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REVIEW

Thyroid Peroxidase Gene Mutations Associated with Thyroid Disorders

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Abstract—The *TPO* gene belongs to the group of genes responsible for the biosynthesis of thyroid hormones and encodes thyroid peroxidase, a key enzyme involved in this process. Mutations in these genes can result in thyroid dysfunction characterized by reduced levels of thyroid hormones. Hypothyroidism caused by *TPO* pathogenic variants typically presents as permanent hypothyroidism and is frequently associated with endemic goiter. This analytical review summarizes and systematizes data from the studies conducted in different regions of the world on mutations identified in the *TPO* gene in patients with hypothyroidism. Particular attention is given to mutations within structural and functional domains of thyroid peroxidase, which has a unique molecular architecture within its family.

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INTRODUCTION

Thyroid peroxidase (TPO) plays a key role in the biosynthesis of thyroid hormones and is one of the principal thyroid autoantigens. Autoantibodies against TPO are detected in the serum of 90-100% patients with autoimmune thyroid diseases (AITDs) [1-3]. One of the underlying causes of autoimmune disorders is a genetically determined defect in immunological surveillance. Mutations in genes involved in thyroid hormone biosynthesis, including the *TPO* gene, may lead to thyroid dysfunction accompanied by thyroid hormone deficiency [4]. Decreased thyroid function manifests as clinical or subclinical hypothyroidism and may develop in the context of both autoimmune and non-autoimmune thyroiditis. Among thyroid pathologies, hypothyroidism with autoimmune thyroiditis (AIT) accounts for 20.0-40.0% cases in adults and ~7.2% cases in children, depending on the iodine endemicity in a given region [1, 2]. Congenital hypothyroidism (CH), the clinical manifestations of which most commonly include goiter and persistent hypothyroidism (PH), occurs in ~1 in 2000-4000 newborns. Thyroid dysgenesis is responsible for ~85% cases of persistent primary goiter, while congenital defects in the thyroid hormone biosynthesis (dyshormonogenesis) account for 10-15% of cases [5, 6]. Lowering the thyroid-stimulating hormone (TSH) cut-offs in neonatal screening, together with demographic changes, have led to an almost twofold increase in the reported incidence of CH – from 1 : 3500 to 1 : 1714. Additional cases identified at the lower TSH cut-offs are generally associated with milder forms of hypothyroidism [7].

TPO is located on the apical membrane of thyrocytes that faces the thyroid follicle lumen. The lumen contains colloid, which is composed primarily of thyroglobulin, the largest and most important thyroid autoantigen. TPO is a glycosylated hemoprotein that catalyzes several essential steps in the synthesis of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Specifically, TPO oxidizes iodide in the presence of hydrogen peroxide to generate molecular iodine, which subsequently iodinates tyrosine residues in the thyroglobulin molecule [3, 8].

Mutations in the *TPO* gene can impair production of thyroid hormones. Inactivating mutations underlie a specific form of thyroid dysfunction, thyroid

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dyshormonogenesis, occurring due to complete (total iodide organification defect, TIOD) or partial (partial iodide organification defect, PIOD) failure of iodide organification. TIOD represents the prevalent phenotype among these autosomal recessive disorders [9-11].

The first publication devoted to the study of mutations in the *TPO* gene dates back to 1992. Abramowicz et al. [12] were the first to describe a *TPO* gene mutation identified in a patient with CH. They demonstrated that the patient was homozygous for a frameshift mutation caused by a 4-bp insertion in exon 8 of the *TPO* gene.

Over the following decade, numerous reports have been published on the identification of *TPO* mutations in the genomes of patients with thyroid diseases, including findings from large population studies, that have led to the publication of several comprehensive review articles. In most cases, *TPO* mutations have been discussed alongside alterations in other genes involved in the thyroid hormone biosynthesis and embryonic thyroid development. For example, a review by Troshina et al. [4] analyzed mutations in the *TSHR* (*thyroid-stimulating hormone receptor), NIS* (*sodium-iodide symporter/SLC5A5)*, *TPO*, *THOX (dual oxidase 2/DUOX2*), and *TG* (thyroglobulin) genes and associated clinical phenotypes, focusing on genetic disorders of thyroid hormone biosynthesis (hormonogenesis) [4].

The prevalence of hypothyroidism caused by mutations in the *TPO* gene varies considerably across different regions of the world. Reported rates include 1 : 66,000 in the Netherlands [13]; 1 : 20,000 in Slovenia, Bosnia, and Slovakia [14]; 1 : 177,000 in Japan [15, 16]; and 1 : 40,000 in Israel [17]. Newborn screening data from China indicate that the incidence of *TPO*-related hypothyroidism in China is higher than the global average. In Xinjiang, a region in the northwest China accounting for approximately one-sixth of the country's territory, the incidence was reported as 1 in 1468 newborns. This rate is higher than that observed in Guangxi (1%), located in southern China, but lower than the rate reported in Shanghai (10%) in eastern China [18, 19]. In Iran (Isfahan), a neonatal screening program for CH was initiated in 2002. The frequency of *TPO* gene mutations in this population was estimated at 1 : 357 newborns, which is approximately ten times higher than the rates reported in comparable studies from North America and Europe [20].

Cases involving combinations of mutations in the *TPO* gene and sensorineural hearing loss have been reported [21]. Evidence suggests that hearing loss associated with *TPO* gene mutations is secondary and results from the thyroid hormone deficiency during critical early developmental stages (embryonic and neonatal periods), leading to the delayed maturation of inner ear structures [22].

TPO plays a crucial role not only in the thyroid hormone synthesis and maintenance of normal thyroid function, but also in the diagnostics and treatment of various thyroid disorders. Studies have shown that TPO is highly expressed in normal thyroid tissue and in benign thyroid diseases, whereas its expression in papillary thyroid carcinoma is weak or absent, which is used to distinguish between benign and malignant thyroid lesions clinically [23]. Certain *TPO* genetic variants have been reported as associated with thyroid carcinoma and hypoechoic thyroid nodules [23, 24].

TPO GENE STRUCTURE

The *TPO* gene was cloned in 1987 its full-length mRNA transcript is 3048 bp long. The gene has an exon–intron organization and is located on the short arm of chromosome 2 (2p25.3) [25]. In addition to the full-length TPO-1 isoform, which comprises 933 amino acid (a.a.) residues and includes all exons, characterization of the exon–intron boundaries in the *TPO* gene revealed at least ten alternatively spliced isoforms that differ from the canonical form by the exclusion of one or more exons. Analysis of TPO mRNA expression in normal thyroid tissue showed that only 19% transcripts were the full-length mRNA, whereas the remaining 81% were truncated mRNA variants. The most prevalent transcript (56%) encodes the TPO-2 isoform, which lacks the residues 533-589 encoded by exon 10. However, subsequent studies demonstrated that this isoform is unable to bind heme, a property essential for the TPO enzymatic activity [26, 27].

The TPO-1 protein includes three main structural regions: an extracellular domain (residues 1-846), a transmembrane domain (residues 847-871), and an intracellular domain (residues 872-933). Analysis of the TPO primary sequence reveals homology between one of the regions and the complement control protein (CCP) domain; another TPO region was homologous to the epidermal growth factor (EGF)-like domain, a feature commonly observed in membrane-bound enzymes. The binding of the heme prosthetic group is mediated by two conserved histidine residues, distal His239 and proximal His494. TPO contains five potential N-glycosylation sites, four of which (Asn129, Asn307, Asn342, and Asn569) are located in the extracellular domain [3, 8].

Comprehensive population analysis and bioinformatic characterization of *TPO* variants cataloged in the Genome Aggregation Database (gnomAD) v.2.1.1 were reported in a 2022 review [28], which identified 183 distinct *TPO* variants. The study analyzed

the distribution of cytosine substitution variants, nonsense mutations, frameshifts, and splice (acceptor/donor) site mutations across different ethnic groups. Based on the gnomAD v2.1.1 dataset, the estimated prevalence of heterozygous carriers of potentially deleterious *TPO* variants was 1 in 77.

Reports of *TPO* mutations in patients with functional thyroid disorders continue to accumulate, making the publication of a dedicated review on the most frequently identified mutations in patients with CH across different regions particularly timely. In the present review, we described the distribution of mutations across various *TPO* domains, including those located near catalytically active sites and within immunodominant regions (IDRs), i.e., TPO fragments binding antibodies in the sera of patients with AITDs, with special attention to the types of mutation and its association with clinical manifestations of hypothyroidism.

