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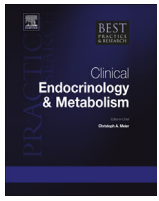

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Iodide handling disorders (NIS, TPO, TG, IYD)

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Iodide Handling Disorders lead to defects of the biosynthesis of thyroid hormones (thyroid dyshormonogenesis, TD) and thereafter congenital hypothyroidism (CH), the most common endocrine disease characterized by low levels of circulating thyroid hormones. The prevalence of CH is 1 in 2000–3000 live births. Prevention of CH is based on prenatal diagnosis, carrier identification, and genetic counseling. In neonates a complete diagnosis of TD should include clinical examination, biochemical thyroid tests, thyroid ultrasound, radioiodine or technetium scintigraphy and perchlorate discharge test (PDT).

Biosynthesis of thyroid hormones requires the presence of iodide, thyroid peroxidase (TPO), a supply of hydrogen peroxide (DUOX system), an iodine acceptor protein, thyroglobulin (TG), and the rescue and recycling of iodide by the action of iodotyrosine deiodinase or iodotyrosine dehalogenase 1 (IYD or DEHAL1). The iodide transport is a two-step process involving transporters located either in the basolateral or apical membranes, sodium iodide symporter (NIS) and pendrin (PDS), respectively. TD has been linked to mutations in the *solute carrier family 5, member 5 transporter* (*SLC5A5*, encoding NIS), *solute carrier family 26, member 4 transporter* (*SLC26A4*, encoding PDS), *TPO*, *DUOX2*, *DUOXA2*, *TG* and *IYD* genes. These mutations produce a heterogeneous spectrum of

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CH, with an autosomal recessive inheritance. Thereafter, the patients are usually homozygous or compound heterozygous for the gene mutations and the parents, carriers of one mutation. In the last two decades, considerable progress has been made in identifying the genetic and molecular causes of TD. Recent advances in DNA sequencing technology allow the massive screening and facilitate the studies of phenotype variability. In this article we included the most recent data related to disorders caused by mutations in NIS, TPO, TG and IYD.

Introduction

Biosynthesis of thyroid hormones requires the integrity of a complex protein group, several sequential steps, and is critically dependent on the three-dimensional structure of thyroglobulin (TG) and a specific enzymatic system [1,2]. The cascade of events leading to the thyroid hormone synthesis takes place at the cell–colloid interface of polarized follicular thyroid cells (Fig. 1) [1,2].

The iodide transport is a two-step process involving transporters located either in the basolateral or apical membranes (Fig. 1). Iodide is accumulated from the blood into the thyroidal cell through the sodium iodide symporter (NIS) localized in the basolateral membrane [1,2]. On the other hand, pendrin (PDS), located in the apical membrane, is responsible for the iodide transport from epithelial cell to

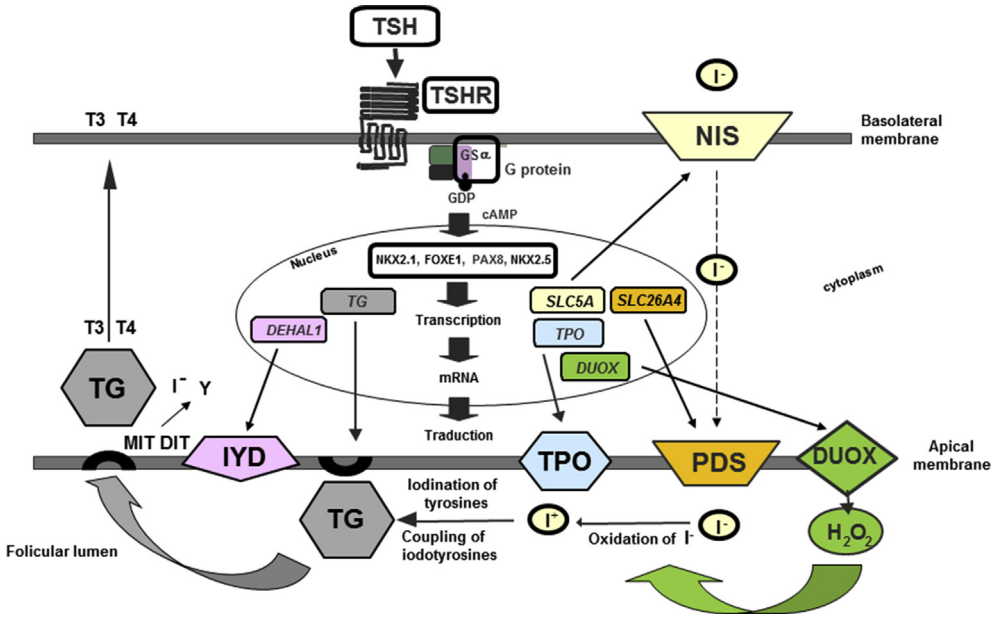

Fig. 1. Schematic representation of the biosynthesis of thyroid hormones and recycling iodide in the thyroid cell. Relevant processes in the different compartments and thyroid proteins (TG, thyroglobulin; TPO, thyroperoxidase; NIS, sodium iodide symporter; PDS, pendrin; DUOX, dual oxidase system; IYD, iodotyrosine deiodinase) are shown. In the follicular lumen oxidation of iodine, iodination of tyrosines (MIT, 3-monoiodotyrosine; DIT, 3,5-diiodotyrosine) and coupling of iodotyrosines takes place on tyrosine residues in TG, resulting in 3,5,3′-triiodothyronine (T_3) and 3,5,3′,5′-tretraiodothyronine (T_4) synthesis. The *TG*, *TPO*, *DUOX*, *SLC5A5* (solute carrier family 5, member 5 transporter), *SLC26A4* (solute carrier family 26, member 4 transporter) and *DEHAL1* (iodotyrosine dehalogenase 1) genes, as well as transcription factors NKX2.1 (also known as TTF1 or T/EBP), FOXE1 (also known as TTF2 or FKHL15), paired box transcription factor 8 (PAX-8) and NKX2.5 are shown into nucleus. TSH: thyrotrophin, TSHR: receptor for TSH, G protein: guanine nucleotide-binding protein, GDP: guanosine diphosphate, cAMP: cyclic adenosine monophosphate.

follicular lumen. Once correctly folded TG homodimers have reached the follicular lumen, several tyrosine residues are iodinated (Fig. 1). The subsequent coupling between either two diiodotyrosine residues (DIT), or between a DIT and a monoiodotyrosine residue (MIT), results in the formation of T_4 or T_3 within the TG molecule, respectively. The key enzymatic system is located on the apical plasma membrane. Thyroid peroxidase (TPO) catalyzes both the iodination and coupling of hormonogenic tyrosyl residues of TG with a strict requirement of hydrogen peroxide, which acts as an electron acceptor (Fig. 1). H_2O_2 is generated by a metabolic pathway, involving two members of the NADPH oxidase family: dual oxidase 1 (DUOX1) and dual oxidase 2 (DUOX2) and two endoplasmatic reticulum (ER)-resident proteins: DUOX maturation factor 1 (DUOXA1) and DUOX maturation factor 2 (DUOXA2), essential for DUOX maturation because its control the translocation to the plasma membrane [1,2]. The thyroid cells produce free thyroid hormones by proteolytic cleavage of the TG, which are delivered to the blood circulation for action at their peripheral target tissues. A certain amount of free MIT and DIT is also released by the thyroid cells. Therefore, the rescue and recycling into thyroid cell of iodide from MIT to DIT by the action of iodotyrosine deiodinase (IYD) (also known as iodotyrosine dehalogenase 1 (DEHAL1)) are essential to prevent loss of this rare element, especially in iodine-deficient areas (Fig. 1) [1,2]. In this review we included the most recent data related to disorders caused by mutations in NIS, TPO, TG and IYD.

