Severe post-partum autoimmune hypothyroidism associated with a novel loss-of-function mutation in intracellular domain of human thyrotropin receptor

Ostra poporodowa autoimmunologiczna niedoczynność tarczycy związana z nową mutacją utraty funkcji w wewnątrzkomórkowej domenie ludzkiego receptora TSH

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Abstract

Several loss-of-function TSHR gene mutations have been reported previously. In the present study, a mutation in exon 10 of the TSHR gene was studied. A 35-year-old mother of two children attended our hospital with symptoms of hypothyroidism after her second delivery. She had elevated levels of thyroid hormones including both the thyroid antibodies and those treated with L-thyroxine. In the present study, exon 10 of the TSHR gene of the patient was studied. Sequencing of exon 10 revealed a G to T transversion, resulting in a loss of function mutation changing glutamic acid 757 to stop codon, (E757Stop) in the intracellular domain of TSH receptor and also C to T transition, leading to no change in amino acid sequence (glycine to glycine at 753 amino acid position). We report a novel sporadic loss of function mutation in TSHR protein at codon 757 (E757Stop).

Streszczenie

Publikacje na temat mutacji utraty funkcji genu TSHR pojawiały się już wcześniej. W prezentowanej pracy zbadano mutację w pozycji eksonu 10 genu TSHR. Do szpitala zgłosiła się 35-letnia matka dwojga dzieci z objawami niedoczynności tarczycy po drugim porodzie. Miała podwyższony poziom hormonów tarczycy, w tym zarówno przeciwciał przeciw tarczycyJak i L-tyroksyny. W pracy przestudiowano ekson 10 genu TSHR pacjentki. Przy sekwencjonowaniu eksonu 10 odkryto transwersję G do T, czego skutkiem jest mutacja utraty funkcji zmieniająca kwas glutaminowy 757 na kodon stop (E757Stop) w wewnątrzkomórkowej domenie receptora TSH, a także przejście C do T nieprowadzące do żadnej zmiany w sekwencji aminokwasu glutaminowego (glycine to glycine w pozycji aminokwasu 753). Przedstawiamy raport na temat nowej sporadycznej mutacji utraty funkcji w białku TSHR w pozycji kodonu 757 (E757Stop).

Introduction

The physiological action of thyroid stimulating hormone (TSH) is mediated by a G protein coupled “seven trans-membrane domain receptor”, the thyroid stimulating hormone receptor (TSHR) [1]. This interaction leads to activation of second messenger pathways involving cAMP, inositol 1, 4, 5-triphosphate, and diacylglycerol (DAG), leading to the production and secretion of T₄ and T₃ [2]. The extracellular part of this receptor is involved in the binding of TSH, and the trans-membrane and intracellular domain has effector properties triggering G protein activation [3]. The gene organisation reflects these dual functions because a single exon (exon 10) encodes the trans-membrane and intracellular domain, whereas the extracellular domain is encoded by the remaining nine exons [4].

Since the first report of Congenital Hypothyroidism caused by a TSHR mutation, several cases of loss-function mutations of TSHR have been reported. Most of the mutations are missense mutations, but deletions and insertions have been identified as well [5].

Upon binding of TSH to its receptor, the main pathway of the signal transduction involves the activation of the adenylate cyclase through G protein
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In a familial study conducted on a Caucasian population, a missense mutation (W546X mutation) in exon 10 was detected in approximately 1 in 180 individuals and it was concluded that it could be a major contributor to hypothyroidism in the Welsh population [7]. The mechanism leading to loss-of-function mutations of TSHR includes abnormal binding affinity, abnormal receptor synthesis, accelerated degradation, defective receptor targeting to the cell membrane, and abnormal signal transduction [8]. Mutations may exert their activity by causing protein misfolding, misassembly or aberrant oligomerisation. Loss-of-function mutations are located all along the TSHR [9].

In India, genetic study on hypothyroidism is scarce, especially on TSHR. Here we report a novel mutation in a post-partum hypothyroid patient at our end.

Case report

The study was conducted in accordance with the Helsinki Declaration [10]. Written informed consent for molecular studies was obtained from the patient. The study was approved by the Choithram Hospital and Research Centre Ethics Committee (DCGI registered ethics committee as per guidelines of Indian Council of Medical Research).

Patient’s background

The patient is a 35-year-old mother of two with no family history of thyroid disorders or any other endocrine diseases.

During pregnancy

During both pregnancies, she did not manifest any symptoms of hypothyroidism. All the haematological biochemical and endocrinological parameters including thyroid profiles were within normal range during both pregnancies. Both children were born by normal delivery without any complications.

