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BRIEF REPORT

A New Case of Congenital Goiter with Hypothyroidism Caused by a Homozygous p.R277X Mutation in the Exon 7 of the Thyroglobulin Gene: A Mutational Hot Spot Could Explain the Recurrence of This Mutation

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Identification of thyroglobulin (TG) gene mutations may provide insight into the structure-function relationship. In this study, we have performed molecular studies in a patient with congenital goiter, hypothyroidism, and impairment of TG synthesis. Genomic DNA sequencing revealed a homozygous c.886C→T mutation in exon 7, resulting in a premature stop codon at amino acid 277 (p.R277X). The same nonsense mutation had been reported previously in two Brazilian families with multiple occurrence of congenital hypothyroidism with goiter. We compared the insertion/deletion polymorphism in intron 18, microsatellites (Tgm1, Tgm2, TGrI29, and TGrI30), and exonic single-nucleotide polymorphism haplotypes identified in the patient with a member of the previously reported family, who also carry the mutation as a compound heterozygous mutation. The single-nucleotide polymorphism and microsatellite analysis revealed that the two affected individuals do not share a common TG allele. This suggests that the p.R277X mutation is a mutational hot spot. No difference in either splicing or abundance of the amplified product was detected by RT-PCR, excluding that an alternative splicing mechanism, by skipping of exon 7, would restore the normal reading frame. In conclusion, we report a new case of congenital goiter and hypothyroidism caused by a p.R277X mutation in the TG gene. Moreover, we show that nucleotide 886 is a mutational hot spot that explains the recurrence of this mutation. **(*J Clin Endocrinol Metab* 90: 3766–3770, 2005)**

CONGENITAL GOITER IS a subtype of primary congenital hypothyroidism caused by a mutation in one of the genes coding for the proteins responsible for thyroid hormone synthesis. These include Na^+/I^- symporter (NIS), thyroglobulin (TG), thyroperoxidase (TPO), thyroid oxidase 2 (THOX 2, also known as DUOX 2), and Pendrine (PDS, also known as SLC26A4) genes (1).

In the last decade, several mutations in the TG gene were reported. In animals, TG mutations have been observed in Afrikander cattle (p.R697X) (2), Dutch goats (p.Y296X) (3), cog/cog mouse (p.L2263P) (4), and rdw rats (p.G2320R) (5). Nine different mutations leading to congenital goiter and hypothyroidism have been identified and characterized in the human TG gene: g.IVS3-3 C→G (6), p.R277X (exon 7) (7, 8), p.362fsX382 (exon 9) (9), p.C1245R (exon 17) (10), p.R1511X (exon 22) (8), g.IVS30+1G→T (11), p.C1977S (exon 33) (10), g.IVS34-1G→C (8), and p.R2223H (exon 38) (9). Mutations in the TG gene also have been reported associated with endemic (12) and nonendemic simple goiter (13, 14).

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Abbreviations: Indel, Insertion/deletion; SNP, single-nucleotide polymorphism; TG, thyroglobulin.

We previously identified two unrelated Brazilian families harboring the p.R277X mutation (7, 8). The affected individuals of these families were homozygous (7) or compound heterozygous (8) (p.R277X/p.R1511X, p.R277X/g.IVS34-1G→C) for the nonsense mutation. The premature stop codon resulted in a grossly truncated protein of only 276 amino acids with marked reduction in the ability to generate thyroid hormone.

We report here a new case of congenital goitrous hypothyroidism, in an Argentinean family, that is homozygous for the p.R277X mutation. The present study suggests that this TG mutation is a mutational hot spot.

Patient and Methods

Clinical report

A detailed clinical and laboratory evaluation of patient 1 has been reported previously (15). Patient 1 had goiter, congenital hypothyroidism, and a marked impairment of TG synthesis.

Written informed consent was obtained from the individuals involved in this study, and the research project was approved by the institutional review board.

Genomic DNA isolation

DNA was obtained from goitrous thyroid tissue in patient 1. Genomic DNA was isolated by the SDS-proteinase K method.

DNA sequencing

The promotor region and the exons 1, 3, 4, 7, 9, 10, 12, 16, 17, 18, 21, 22, 29, 30, 33, 35, 38, 43, 44, 46, and 48 of the human TG gene, including splicing signals and the flanking intronic regions of each intron, were amplified using the primers and PCR conditions reported previously (8, 9). Both the sense and antisense strands were sequenced using the same TG-specific primers used in the amplification with the BigDye Terminator cycle sequencing kit (Applied Biosystems, Weiterstadt, Germany). The samples were analyzed on the ABI Prism 3100 DNA sequencer (Applied Biosystems).