Clinical genetics of the thyroid dyshormonogenesis

Congenital hypothyroidism (CH) is the most common endocrine disease in childhood that exhibits marked intra and interfamilial phenotypic and genotypic variability [3]. The prevalence is 1 in 2000–3000 live births. Untreated CH can result in abnormal growth and development as well as of severe mental retardation. Whereas, early diagnosis and treatment with L-thyroxine as a consequence of the neonatal screening programs led to normal development in nearly all cases.

Based on genetic alterations, the congenital hypothyroidism (CH) can be classified into two main groups: (i) those caused by disorders of thyroid gland development (dysembriogenesis or dysgenesis), which accounts for 80–85% of the cases; and (ii) by defects in any step of thyroid hormone synthesis (dyshormonogenesis), which accounts for the remaining 15–20% of the cases [3]. Thyroid dyshormonogenesis has been linked to mutations in the *solute carrier family 5*, *member 5 transporter* (*SLC5A5*, encoding NIS), *solute carrier family 26*, *member 4 transporter* (*SLC26A4*, encoding PDS), *TPO*, *DUOX2*, *DUOXA2*, *TG*, *and IYD* genes [1–3]. In general, because mutations in each of the genes causing dyshormonogenesis are inherited in an autosomal recessive manner, the patients should be homozygous or compound heterozygous for the gene mutations and the parents should be carriers of one mutation. Iodide organification defects (IOD) are associated with mutations in the *TPO*, *SLC26A4*, *DUOX2* or *DUOXA2* genes. IOD are characterized by high levels of serum TG and TSH with simultaneous low levels of circulating thyroid hormones and a positive perchlorate discharge test (PDT), indicating that the iodide is taken up by thyroid cells but it is not incorporated into the TG protein. PDT is used to distinguish total iodide organification defect (TIOD) from partial thyroid organification defect (PIOD, between 10 and 90%) [1–3]. *TPO* gene mutations in patients with PIOD usually affect a single allele, whereas homozygous or compound-heterozygous *TPO* gene mutations are associated to TIOD. In contrast, both biallelic and monoallelic *DUOX2* gene mutations could be associated with TCH or PCH. Instead, TCH can be caused by iodine excess or deficiency and exposure to maternal antithyroid drugs or transplacental antibodies. Mutations in *SLC26A4* gene cause Pendred syndrome characterized by congenital sensorineural hearing loss, goiter with or without hypothyroidism and usually PIOD. In patients with IYD deficiency the organification process was not affected whereas the serum TG levels were elevated [1–3]. In contrast, the presence of low TG level and also negative PDT in a goitrous individual suggest a TG defect.

Disorders caused by NIS mutations

NIS is a glycoprotein that resides in the basolateral membrane of thyroid epithelial cells with a molecular mass of approximately 80–90 kDa [4]. It is a highly specialized and efficient protein mediating the active uptake of iodide from the bloodstream into the thyroid gland using the sodium gradient generated by Na^+/K^+-ATPase. NIS transports two sodium cations (Na^+) for each iodide anion (I^-) into the

cell. Apart from thyroid cells NIS can also be found in other tissues such as salivary and lacrimal glands, mammary tissue during lactation, lung airway epithelial cells, intestinal enterocytes, epithelial and parietal stomach cells, placenta and testicular cells [4]. The human *SLC5A5* gene is located on chromosome 19 (19p12–13.2), spanning 23,2 Kb of genomic DNA and containing 15 exons [4]. Full-length human mRNA transcribed from the gene consists of 3576 nucleotides long (GenBank Accession Number: NM_000453.2). An open reading frame of 1929 nucleotides encodes a protein of 643 amino acids residues. The structure of the NIS is composed of 13 transmembrane segments (TMS), an extracelular N-terminus extremity and an intracellular C-terminus with 3 extracellular N-linked glycosylation sites (Fig. 2), 1 in the loop between TMS VI and VII (at position 225) and 2 in the loop between TMS XII and XIII (at positions 485 and 497) [4]. Remarkably, a typical N-terminal signal peptide is missing.

Changes in the expression and/or function of NIS, due to mutations in the gene, cause a wide spectrum of thyroid disorders. The suggested diagnostic criteria for iodide transport defect includes: small or large goiter with hypothyroidism or compensated hypothyroidism, reduced or absent thyroid uptake of radioiodide or pertechnetate, inability to concentrate iodide in salivary glands with low I^- saliva to plasma ratio and a positive response to therapy with high dose of iodide. In these patients 15 different *SLC5A5* inactivating gene mutations have been reported [4–16] (Table 1). After the gene was cloned, the first mutation was identified by Fujiwara et al. in a patient who presented CH caused by defects in iodide transport [5]. Sequencing revealed a homozygote c.1060A>C transversion, responsible for the substitution p.T354P in the ninth TMS of the NIS [5]. *In vitro* analysis evidence complete loss of iodide transport activity. Interestingly, this mutant protein was overexpressed in the basolateral membrane of thyrocytes. It was documented that the lack of activity by p.T354P mutant is due to the loss of a hydroxyl group at position 354 by the incorporation of a proline [4]. The β-hydroxylated residue T^{354}, play a key role in binding and/or translocation of Na^+. p.T354P is a highly prevalent NIS mutation in the Japanese population. In the following years other seven missense mutations (p.V59E, p.G93R, p.R124H, p.Q267E, p.V270E, p.G395R, p.G543E) were identified and characterized in the *SLC5A5* gene [7–10,13,16]. Pohlenz et al. described a patient with iodide trapping defect caused by a complex compound heterozygous mutations [8]. The paternal allele was a single amino acid substitution (p.Q267E), whereas the maternal allele consists of a heterozygous cytosine-to-guanine transversion at nucleotide position 1593 (c.1593C>G) in exon 13, which replaces a tyrosine residue at position 531 by a stop codon (TAC>TAG, p.Y531*) [8]. Remarkably, the stop codon generate also creates a cryptic 3′ splice site (AC to AG) in exon 13 at 67 nucleotides (1527_1593) downstream of the authentic 3′ splice site in intron 12, resulting in a frameshift at amino acid 509 with a putative premature stop at 6 codons downstream (position 515), located in the same exon 13 (p.S509Rfs*7) [8]. The resulting protein lack the 129 C-terminal amino acids. Moreover, three cases of iodide transport defects with identified deletion in the *SLC5A5* gene have been reported. Kosugi et al. demonstrated, in a Spanish family, a homozygous large deletion of 6192 bases spanning from exon 3 to intron 7 and an inverted insertion of a 431 base fragment spanning from exon 5 to intron 5 of the *SLC5A5* gene [11]. The deletion resulting in an in-frame 182-amino acid deletion from M^{142} in the fourth TMS to Q^{323} in the fourth exoplasmic loop (p.M142_Q323del) [11]. Another complex homozygous deletion was observed by Tonacchera et al. in a patient with CH and mental retardation [12]. Sequencing revealed a 15 nucleotide deletion between nucleotides positions 1315 and 1329 (c.1315_1329delGCCTGCAACACACCG) of the exon 11 and the insertion of 15 nucleotide duplicating the first 15 nt of the adjacent intron. This resulted in a deletion of the last five amino acids of exon 11 located in the sixt intracelular loop (p.A439_P443del) [12]. More recently, a new deletion was identified by Montanelli et al. consisting of in-frame six-nucleotide deletion of the coding sequence (c.859_864delGTCGGC) corresponding to the deletion of amino acids V^{287} and G^{288} (p.V287_G288del) located at the beginning of the eighth TMS [14]. Completing the universe of mutations identified in the *SLC5A5* gene a -54C>T mutation located in the 5-untranslated region associated with a substantial decrease in iodide uptake [15] and p.C272* mutation were described [6].