Post pregnancy

At the time of diagnosis of hypothyroidism

The patient arrived at our hospital outpatient department 6 months after she delivered her second child. She complained of weight gain, numbness in the hand, lethargy, and hair loss. Swelling on the face and trunk was also observed. Her height and weight was measured and the body mass index (BMI) was calculated. At the time of diagnosis her BMI was above normal (Table 1). The haematological, biochemical, and endocrinological parameters were measured using Sysmax XT-1800 (based on DC method with Hydrodynamic Focusing, Flow Cytometry method, and SLS-Haemoglobin method), Cobus C311 (based on CHOD-PAP method for total Cholesterol and Roche III gen method for low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol), and Abbott Architect i1000SR (based on Chemiluminescent Microparticle Immunoassay for thyroid hormone profile and thyroid antibodies estimation), respectively. At the time of diagnosis the estimated values of thyroid hormones in serum (Table I) indicated that the patient was suffering from hypothyroidism. Marked elevation of both the thyroid antibodies (thyroglobulin and thyroid peroxidase antibodies) in the serum (Table I) suggested autoimmune hypothyroidism. Borderline elevations of serum lipids were also observed, and the haematological values were within normal range (Table 1). It was recommended that the patient be treated with a dose of 100 µg L-thyroxine per day.

Patient follow-up

After 15 days follow-up, symptomatic improvement was seen in the patient, and reassessment of her serum TSH and serum cholesterols every 6 months was advised. During the first 6-month follow-up the serum TSH value was still elevated and the serum cholesterols were within normal range (Table I). The dose of L-thyroxine was increased to 125 µg per day. The thyroid hormone levels were within normal range in the last two 6-month follow-ups of the patient (Table 1).

Molecular biological findings of the patient

Methodology

In the present study, exon 10 of the TSHR gene of the patient, her mother, and her brother was studied. DNA was isolated from whole blood by using a DNA extraction Kit (DNA extraction Kit, Qiagen, USA). Extracted DNA was amplified by polymerase chain reaction (PCR) method. Due to a large sequence of exon 10 of the TSHR gene, two sets of DNA primers (each set of primers consisted of one forward and one reversed sequence) were used to amplify the DNA products in two parts (part I and part II) (Table 2) [11]. The PCR products were analyzed by running a 1% agarose gel, stained with ethidium bromide. The amplified products were purified by Qiagen Gel Extraction Kit and were sequenced using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and the results in the form of a biogram were analysed using the computer software BioEdit. Part I and Part II sequences of exon 10 were submitted to GenBank and the provided accession numbers were KT240093 and KT318753, respectively, for the patient, KT318754 and KT318755 for her mother, and KT318756 and KT318757 for her brother.
Results

Sequencing of exon 10 of the TSHR gene in the patient revealed a G to T transversion at nucleotide position 177035 (emb|AL136040.5|), predicted to result in a loss of function mutation changing glutamic acid 757 to stop codon, (E757Stop) in the intracellular domain of TSH receptor and also C to T (Transition) silent mutation at 177025 nucleotide position (emb|AL136040.5|), which leads to no change in amino acid sequence (glycine to glycine at 753 amino acid position).

No mutational changes were observed in the gene analysis of the mother and brother of the patient, and their thyroid hormone parameters including thyroid antibodies were within normal range.

Discussion

Indore is located in the central part of India. Although this is an iodine sufficient region, previous studies reported that the incidence of hypothyroidism including subclinical and autoimmune thyroiditis is very high in this region [12]. Post-partum thyroiditis (PPT) appears to be identical to a transient form of autoimmune thyroiditis, and by the presence of thyroid antibodies is considered secondary to an

Table 1. The physiological parameters of the patient during diagnosis and follow-up