TPO GENE MUTATIONS

Mutations in the TPO extracellular domain. The extracellular domain of TPO, located on the outer surface of the thyrocyte, was reconstructed using computer modeling. This domain is characterized by the presence of myeloperoxidase (MPO)-like region (a.a. 142-733), which exhibits the enzymatic activity and is highly homologous to the human MPO. It is followed by the CCP-like region (a.a. 739-795), which enables TPO to directly activate complement without the involvement of immunoglobulins [29]. The last fragment of the TPO extracellular domain is the epidermal growth factor (EGF)-like region (a.a. 796-841) [30].

The extracellular domain is a hotspot for numerous mutations in the *TPO* gene.

Mutations in the TPO gene sequence encoding residues 1-141 of the extracellular domain. This fragment contains the catalytically active sites and the first glycosylation site of the extracellular domain (Asn129). The data on *TPO* mutations identified in the genomes of patients with hypothyroidism from various regions worldwide are summarized in Table 1, where they are organized according to their position within the domain and the chronological order of their discovery. Associated clinical complications, including goiter, multinodular goiter (MNG), Pendred syndrome, and others, are reported for each case.

Ten mutations in the *TPO* gene were identified in 21 individuals in a cohort of 83 Turkish and 21 Pakistani patients with CH. Among these, two novel mutations, Ala5Thr and Tyr55X, were located within the 1-141 a.a. fragment of the extracellular domain [31]. Clinical features observed in patients carrying the Ala5Thr mutation were consistent with a partial loss-of-function effect [32]. The nonsense mutation Tyr55X was detected in two affected siblings from a single family, both of whom exhibited TIOD. Nonsense mutations occurring in the N-terminal sequence-coding region of the *TPO* gene are known to entirely abolish the enzymatic activity of TPO, leading to severe impairment of iodide organification and consequent thyrotoxicosis [33].

A multicenter genomic study of 49 patients with CH from the UK and Middle Eastern countries identified the frameshift mutation Glu17AspfsX77 located in the 1-141 a.a. fragment of the extracellular domain was identified in a patient with hypothyroidism complicated by the Pendred syndrome [34].

Analysis of genomes of 230 Chinese patients with CH identified 35 mutations in the *TPO* gene in 23 individuals (10.0%). Among these patients, biallelic mutations were detected in 13 individuals, while heterozygous mutations were found in 10 patients. A novel mutation (Ser37Pro) located at the extracellular domain N-terminus was identified in one patient [35]. A frameshift mutation (a 20-bp duplication in exon 2) was identified in a genomic study of a TIOD patient from the Netherlands [36]. In a subsequent publication, the same authors reported that among 16 *TPO* gene mutations identified in 45 Dutch patients with TIOD, the frameshift mutation *ins20bp141* was detected in five individuals. This mutation introduces a premature stop codon in exon 3 [13].

A molecular genetic study of 244 Russian patients with CH identified 20 *TPO* variants representing different types of nucleotide changes (including 15 novel variants) in 30 patients (12.3%). Compound heterozygous mutations were detected in 9 patients, while heterozygous mutations were identified in 21 patients. These *TPO* gene defects included frameshift-inducing insertions and deletions (n = 4), a nonsense mutation (n = 1), missense mutations (n = 13), and intronic mutations leading to aberrant splicing (n = 2). Five mutations were localized within the N-terminal extracellular domain of TPO (residues 1-141). The Pro70Ala mutation was identified in two patients; in one case, it occurred as part of a compound heterozygous mutation (Pro70Ala/Cys808AlafsX24, exon 14). The heterozygous nonsense mutation Arg89X was detected in a patient with CH without goiter. The compound heterozygous mutation Arg89X/Ala397ProfsX76, affecting the 1-141 a.a. region of TPO, was identified in a patient with hypothyroidism complicated by the Pendred syndrome [17, 33], as well as in another patient with hypothyroidism and MNG. Additionally, the compound heterozygous mutation Met94Thr/Asp240Val was detected in a patient with hypothyroidism complicated by goiter. The heterozygous Ser97Pro mutation was found in two patients with CH, including one case complicated by goiter [37].

Table 1. Mutations in the *TPO* gene fragment encoding residues 1-141 of the extracellular domain

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Ala5Thr	Turkey	1	Hom	TIOD	[31]
Ala5Thr	Turkey	1	Hom	PIOD	[32]
Glu17AspfsX77	UK (+)	1	ComHet	Pendred syndrome	[34]
Ser37Pro	China	1	Het	HP	[35]
ins20 bp141	Netherlands	1	ComHet	TIOD	[36]
ins20 bp141	Netherlands	5	ComHet	TIOD	[13]
Tyr55X	Turkey	2	Hom	hypoplasia/ Tourette syndrome	[31]
Tyr55X	Turkey	2	Hom	TIOD	[33]
Pro70Ala	Russia	1	Het	PH	[37]
Pro70Ala	Russia	1	ComHet	PH	[37]
Glu72fsX86	Argentina	1	ComHet	TIOD	[38]
Arg89X	Turkey	1	Het	goiter	[39]
Arg89X	Russia	1	ComHet	Pendred syndrome	[22]
Arg89X	Russia	2	Het	hypoplasia	[37]
Arg89X	Russia	2	Het	goiter	[37]
Arg89X	Russia	1	ComHet	MNG/Pendred syndrome	[37]
Met94Thr	Russia	1	ComHet	goiter	[37]
Ser97Pro	Russia	1	Het	goiter	[37]
Ser97Pro	Russia	1	Het	PH	[37]
Asn129fsX208	Argentina	2	Het	TIOD/goiter	[9]
Asn129fsX208	Argentina	1	ComHet	TIOD	[38]
Ser131Pro	Portugal	1	ComHet	goiter	[40]
Ser131Pro	Russia	1	ComHet	MNG	[37]
insGGCC395	Italy	1	Hom	MNG/BRRS	[41]
Pro135His	China	1	Het	PH	[19]

Note. Mutations (or amino acid substitutions) identified repeatedly in different countries are shown in bold; mutations in the *TPO* nucleotide sequence are shown in *italic*; *n*, the number of carriers of the identified mutation in the study; UK (+) represents United Kingdom, Oman, Saudi Arabia, UAE, and Turkey; Het, heterozygous mutation; Hom, homozygous mutation; ComHet, compound heterozygous mutation; BRRS, Bannayan–Riley–Ruvalcaba syndrome. For other abbreviations, see the text of the article.

Rivolta et al. [38] described the compound heterozygous mutation *c.215delA/c.2422T>C* (p.Glu72fsX86/p.Cys808Arg) identified in the genome of an Argentinian patient with hypothyroidism associated with the iodide organification defect [38]. The heterozygous mutation Arg89X was detected *in utero* in an embryo of a patient from Turkey with hypothyroidism complicated by goiter. The nonsense mutation c.*265C>T* located in exon 4, leads to the formation of a stop codon at position 89 and, as a consequence, to a severe truncation of the TPO protein with a complete loss of its heme-binding site [39].

In a cohort of 40 Argentinian patients with hypothyroidism and goiter, 14 individuals with an iodide organification defect were selected for genomic analysis. Mutations in the *TPO* gene were identified in seven of these patients. One patient was a compound heterozygote carrying two *TPO* mutations, whereas the remaining patients were heterozygous for a single *TPO* mutation. Five novel mutations were detected, including one single-nucleotide deletion and four single-nucleotide substitutions. The *c.387delC* (p.Asn129fsX208) frameshift mutation was identified in exon 5 [9]. The same mutation was later reported in the compound heterozygous genotype Asn129fsX208/Gly387Arg [38]. Notably, this mutation affects Asn129 residue, the first glycosylation site in the TPO extracellular domain.

Among 55 patients with CH treated at clinics in the Portugal's capital, the novel missense mutation *c.391T>C* (p.Ser131Pro) was identified in one patient in a compound heterozygous state with Cys808AlafsX23 [40]. The same p.Ser131Pro variant was also reported as a compound heterozygous mutation with *g.IVS13+2T>G* in a Russian patient with hypothyroidism complicated by MNG [37]. The homozygous *insGGCC395* mutation was found in an Italian patient with CH and Bannayan–Riley–Ruvalcaba syndrome (BRRS), who also developed MNG [41]. Finally, the heterozygous Pro135His mutation was identified in the genome analysis of 219 patients with hypothyroidism from northwestern China [19].

In conclusion, mutations affecting the TPO extracellular domain of (residues 1-141) are rare and usually observed in isolated cases. Fourteen such mutations have been identified, including three homozygous mutations, a missense mutation (Ala5Thr), a nonsense mutation (Tyr55X), and a frameshift mutation (*insGGCC395*), as well as eight compound heterozygous mutations.