Disorders caused by TPO mutations

TPO is a membrane-bound glycoprotein located at the apical membrane of the thyroid follicular cells. The *TPO* gene is located on the short arm of chromosome 2 [2p25] [17]. It comprises 17 exons,

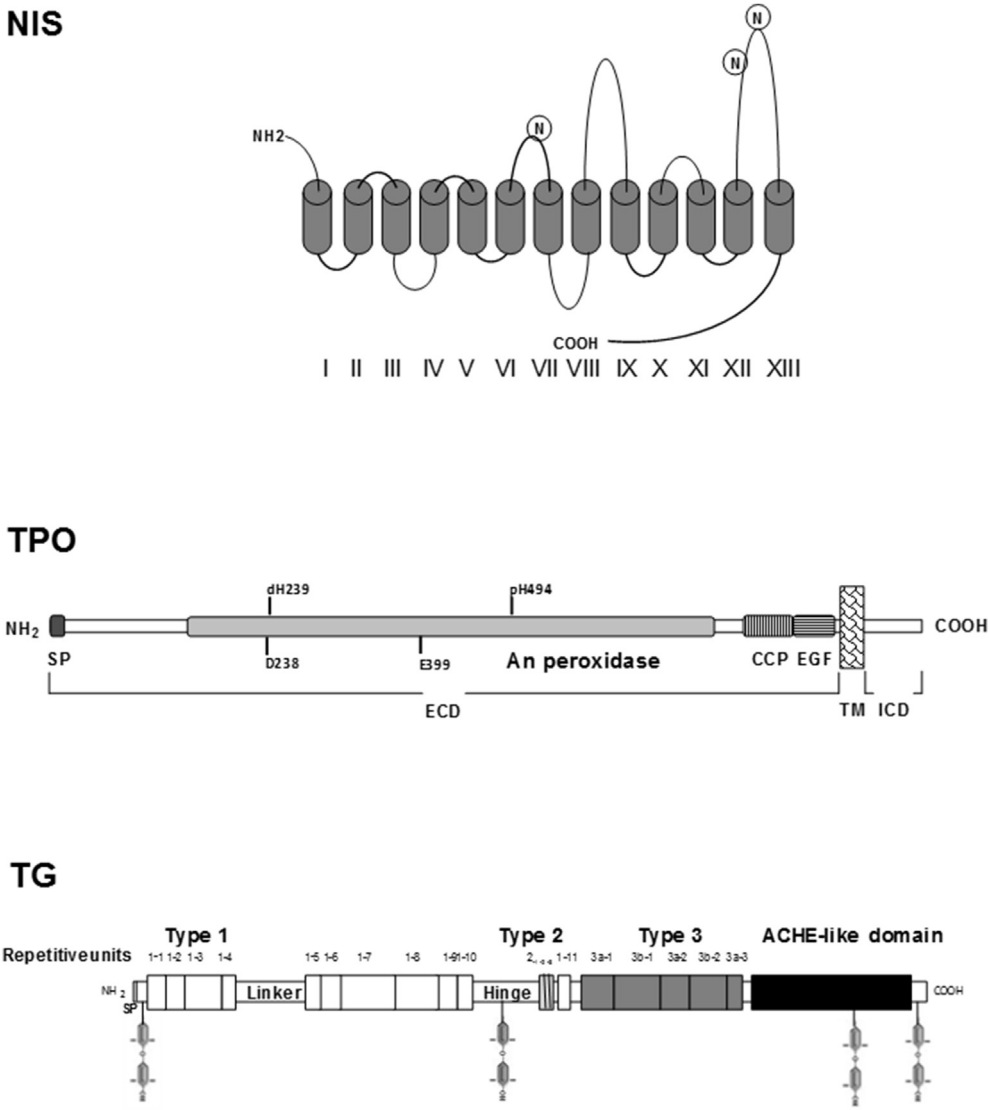

Fig. 2. Schematic representation of the human NIS, TPO and TG domains. NIS secondary structure model. I–XIII: transmembrane domains, N: N-linked glycosylation sites, at positions 225, 485 and 497. **TPO domains.** The signal peptide (SP), complement control protein (CCP) module, calcium-binding EGF-like (EGF) domain, animal haem peroxidase (An peroxidase) region and heme binding sites (proximal histidine (H) 494, distal histidine (H) 239, aspartic acid (D) 238, glutamic acid (E) 399) are shown. ECD, extracellular domain; TMS, transmembrane segment; ICD, intracellular domain. **TG domains.** The signal peptide (SP), repetitive units of type 1, 2 and 3 and the acetylcholinesterase-homology domain (ACHE-like domain) are represented by boxes. The N-terminal limit of repeat type 1–5 is ambiguous. The positions of T_4 (5, 1291 and 2747) and T_3 (2554) are shown.

covers approximately 150 kb of genomic DNA and encodes 933 amino acids. The mRNA is 3048 nucleotides long (GenBank Accesion Number: NM_000547.5) and the pre-protein is composed of a putative 14 amino acids signal peptide followed by a 919 amino acids polypeptide which codifies a large extracellular domain, a transmembrane domain, and a short intracellular tail (Fig. 2) [17]. H_2O_2 is used as a substrate by TPO in the organification of iodide.

The amino acid sequences between 149 and 711, corresponding to exons 5–12 in the human *TPO* gene show some significant similarities with animal haem peroxidase (An peroxidase) consensus

Table 1
NIS mutations.

Exon/intron position	Nucleotide position	Amino acid position	References
Exon 1	−54C>T	5′ untranslated region	[15]
Exon 1	c.176T>A	p.V59E	[10]
Exon 1	c.277G>C	p.G93R	[7]
Exon 2	c.371G>A	p.R124H	[13]
Exon 3 to intron 7	A deletion of 6192 bases spanning from exon 3 to intron 7 and an inverted insertion of a 431 base spanning from exon 5 to intron 5	p.M142_Q323del	[11]
Exon 6	c.799C>G	p.Q267E	[8]
Exon 6	c.816C>A	p.C272*	[6]
Exon 6	c.809T>A	p.V270E	[16]
Exon 7	c.859_864delGTCGGC	p.V287_G288del	[14]
Exon 9	c.1060A>C	p.T354P	[5]
Exon 10	c.1183G>A	p.G395R	[9]
Exon 11	c.1315_1329delGCCTGCAACACACCG	p.A439_P443del	[12]
Exon 13	c.1593C>G Cryptic 3′ splice site in exon 13 at 67 nucleotides (1527_1593) downstream of the authentic 3′ splice site in intron 12	p.Y531* p.S509Rfs*7	[8]
Exon 13	c.1628G>A	p.G543E	[7]

The nucleotide position is designated according to *SLC5A5* mRNA reference sequences reported in National Center for Biotechnology Information (NCBI), accession number: NM_000453.2. The 'A' of the ATG start codon is denoted as nucleotide +1 being the initiator methionine, the codon 1. Frameshifting mutations are designated by "fs" after a description of the first amino acid affected by the nucleotide change (insertion or deletion) and the stop codón with "*", followed by indication of the length of the shifted open reading frame from the first affected codon to the new stop codon.

sequence (Fig. 2) [17]. Exons 8, 9 and 10 encode the catalytic center of the TPO protein (heme-binding region) which is crucial for the enzymatic activity (Fig. 2) [17]. The next two exons, 13 and 14, belong to the complement control protein (CCP)-like (residues 742–795) and calcium-binding epidermal growth factor (EGF-Ca^{2+})-like (residues 796–839) gene families, respectively (Fig. 2) [17]. Exon 15 encodes for the transmembrane part of the protein and exons 16 and 17 for its cytoplasmic tail [17]. The TPO enzyme activity depends on both proper folding and membrane insertion, and an intact catalytic site.