Insertion/deletion (Indel) polymorphism analysis

The large Indel polymorphism of 1464 bp localized in intron 18 of the human TG gene (16) was analyzed by multiplex PCR, using the primers and PCR conditions described previously. The amplified fragments were analyzed in a 2% agarose gel.

Microsatellite analysis

The Tgm1, Tgm2, TGrI29, and TgrI30 microsatellites, localized in introns 10, 27, 29, and 30 of the human TG gene, respectively, were typed as reported elsewhere (17, 18). PCR products were resolved by electrophoresis in 6% polyacrylamide denaturing gels.

RT-PCR amplification

The primers and conditions used for RT-PCR and the amplified regions were described previously (11). DNA sequences from generated fragments were analyzed with the *Taq* polymerase-based chain terminator method (fmol sequencing system; Promega, Madison, WI) and using the TG-specific forward and reverse primers used in the RT-PCR.

Results

The first and last exon of the TG gene, the exons and their intronic flanking regions where previously mutations and polymorphisms were detected, as well as 180 bp of the TG promoter, were analyzed from patient 1. The GT-AG splicing consensus sequences are rigorously respected in all introns analyzed. Direct sequencing of exon 7 revealed a homozygous cytosine-to-thymine transition at nucleotide position 886, near the end of the exon. Instead of encoding for an arginine residue on position 277, the triplet harboring the mutation encodes a stop codon. The same nonsense mutation (p.R277X) had been reported previously in two Brazilian families with multiple occurrence of congenital hypothyroidism with goiter (7, 8).

The previously characterized Indel (located in intron 18) (16); the microsatellites located in introns 10 (Tgm1), 27 (Tgm2), 29 (TGrI29), and 30 (TgrI30) (17, 18); and the exonic TG single-nucleotide polymorphisms (SNPs) [c.229G→A (p.G58S), c.2200T→G (p.S715A), c.2334T→C (p.P759), c.2488C→G (p.Q811E), c.3082A→G (p.M1009V), c.3474T→C (p.S1139), c.3935G→A (p.G1293D), c.4506C→T (p.A1483), c.5512A→G (p.N1819D), c.5995C→T (p.R1980W), c.6695C→T (p.P2213L), c.7408C→T (p.L2451), c.7501T→C (p.W2482R), c.7589G→A (p.R2511Q), and 7920C→T (p.Y2621)] (19) proved to be interesting and informative polymorphisms for investigating whether a common ancestral chromosome or a mutational hot spot accounted for the occurrence of the same mutation in all of the affected individuals. We compared the haplotypes identified in patient 1 with the haplotypes previously reported from the III-2 member of the previously reported MA family (8), who also carry the mutation as a compound heterozygous mutation (p.R277X/g.IVS34-1G→C). The Indel 18 and TGrI29 and TGrI30 microsatellite results showed that the two affected individuals shared a common Indel and TGrI29 and TGrI30 microsatellite haplotypes associated with the p.R277X mutation. Patient 1 is homozygous for the 541-bp Indel allele, 201-bp TGrI29, and 538-bp TGrI30 alleles, whereas III-2 is homozygous for the 541-bp Indel allele, heterozygous for the 199-bp and 201-bp TGrI29 alleles, and homozygous for the 538-bp TGrI30 allele (Fig. 1). In III-2, the 201-bp TGrI29 allele is associated with the presence of the p.R277X mutation, whereas the 199-bp TGrI29 allele is associated with the g.IVS34-1G→C mutation (8). However, the Tgm1 and Tgm2 microsatellites and SNP analysis reveal that the two affected individuals do not share any TG alleles. As shown in Fig. 1, patient 1 was homozygous for C, G, T, and C in the SNPs localized in the nucleotide positions 2334, 3082, 7501, and 7920, respectively. In contrast, III-2 was homozygous for T, A, C, and T in the same SNPs. Patient 1 was homozygous for 307-bp Tgm1 and 340-bp Tgm2 alleles, whereas III-2 was heterozygous for the 305-bp and 307-bp Tgm1 and 328-bp and 338-bp Tgm2 alleles. In III-2, the 305-bp Tgm1 and 338-bp Tgm2 alleles are associated with the presence of the p.R277X mutation, whereas the 307-bp Tgm1 and 328-bp Tgm2 alleles are associated with the g.IVS34-1G→C mutation (data not shown). Patient 1 was heterozygous for the c.5995C→T SNP (Fig. 1). According to these results, it is very likely that the p.R277X mutation is a mutational hot spot.