The Arg89X mutation was detected in patients with CH from Russia and Turkey, whereas the Ser131Pro mutation was found in patients with CHC from Russia and Portugal. The Arg89X mutation was detected in seven patients presenting with diverse hypothyroidism phenotypes and associated complications, including goiter, MNG, and Pendred syndrome.

Mutations in the MPO-like fragment (residues 142–733) of the TPO extracellular domain. The MPO-like fragment of the TPO extracellular domain (residues 142-733) contains sequences primarily responsible for the TPO enzymatic activity, three N-linked glycosylation sites (Asn307, Asn342, and Asn569), two histidine residues (distal His239 and proximal His494) directly involved in coordination of the heme prosthetic group essential for the TPO catalytic function [3, 8], and an IDR that binds anti-TPO antibodies in the serum of patients with AITDs. It was found that the IDR comprises two overlapping regions, designated as domains A and B. Domain A (IDR/A; residues 456-631) contains the main binding site for IDR/A-specific monoclonal antibodies (mAbs) located within residues 599-617. Domain B (IDR/B) is composed of five discrete regions: a.a. 210-225, 353-363, 549-563, 713-717, and 766-775. Notably, truncation of TPO after residue 771 had virtually no impact on the protein recognition by autoantibodies in all tested patient serum samples [3, 42].

Various strategies have been employed to studying the TPO structure, including the use of recombinant TPO proteins and anti-TPO mAbs. Serum autoantibodies from patients with AITD (n = 10) were tested against peptides corresponding to the TPO fragments 590-622 and 710-722, revealing two surface epitopes [43]. These results were obtained by ELISA with Fab fragments of human mAbs against IDR/A and IDR/B, which were tested against both native TPO and recombinant proteins immobilized on a solid phase. The recombinant proteins included point mutations at specific amino acid positions, such Arg225, Glu604, Asp620, Asp624, Lys627, Arg646, and Asp707 [44]. Using ten mAb variants from a panel of 36, five groups of antigenic determinants were identified in the TPO molecule, encompassing both linear and conformational epitopes. Linear epitopes (residues 606-617 and 706-720) were recognized by mAb1 and contributed to the formation of the conformational epitope 3, consistent with the three-dimensional models of TPO showing that these fragments are spatially close despite being separated in the primary sequence [45].

The data on mutations in the MPO-like region identified in the genomes of patients with hypothyroidism from different world regions are presented in Table 2.

Of 16 mutations identified in the genomes of 45 Dutch patients with TIOD, three were in the MPO-like region and corresponded to three alternative splicing variants: *G3C 439 GG/gt3GC*/gt (exon 4), *G3A 1858 AG/gt3AA*/gt (exon 10), and *G3A 11 AG/gt3AG/at* (intron 10). The most common mutations

Table 2. Mutations in the MPO-like region (residues 142-733) of the extracellular domain of TPO

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
G3C 439 GG/gt3GC/gt	Netherlands	1	Het	TIOD	[13]
Ala148Tyr	Russia	1	Het	PH	[37]
483-2A>G	Sudan	1	ComHet	goiter	[46]
Arg175Gln	Japan	2	ComHet	goiter/PH	[47]
Arg175X	Bosnia	1	ComHet	goiter/Gardner syndrome	[14]
Arg189Gln	China	1	Het	PH	[35]
Asp223del	Russia	2	ComHet	PH	[37]
670_672del	Malaysia	2	ComHet	goiter/PH	[48]
Asp224del	China	1	Het	goiter	[49]
Asp224del	Japan	1	ComHet	goiter	[50]
224_224del	China	3	ComHet	goiter	[35]
Gln235X	Germany	1	ComHet	Pendred syndrome	[21]
Asp240Val	Russia	1	ComHet	PH	[37]
Ala257Ser	Tunisia	9	Hom	goiter/Gardner syndrome	[51]
Ala257Ser	Tunisia	2	Hom	Gardner syndrome	[51]
Ala257Ser	Tunisia	2	Het	goiter/Gardner syndrome	[51]
859G>T	Iran	1	Het	PH	[20]
Gln266X	Argentina	1	ComHet	PH	[52]
Cys269Ser	China	1	ComHet	PH	[35]
820-2 A>G	Japan	1	ComHet	Goiter	[50]
820-1G>A	China	1	ComHet	PH	[19]
Arg279Trp	China	1	Het	goiter/PH	[19]
843delC	China (Taiwan)	1	ComHet	TIOD	[53]
Arg291His	UK (+)	1	ComHet	goiter	[35]
Ser292Phe	Israel	4	ComHet	TIOD/goiter	[17]
Ser292Phe	Tunisia	2	Hom	TIOD/MNG	[51]
Asn307Ser	Israel	1	Het	TIOD/goiter	[17]
Asn307Thr	Argentina	2	Het	TIOD/goiter	[9]

Table 2 (*cont.*)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Ser309Pro	China	1	Het	PH	[19]
Glu319Arg	Turkey	2	Het	goiter	[54]
Glu319Arg	Turkey	5	ComHet	goiter	[31]
Glu319Arg	Turkey	2	Hom	goiter	[31]
Ala326Thr	Netherlands	1	Het	TIOD	[13]
Gly331Val	UK (+)	1	ComHet	goiter	[35]
Asp333Asn	Pakistan	3	Hom	TIOD	[31]
Glu337Lys	China	1	Het	PH	[19]
Arg341Gln	Japan	2	ComHet	PH	[15]
Gly348Arg	Russia	1	ComHet	PH	[37]
Arg361Leu	China	1	Het	hypoplasia	[19]
Pro368Leu	Chile	2	ComHet	goiter	[55]
1207G>T	Iran	1	Het	PH	[20]
Gly387Arg	Argentina	1	ComHet	TIOD	[38]
Gly387Arg	Japan	2	Hom	goiter	[50]
1182_1183ins CGGC	Finland	3	Hom	PH	[56]
1182_1183ins CGGC	Finland	1	ComHet	PH	[56]
Arg396fsX472	Netherlands	1	ComHet	TIOD	[36]
Arg396fsX472	Netherlands	2	ComHet	TIOD	[13]
Arg396fsX472	Argentina	1	Het	TIOD/goiter	[9]
Arg396fsX472	Argentina	1	ComHet	TIOD/goiter	[9]
Arg396fsX472	Argentina	4	ComHet	PH	[52]
Ala397ProfsX76	Argentina	1	Hom	TIOD/goiter	[12]
Ala 397ProfsX76	Portugal	2	ComHet	goiter	[40]
Ala397ProfsX76	Portugal	2	Hom	goiter	[40]
Ala397ProfsX76	Bosnia	1	Het	goiter/Gardner syndrome	[14]
Ala397ProfsX76	Slovenia	4	Het	goiter/Gardner syndrome	[14]

Table 2 (*cont.*)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Ala397Prof sX76	Slovenia	2	Het	Gardner syndrome	[14]
Ala397ProfsX76	Slovakia	1	Het	follicular adenoma	[14]
Ala397ProfsX76	Bosnia	3	ComHet	goiter	[14]
Ala397ProfsX76	Slovenia	3	Hom	goiter/Gardner syndrome	[14]
1184_1187 dup.GCCG	Turkey	1	Hom	Goiter	[31]
Ala397ProfsX76	UK (+)	1	Het	TIOD/Goiter	[34]
Ala397Pro fsX76	Russia	1	ComHet	Pendred syndrome	[22]
Ala397ProfsX76	Russia	1	Het	goiter	[37]
Ala397ProsX76	Russia	2	ComHet	MNG	[37]
Ala397ProfsX76	Russia	1	Het	hypoplasia	[37]
1283G>C	Iraq	1	Het	PH	[20]
1242G>T	Brazil	2	ComHet	PIOD/goiter	[10]
Asn425Ser	Portugal	1	ComHet	goiter	[40]
insGGCC1277	Netherlands	3	ComHet	TIOD	[36]
insGGCC1277	Netherlands	6	Hom	TIOD	[36]
insGGCC1277	Netherlands	13	ComHet	TIOD	[13]
insGGCC1277	Netherlands	12	Hom	TIOD	[13]
insGGCC1277	Brazil	4	Hom	TIOD/goiter	[10]
insGGCC1277	Brazil	2	ComHet	TIOD/goiter	[10]
Ala426Gly	Sudan	3	Hom	goiter	[46]
Ala426Gly	Sudan	1	ComHet	goiter	[46]
Trp428Arg	China	1	ComHet	PH	[35]
Ala430Glu	China	1	ComHet	goiter	[35]
Val433Met	Argentina	1	ComHet	TIOD/goiter	[9]
His438Arg	Finland	1	ComHet	PH	[56]
Ala443Val	China	1	ComHet	PH	[19]
Ala443Pro	China	2	Hom	goiter	[35]
Ala443Pro	China	1	ComHet	goiter	[35]