Organification defects due to *TPO* gene mutations are characterized by intact iodide trapping, normal TG protein expression and aberrant organification of iodide. Up to now, one hundred three properly annotated deleterious mutations in the human *TPO* gene that cause iodide organification defect have been identified and characterized: 5 splice site and intronic mutations, 9 nonsense mutations, 66 missense mutations, 17 deletions (9 single, 7 multiple, one involving a large number of nucleotides), 5 single nucleotide insertions or duplication and one insertion/deletion (Table 2) [17–65]. The majority of *TPO* mutations are localized in exons 8, 9 and 11 of the gene (Table 2). The first description of the human *TPO* mutation was conducted in an Argentinian boy with iodide organification defect, who presented with hypothyroidism at the age of 4 months and developed a compressive goiter at the age of 12 years [18]. Homozygous duplication of a tetranucleotide – GGCC – in exon 8 of the *TPO* gene was identified, 152 bp upstream from the junction with intron 8 [18]. The resulting frameshift generates a stop codon in exon 9, which would result in a truncated protein with mutation of the proximal and deletion of the distal putative heme binding histidine residues and TPO activity is expected to be absent [18]. Interestingly, alternative splicing restores disrupting the normal reading frame [18]. The result is a nearly full-length protein with a 91-amino acid segment replaced by a 51 residue unrelated segment which is read in a different frame [18]. This translation product is expected to have an unchanged distal putative heme binding His, but to lack the proximal putative heme binding His [18]. The presence of the NaeI site in the *TPO* alleles amplified by PCR provides a rapid test for direct genetic diagnosis of this mutation. The duplication GGCC in exon 8 is a common alteration of the *TPO* gene in Caucasian population.

Table 2
Thyroid peroxidase mutations.

Exon/intron position	Nucleotide position	Amino acid position	First reference
Acceptor and donor splice site and intronic mutations			
Intron 8	c.1338+1G>T (g.IVS8+1G>T)	NA	[52]
Intron 10	c.1768+1G>A (g.IVS10+1G>A)	NA	[25]
Intron 12	c.2216+144_148delGGGC (g.IVS12+144_+148delGGGGC) c.2216+144_153delGGGGCGGGGC (g.IVS12+144_+153delGGGGCGGGGC)	NA	[55]
Intron15/Exon 16	c.2619-6_2622delCCACAGGACA	NA	[37]
Exon 16	c.2748G>A splice site	NA	[36]
Nonsense mutations			
Exon 3	c.165C>A	p.Y55*	[53]
Exon 6	c.523C>T	p.R175*	[40]
Exon 7	c.703C>T	p.Q235*	[38]
Exon 7	c.796C>T	p.Q266*	[50]
Exon 8	c.943C>T	p.Q315*	[47]
Exon 10	c.1618C>T	p.R540*	[20]
Exon 11	c.1786G>T	p.E596*	[53]
Exon 16	c.2619G>A	p.W873*	[46]
Exon 16	c.2665G>T	p.G889*	[58]
Missense mutations			
Exon 2	c.13G>A	p.A5T	[53]
Exon 3	c.157G>C	p.A53P	[27]
Exon 4	c.349G>C	p.D117H	[25]
Exon 5	c.391T>C	p.S131P	[36]
Exon 6	c.524G>A	p.R175Q	[34]
Exon 6	c.566G>A	p.R189Q	[48]
Exon 7	c.718G>A	p.D240N	[22]
Exon 8	c.872G>A	p.R291H	[64]
Exon 8	c.875C>T	p.S292F	[42]
Exon 8	c.920A>C	p.N307T	[32]
Exon 8	c.940C>T	p.R314W	[44]
Exon 8	c.955G>A	p.G319R	[45]
Exon 8	c.976G>A	p.A326T	[25]
Exon 8	c.992G>T	p.G331V	[64]
Exon 8	c.1022G>A	p.R341Q	[49]
Exon 8	c.1132G>A	p.E378K	[37]
Exon 8	c.1152G>T	p.E384D	[31]
Exon 8	c.1159G>A	p.G387R	[41]
Exon 8	c.1186C>T	p.R396C	[57]
Exon 8	c.1219C>T	p.H407Y	[62]
Exon 8	c.1235G>A	p.R412H	[63]
Exon 8	c.1274A>G	p.N425S	[36]
Exon 8	c.1297G>A	p.V433M	[32]
Exon 8	c.1313G>A	p.R438H	[61]
Exon 8	c.1315A>G	p.K439E	[48]
Exon 8	c.1327G>C	p.A443P	[60]
Exon 8	c.1338G>C	p.Q446H	[46]
Exon 9	c.1339A>T	p.I447F	[21]
Exon 9	c.1357T>G	p.Y453D	[20]
Exon 9	c.1373T>C	p.L458P	[26]
Exon 9	c.1471C>T	p.R491C	[60]
Exon 9	c.1472G>A	p.R491H	[26]
Exon 9	c.1477G>A	p.G493S	[29]
Exon 9	c.1496C>T	p.P499L	[32]
Exon 9	c.1502T>G	p.V501G	[54]
Exon 9	c.1581G>T	p.W527C	[25]
Exon 9	c.1597G>T	p.G533C	[30]

(*continued on next page*)

Table 2 (*continued*)

