Monoallelic thyroid peroxidase gene mutation in a patient with congenital hypothyroidism with total iodide organification defect

Mutação monoalélica no gene da tireoperoxidase em paciente com hipotireoidismo congênito com defeito total de incorporação de iodeto

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SUMMARY

The aim of this study was to identify the genetic defect of a patient with dyshormonogenetic congenital hypothyroidisms (CH) with total iodide organification defect (TIOD). A male child diagnosed with CH during neonatal screening. Laboratory tests confirmed the permanent and severe CH with TIOD (99% perchlorate release). The coding sequence of *TPO, DUOX2*, and *DUOXA2* genes and 2957 base pairs (bp) of the *TPO* promoter were sequenced. Molecular analysis of patient's DNA identified the heterozygous duplication GGCC (c.1186_1187insGGCC) in exon 8 of the *TPO* gene. No additional mutation was detected either in the *TPO* gene, *TPO* promoter, *DUOX2* or *DUOXA2* genes. We have described a patient with a clear TIOD causing severe goitrous CH due to a monoallelic *TPO* mutation. A plausible explanation for the association between an autosomal recessive disorder with a single *TPO*-mutated allele is the presence of monoallelic *TPO* expression. Arq Bras Endocrinol Metab. 2010;54(8):732-7

SUMÁRIO

O objetivo deste estudo foi identificar defeitos genéticos em paciente com hipotireoidismo congênito (HC) por disormonogênese e defeito total de incorporação de iodeto (DIIT). Neonato do sexo masculino com HC diagnosticado pelo rastreamento neonatal. Exames clínicos e radiológicos confirmaram que o paciente apresentava HC severo e permanente com DIIT (teste de perclorato: 99%). A região codificadora dos genes *TPO, DUOX2, DUOXA2* e 2957 pares de bases (pb) do promotor de *TPO* foram sequenciados. No paciente foi identificada a duplicação em heterozigose GGCC no éxon 8 do gene *TPO* (c.1186_1187insGGCC). Nenhuma outra mutação foi localizada nos genes *TPO*, incluindo o promotor, *DUOX2* ou *DUOXA2*. Descrevemos paciente com grave defeito de organificação de iodeto, provocando HC severo com bócio, em consequência de uma única mutação monoalélica no gene *TPO*. A expressão monoalélica no tecido tireoideano explicaria a associação de uma doença autossômica recessiva com uma única mutação monoalélica. Arq Bras Endocrinol Metab. 2010;54(8):732-7

INTRODUCTION

Congenital hypothyroidism (CH) is the most common cause of preventable mental retardation with prevalence ranging from 1:2000 to 1:3000 newborns. In recent years prevalence has increased due to lower TSH cutoff values (10 mU/mL) used in the newborn screening programs (1).

Confirmatory biochemical exams associated with imaging and perchlorate tests are performed when the child reaches three years of age. The tests are conducted after four weeks of L-thyroxine withdrawal, when there is no risk of brain damage.

The most common cause of congenital hypothyroidism is thyroid dysgenesis (athyreosis, hemiagenesis, hypoplasia, and ectopia) seen in approximately 75% of the cases. Defects in thyroid hormone synthesis, or dyshormogenesis, accounts for 15%-20% of the cases and is caused by defects in genes involved in the thyroid-hormone synthesis pathway (2). Mutations in thyroid peroxidase (*TPO*) are the most frequent genetic alterations in dyshormonogenesis, with prevalence of 1/40,000 newborns (3). Mutations in thyroglobulin (*TG*) (4), sodium iodide symporter (*NIS*), pendrin (*PDS*), dual oxidase 2 (*DUOX2*) (5), dual oxidase maturation factor 2 (*DUOXA2*) (6), and iodotyrosine deiodinase (*DEHAL1*) (7) genes have been also identified in patients with dyshormonogenesis.

TPO is a heme-containing enzyme localized on the apical membrane of the follicular cell. TPO catalyzes the iodination of tyrosine residues in thyroglobulin, and couples these residues to form thyroid hormones (8).

TPO mutations have been associated with total iodide organification defect (TIOD), where more than 80% of iodide is discharged from the intrathyroidal iodide pool, indicating that the iodide that is taken up was not incorporated into the TG protein. Partial iodide organification defects are associated with iodide discharge levels of between 25% and 79% (9). More than 60 *TPO* gene mutations have been previously described. Linkage studies have characterized the autosomal recessive mode of inheritance of *TPO* gene disorders (3).

The aim of this study was to identify the genetic defect of a patient with dyshormonogenetic CH and TIOD.