Table 2 (*cont.*)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Ile447Phe	Netherlands	1	Het	TIOD	[13]
Tyr453Asp	Netherlands	1	ComHet	TIOD	[36]
Tyr453Asp	Netherlands	9	ComHet	TIOD	[13]
Tyr453Asp	Germany	1	ComHet	goiter/Pendred syndrome	[21]
Tyr453Asp	UK (+)	1	ComHet	Goiter	[34]
1425delC	Netherlands	1	Het	TIOD	[13]
Ala477Asn483del	Slovenia	2	Het	goiter/Gardner syndrome	[14]
Ala477Asn483del	Bosnia	2	Het	goiter/Gardner syndrome	[14]
Asn483Lys	Russia	1	Het	PH	[37]
Ala489Thr	Russia	2	ComHet	PH	[37]
Ala489Thr	China	1	ComHet	PH	[35]
Arg491His	UK (+)	1	ComHet	TIOD/goiter	[34]
Arg491His	UK (+)	1	Hom	TIOD/goiter	[34]
Gly493Ser	China (Taiwan)	1	ComHet	TIOD	[53]
Gly493Ser	Portugal	2	Hom	goiter	[40]
Gly493Ser	Israel	9	ComHet	TIOD/goiter	[17]
Gly493Ser	Iraq	1	ComHet	goiter	[57]
Pro499Lys	Argentina	1	Het	TIOD/goiter	[9]
1502T>G	Malaysia	2	Hom	MNG	[58]
Glu510Ala	UK (+)	1	Het	PH	[34]
Pro512His	Sudan	2	ComHet	goiter	[46]
Trp527Cys	Netherlands	1	Het	TIOD	[13]
Trp527Cys	Japan	1	Hom	TIOD/goiter	[50]
Trp527Cys	Russia	1	Het	goiter	[37]
1597+1G>T	Brazil	1	Het	PIOD/goiter	[10]
Arg540X	Netherlands	2	ComHet	TIOD	[36]
Arg540X	Netherlands	4	ComHet	TIOD	[13]
Arg540X	Israel	9	ComHet	TIOD/goiter	[17]

Table 2 (*cont.*)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Arg540X	Turkey	3	Het	goiter	[54]
Arg540X	Japan	2	ComHet	PH	[15]
Arg540X	Iraq	2	ComHet	goiter	[57]
Arg540X	Turkey	8	ComHet	TIOD	[31]
Arg540X	Turkey	2	Hom	TIOD	[59]
Gly553Cys	Japan	3	ComHet	goiter	[50]
Thr561Met	China	1	ComHet	goiter	[18]
Ser571Arg	China	1	Het	goiter	[19]
Asp574 Lys575 del	Japan	3	ComHet	goiter	[50]
Ala576Val	Germany	1	ComHet	MNG	[60]
Arg584Gln	UK (+)	2	Hom	PH	[34]
Arg584Gln	Russia	1	Het	PH	[37]
Gly587Arg	Sudan	2	ComHet	goiter	[46]
Asn592Ser	China	1	ComHet	PH	[19]
1780C>A	Brazil	2	Het	PIOD/goiter	[10]
1780C>A	Brazil	2	ComHet	PIOD/goiter	[10]
Arg595Lys	Argentina	4	ComHet	PH	[52]
Arg595Lys	Argentina	2	Hom	PH	[52]
Glu596X	Pakistan	3	Hom	TIOD	[31]
Ser617Arg fsX23	Russia	1	Het	PH	[37]
Ser617Arg fsX23	Russia	1	ComHet	PH	[37]
G3A 1858 AG/gt3AA/gt	Netherlands	3	Het	TIOD	[13]
G3A 11 intron 10 AG/gt3AG/atT/	Netherlands	1	Het	TIOD	[36]
G3A 11 intron 10 AG/gt3AG/atT/	Netherlands	1	Het	TIOD	[13]
1978 C>G	Iraq	2	Het	goiter	[57]
Asp633Val	Russia	1	Het	PH	[37]
Asp633Asn	Pakistan	3	Hom	TIOD	[31]
Arg648Gln	USA	3	ComHet	TIOD	[61]

Table 2 (*cont.*)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Arg648Gln	China	1	ComHet	goiter	[13]
Ile657Thr	China	1	ComHet	goiter	[49]
Ile657Thr	China	1	Het	PH	[49]
Gln660Glu	Brazil	1	ComHet	TIOD/goiter	[10]
Gln660 Glu	Portugal	5	ComHet	goiter	[40]
Gln660 Glu	Portugal	1	Hom	goiter	[40]
Arg665Thr	Japan	1	ComHet	TIOD/goiter	[62]
Arg665Thr	Japan	2	ComHet	TIOD/goiter	[47]
Arg665 Gln	Argentina	1	Het	TIOD/goiter	[9]
Arg665 Gln	Pakistan	4	ComHet	TIOD	[31]
Arg665 Gln	Russia	1	Het	PH	[37]
2088C>T	Iran	1	Het	PH	[20]
Gly667Asp	Argentina	1	ComHet	PH	[52]
Gly667Ser	Slovenia	1	Het	MNG	[14]
Glu673Lys	Russia	4	Het	PH	[37]
Glu673Lys	China	1	Het	PH	[19]
Asn674Ser	China	1	Het	goiter	[19]
2068G>C	Brazil	2	ComHet	TIOD/goiter	[10]
Arg693Trp	Netherlands	2	Het	TIOD	[13]
2084G>A	Brazil	2	Het	PIOD/goiter	[10]
2090G>A	China	2	ComHet	MNG	[24]
Phe718X	Netherlands	1	Het	TIOD	[13]

Note. For designations, see Note to Table 1.

were *insGGCC1277*, Tyr453Asp, Arg540X, and Arg396fsX472 [13]. Previously, Dutch researchers [36] reported seven different mutations among 15 patients from nine apparently unrelated families. These included three frameshift mutations and four single nucleotide substitutions. Among the patients, nine individuals from five families were compound heterozygotes, while six patients from four families were homozygous for the *insGGCC1277* mutation [36]. In a cohort of 43 patients with the Gardner syndrome and PH, representing 39 unrelated families from Slovenia (30 patients), Bosnia (11 patients), and Slovakia (2 patients), mutations in the *TPO* gene were detected in 46% of participants. Seven different mutations were identified, four of which (Arg175X, Ala397ProfsX76, Ala477Asn483del, Gly667Ser) were

located in the MPO-like region [14]. In a consanguineous Israeli population with CH and persistent primary hypothyroidism, one novel mutation (Ser292Phe) and two previously reported mutations (Gly493Ser and Arg540X) were associated with idiopathic organification defects (IODs). The latter two mutations were present in 90% of patients. Thyroid enlargement, mostly multinodular and sometimes retrosternal, was observed in 64% of patients, while neurological complications occurred in 59% (13 patients). Four subjects carrying two different TPO mutations exhibited sensorineural hearing loss, highlighting the need for long-term follow-up in patients with TPO mutations [17]. A 12-year clinical study of 13 patients (10 men and 3 women) with thyroid dyshormonogenesis and Gardner syndrome from three related Tunisian families revealed significant phenotypic variability, including goiter (9 patients), sensorineural hearing loss (4 patients), and mental retardation (6 patients). The Ala257Ser mutation in a homozygous state was detected in 11 subjects. Additionally, the previously described Ser292Phe mutation was detected in homozygosity in two patients, both presenting with multinodular thyroid enlargement. Missense mutations are the most annotated changes in the *TPO* gene; however, due to limited functional data, their interpretation often involves *in silico* analysis. Thus, according to *in silico* analysis, the Ser292Phe mutation may reduce the TPO catalytic cavity, thereby limiting substrate access to the enzyme's active site [51]. In a study of 219 patients with hypothyroidism from northwestern China, the use of high-throughput sequencing and bioinformatic analysis allowed to identify 19 rare *TPO* variants in 17 individuals (7.8%), including 7 novel variants. Most were heterozygous, with two compound heterozygotes (*g.IVS7-1G>A*/Ala443Val and Asn592Ser/Asn798Lys). The splicing variant *c.820-1G>A* (*g.IVS7-1G>A*) in intron 7 may impair mRNA splicing, producing an inactive protein [19]. The study of 26 Sudanese families with CH and goiter and suspected Gardner syndrome revealed rare mutations in the MPO-like region, including *c.483-2A>G* (g.*IVS5-2A>G*), Ala426Gly, Pro512His, and Gly587Arg. The Ala426Gly mutation was observed in three patients in a homozygous state and in one compound heterozygote (Ala426Gly/Cys808AlafsX24) [46]. Two siblings with goitrogenic CH from Japan carried two missense mutations, including novel *c.614G>A* (p.Arg175Gln) mutation. In cotransfection experiments in CHO-K1 cells, mRNA transcribed from the mutant *c.614G>A* gene encoded TPO with a molecular weight similar to that of the wild-type protein [47]. As mentioned above, mutations affecting the first extracellular glycosylation site (Asn129) were reported in Argentina, along with mutations in the MPO-like region. The *c.920A>C* (Asn307Thr) mutation affecting the second glycosylation site, was detected in two patients. Additionally, a GGCC duplication in exon 8 (*c.1186_1187insGGCC*, p.Arg396fsX472) was reported, with one patient being a compound heterozygote (Arg396fsX472/Val433Met) [9]. The Glu319Arg mutation has also been intermittently reported in Turkish patients [31, 54]. In a combined cohort of 83 Turkish and 21 Pakistani patients with PH and dyshormonogenesis, seven TPO mutations were identified, including two novel mutations (Glu596X, Asp633Asn) and five previously described ones, all located in the 142-733 fragment of the extracellular domain [31].

