Variable Clinical Phenotypes in a Family with Homozygous c.1159G>A Mutation in the Thyroid Peroxidase Gene

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Abstract

Background: Defects in the thyroid peroxidase (TPO) gene have been associated with goitrous congenital hypothyroidism (CH). Case Report: In this study, we report 3 siblings possessing a homozygous mutation, c.1159G>A, but exhibiting different clinical phenotypes in a Malaysian-Malay family. The index patient was diagnosed with CH during a routine neonatal screening but the other 2 siblings appeared to be asymptomatic until the ages of 19 and 12.5, respectively, when they started to develop goiter. Results and Conclusion: The mutation was predicted to interrupt the correct splicing of pre-mRNA and also leads to structural alterations in the functional sites of the mutant TPO.
suggest the association of goiter development with a homozygous c.1159G>A mutation, but the CH in the index patient could be triggered by other genetic and epigenetic factors distinct from the c.1159G>A mutation. © 2014 S. Karger AG, Basel

Introduction

Congenital hypothyroidism (CH) is reported to affect 1 in every 3,000–4,000 newborn babies [1]. About 10–20% of cases of CH are due to thyroid dyshormonogenesis or defects in intermediary steps of thyroid hormone synthesis [2]. The concern for thyroid dyshormonogenesis increases with the presence of thyroid nodule or a goiter which can possibly lead to thyroid cancer [3, 4], although it is rare. Mutation in thyroid peroxidase (TPO) gene is known to be responsible for the majority cases of CH with dyshormonogenetic goiter [5]. TPO gene contains 17 exons which encode a protein with 933 amino acids in length that plays important roles in T₃ and T₄ hormone synthesis [6]. TPO defects are commonly inherited in an autosomal recessive mode [7, 8] but autosomal dominant inheritance had also been reported [9]. In 2007, Rivolta et al. [10] reported a patient with CH who was a compound heterozygote for a c.1159G>A mutation in exon 8 and a frameshift mutation in exon 5 of the TPO gene. In the present study, we describe variable phenotypes of members in a Malaysian-Malay family who were homozygotes for the c.1159G>A mutation in the TPO gene. The effects of the mutation on pre-mRNA splicing and protein function were analyzed using in silico methods.

Subjects and Methods

An infant (II-4) was diagnosed with CH during a routine neonatal screening with serum TSH 57.9 µU/ml and free T₄ 5.4 pmol/l. ⁹⁹Tm scintigraphy confirmed that he had a thyroid gland but later developed a diffuse goiter (right lobe 3.5 × 1.6 × 1.4 cm; left lobe 4.5 × 1.6 × 1.3 cm) when he was 7 years. Meanwhile, his elder sister (II-3), who was not diagnosed with CH, was noted to have a large multinodular goiter (right lobe 3.5 × 2.2 × 8.0 cm; left lobe 3.0 × 1.9 × 7.1 cm) at 12.5 years when the family members were examined and screened for this study. Her serum levels of TSH and free T₄ were initially normal but she developed hypothyroidism a year later. Her thyroid antibodies were negative but the human thyroglobulin levels were markedly increased at 999 ng/ml (normal range 0–55 ng/ml) 4 years later when she was followed regularly at the outpatient clinic. Both of them had normal growth and development. CH was also not detected in the eldest sister, II-1, initially. However, she also developed diffuse goiter with negative thyroid antibodies, and was diagnosed with biochemical hypothyroidism at the age of 19 years. No other congenital anomalies were detected in the proband, II-1 and II-3. Another sibling, II-2 and their parents (I-1 and I-2) who were consanguineous (second cousins) were all healthy, euthyroid, without goiters and no congenital anomalies. Iodine levels were optimum for all of them except for the proband who had moderate iodine deficiency (urinary iodine <18.75 µg/l) and his asymptomatic father (I-1) who had mild iodine deficiency (urinary iodine 56.54 µg/l). Genomic DNA was extracted from peripheral venous blood from the proband, the affected sisters and his other healthy family members using QIAamp® DNA Blood Mini Kit (Qiagen, Germany). The TPO gene was PCR-amplified with flanking intronic primers covering all the 17 exons using previously described protocols [8]. The effect of the detected mutation on enzyme activity was evaluated using PolyPhen-2 [11] and SIFT [12] algorithms. For structural comparison purpose, homology models of the human TPO including wild-type and mutant proteins were generated as described before [8]. Human splicing finder [13] analysis was performed to predict the impact of the nucleotide substitution on splicing signals of TPO transcript. This study was approved by the University of Malaya Medical Centre (UMMC) Ethical Committee (Institutional Review Board) in accordance to the ICH-GCP Guideline and the Declaration of Helsinki (Ref. No. 654.16).