Exon/intron position	Nucleotide position	Amino acid position	First reference
Exon 10	c.1682C>T	p.T561M	[59]
Exon 10	c.1690C>A	p.L564I	[31]
Exon 10	c.1727C>T	p.A576V	[65]
Exon 10	c.1751G>A	p.R584Q	[64]
Exon 10	c.1768G>A	p.G590S	[20]
Exon 11	c.1784G>A	p.R595K	[50]
Exon 11	c.1858G>A	p.D620N	[56]
Exon 11	c.1897G>A	p.D633N	[53]
Exon 11	c.1921G>A	p.E641K	[56]
Exon 11	c.1943G>A	p.R648Q	[23]
Exon 11	c.1970T>C	p.I657T	[58]
Exon 11	c.1978C>G	p.Q660E	[24]
Exon 11	c.1993C>T	p.R665W	[28]
Exon 11	c.1994G>A	p.R665Q	[31]
Exon 11	c.1999G>A	p.G667S	[40]
Exon 11	c.2000G>A	p.G667D	[50]
Exon 12	c.2077C>T	p.R693W	[25]
Exon 12	c.2173A>C	p.T725P	[56]
Exon 13	c.2266T>C	p.C756R	[52]
Exon 13	c.2311G>A	p.G771R	[28]
Exon 13	c.2327G>A	p.G776D	[62]
Exon 13	c.2386G>T	p.D796Y	[29]
Exon 14	c.2395G>A	p.E799K	[20]
Exon 14	c.2398T>C	p.C800R	[35]
Exon 14	c.2422T>C	p.C808R	[32]
Exon 14	c.2512T>A	p.C838S	[36]
Exon 15	c.2578G>A	p.G860R	[40]
Exon 15	c.2587G>A	p.A863T	[48]
Exon 16	c.2647C>T	p.P883S	[33]
Single nucleotide insertion or duplication			
Exon 11	c.1955_1956insT (c.1955dupT)	pL652Lfs*17	[43]
Exon 13	c.2268_2269insT (c.2268dupT)	p.E757*	[27]
Exon 14	c.2421_2422insC (c.2421dupC)	p.C808Lfs*72	[20]
Multiple nucleotide insertion or duplication			
Exon 2	c.50_51insCTGGTTATGGCCTGCACAGA (c.31_50dupCTGGTTATGGCCTGCACAGA)	p.E17Dfs*77	[19]
Exon 8	c.1186_1187insGGCC or 1187_1188insGCCG (c.1183_1186dupGGCC or c.1184_1187dup GCCG) Alternative splicing restores the normal reading frame disrupted by the mutation.	p.R396Rfs*77	[18]
Single nucleotide deletion			
Exon 4	c.215delA	p.Q72Rfs*15	[41]
Exon 5	c.387delC	p.N129Lfs*80	[32]
Exon 8	c.843delC	p.A281Afs*37	[29]
Exon 8	c.1335delC	p.H445Hfs*6	[25]
Exon 9	c.1496delC	p.P499Rfs*3	[43]
Exon 13	c.2243delT	p.V748Gfs*50	[27]
Exon 14	c.2413delC	p.H805Tfs*27	[29]
Exon 14	c.2421delC	p.P808Pfs*25	[64]
Exon 14	c.2422delT	p.C808Afs*24	[25]
Multiple nucleotide deletion			
Exon 7	c.670_672delGAC	p.D224del	[57]
Exon 9	c.1429_1449delGCCAACCCCACTGTGTCCAAC	p.A477_N483del	[40]
Exon 9	c.1529_1564delAGCACCCCGACCTGCCCGGG CTGTGGCTGCACCAGG	p.E510A – H511_A522del	[64]
Exon 10	c.1718_1723delTGGATC	p.D574_L575del	[30]
Exon 12	c.2153_2154delTT	p.F718*	[25]
Exon 16	c.2622_2638delACGCACTGGCACTAAAT	p.T874Tfs*101	[48]
Exon 16	c.2722_2731delCGGGCCGCAG	p.R908Lfs*64	[39]

Table 2 (*continued*)

Exon/intron position	Nucleotide position	Amino acid position	First reference
Large nucleotide deletion			
Exons 11-15	Deletion encompassing exon 11 (position 1769) - exon 15 (position 2386)	p.G590Gfs*22	[51]
Insertion/deletion			
Exon 8	c.1188_1193delCGCCAGinsGCCGCCCCAC	p.R396Rfs*77	[64]

The nucleotide position is designated according to *TPO* mRNA reference sequences reported in National Center for Biotechnology Information (NCBI), accession number: NM_000547.5. The 'A' of the ATG start codon is denoted as nucleotide +1 being the initiator methionine, the codon 1. Intronic nucleotides located upstream of the exon have negative numbering, while those located downstream have positive numbering. Splicing mutations are annotated by using cDNA sequences and old nomenclature (g.IVS) is included. Frameshifting mutations are designated by "fs" after a description of the first amino acid affected by the nucleotide change (insertion or deletion) and the stop codón with "*", followed by indication of the length of the shifted open reading frame from the first affected codon to the new stop codon. NA, Not Available.

In EGF-Ca^{2+} binding domain of human TPO (corresponding to exon 14) are located 6 cystein residues responsible for the formation of 3 disulfide bridges essential for protein structure: C800-C814, C808-C823 and C825-C838. Disruption of the C808-C823 disulfide bond, as a result of p.C808R [32] or p.C838S [36] mutation, would generate significant conformational changes which could on the one hand partially retain the TPO on the endoplasmic reticulum, or it might be located within the membrane with conformational defects responsible for a lower net activity of the enzyme.

Interestingly, the literature informs patients with TIOD phenotype and a single *TPO*-mutated allele [17]. Among then, maternal isodisomy for chromosome 2p associated with severe congenital hypothyroidism was reported in one case and 2p25-deleted null alleles segregated from her father in another one [17]. Furthermore, Fugazzola et al. identified a monoallelic expression of a mutant *TPO* allele involved in the other TIOD cases with a single *TPO* mutation [17].

Disorders caused by TG mutations

TG is a large homodimeric secretory protein (660 kDa) with a high degree of glycosylation. Its main function is to provide the precursor for synthesis and storage of thyroid hormones. It is also an important storage of iodine when external iodine availability is limited. Human *TG* gene is a single copy gene of 270 kb long that maps on chromosome 8q24.2-8q24.3 and contains an 8453 nucleotides coding sequence (GenBank Accesion Number: NM_003235.4) divided into 48 exons [1,2]. The human TG mRNA codes for a polypeptide chain of 2767 amino acids. A leader peptide of 19 aminoacids is followed by a 2748-amino-acid polypeptide, corresponding to the monomeric human TG (Fig. 2) [1,2]. Four hormonogenic acceptor tyrosine residues have been identified and localized at positions 5, 1291, 2554 and 2747 in human TG (Fig. 2) [1,2]. The internal protein organization makes *TG* an example of gene evolution by intragenic duplication events and gene fusions. The TG protein is composed of four structural and functional regions (Fig. 2) [1,2]. The N-terminal and the central part of the monomer includes three types of repetitive motifs, called TG type-1, TG type-2, and TG type-3, organized in three regions (I, II and III), comprising Cys-rich repeat domains covalently bound by disulfide bonds (Fig. 2) [1,2]. Interestingly, type-1 repeats could function as binder and reversible inhibitors of the protease. TG type-1 domains have been found as parts of six architecturally distinct protein groups. Region I comprises 10 of the 11 TG type-1 repeats, a linker and hinge segments. Region II contains 3 TG type-2 repeats and the 11th TG type-1 repeat, whereas region III contains five TG type-3 repeats. The fourth region located in the carboxy-terminal, between residues 2192 to 2716, is a nonrepetitive domain that shows significant homology with the acetylcholinesterase (ACHE), named the ACHE-like or CheL domain (Fig. 2) [1,2]. ACHE-like domain is required for protein dimerization and consequently plays a critical structural and functional role in the TG protein, that is essential for intracellular transport of TG to the site of its hormonogenesis. This region functions as an intramolecular chaperone and as a molecular escort for TG regions I, II, and III [1,2].

The cases with TG synthesis defects present a congenital goiter or goiter appearing shortly after birth, clinical spectrum ranges from euthyroid to mild or severe hypothyroidism, high iodide uptake,

normal organification of iodide, elevated serum TSH with simultaneous low or normal serumT_4 and T_3 levels, and low serum TG concentration. *TG* gene mutations is a relatively common cause of thyroid dyshormonogenesis with an estimated incidence of approximately 1 in 100,000 newborns [1,2].

The first-described human mutation causing a TG defect associated with CH was the mutation g.IVS3-3C>G [66]. To date, one hundred seventeen deleterious mutations in the human *TG* gene have been identified and characterized: 19 splice site mutations, 23 nonsense mutations, 57 missense mutations, 13 deletions (9 single, 2 multiple and 2 involving a large number of nucleotides) and 4 single nucleotide insertions or duplication (Table 3) [1,2,49,60,61,64,66–97]. Mutations of *TG* gene have been also associated with endemic and nonendemic simple goiter [67]. The p.C1058R and p.C1977S mutations are the most frequently identified *TG* mutations in Japanese population, whereas the frequent mutation p.R277* is found in Caucasian populations.