Screening by next-generation sequencing (NGS) identified 49 cases of CH across 34 ethnically diverse families from the United Kingdom and the Middle East. Putative CH-associated mutations were found in 29 cases. Monogenic defects, four of which were associated with TPO, were identified in 19 cases. In ten cases, mutations were observed in different genes, including six mutations in the *TPO* gene. These included two known pathogenic missense mutations (Arg491His, Arg665Gln), two novel frameshift mutations (Cys808AlafsX24; Ala397ProfsX76), and two novel missense mutations (Arg291His, Gly331Val) [34]. Sequencing of the *TPO* gene in a German patient revealed heterozygous mutations that resulted in a stop codon at position 235 (novel nonsense mutation Gln235X) and an amino acid substitution at codon 453 (known missense mutation Tyr453Asp) [21]. In a cohort of 102 patients with CH from Japan, autosomal recessive hypothyroidism was diagnosed in 14 individuals. Biallelic mutations in the *TPO* gene were detected in the genomes of two patients: one mutation led to the amino acid substitution at position 341 (novel missense mutation Arg341Gln) and the other (*c.1618C>T*) resulted in a stop codon at position 540 (known nonsense mutation Arg540X) [15].

The genomes of twelve biologically unrelated Malaysian-Chinese patients with CH were analyzed for mutations in the *TPO* gene. Two novel mutations, *c.670_672del* and *c.1186C>T*, were identified in four of the twelve patients. *In silico* analysis indicated that these mutations are likely to disrupt the structure and/or function of the TPO protein [48].

Analysis of the *TPO* gene in thirty Chinese children with CH revealed six genetic variants in six patients, including two inactivating heterozygous mutations located in the 142-733 fragment of the extracellular domain: *c.670_672del* (p.Asp224del) and *c.1970T>C* (p.Ile657Thr) [49]. NGS-based genetic screening showed that one Japanese patient was a compound heterozygote for two *TPO* mutations (p.Asp224del/*g.IVS7-2 A>G*), while another patient was homozygous for the known Trp527Cys mutation. *In vitro* functional assays in HEK293 cells demonstrated that both mutations led to a partial loss of

enzymatic activity. These findings suggest a previously unrecognized correlation between clinical phenotypes and residual TPO enzymatic activity in patients with the TPO deficiency. Additionally, three patients from three different families were found to be compound heterozygous for two TPO mutations (Gly553Cys/Asp574Lys575del), while two other patients were homozygous for the known Gly387Arg mutation [50].

Three novel mutations, Gln266X, Arg595Lys, and Gly667Asp, and a previously described mutation, *c.1186_1187insGGCC* (p.Arg396fsX472), were identified in seven patients from four unrelated Argentine families with CH. Four patients were compound heterozygotes for the Arg396fsX472/Arg595Lys mutations, two patients were homozygotes for Arg595Lys, and one patient had the compound heterozygous Gln266X/Gly667Asp mutation [52]. Single-strand conformation polymorphism (SSCP) method was used to detect *TPO* mutations in five unrelated patients with TIOD in Taiwan. Five novel mutations were detected; three of them were frameshift mutations and two were single deletions. The other two substitutions were single nucleotide substitutions. A deletion of C at position 843 (*c.843 del.C*) in exon 8 and a single nucleotide substitution *c.1477G>A*, which results in the Gly493Ser substitution, are located in the MPO-like region of TPO [53]. In Brazil, genome analysis of 14 patients with goiter, including seven individuals with TIOD and seven with PIOD, identified various *TPO* mutations. Among TIOD patients from three families, a homozygous GGCC insertion in exon 8 at position 1277 was observed in family 1; a compound heterozygous GGCC insertion in exon 8 at position 1277 together with the *c.2068G>C* mutation in exon 11 was observed in family 2; and a compound heterozygous *c.2068G>C* mutation in exon 11 with a C nucleotide insertion of in exon 14 between positions 2505 and 2511 was found in family 3. In PIOD patients, heterozygous mutations were detected in exon 10 (*c.1780C>A*) and exon 11 (*c.2084G>A*) in patients from families 4 and 5, respectively, while a compound heterozygous variant with mutations in exons 8 and 10 (*1242G>T* and *1780C>A*) was identified in a patient from family 6. A heterozygous mutation at the first nucleotide of the exon/intron boundary (*c.1597+1G>T*, *g.IVS9+1G>T*) was detected in a patient from family 8 [10].

Two different mutations were detected in five children with congenital goiter from three consanguineous families from Turkey. A nonsense mutation in exon 10 (Arg540X) was identified in affected children from families I and II, and a novel missense mutation in exon 8 (Glu319Arg) was identified in the third family [54]. In a cohort from Chile, two potentially pathogenic *TPO* mutations resulting in Pro368Leu and Val748Met substitutions were identified in 2 of 12 patients (16.6%). One patient carried compound heterozygous mutations *c.1103C>T* and *c.2242G>A*, while the other patient was heterozygous for *c.2242G>A*. Both patients presented with diffuse goiter [55]. NGS performed in 15 patients with sporadic disease and 11 patients with familial disease from Finland revealed Asp394fs and His438Arg mutations in four patients with the familial disease [56].

The screening of 63 Arab patients from Iraq (16 men and 47 women) with toxic and nontoxic thyroid goiter revealed a total of ten heterozygous mutations, including C→T substitution at position 1708 in exon 10 (*c.1708C*>T) and C→G substitution at position 1978 in exon 11 (*c.1978C>G*). Among the ten mutations detected, the *c.1978C>G* mutation was observed in two patients with nontoxic goiter, whereas the *c.1708C>T* and *c.1978C>G* mutations were found in 2 and 6 patients with toxic goiter, respectively. Overall, mutations in the *TPO* gene were predominantly observed in women (90%) and in adults aged 30-50 years (80%) [57].

The screening for *TPO* mutations in two siblings with CH and MNG and in healthy members of their Malaysian families demonstrated that both sisters were homozygous for the novel mutation *c.1502T>G*. This variant is predicted to result in the replacement of highly conserved Val501 residue with glycine (p.Val501Gly). *In silico* analysis using the PolyPhen-2 and SIFT programs showed that the Val501Gly substitution is functionally damaging to the protein. Furthermore, tertiary structure modeling demonstrated alterations in the TPO active site [58]. The homozygous nonsense mutation *c.1618C>T* (p.Arg540X), was identified in the *TPO* genes of two Turkish patients with TIOD. All unaffected family members were either heterozygous carriers or homozygous for the wild-type allele, supporting the pathogenic role of this mutation [59].

The Arg665Trp mutation was identified in a compound heterozygous state together with Arg175Gln (Arg175Gln/Arg665Trp) in two Japanese patients [47]. In a separate study, direct sequencing of all exons and flanking regions of the *TPO* gene was performed in a 26-year-old German man of Thai origin with CH complicated by MNG and in his family members. This analysis revealed compound heterozygosity for two *TPO* mutations: the novel missense mutation *c.1727C>T* in exon 10, resulting in the Ala576Val substitution, and the *c.2268_2269insT* insertion in exon 13, causing a frameshift and introduction of a premature stop codon at position 757 (Glu757X) [60].