Finally, RT-PCR of the TG mRNA was performed on total RNA isolated from patient 1 and from thyroid tissues from a wild-type control, resulting in 12 overlapping fragments that range from 638-1076 bp, covering the total coding region of 8307 bp. No difference in either splicing or abundance of the amplified product was detected. The homozygous state of the 886C→T substitution was confirmed in the proposita, by the sequencing of the 5′ region that mapped between nucleotide positions 57 and 2399.

Discussion

We report a patient of Argentinean origin with congenital hypothyroidism and goiter caused by TG deficiency. The diagnosis of dyshormonogenesis was based on the high ^{131}I uptake, lower normal limit serum TG, elevated serum TSH with simultaneous low serum T_4 and T_3 levels, and perchlorate discharge test interpreted as negative (15). Molecular analyses revealed a homozygous p.R277X, which has been found before to cause TG deficiency. Congenital hypothyroidism as a result of impaired TG synthesis was also the initial diagnosis made for the sister of the propositus (15). Because the DNA samples from father, mother, and sister of patient 1 are unavailable at present, it is not possible to confirm the presence of the p.R277X mutation in them.

TG is a homodimeric glycoprotein of 660 kDa synthesized and secreted by the thyroid cells into the follicular lumen. TG is encoded by a single gene of 270 kb located on chromosome 8q24 and contains an 8.5-kb coding sequence divided into 48 exons (19). The preprotein monomer is composed of a 19-amino-acid signal peptide followed by a 2749-residue polypeptide. The monomeric primary structure is characterized by the presence of three types of repetitive units that

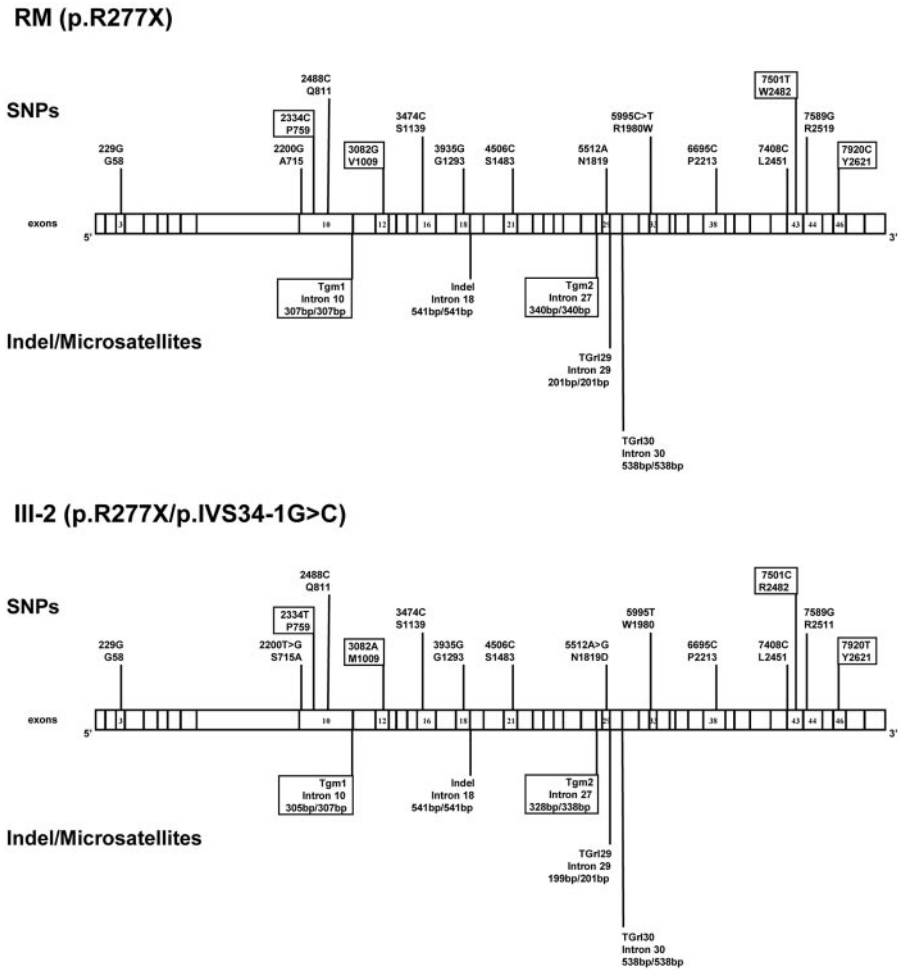

FIG. 1. Comparative haplotype analysis of patient 1 and III-2, using SNP, Indel, and microsatellite polymorphisms of the TG gene. The informative variations between patient 1 and III-2 are *boxed*. The nucleotide position of the SNPs is designated according to TG mRNA reference sequences (GenBank accession no. NM_003235). The A of the ATG of the initiator methionine codon is denoted nucleotide +1. The amino acid positions are numbered after subtracting the 19-amino-acid signal peptide.