Exon skipping in the *TG* gene can be caused by nucleotide substitutions or deletion in acceptor or donor splice sites involving the −3/−2/−1 (c.275-3C>G, c.6563-2A>G, c.2762-1G>A, c.6200-1G>C, c.7998-1G>A) or +1/+2/+3/+4/+5/+6 position (c.638+1G>A, c.745+1G>A, c.4932+1G>C, c.5686+1G>T, c.5686+1G>A, c.5686+1G>C, c.6262+1delG, c.6876+1delG, c.274+2T>G, c.7036+2T>A, c.7862+2T>A, c.4159+3_+4delAT; c.3433+3_+6delGAGT, c.638+5G>A), respectively (Table 3) [49,64,66,72,74,76,77,81,82,84,91,94,96,97]. Recently, it has been identified two exonic cryptic 5′ splicing sites in exons 6 (c.745+1G>A) [96] and 19 (c.4159+3_+4delAT) [91] of the *TG* gene. The elimination of exons in the *TG* gene by aberrant splicing results in an altered ability to transfer an iodophenoxyl group from the donor site to the acceptor iodotyrosine.

The 23 inactivating mutations that generates truncated proteins have been localized in exons 4 (p.Y107*, p.R140*), 7 (p.R277*), 9 (p.R432*, p.S509*, p.Q611*, p.W618*, p.Q636*, p.Q692*), 10 (p.Q717*, p.Q752*, p.R768*, p.Q810*), 13 (p.C1032*), 20 (p.W1418*), 22 (p.R1511*), 27 (p.Q1765*, p.Q1777*), 31 (p.Y1903*) 37 (p.Q2142*), 40 (p.R2317*), 46 (p.Q2638*) and 47 (p.R2688*) of the *TG* gene (Table 3) [49,61,64,68,70,77,81–83,85–87,92,95,97]. These truncated proteins could represent adequate targets for nonsense mediated mRNA decay (NMD) pathway, a known RNA surveillance mechanism that detects and rapidly degrades selectively mRNAs that contains premature terminated codons. The p.R277* mutation affected individuals have either homozygous or compound heterozygous mutations. This mutation has been found in families from Brazil, Argentina, Spain and France [2,64,70,74,79,82–84,86,87,92]. The functional consequences of p.R277* truncated protein are a complete loss of the central and carboxy-terminal hormonogenic domains and consequently, limited ability to generate thyroid hormone. However, p.R277* TG peptide retains its ability for T_4 synthesis because it still harbors both the acceptor Tyr 5 and the donor Tyr 130. The p.R1511* mutation was identified in members of unrelated families with history of CH from Brazil, Argentina and France [2,68,74,85]. The p.R1511* mutation is removed from the transcripts by exon skipping using an alternative splicing [68]. The elimination of mutated exon 22 in the pre-mRNA restores the reading frame allowing translation to reach the normal stop codon and results in an in-frame deletion of 57 amino acid residues [68]. Truncated protein can be also caused by nucleotide deletions (Table 3) [49,64,73,77,81,92,97] and insertions or duplication (Table 3) [49,79,86,91] in the *TG* gene (Table 3). Recently, genetic analysis in three brothers of Turkish origin born from consanguineus parents and affected by CH, goiter and low levels of serum TG, showed a DNA inversion of 16,962 bp in the *TG* gene associated with two deleted regions at both sides of the inversion limits [93].

Sequencing analysis of the *TG* gene revealed sixteen missense mutations that involved wild-type Cys residue: p.C141S, p.C164Y, p.C175G, p.C707Y, p.C1058R, p.C1245R, p.C1262Y, p.C1474Y, p.C1491F, p.C1588F, p.C1878Y, p.C1885G, p.C1977S, p.C1981W, p.C1987Y and p.C2135Y (Table 3) [49,64,69,75,77–79,92,97]. The loss of Cys residues can eliminate disulfide bonds and alter the normal conformational structure of the TG, possibly preventing the interaction of hormonogenic acceptor and donor sites.

Finally, nine missense mutations were reported in the ACHE homology domain: p.A2215D, p.R2223H, p.G2300D, p.R2317Q, p.G2355V, p.G2356R, p.L2528Q, p.R2566W and p.W2666L (Table 3) [64,73,75,77–79,97]. Functional analysis suggests that the p.A2215D mutation results in retention of the TG protein inside the ER and degradation via the proteasome system [82], as already observed in the *cog/cog* congenital goiter mouse and the rdw/rdw non-goitrous CH rat [1].

Table 3
Thyroglobulin mutations.

Exon/intron position	Nucleotide position	Amino acid position	First reference
Acceptor and donor splice site mutations			
Intron 3	c.274+2T>G (g.IVS3+2T>G)	Skipping of exon 3	[81]
Intron 3	c.275-3C>G (g.IVS3-3C>G)	Skipping of exon 4	[66]
Intron 5	c.638+1G>A (g.IVS5+1G>A)	Skipping of exon 5	[76]
Intron 5	c.638+5>A (g.IVS5+5G>A)	NA	[64]
Intron 6	c.745+1G>A (g.IVS6+1G>A)	Skipping of exon 6 or partially included by use of cryptic 5′ splice site	[96]
Intron 10	c.2762-1G>A (g.IVS10-1G>A)	NA	[77]
Intron 15	c.3433+3_+6delGAGT (g.IVS15+3_+6delGAGT)	NA	[64]
Intron 19	c.4159+3_+4delAT (g.IVS19+3_+4delAT)	Skipping of exon 19 or partially included by use of cryptic 5′ splice site	[91]
Intron 24	c.4932+1G>C (g.IVS24+1G>C)	NA	[77]
Intron 30	c.5686+1G>T (g.IVS30+1G>T)	Skipping of exon 30	[72]
Intron 30	c.5686+1G>A (g.IVS30+1G>A)	NA	[77]
Intron 30	c.5686+1G>C (g.IVS30+1G>C)	Skipping of exon 30	[94]
Intron 34	c.6200-1G>C (g.IVS34-1G>C)	Skipping of exon 35	[74]
Intron 35	c.6262+1delG (g.IVS35+1delG)	Skipping of exon 35	[84]
Intron 37	c.6563-2A>G (g.IVS37-2A>G)	NA	[49]
Intron 39	c.6876+1delG (g.IVS39+1delG)	NA	[97]
Intron 40	c.7036+2T>A (g.IVS40+2T>A)	Skipping of exon 40	[96]
Intron 45	c.7862+2T>A (g.IVS45+2T>A)	NA	[77]
Intron 46	c.7998-1G>A (g.IVS46-1G>A)	NA	[82]
Nonsense mutations			
Exon 4	c.378C>A	p.Y107*	[92]
Exon 4	c.475C>T	p.R140*	[64]
Exon 7	c.886C>T	p.R277*	[70]
Exon 9	c.1351C>T	p.R432*	[81]
Exon 9	c.1583C>A	p.S509*	[64]
Exon 9	c.1888C>T	p.Q611*	[95]
Exon 9	c.1911G>A	p.W618*	[95]
Exon 9	c.1963C>T	p.Q636*	[61]
Exon 9	c.2131C>T	p.Q692*	[77]
Exon 10	c.2206C>T	p.Q717*	[87]
Exon 10	c.2311C>T	p.Q752*	[64]
Exon 10	c.2359C>T	p.R768*	[86]
Exon 10	c.2485C>T	p.Q810*	[49]
Exon 13	c.3153T>A	p.C1032*	[97]
Exon 20	c.4310G>A	p.W1418*	[77]
Exon 22	c.4588C>T	p.R1511* Skipping of exon 22	[68]
Exon 27	c.5350C>T	p.Q1765*	[81]
Exon 27	c.5386C>T	p.Q1777*	[85]
Exon 31	c.5766C>A	p.Y1903*	[97]
Exon 37	c.6481C>T	p.Q2142*	[82]
Exon 40	c.7006C>T	p.R2317*	[83]
Exon 46	c.7969C>T	p.Q2638*	[77]
Exon 47	c.8119C>T	p.R2688*	[97]
Missense mutations			
Exon 2	c.113G>A	p.R19K	[80]
Exon 3	c.262C>T	p.R69W	[97]
Exon 7	c.799C>T	p.L248F	[97]
Exon 8	c.925A>G	p.T290A	[97]
Exon 8	c.986A>C	p.Q310P	[77]
Exon 9	c.1382C>T	p.T442I	[97]
Exon 10	c.2222C>T	p.T722M	[97]
Exon 10	c.2281C>T	p.P742S	[60]
Exon 10	c.2610G>T	p.Q851H	[67]
Exon 10	c.2687G>A	p.R877Q	[90]