NGS analysis of the *TPO* gene in 192 Chinese patients with CH identified three distinct mutation variants in two individuals. Further sequencing of other hemochromatosis-associated candidate genes revealed that patient 1 was homozygous for the *c.2422delT* mutation and also carried two heterozygous pathogenic

variants in *DUOX2*. Patient 2 harbored pathogenic *TPO* variants *Arg648Gln* and *Thr561Met*. This study identified one novel mutation (Thr561Met) and two previously reported mutations (Cys808AlafsX24 and Arg648Gln), demonstrating a 1% prevalence of TPO mutations among the examined Chinese CH cohort [18]. In a separate study from Portugal, eight different *TPO* mutations, including three novel missense variants, were identified in 13 patients (seven homozygotes and six compound heterozygotes) selected from a cohort of 723 individuals with CH. Notably, four mutations (Ala397ProfsX76, Asn425Ser, Gly493Ser, and Gln660Glu) were located within the MPO-like fragment [40].

TPO gene mutations in the genomes of CH patients with dyshormonogenesis from Isfahan were less frequent compared to other studies. Using the SSCP method and sequencing, six known single nucleotide polymorphisms (SNPs) were detected in a cohort of 41 patients. Two SNPs (G11A, A35G) were located in the promoter region and exon 1; the other four were in the sequence encoding the MPO-like region: *c.859G>T*, *c.*1207G>*T*, *c.1283G>C*, and *c.2088C>T* (Table 2) [20]. In a study of 15 members of a Chinese family, the compound heterozygous mutation c.*2268-2269insT*/c.2090G>A* was detected in the genomes of two patients with congenital goiter [24]. The *TPO* genes of the patient with CH from Japan and her parents were sequenced directly, and two missense mutations, Arg665Trp and Gly771Arg, were detected. The first mutation was inherited from the patient's father, and the second mutation from her mother [62].

In conclusion, mutations in *TPO* gene sequence encoding the MPO-like region (a.a. 142-733) of the extracellular domain are rare and occur sporadically. Among the 95 mutations identified in this region, 20 were observed in a homozygous state, while 56 occurred as compound heterozygous variants.

Homozygous mutations are Ala257Ser, Ser292Phe (Tunisia); Glu319Arg, Ala397ProfsX76, Arg540X (Turkey); Asp333Asn, Glu596X, Asp633Asn (Pakistan); c.*1182_1183insCGGC* (Finland); Gly387Arg, Trp527Cys (Japan); Ala397ProfsX76, Gly493Ser, Gln660Glu (Portugal); Ala397ProfsX76, Arg595Lys (Argentina); *insGGCC1277* (Netherlands); *insGGCC1277* (Brazil); Ala426Gly (Sudan); Ala443Pro (China); Arg491His, Ala584Gln (UK+); Val501Gly (Malaysia).

Each country exhibits its own spectrum of mutations identified in patients with CH. Several identical mutations have been reported in CH patients from different regions of the world. These include Ser292Phe (Tunisia, Israel); Arg396fsX472 (Argentina, Netherlands); Ala397ProfsX76 (Argentina, Portugal, Russia, Slovenia, UK+); *insGGCC1277* (Brazil, Netherlands); Tyr453Asp (Netherlands, Germany, UK+); Ala489Thr (Russia, China); Gly493Ser (Portugal, Taiwan, Israel, Iraq); Trp527Cys (Russia, Netherlands, Japan); Arg540X (Netherlands, Israel, Iraq, Turkey, Japan); Arg584Gln (UK+, Russia); Arg648Gln (USA, China); Arg665Gln (Argentina, Russia, Pakistan); Gly673Lys (Russia, China).

A total of 29 mutations have been identified in the TPO fragment corresponding to IDR/A (residues 456-631). Five of these mutations (Ala489Thr, Gly493Ser, Trp527Cys, Arg540X, and Arg584Gln) were detected repeatedly.

Three mutations were identified in the *TPO* sequence encoding the MPO-like fragment corresponding to IDR/B (residues 210-225, 353-363, 377-386, 549-563, and 713-717). Among them, the *c.670_672del* (p.Asp224del) mutation were detected repeatedly.

No recurrent mutations have been reported in the TPO fragments 561-575, 590-622, and 710-722, i.e., regions representing the greatest interest to immunologists. Three mutations were identified in the 561-575 region, including the compound heterozygous variant Asp574_Lys575del. The 590-622 region harbored five mutations, in particular, the homozygous variants Arg595Lys and Glu596X. The 710-722 region contained only a single heterozygous mutation (Phe718X).

It is necessary to highlight two mutations in the MPO-like region: Ala397ProfsX76 and Arg540X, both of which are located outside of IDRs.

The frameshift mutation *c.1184_1187insGCCG* is a 4-bp insertion in exon 8, resulting in p.Ala397ProfsX76. This mutation has been detected in 26 patients with CH from eight countries across two continents. Among these cases, 22 presented with the goitrous form of CH. Seven patients were homozygous, and another seven patients were compound heterozygous. The *c.1184_1187insGCCG* (p.Ala397ProfsX76) mutation was first reported in 1992 in a patient from Argentina [12] and has since been repeatedly identified in CH patients from multiple European and Asian countries [14, 22, 31, 34, 37, 40].

The nonsense mutation *c.1618C>T* in exon 10, resulting in a premature stop codon at position 540 (p.Arg540X), was identified in 30 patients with CH from six Eurasian countries. The variant was homozygous in 2 patients and compound heterozygous in 25 patients. Goiter was observed in 14 patients. The Arg540X mutation was first reported in a Dutch patient in 1995 [36] and has since been repeatedly detected in CH patients from multiple European and Asian populations [13, 15, 17, 31, 54, 57, 59].

Mutations in the TPO gene sequence encoding the CCP-like region (residues 739-795) of the extracellular domain. The data on mutations in the TPO region 739-795 are summarized in Table 3.

The IDR/B includes the antigenic determinant 766-775.

Table 3. Mutations in the *TPO* sequence encoding the CCP-like region (residues 739-795) in the TPO extracellular domain

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
del TT 2243/2244	Netherlands	1	ComHet	TIOD	[13]
Val748 Met	China	1	Het	PH	[34]
Val748Met	Chile	1	Het	goiter	[55]
Val748Met	Chile	1	ComHet	goiter	[55]
2266T>C	Malaysia	1	ComHet	goiter/PH	[48]
Cys756Arg	China	1	ComHet	goiter	[49]
Cys756 Arg	Germany	2	ComHet	goiter	[63]
Cys756fsX	China	4	ComHet	goiter	[35]
Cys756fsX	China	3	Hom	goiter	[35]
Cys756fsX	China	1	Het	goiter	[35]
2268dup.	Malaysia	2	Hom	MNG	[65]
2268dup.	Malaysia	4	ComHet	goiter/PH	[48]
Glu757X	China	2	Hom	goiter	[49]
Glu757X	China	2	ComHet	goiter	[49]
Glu757X	China	1	Het	PH	[19]
2268-2269 insT*	China (Taiwan)	4	ComHet	TIOD	[53]
2268-2269 insT*	China	4	Het	MNG	[24]
2268-2269 insT*	China	2	ComHet	goiter	[24]
2268-2269 insT*	China	1	Het	papillary cancer	[24]
2268-2269 insT*	Germany	1	ComHet	MNG/PH	[60]
Leu764Pro	China	1	Hom	PH	[35]
Arg769Trp	China	1	ComHet	PH/LBV	[19]
Gly771Arg	Japan	1	ComHet	TIOD	[62]
Gly771Arg	Pakistan	4	ComHet	TIOD	[31]

Note. For designations, see Note to Table 1; LBV, likely benign variant.

Out of 16 mutations identified in a study of genomes from 45 Dutch patients with TIOD, only the *c.del.TT2243/2244* deletion was located in the extracellular domain region 739-795 [13].

Among the 35 mutations identified across 23 of 230 Chinese patients with CH, Table 3 highlights three specific mutations: one heterozygous variant, Val748Met, and two homozygous variants, Cys756fsX

and Leu764Pro [35]. Notably, the Val748Met mutation was also observed in 2 of 12 CH patients from Chile [55].

Two Malaysian sisters with goiter were found to carry the homozygous *c.2268dup* mutation, which generates both normal and alternatively spliced mRNA transcripts, ultimately leading to the loss of the TPO enzymatic activity. The *c.2268dup* mutation is predicted to introduce a premature stop codon at position 757 (p.Glu757X). Rather than restoring the normal reading frame, the alternatively spliced transcript contains a different premature stop codon at position 740 (p.Asp739ValfsX740). In the initial phase of this study, a shorter native form of TPO was detected in both patients using the MoAb47 antibody, which recognized an epitope within the 713-721 a.a. region [65].