include 11 type-1, three type-2, and five type-3 repeat motifs (19). However, the carboxyl-terminal domain of the molecule is not repetitive and shows a striking homology with acetylcholinesterase. A correct three-dimensional structure is essential for thyroid hormonogenesis. The p.R277X mutation results in a grossly truncated protein of 276 amino acids with limited ability to generate thyroid hormone (Fig. 2). Four hormonogenic acceptor tyrosines have been identified and localized at positions 5, 1291, 2554, and 2747, and several other tyrosines localized at positions 130, 847, and 1448 have been proposed as donor sites that transfer an iodophenoxyl group to an acceptor iodotyrosine (19). The most important hormonogenic acceptor site is at tyrosine 5, which couples with the donor tyrosine at position 130. The truncated p.R277X form of TG still harbors both the acceptor tyrosine 5 and the donor tyrosine 130 (Fig. 2). However, the premature stop codon eliminates the carboxyl-terminal hormonogenic domain, resulting in the loss of thyroid hormone formation. Interestingly, a 26-kDa amino-terminal TG peptide with the hormonogenic acceptor site at tyrosine 5 retained its ability for T_4 synthesis (20). In agreement with this observation, it should be considered that the small p.R277X peptide is sufficient for the synthesis of T_4 in the N-terminal domain. The glycosylation of the TG is an essential process that permits the migration from the endoplasmic reticulum to the Golgi. *In vitro* expression of the truncated p.R277X TG cDNA showed that the mutated TG protein can be glycosylated (7) (Fig. 2), indicating their possible exportation to the apical surface of thyrocytes. Several endoplasmic reticulum chaperones, such as calnexin, Grp94, and Bip, interact with TG during its maturation and may serve to prevent exportation of improperly folded TG proteins (19). This process is known as endoplasmic reticulum quality control. The p.R277X TG protein would have reduced ability to be exported from the endoplasmic reticulum because of massive induction of molecular chaperones, which bind to the exportable truncated protein. The present case, together with two other p.R277X mutations described in a previous study (7, 8), excluded an alternative splicing mechanism, by skipping exon 7, to restore the normal reading frame disrupted by the nonsense mutation and eliminate the stop codon that would truncate the protein.

It is of clinical and public health interest to know whether this mutation is an independently recurrent mutation or whether it is a result of a founder effect. To discriminate between a *de novo* recurrence of the p.R277X mutation and a founder effect, we genotyped a set of 15 exonic SNPs, one Indel, and four microsatellites localized in the TG gene. Two affected individuals from two unrelated families with congenital goiter and hypothyroidism harboring the mutation