Results and Discussion

A documented mutation, c.1159G>A, which is located in exon 8 of the TPO gene, was detected in the proband (fig. 1a). He was shown to carry the mutation in a homozygous form. The nucleotide change was predicted to cause an amino acid substitution of glycine by arginine at codon 387 (p.Gly387Arg). SIFT and PolyPhen analyses predicted that the mutation is ‘benign’ to the TPO function. Protein secondary structure prediction analysis carried out by Rivolta et al. [10] showed that the mutation causes a reduced stretch of the β-sheet structure. In this study, 3-D homology models of the wild-type and p.Gly387Arg mutant TPO were generated and compared for further evaluation of the effect of the mutation on protein function. Early prediction for conformational changes in the β-sheet structure of the mutant was also reflected in the current homology model analyses, even though at different regions (fig. 1b). It was also found that the area around the heme-binding site (Glu-399) [14] and the transition state stabilizer site (Arg-396) [15] of the mutant are less negatively charged if compared to the wild-type protein (fig. 1b). Depolarization of this area could be attributable to the induced conformational change in the binding pocket when Gly387 is replaced by Arg, and it is highly likely that the heme-binding process and/or the stability of the protein are affected.
**Homozygous c.1159G>A Mutation Detected in a Malaysian-Malay Family**

**Fig. 1.** 

**a** DNA sequencing profiles. Electropherogram profiles of a control with a wild-type allele (i), and II-4 (proband) who is a homozygote for the c.1159G>A mutation (ii). The single nucleotide transition c.1159 is indicated by the arrow. The nucleotide change is predicted to cause an amino acid substitution of glycine to arginine at residue 387 (p.Gly387Arg).

**b** Computer-generated models illustrating the 3-D orientation of the wild-type (i) and mutant p.Gly387Arg (ii) TPO proteins. The protein backbones are presented as ribbons (α-helix in red, β-pleated sheet in cyan, coils in grey, and turns in green). Residues Pro-377 to Glu-399 are represented as Connolly surface to allow the visualization of the conformational changes in the binding pocket. The Connolly surface is colored according to electrostatic potential spectrum (negative potential, in red, to neutral, in white, to positive, in blue). Regions in yellow rings highlight the reduced stretch of β-sheet structure observed when the wild type (i) changes to the mutant TPO protein (ii).

**c** Family pedigree of the index patient. A family pedigree of siblings with hypothyroid goiter. The proband is indicated by an arrow.
Recent reports have shown that mutations in exonic sequences might disrupt the sequence recognized by splicing regulators and lead to abnormal splicing or exon skipping [16, 17]. In the present study, human splicing finder analysis revealed that sequence alteration caused by the c.1159G>A mutation is predicted to create a new acceptor splice site at c.1150 for exon 8 of the TPO gene and possibly give rise to the production of a TPO mRNA transcript 341 bp shorter than that of the wild type. Alternatively, the alteration in the ESE-binding site sequence due to the c.1159G>A mutation might interrupt the correct splicing of pre-mRNA and lead to the activation of other potential splice sites in exon 8 which subsequently give rise to the production of various TPO protein isoforms. Since exon 8 of the TPO gene has been suggested to code for a site that participates in catalytic mechanism [18], any modifications or absence of this critical region can thus cause the translated TPO protein devoid of, or reduced in, enzymatic activity.

In this study the c.1159G>A mutation was also detected in other family members of the proband. DNA sequencing analysis revealed that the healthy proband’s father, mother and 1 of the sisters (II-2) were all heterozygotes for the c.1159G>A mutation. Meanwhile, his sisters (II-1 and II-3) were shown to carry the c.1159G>A mutation. In summary, we report different clinical phenotypes in a Malaysian-Malay family with dys hormonogenetic goiter associated with a homozygous c.1159G>A mutation. This project was financially supported by grants from the Ministry of Higher Education, Malaysia (FP050/2010B) and Postgraduate Research Fund, University of Malaya (PV116-2012A).

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Disclosure Statement

The authors have no conflict of interests to disclose.

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