SUBJECTS

The index patient is a boy who was diagnosed with CH during neonatal screening with serum TSH of 35.9 µUI/mL. His parents were not consanguineous. He was started on a daily dose of L-thyroxine (12.5 mg/kg) at 30 days of life. CH was confirmed at the age of 3 years, when the medication was suspended for 4 weeks and laboratory tests showed: serum TSH: 635.3 µUI/mL (reference value: 0.5 – 4.5 µUI/mL); total T4 (T4T): 1.0 µg/dL (reference value: 4 – 11 µg/dL), Free T4 (FT4): 0.1 ng/dL (reference value: 0.7 – 1.7 ng/dL); TG: 129.6 ng/mL (reference value: 5 – 30 ng/mL) and iodide discharge after perchlorate: 99% (reference value < 15%). The scintigraphy and ultrasound examinations indicated a heterogeneous eutopic, larger than normal thyroid gland.

This study was approved by the Ethical Committee of the Hospital das Clínicas, University of São Paulo Medical School. Informed consent was obtained from the parents of the patient.

METHODS

Thyroid function tests

Serum Total T4 (TT4), free T4 (FT4), total T3, TSH, and TG levels were determined by electrochemiluminescence immunoassay (Roche Corporation, Indianapolis, IN). Perchlorate discharge test was performed with 25 mCi of radiodine ^{131}I administered orally and thyroid uptake was measured after 1 hour. At this point 0.5 g of potassium perchlorate was orally administered and sequential uptakes were measured at 15-minute intervals for an additional hour. The effect of perchlorate was expressed as a percentage of radioiodine uptake at 1 hour. Thyroid volume was calculated after echographic studies. Scintigraphy was performed with ^{131}I.

DNA sequencing

Peripheral blood DNA from the patient, his parents and his brother was isolated by the sodium dodecyl sulfate/proteinase K method. The complete coding sequence of the human *TPO* gene, including splice sites and flanking intronic regions of each exon, was amplified using primers and conditions reported previously (9). A *TPO* promoter region of 2957 bp was also sequenced with specific primers (Table 1).

DNA sequencing of each amplified fragment was performed with a ABI 377 system (Applied Biosystems Corp., Foster City, CA) using the same *TPO*-specific primers used in the amplification step. The sequences were compared with those of the human *TPO* gene sequence (GeneID: 7173).

Table 1. Primers used to amplify 2957 bp of the *TPO* promoter

Name	Primer forward	Primer reverse	PCR product size (bp)
ProTPO 1	5' gccccttttcacagggtat 3'	5 'AACTGCACTGCTGAGTA 3'	375
ProTPO 2	5' tgaggattgaggggagaatg 3'	5' ctctggccgaatgagttagg 3'	342
ProTPO 3	5' gaagcctttgcatcgtgttt 3'	5' cccaagcccctagttttctt 3'	451
ProTPO 4	5' aggatggctgaatcctcaga 3'	5' gacttggagcctcttcatgc 3'	482
ProTPO 5	5' cctagacgctggtgctctg 3'	5' ccagactcggtggctcatta 3'	425
ProTPO 6	5' gagacttttggcagcaaggt 3'	5' agctgttgggtgaagtccag 3'	466
ProTPO 7	5' ggctacaaaacgacctggag 3'	5' ccatgagcctccagaaactg 3'	406

In order to amplify and sequence the coding region and intron-exon borders of the *DUOX2* and *DUOXA2* gene we used the primers and conditions previously described (5,6). These sequences were compared with those of human *DUOX2* and *DUOXA2* (GeneID: 50506 and GeneID: 405753, respectively).

A control group of 100 healthy subjects without thyroid disease was evaluated to determine whether the observed DNA substitutions were *bona fide* mutations or polymorphisms. All control subjects had normal FT4 (0.7-1.5 ng/dL) and TSH levels (0.5-4.5 U/mL).

RESULTS

Screening for *TPO* gene mutations identified the known insertion of 4 base pairs GGCC in position 1186 of the cDNA (c.1186_1187insGGCC) (or in position 1227 of the DNA gene), in exon 8, which introduces a new stop codon in exon 9 (p.Ala396fsX472) (10). This mutation was present in a heterozygous form (Figure 1) and was the only mutation identified in the *TPO* gene. Polymorphisms were also found in the promoter, intronic and coding sequences of the *TPO* gene (Table 2).

The 33 exons of the *DUOX2* gene were sequenced revealing only polymorphisms in this gene. These *DUOX2* alterations were also found in more than 1% of the control subject's DNAs (Table 3). The sequence analysis of *DUOXA2* gene did not show sequence alterations in the patient's DNA.

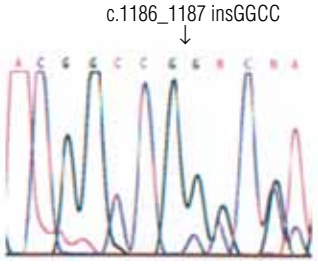

Figure 1. Sequence chromatograms showing the heterozygous mutation (c.1186_1187insGGCC) in exon 8.