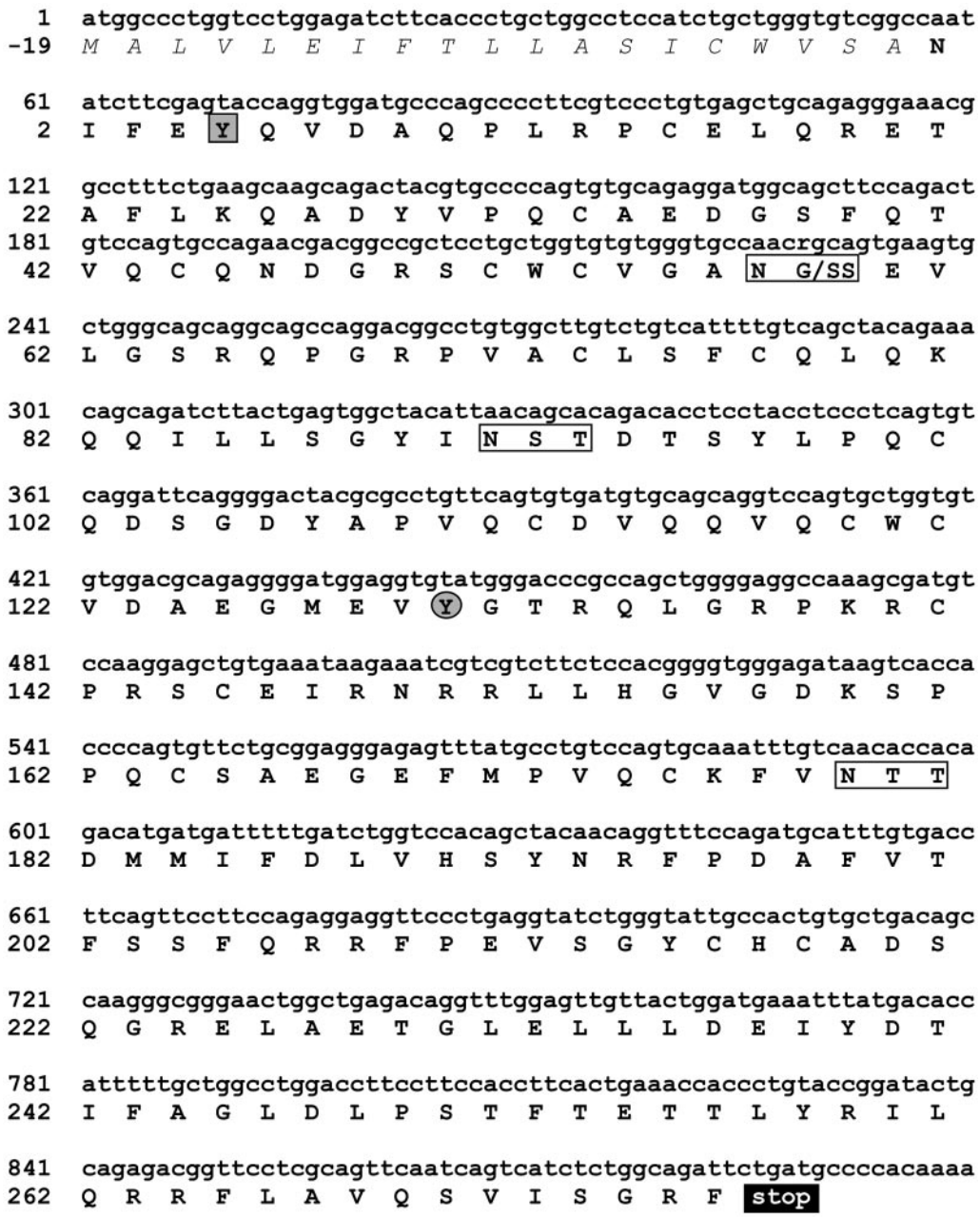

FIG. 2. Deduced amino acid sequences from p.R277X truncated TG protein. The nucleotide sequence is given in the *upper line*, and the amino acid translation (represented by single-letter code) is given below their respective codons. The 19-amino-acid signal peptide is in *italic letters*. The nucleotide position is designated according to TG mRNA reference sequences (GenBank accession no. NM_003235). The A of the ATG of the initiator methionine codon is denoted nucleotide +1. The amino acid positions are numbered after subtracting the 19-amino-acid signal peptide. *Gray shaded square*, Acceptor tyrosine 5; *gray shaded circle*, donor tyrosine 130. Putative N-glycosylation sites are *boxed*.

p.R277X, either in a homozygous state (patient 1) or as a compound heterozygous mutation (III-2) (8) do not share common TG alleles (Fig. 1). We therefore suggest that the p.R277X mutation is a mutational hot spot. Interestingly, patient 1 displays only one heterozygous polymorphism of the 20 investigated, at nucleotide position 5995 (C→T). This result indicates that patient 1 inherited from his parents different TG alleles, supporting that a mutational hot spot mechanism is responsible for the p.R277X mutation. However, in the absence of haplotype data from the father and mother of the patient, we cannot exclude that a *de novo* mutation could be considered as a possible event for the 5995C→T polymorphism. The nonsense mutation in exon 7 occurs in a CpG dinucleotide sequence and could be caused by deamination of a methylated cytosine resulting in a thymine. In this sense, the CGA arginine codon is considered a hot spot for mutations in mammalian DNA.

In conclusion, we report a new case with congenital goitrous hypothyroidism caused by the p.R277X mutation of the TG gene. Haplotype studies indicate that a mutational hot spot

could explain the recurrence of this mutation. Because the p.R277X mutation is the most frequently reported mutation in the TG gene, it would be helpful to investigate additional cases with congenital hypothyroidism regarding this mutation.

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