Genomic analysis revealed the *c.2268dup* mutation in 4 out of 12 unrelated Malaysian-Chinese patients with CH. Notably, one patient also harbored a second mutation, *c.2266T>C*, in addition to *c.2268dup* [48].

Six mutations were identified by molecular analysis in six patients with goiter in a study of thirty Chinese children with CH. Two of these mutations, corresponding to the Cys756Arg and Glu757X substitutions, had been previously reported and were in the 739-795 a.a. fragment of the extracellular domain. The Glu757X mutation was found in four patients: as a homozygous variant in the twins and as a compound heterozygous variant in two unrelated patients [49].

Compound heterozygous mutations were identified in two children with CH from Germany. Both children harbored an identical novel mutation in exon 13 (Cys756Arg). One of the children exhibited dominant inheritance of thyroid dyshormonogenesis [60]. As previously mentioned, five novel *TPO* mutations were detected in five unrelated patients with TIOD from China (Taiwan). Among these, the frameshift mutation *c.2268-2269insT** (a single insertion of T between nucleotides 2268 and 2269) in exon 13 is presented in Table 3. Of all the *TPO* mutations identified, *c.2268-2269insT** was the most common, occurring in a heterozygous state in all TIOD patients except one. All five patients with TIOD were compound heterozygotes [53]. Further investigation revealed the *c.2268-2269insT** mutation in four individuals from a Chinese family of 15 members with normal thyroid hormone levels; heterozygotes displayed degenerative hypoechoic thyroid nodules. Compound heterozygous mutations *c.2268-2269insT*/c.2090G>A* were identified in two patients with congenital goiter. Additionally, the *c.2268-2269insT** mutation was detected in a patient with a multifocal papillary thyroid carcinoma with lymph gland and nerve invasion in the left lobe of thyroid gland. [24]. Genomic analysis revealed the compound heterozygous mutation *c.2268_2269insT*/c.1727C>T* in a German patient of Thai origin with CH complicated by MN, which was inherited from both parents as a novel mutation in exon 10 from his German mother and mutation in exon 13 from his Thai father. Bioinformatic analyses using two programs predicted that this variant likely disrupts the structure of the TPO protein [63].

The study analyzing genomes of 219 Chinese patients with hypothyroidism revealed the heterozygous variant Glu757X and the compound heterozygous variant Arg769Trp in the TPO fragment 739-795 a.a. [19]. The compound heterozygous mutation Arg665Trp/Gly771Arg was identified in a Japanese patient with CH, where the Arg665Trp was inherited from the father and Gly771Arg was inherited from the mother [66].

A study of 21 patients from Pakistan with CH complicated by the tetra-amelia syndrome identified ten mutations, one of them being Gly771Arg (Table 3) [31].

In conclusion, among the 11 identified mutations, two were homozygous (Cys756fsX and Leu764Pro, both reported in China) and the remaining nine were compound heterozygous. Additionally, four identical mutations were observed in patients with CH from different regions worldwide: Val748Met (Chile, China), Cys756Arg (Germany, China), *p.2268-2269insT** (Germany, China), and Gly771Arg (Japan, Pakistan).

The *c.2268-2269insT** mutation was identified in 12 patients with diverse hypothyroidism phenotypes and various complications (goiter, MNG, cancer, etc.).

Therefore, in certain cases, *TPO* genetic variants may be associated with thyroid carcinoma and hypoechoic thyroid nodules. Two mutations were identified in the *TPO* nucleotide sequence, affecting the 766-775 a.a. fragment of IDR/B, including recurrently observed Gly771Arg mutation.

Mutations in the TPO nucleotide sequence encoding the EGF-like region (residues 796-841) of the extracellular domain. The data on mutations in the TPO region 796-841 a.a. are presented in Table 4. This region is not classified as an IDR.

Five compound heterozygous mutations were identified in five unrelated patients with TIOD from Taiwan. Among these, a single nucleotide substitution, *c.2386 G>T*, either results in an amino acid substitution (Asp796Tyr) or affects splicing, while a single nucleotide deletion, *c.2413delC*, is located within the region encoding the EGF-like domain (residues 796-841) [53]. In addition, mutations in intron 13, including *c.2386+2 T>G* (*g.IVS13+2 T>G*), have been reported in a patient with MNG from Russia [37]. The rare Asn798Lys mutation was identified in a patient with congenital goiter from a 15-member family in China [19]. Furthermore, Glu799Lys and the insertion mutation *insC2505-2511* were detected as part of

Table 4. Mutations in the *TPO* gene sequence coding for the EGF-like region (residues 796-841) of the extracellular domain

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
2386G>T	China (Taiwan)	2	ComHet	TIOD	[53]
2386+2T>G	Russia	1	ComHet	MNG	[37]
Asn798Lys	China	1	ComHet	goiter	[21]
Glu799Lys	Netherlands	1	ComHet	TIOD	[36]
Glu799Lys	USA	3	ComHet	TIOD	[61]
Glu799Lys	USA	11	Hom	TIOD	[61]
Glu799Lys	Netherlands	1	ComHet	TIOD	[13]
Glu799Lys	Slovenia	1	ComHet	MNG/Gardner syndrome	[14]
Glu799Asp	China	3	Het	PH	[35]
2413delC	China (Taiwan)	1	ComHet	TIOD	[53]
Cys808fsX23	Portugal	1	ComHet	goiter	[40]
Cys808fsX23	Portugal	1	Hom	goiter	[40]
Cys808fsX23	Japan	1	Het	goiter	[15]
2422delT	Netherlands	1	ComHet	TIOD	[13]
2422delT	Turkey	1	Het	n.d.	[31]
2422delT	China	1	Hom	PH	[18]
Cys808AlafsX24	Bosnia	2	ComHet	goiter/Gardner syndrome	[14]
Cys808AlafsX24	UK (+)	1	Hom	PH	[34]
Cys808AlafsX24	China	1	Hom	PH	[18]
Cys808AlafsX24	Russia	4	ComHet	PH	[37]
Cys808AlafsX24	Sudan	1	ComHet	PH/goiter	[47]
Cys808Arg	Argentina	1	Het	TIOD/goiter	[9]
Cys808Arg	Argentina	1	ComHet	TIOD	[38]
ins C2505-2511	Netherlands	1	ComHet	TIOD	[36]
ins C2505-2511	Netherlands	3	ComHet	TIOD	[13]
ins C2505-2511	Brazil	1	ComHet	goiter/TIOD	[10]
2512delT	Netherlands	4	ComHet	TIOD	[13]
Cys838Ser	Portugal	2	Hom	goiter	[40]
2519-19 G>C	India	1	Het	goiter	[64]

Note. For designations, see Note to Table 1; n.d., not determined.

Table 5. Mutations in the *TPO* gene sequence encoding the TPO transmembrane domain (residues 846-871)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
Arg846Trp	China	1	Het	PH	[35]
Arg846Trp	China	2	Het	PH/goiter	[19]
Ser853Leu	China	1	Het	PH	[19]
Gly860Arg	Slovenia	1	ComHet	MNG	[14]
Gly860Arg	Iran	1	Hom	PH	[20]
Gly860Arg	China	1	ComHet	PH	[35]
Gly860Arg	Sudan	1	Hom	PH/goiter	[46]

Note. For designations, see Note to Table 1.

a compound heterozygous variant among seven different TPO gene mutations in 15 patients from the Netherlands [36]. The same authors later expanded their cohort to 45 patients to identify additional mutations, including *c.2422delT* and *c.2512delT* [13].

A high incidence of severe hypothyroidism caused by TIOD was observed in a younger generation of five related families within an inbred Amish population. Sequencing identified two missense mutations: Glu799Lys and Arg648Gln. The Glu799Lys mutation was found in both alleles of 11 affected homozygotes, while both mutations were present in each of the three affected compound heterozygotes. These findings highlight the effectiveness of DNA pooling strategies for localizing defective genes in inbred populations harboring two relatively rare mutations [60]. Notably, the Glu799Lys mutation was also identified in one patient among a cohort of 30 patients from Slovenia [14]. Analysis of the *TPO* gene in 14 unrelated patients presenting with clinical signs of iodide organification disorder identified a single case of the *c.2422T>C* mutation in exon 14 resulting in the amino acid substitution Cys808Arg [9]. The same research group from Argentina reported Cys808Arg as a part of a compound heterozygous mutation in a patient with TIOD [38]. The Cys808fsX23 mutation has been detected in various genetic contexts, including heterozygous [15], compound heterozygous [40], and homozygous states [18, 40]. Similarly, the *c.2422delT* (p.Cys808AlafsX24) mutation has been reported as compound heterozygous [13, 14, 37, 46] and homozygous [18, 34]. The rare Cys838Ser mutation was identified in two twins with congenital goiter in Portugal [40]. To evaluate the contribution of common genetic variants in *TSHR*, *TPO*, *TG*, and *DUOX2* genes to CH associated with thyroid agenesis or goiter, a cohort of 1,144 newborns from India was screened. This study identified a rare variant *c.2519-19G>C* (*g.IVS14-19G>C* in intron 14 [64].