The c.1186_1187insGGCC mutation was not identified in his mother, stepfather or brother's DNA. Polymorphism's analysis confirmed that there was not genetic relation between the patient and the stepfather.

Table 2. Polymorphisms identified in the promoter, intronic and coding sequences of the *TPO* gene of the patient reported in this study

Region	DNA position	Nucleotide change	Amino acid substitution
Promoter	-1197	A/A	-
Promoter	-706	G/G	-
Promoter	-35	A/A	-
Exon 1	16	A/A	-
Exon 2	102	C/G	-
Intron 4	+31	T/C	-
Exon 7	859	G/T	Ala257Ser
Exon 8	1207	G/T	Ala373 Ser
Exon 8	1283	G/C	Ser398Thr
Exon 11	2088	C/T	-
Exon 12	2235	C/T	-
Exon 12	2263	A/C	Thr725Pro
Exon 15	2630	C/C	Val847Ala

Table 3. Polymorphisms identified within *DUOX2* gene sequence

DNA position	Nucleotide change	Amino acid substitution
413	C>T	Pro100Leu
567	C>T	Gly131Arg
598	G>A	-
633	C>T	-
1461	G>C	-
2033	A>G	His678Arg
2102	G>A	Arg701Gln
2281	G>A	-
3200	C>T	-
gIVS14 +65C>A	-	-
gIVS17+10C>T	-	-
gIVS25+15T>A	-	-
gIVS33+29G>T	-	-

DISCUSSION

TPO enzymatic activity is essential for thyroid hormonogenesis. Inactivating TPO mutations are the most frequent molecular basis for dyshormonogenetic congenital goitrous hypothyroidism due to iodide organification defect (3).

The most frequently studied phenotype is the TIOD, characterized by iodide discharge after perchlorate > 80%. Most affected patients present with severe and permanent hypothyroidism with mental retardation if not treated in the neonatal period. Discharge levels between 25% and 79% are denominated partial iodide organification defects (PIOD). The variety of clinical features in PIOD patients suggests that diverse mechanisms such as delayed or reduced activity of enzymes involved in hormonogenesis, or defects in iodine storage and release (11) may lead to PIOD.

The established molecular basis of TIOD is either due to biallelic (homozygous or compound heterozygous) inactivation mutations in the *TPO* gene, or biallelic inactivation mutations in the *DUOX2* gene. Biallelic inactivation of *DUOX2* results in a complete block of H_2O_2 generation, a relatively rare genotype (5). More frequent, are the inactivating mutations in the *TPO* gene resulting in a TIOD (3).

Few studies have described cases of PIOD due to *TPO* biallelic mutations (9,12,13). Mutations in *DUOX2* and *DUOXA2* have also been reported in PIOD patients (5,6). Inactivating monoallelic *DUOX2* mutations have also been reported in relation to transient forms of relatively mild hypothyroidism (5).

In this study we described a male child with severe congenital goitrous hypothyroidism and TIOD, extremely low serum FT4 and TT4 coexisting with very high levels of serum TSH and TG. Moreover a high iodine uptake and 99% of iodide discharge after perchlorate confirmed the absence of organification of iodide. Molecular analysis of the patient's DNA indicated presence of duplication of the tetranucleotide GGCC (c.1186_1187insGGCC) in exon 8 of the *TPO* gene, in heterozygous state. Interestingly, this was the only mutation identified in the whole *TPO* coding region, in the intron-exon borders and in ~3000bp of the *TPO* promoter. No additional mutation was found either in DUOX2 or DUOXA2 genes.

This mutation has been previously identified in other Brazilian TIOD patients as well as in other reports (3). The resulting frameshift introduces a new stop codon in exon 9, which could result in a grossly truncated protein and promote a drastic reduction of TPO activity in the affected thyroid tissue (Figure 2) (10).

Cases with a typical TIOD phenotype and a single *TPO*-mutated allele have also been described. The frequency of this phenomenon is unexpectedly high. It has been found in around 9% to 65% of the reported TIOD or PIOD patients of different geographic origin (9,14-18). In our experience, three out of four TIOD Brazilian patients and three out of six PIOD patients, all with *TPO* mutations, had only one mutant allele (data not shown).

Genetic explanations for monoallelic *TPO* mutations in TIOD patients may be the presence of intronic mutations creating an alternative splicing site or of mutations located in the distal promoter, more than 3000 bp upstream of the transcription initiation site. The presence of large gene deletions has also been advocated. Polymorphisms, however, both in the coding and noncoding regions of *TPO* patient's gene deny this possibility.