(*continued on next page*)

Table 3 (*continued*)

Exon/intron position	Nucleotide position	Amino acid position	First reference
Exon 11	c.2969G>A	p.S971I	[77]
Exon 12	c.3035C>T	p.P993L	[77]
Exon 13	c.3149G>T	p.W1031L	[64]
Exon 15	c.3332C>G	p.T1092R	[97]
Exon 15	c.3416C>T	p.S1120L	[61]
Exon 20	c.4378G>A	p.V1441I	[61]
Exon 21	c.4397G>A	p.S1447N	[77]
Exon 21	c.4493C>T	p.T1479M	[60]
Exon 22	c.4575G>T	p.Q1506H	[97]
Exon 22	c.4604A>G	p.D1516G	[97]
Exon 24	c.4859C>T	p.T1601M	[60]
Exon 24	c.4930C>G	p.Q1625E	[64]
Exon 26	c.5176C>T	p.L1707F	[97]
Exon 27	c.5318C>A	p.A1754D	[97]
Exon 31	c.5791A>G	p.I1912V	[77]
Exon 38	c.6605C>G	p.P2183R	[92]
Missense mutations involved in the wild type cysteine residues			
Exon 5	c.479G>C	p.C141S	[64]
Exon 5	c.548G>A	p.C164Y	[79]
Exon 5	c.580T>G	p.C175G	[77]
Exon 10	c.2177G>A	p.C707Y	[64]
Exon 14	c.3229T>C	p.C1058R	[75]
Exon 17	c.3790T>C	p.C1245R	[69]
Exon 17	c.3842G>A	p.C1262Y	[92]
Exon 21	c.4478G>A	p.C1474Y	[64]
Exon 22	c.4529G>T	p.C1491F	[49]
Exon 24	c.4820G>T	p.C1588F	[77]
Exon 31	c.5690G>A	p.C1878Y	[78]
Exon 31	c.5710T>G	p.C1885G	[97]
Exon 33	c.5986T>A	p.C1977S	[69]
Exon 33	c.6000C>G	p.C1981W	[92]
Exon 33	c.6017G>A	p.C1987Y	[77]
Exon 37	c.6461G>A	p.C2135Y	[77]
Missense mutations originating new cysteine residues			
Exon 8	c.967G>T	p.G304C	[89]
Exon 10	c.2276A>G	p.Y740C	[64]
Exon 12	c.3022C>T	p.R989C	[77]
Exon 17	C.3808C>T	p.R1251C	[97]
Exon 26	c.5071C>T	p.R1672C	[64]
Exon 34	c.6130C>T	p.R2025C	[61]
Missense mutations in the ACHE-homology domain			
Exon 38	c.6701C>A	p.A2215D	[79]
Exon 38	c.6725G>A	p.R2223H	[73]
Exon 40	c.6956G>A	p.G2300D	[77]
Exon 40	c.7007G>A	p.R2317Q	[78]
Exon 41	c.7121G>T	p.G2355V	[77]
Exon 41	c.7123G>A	p.G2356R	[75]
Exon 44	c.7640T>A	p.L2528Q	[64]
Exon 44	c.7753C>T	p.R2566W	[97]
Exon 47	c.8054G>T	p.W2666L	[64]
Single nucleotide insertion or duplication			
Exon 7	c.759_760insA (c.759dupA)	p.L235Tfs*3	[79]
Exon 9	c.1345_1346insC (c.1345dupC)	p.P430Pfs*36	[86]
Exon 9	c.2115_2116insT (c.2115dupT)	p.V687Cfs*2	[49]
Exon 17	c.3788_3789insT (c.3788dupT)	p.I1244Ifs*3	[91]
Single nucleotide deletion			
Exon 9	c.1143delC	p.G362Gfs*21	[73]
Exon 9	c.1348delT	p.S431Pfs*29	[81]
Exon 9	c.1712delT	p.L552Pfs*25	[81]
Exon 10	c.2736delG	p.R893Rfs*54	[92]
Exon 17	c.3780delG	p.G1241Gfs*3	[49]

Table 3 (*continued*)

Exon/intron position	Nucleotide position	Amino acid position	First reference
Exon 22	c.4537delG	p.D1494Tfs*54	[77]
Exon 28	c.5466delA	p.K1803Kfs*30	[92]
Exon 33	c.6047delA	p.Q1997Rfs*2	[81]
Exon 36	c.6360delC	p.T2101Tfs*33	[64]
Multiple nucleotide deletion			
Exon 27	c.5299_5301delGAT	p.D1748del	[86]
Exon 36	c.6391_6394delTTGT	p.L2112Rfs*21	[97]
Large nucleotide deletion			
Deletion in the 5′ region of the TG gene that involves promoter region and 11 first exons.			[71]
Deletion of 9908 bp that includes exon 45			[88]
Imperfect DNA inversion			
DNA inversion of 16,962 bp from exon 48 to intron 45 in the TG gene associated with two deleted regions at both sides of the inversion limits			[93]

The nucleotide position is designated according to *TG* mRNA reference sequences reported in National Center for Biotechnology Information (NCBI), accession number: NM_003235.4. 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. Intronic nucleotides located upstream of the exon have negative numbering, while those located downstream have positive numbering. Splicing mutations are annotated by using cDNA sequences and old nomenclature (g.IVS) is included. Frameshifting mutations are designated by "fs" after a description of the first amino acid affected by the nucleotide change (insertion or deletion) and the stop codón with "*", followed by indication of the length of the shifted open reading frame from the first affected codon to the new stop codon. NA, Not Available.

Disorders caused by IYD mutations

Secretion of T3 and T4 by the thyroid requires proteolysis of TG, during which MIT and DIT are release from the peptide linkage. MIT and DIT can not be reused as such for the synthesis of thyroid hormones [98]. Both are enzymatically deiodized by IYD. It is a redox process leading to the formation of iodide and tyrosine, which can be recycled for hormonogenesis. It was thought that the deiodination of free MIT and DIT occurred after the formation of colloid drops in the phagolysozomes. However, Gnidehou et al. provided direct evidence that IYD is a membrane protein located in the apical pole of the thyrocyte, near to the TG iodination site, with a large extracellular N-terminus, a single transmembrane segment and a short intracellular C-terminus [99]. According to these findings IYD may be a rapid iodide-recycling process at the organification site. IYD is a member of the superfamily of NADH oxidase/flavin reductases. This enzyme contains an area of putative binding to the prosthetic group, the flavin mononucleotide (FMN), at the nitroreductase catalytic domain of the protein. The nitroreductases are proteins that can reduce nitroaromatic compounds using FMN as cofactor. *DEHAL1* mRNA has been also found with low expression levels in human kidney and liver.