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal range</th>
<th>At the time of diagnosis</th>
<th>6 monthly follow-up</th>
<th>Yearly follow-up</th>
<th>1.5 yearly follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical parameters:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height [cm]</td>
<td>NA</td>
<td>154</td>
<td>154</td>
<td>154</td>
<td>154</td>
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<tr>
<td>Weight [kg]</td>
<td>43.87–59.05</td>
<td>68.3</td>
<td>72.1</td>
<td>76.3</td>
<td>82.1</td>
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<tr>
<td>BMI [kg/m²]</td>
<td>18.5–24.9</td>
<td>28.8</td>
<td>30.4</td>
<td>32.2</td>
<td>35</td>
</tr>
<tr>
<td>Endocrinological, biochemical, and haematological parameters:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total T₃ [ng/dl]</td>
<td>60–181</td>
<td>53.8</td>
<td>–</td>
<td>–</td>
<td>93.2</td>
</tr>
<tr>
<td>Total T₄ [µg/dl]</td>
<td>4.5–12.6</td>
<td>2.3</td>
<td>–</td>
<td>–</td>
<td>8.6</td>
</tr>
<tr>
<td>TSH [µIu/ml]</td>
<td>0.35–4.94</td>
<td>52.0</td>
<td>8.7</td>
<td>1.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Free T₄ [ng/dl]</td>
<td>0.7–1.48</td>
<td>0.53</td>
<td>–</td>
<td>–</td>
<td>1.43</td>
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<tr>
<td>Anti Tg Ab [IU/ml]</td>
<td>≤ 4.11</td>
<td>≥ 1000</td>
<td>–</td>
<td>–</td>
<td>56.88</td>
</tr>
<tr>
<td>Anti TPO Ab [IU/ml]</td>
<td>≤ 5.61</td>
<td>≥ 1000</td>
<td>–</td>
<td>–</td>
<td>658.93</td>
</tr>
<tr>
<td>Total cholesterol [mg/dl]</td>
<td>120–220</td>
<td>234</td>
<td>216</td>
<td>–</td>
<td>220</td>
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<td>LDL cholesterol [mg/dl]</td>
<td>80–130</td>
<td>133</td>
<td>129</td>
<td>–</td>
<td>126</td>
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<tr>
<td>HDL cholesterol [mg/dl]</td>
<td>40–80</td>
<td>40</td>
<td>42</td>
<td>–</td>
<td>42</td>
</tr>
<tr>
<td>Hb [gm%]</td>
<td>11.5–16</td>
<td>11.0</td>
<td>–</td>
<td>–</td>
<td>12.9</td>
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<tr>
<td>RBC [million/mm³]</td>
<td>3.8–4.8</td>
<td>4.4</td>
<td>–</td>
<td>–</td>
<td>4.97</td>
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<tr>
<td>WBC [mm³]</td>
<td>4,000–10,000</td>
<td>10,000</td>
<td>–</td>
<td>–</td>
<td>7,100</td>
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<tr>
<td>Platelets [Lacs/mm³]</td>
<td>1.5–4.5</td>
<td>2.6</td>
<td>–</td>
<td>–</td>
<td>3.69</td>
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<tr>
<td>PCV (%)</td>
<td>36–46</td>
<td>34</td>
<td>–</td>
<td>–</td>
<td>39.4</td>
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<tr>
<td>MCHC (%)</td>
<td>28–40</td>
<td>32.7</td>
<td>–</td>
<td>–</td>
<td>32.7</td>
</tr>
</tbody>
</table>

Dose of L-thyroxine [µg/day]:

| | NA | NA | 0.0 | 100.0 | 125.0 | 125.0 |

Table 2. Working DNA primer sequence

<table>
<thead>
<tr>
<th>Name of exon</th>
<th>Primer sequence</th>
<th>Ta [ºC]</th>
<th>PCR product size [bp]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 10 (part I)</td>
<td>F – ACT GTC TTT GCA AGC GAG TT R – GTG TCA TGG GAT TGG AAT GC</td>
<td>50</td>
<td>875</td>
</tr>
<tr>
<td>Exon 10 (part II)</td>
<td>F – TGG CAC TGA CTC TTT TCT GT R – GTC CAT GGG CAG GCA GAT AC</td>
<td>50</td>
<td>868</td>
</tr>
</tbody>
</table>

Ta (Tm-S) – Annealing temperature, F – forward sequence (5’ → 3’), R – reversed sequence (3’ → 5’), bp – base pair.
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The thyroidal compensation in mild to moderate RTSH is expected to be clinically stable with no progression toward true hypothyroidism or spontaneous regression toward normal TSH levels. In contrast, development of overt hypothyroidism at the age of 15 years was shown in a patient homozygous for the R540H mutation presenting with compensated hypothyroidism in infancy, but not in an additional four subjects with the same genotype after long-term follow-up [21].

A mutation in the TSHR gene is very rare. To our best knowledge, we are reporting a novel loss of function mutation in TSHR protein at codon 757 (E757Stop), which has not been reported previously. Further study is needed to enumerate the prevalence and frequency of the incidence of the reported mutation in the population, especially in the Indian population.

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Conflict of interest

The authors declare no conflict of interest.

References


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