Other mechanisms that may explain our's and other's TIOD cases with a single *TPO*-mutated allele is the presence of monoallelic expression in the thyroid tissue, as has been shown by Fugazzola and cols. (18). This group reported one family with TIOD due to monoallelic expression of a maternal mutant *TPO* allele in the thyroid tissue.

In diploid eukaryotic organisms, there are three different classes of monoallelically expressed genes. One class is the autosomal imprinted genes whose monoallelic expression is regulated in a parent-of-origin-specific manner. A second class is X-inactivated genes regulated by a random process. The third class is autosomal genes subject to random monoallelic expression (such as odorant receptor, immunoglobulins, T-cell receptors, interleukins, and natural killer cell receptors) (19). A recent study has shown that this last class of genes is relatively frequent. Using a general strategy for genome-wide analysis of monoallelic expression, Gimelbrant and cols. (19) showed that ~ 5% to 10% of 4000 assessed autosomal genes display random monoallelic transcription in human cells with stability in each clonal cell line.

Unusual regulatory mechanisms have been observed for monoallelically expressed genes: e.g., DNA rearrangement and allele-specific DNA methylation for the immunoglobulin gene; a single enhancer associated with multiple odorant receptor gene promoters located on different chromosomes (19). Other transcriptional or post-transcriptional mechanisms may also be considered, such as alternative polyadenylation, chromatin remodeling, histone modifications, RNA edditing and microRNA regulation.

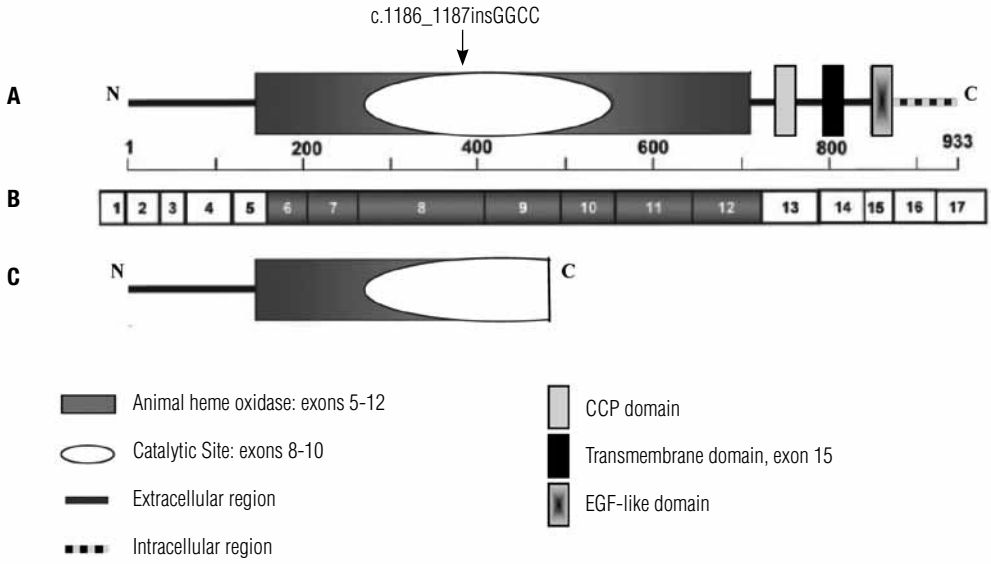

Figure 2. (**A**) Schematic normal TPO, showing protein domains: heme oxidase region (exons 5-12); catalytic site (exons 8-10); transmembrane domain (exon 15); CCP; and EGF-like domain; intra and extracellular regions of the protein (adapted from (20). (**B**) cDNA of human TPO showing the localization of the mutation (c.1186_1187insGGCC) which introduces a new stop codon in exon 9 (p.Ala396fsX472). (**C**) Small inactive mutant protein, that lost important domains.

The mechanism resulting in monoallelic *TPO* expression may also be independent of parental origin. Fugazzola and cols. (18) detected the maternal mutant *TPO* allele expression associated to the lack of paternal allele transcripts at the thyroid tissue level. In our study, despite the fact that we were not able to study the biological father's DNA, the exclusive expression of the paternal mutated allele may be considered. The stochastic origin of monoallelic *TPO* expression may be also supported by the detection of the same mutant *TPO* allele in the healthy mother and in her severe congenital hypothyroid daughter with TIOD (data not shown). This was the case of another patient studied in our laboratory with monoallelic *TPO* mutation associated with silencing of the paternal allele.

In conclusion we have described a patient with 99% of iodide discharged after perchlorate with a clear organification defect causing severe congenital goitrous hypothyroidism due to a monoallelic *TPO* mutation (c.1186_1187insGGCC) in exon 8.

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