DEHAL1 gene is located on human chromosome 6 (6q24-25), spanning 35,7 Kb of genomic DNA and containing 6 exons comprising in total 8742 nucleotides coding sequence [98]. The *DEHAL1* gene leads to different transcribed as a result of alternative splicing of exons 5 and 6 [100]. The three isoforms identified in the thyroid cells, DEHAL1, DEHAL1B and DEHAL1C, share the same signal peptide and transmembrane and extracytoplasmic domains, encoded by exons 1 to 4, but have different cytoplasmic tails encoded by exon 5 and/or 6 (Fig. 3) [100]. The exon 5 is completely removed in the DEHAL1 isoform. The human *DEHAL1* mRNA (7419 nucleotides, GenBank Accession Number: NM_203395) codes for a polypeptide chain of 289 amino acids. A leader peptide of 23 aminoacids is followed by a 266-amino-acid polypeptide. DEHAL1B isoform is a variant resulting from splicing of the part of exon 5, whereas the terminal carboxyl región of the DEHAL1C isoform includes the complete exon 5 with lacking exon 6 of *DEHAL1* gene [100]. In the thyroid mRNA levels of *DEHAL1C* and *DEHAL1B* are lower than *DEHAL1*. The DEHAL1 isoform turned out to be the only active isoform in the presence of NADPH. This suggests that the modification of the cytoplasmic tail could affect the structure and therefore its interaction with FMN.

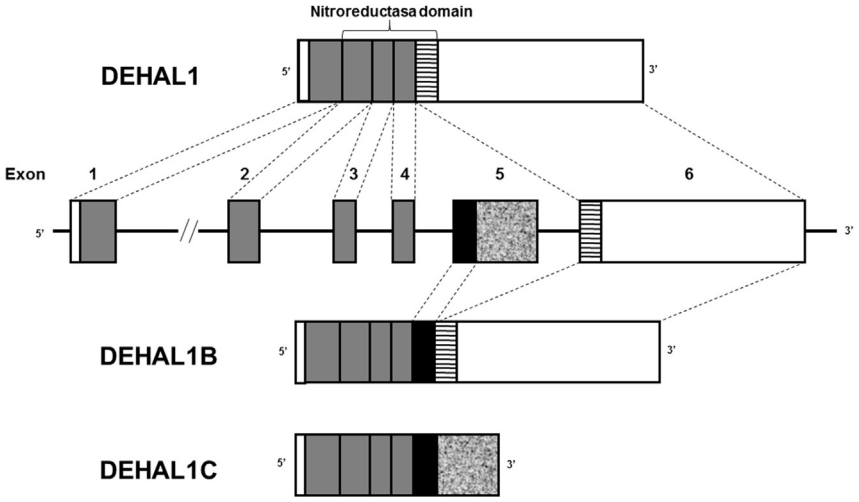

Fig. 3. Splice variants of human DEHAL1. Exons are denoted by boxes. The alternatively exon 5 is in black (5′ región) and grey dots (3′ región). The alternatively terminal exon 6 is shown in hatched (coding sequences) and white (3′ untranslated sequences). DEHAL1, DEHAL1B and DEHAL1C, share the same signal peptide and transmembrane and extracytoplasmic domains, encoded by exons 1 to 4. The exon 5 is completely removed in the DEHAL1 isoform. DEHAL1B isoform is a variant resulting from splicing of part of exon 5, whereas the terminal carboxyl región of the DEHAL1C isoform includes the complete exon 5 and lacks the exon 6 of *DEHAL1* gene.

The lack of DEHAL1 activity does not directly interfere with thyroid hormone synthesis or secretion. However, when this happens, the iodotyrosines pass to blood circulation and there is loss of them in the urine. The main consequence of this defect is, therefore a great loss of iodine, which enhances stimulation of thyroid, hyperplasia and goiter, and loss of the precursors of thyroid hormones. *DEHAL1* gene mutations were first described by Moreno et al. in three different consanguineous families of Turkish, Scottish and Moroccan descent [101]. All the patients had homozygous *DEHAL1* gene mutations, and presented severed goitrous hypothyroidism. Two missense mutations (c.301C>T [p.R101W], c.347T>C [p.I116T]) and a deletion of three nucleotides (c.315–317delCAT [p.F105-I106L]) were identified in exon 2 [101]. The p.R101W mutation was localized at the 10th position of the catalytic nitroreductase domain of the IYD protein, between two predicted FMN binding amino acids (R^{100}-S^{102}), whereas the p.I116T was identified in the nitroreductase domain but outside the predicted FMN binding sites [101]. The replacement of both phenylalanine at position 105 and isoleucine at position 106 by leucine was localized also within the nitroreductase domain near to putative FMN binding amino acids (R^{104}). All mutations disrupt the capacity of IYD to deiodinate.

Affink et al. by application of the HPLC tandem mass spectrometry assay identified two patients (mother and daughter) from a consanguineous Moroccan family with goitrous hypothyroidism [102]. The mother showed high-plasma TG and increased urinary DIT and MIT levels. Sequence analysis revealed a homozygous missense mutation (c.658G>A [p.A220T]) in exon 4 of *DEHAL1* gene [102]. In vitro expression studies of the mutant demonstrated that the p.A220T mutation abolishes enzymatic dehalogenase activity. A^{220} might be involved in FMN binding. Interestingly, a sibling who is a 14-yr-old boy heterozygous for the p.A220T mutation showed goiter, hypothyroidism with increased serum TG and urinary MIT and DIT excretion [102]. However, other heterozygous sibs have normal thyroid size, plasma TG levels and MIT or DIT excretion. Finally, a second consanguineous Moroccan family with IYD deficiency by homozygous p.A220T mutation was identified via a genome-wide approach [103]. Elevated MIT and DIT excretion was observed in hypothyroid 20-yr-old index patient and his still-euthyroid 4.5-yr-old sister.

Practice points

- Perchlorate descarge test and the measurement of thyroglobulin serum concentration represented an important diagnostic tool that help to differentiate patients with iodide organification disorder from those with iodotyrosine dehalogenase 1 or thyroglobulin deficiencies.
- The diagnostic criteria for iodide transport defect are: reduced or absent thyroid uptake, low I^- saliva to plasma ratio and a positive response to therapy with high dose of iodide.
- The diagnostic criteria for thyroid peroxidase defect are: intact iodide trapping, normal thyroglobulin protein expression and positive perchlorate descarge test. The duplication GGCC in exon 8 is a common alteration of the *thyroid peroxidase* gene in Caucasian population.
- The diagnostic criteria for thyroglobulin defect are: intact iodide trapping, negative perchlorate descarge test and low serum thyroglobulin levels. The p.C1058R and p.C1977S mutations are the most frequently identified *thyroglobulin* gene mutations in Japanese population, whereas the mutation p.R277* is the most frequently found in Caucasian populations.
- The diagnostic criteria for iodotyrosine dehalogenase 1 defect are: intact iodide trapping, negative perchlorate descarge test, elevated serum thyroglobulin levels with simultaneous elevated MIT and DIT concentrations in blood or urine.

Research agenda

- The intrafamilial phenotype variability or between families carrying the same mutation which implies the idea of additional genetic and epigenetic changes and/or environmental/nutritional factors. Recent advances in DNA sequencing technology (Next Generation Sequencing) allow us to study such phenotype variability.
- Gene panel sequencing also represents a valuable tool to investigate in the same patient the coexistance of multiple inactivacting mutations or functional polymorphisms in different thyroid genes.

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