocrinol (Oxf) 2004;60(2):220-7.


APPENDIX A
Diagnosis algorithm

Patient with mildly increased TSH level, normal thyroid anatomy and no autoimmunity

*** Wait to collect > 5 samples

Amplification and sequencing of exon 10 of the *TSHR* gene [3 fragments]
MLPA using MRC-Holland of the *TSHR* gene [5 hypothyroidism genes]

1. No mutation detected
2. Variation in sequence of unknown significance
3. No variation in TSHR gene copy number

Amplification and sequencing of exon 1–9 of the *TSHR* gene [10 fragments]

1. No mutation detected
2. Variation in sequence of unknown significance

1. Detection of a known or truncated or presumed pathogenic mutation using SIFT, PolyPhen or MutationTaster
   or
2. Detection of a variation in the copy number (e.g., exon deletion) not listed in the Database of Genomic Variants (http://dgv.tcag.ca/dgv/app/home) and validated using PCR

Report positive for detection of pathogenic mutation (or two recessive mutations)

Detection of a known or truncated or presumed pathogenic mutation using SIFT, PolyPhen or MutationTaster

Report negative for detection of pathogenic mutation