In conclusion, among the 13 mutations identified, five were homozygous: Glu799Lys (USA); Cys808fsX23 and Cys838Ser (Portugal); and *c.2422delT* (Cys808AlafsX24) (UK+, China). Ten mutations were found in a compound heterozygous state. Notably, four identical mutations were observed in patients with CH from different regions worldwide: Glu799Lys (Netherlands, USA, Slovenia); Cys808fsX23 (Portugal, Japan); *c.2422delT* (Cys808AlafsX24) (Netherlands, Turkey, Bosnia, UK+, Russia, China, Sudan); and *insC2505-2511* (Netherlands, Brazil).

Two mutations warrant special attention. The missense Glu799Lys mutation has been identified in 17 patients with CH from three countries across two continents: the Netherlands, the USA, and Slovenia. Of these patients, 11 were homozygous and 6 were compound heterozygous [13, 14, 36, 61]. MNG was reported in one patient. The mutation was first described in 1995 in a patient from the Netherlands [36].

The frameshift mutation *c.2422delT* (p. Cys808AlafsX24), caused by a deletion in exon 14, was identified in 12 patients with CH from seven countries across two continents: the Netherlands, Turkey, Bosnia, the United Kingdom, Russia, China, and Sudan. Among these patients, three were homozygous and eight were compound heterozygous for the mutation. Goiter was observed in three patients [13, 14, 18, 34, 37, 46]. This mutation was first reported in 2000 in a patient from the Netherlands [13].

Mutations in the TPO transmembrane domain (residues 846-871). The data on mutations in the TPO transmembrane domain (residues 846-871) are summarized in Table 5.

Table 6. Mutations in the *TPO* gene sequence coding for the intracellular domain (residues 872-933)

Mutation and/or change in the TPO protein sequence resulting from *TPO* mutations	Region, country	*n*	Mutation type	Clinical manifestation of hypothyroidism	Reference
2618+1G>T	Russia	1	Het	hypoplasia	[37]
Pro883Ser	Japan	1	Het	PH	[67]
2647C> T	Malaysia	1	ComHet	PH/goiter	[48]
Pro883Ser	China	1	ComHet	goiter	[49]
Pro883Ser	China	1	Het	PH	[35]
Pro883Ser	China	1	Het	PH/athyreosis	[19]
Pro883Ser	China	1	Het	goiter	[19]
Pro883Ser	China	1	ComHet	PH	[65]
Gly889Arg	China	1	Het	PH	[19]
Gly889X	China	1	ComHet	goiter	[49]
Arg908fsX	USA	n.d.	n.d.	n.d.	[68]
Gln912fsX	USA	n.d.	n.d.	n.d.	[68]
2748 G>A	Portugal	2	ComHet	PH	[40]
Ser918Cysfs*62	Argentina	1	ComHet	goiter	[69]
del 10 bp 2812	Turkey	1	Hom	MNG	[70]
2749-2 A>C	Argentina	1	ComHet	goiter	[69]
Glu917Lys	USA	n.d.	n.d.	n.d.	[68]

Note. For designations, see Note to Table 1; n.d., not determined.

Among 35 mutations identified in the *TPO* gene during genomic analysis of 230 Chinese patients with CH, two mutations (Arg846Trp and Gly860Arg) were located in the transmembrane domain and were detected in 23 individuals. The functional impact of TPO mutations was assessed *in vitro* by expressing 14 mutant TPO constructs in human embryonic kidney (HEK) cells using the pcDNA3.1 plasmid. Notably, a patient harboring the Gly860Arg mutation exhibited the loss of thyroid function [35].

In a genome-wide study of 219 hypothyroid patients from northwest China, 17 individuals carried 19 rare variants of the *TPO* gene. Two of these mutations were located within the transmembrane domain: the previously reported Arg846Trp mutation and one of seven newly identified variants Ser853Leu [19].

In a genomic study of 43 patients from Slovenia, Bosnia, and Slovakia with persistent hypothyroidism and orthotopic thyroid glands, representing 39 unrelated families, mutations in the *TPO* gene were identified in 20 patients. A total of seven distinct mutations were detected, four of which were novel, including Gly860Arg [14].

A mutation in the *TPO* gene was identified in only one of 41 Iranian patients with PH. This mutation causes the Gly860Arg substitution and was detected in a homozygous state in the affected patient. To further characterize this variant, SSCP analysis and DNA sequencing of the corresponding *TPO* exon were performed in the patient's family, which showed that both patient's parents were heterozygous mutation carriers [20].

Six *TPO* mutations were identified in affected members of four families in the genomic analysis of patients with CH and goiter from 26 Sudanese families. One of these mutations, Gly860Arg located in the transmembrane domain, was detected in a patient in a homozygous state [46].

In conclusion, only three heterozygous mutations have been reported in the TPO transmembrane domain (residues 846-871). Among these, the missense mutation Gly860Arg has been identified in patients with CH from four countries (Iran, Sudan, Slovenia, and China) spanning two continents. Reported cases include two homozygous patients and two compound heterozygotes. Clinically, one patient presented with goiter, while another had MNG [14, 20, 35, 46]. The Gly860Arg mutation was first described in 2007 in a patient from Slovenia [14].

Mutations in the intracellular domain of TPO (residues 872-933). The data on mutations in the TPO intracellular domain are summarized in Table 6.

A rare splice site mutation, *c.2618+1G>T* (*g.IVS15+1G>T*), was identified in intron 15 of the *TPO* gene in a patient from Russia [37]. In a study of nine Japanese children with transient thyroid dysfunction or subclinical hypothyroidism detected by neonatal screening, one child was heterozygous for the Arg450His mutation in *TSHR*, while another carried a novel *TPO* mutation (Pro883Ser). No *DUOX2* mutations were detected in this cohort [66]. Identification of the c.*2647C>T* mutation in a Malaysian patient [48] was followed by multiple reports describing the same mutation in five patients with CH from China [19, 35, 49, 64]. Molecular analysis of the *DUOXA2* and *TPO* genes in 30 Chinese children with CH identified six *TPO* variants, including two heterozygous mutations in the intracellular domain – Pro883Ser and Gly889X, the latter representing a novel inactivating mutation. Germline mutations identified in four unrelated families were consistent with an autosomal recessive pattern of inheritance, while no *DUOXA2* mutations were detected [63]. Further analysis of 219 patients with CH from northwestern China identified *TPO* mutations in 17 individuals, including two mutations affecting the intracellular domain (Pro883Ser and Gly889Arg) [24]. In addition, a retrospective study of ten Chinese patients with dyshormonogenesis revealed heterozygous variants in two pathogenic genes in each patient. Overall, pathogenic alterations were identified in five genes (*TSHR*, *TG*, *TPO*, *DUOX2*, and *DUOXA2*), all of which participate in the thyroid hormone biosynthesis. In one patient, mutations were detected in both the *TPO* (*c.2647C>T*; p.Pro883Ser) and *TG* genes [64].

The summarized information on the hypothyroidism-associated mutations in *TPO* is available in the ClinVar database. Currently, ClinVar reports 296 variants of the *TPO* gene sequence, 83 of which are classified as pathogenic. The majority of identified variants are missense mutations (62.76%), followed by synonymous substitutions (14.39%). Nonsense mutations account for 1.55% of cases, while frameshift mutations resulting from deletions and insertions represent 0.67% and 0.38%, respectively. Mutations most frequently associated with CH include *c.2749G>A* (p.Glu917Lys) in exon 17 and several frameshift mutations in exon 16, namely *2736_2748+3delGGACTCGGAGCAGGT* (Gln912fs) and *2723_2732delGGGCCGCAGC* (Arg908fs) [67].

The c.2748G>A mutation was detected in two patients from Portugal [40]. In a cohort of 17 Argentine patients with CH caused by thyroid dyshormonogenesis, rational clinical diagnostics led to the identification of two novel *TPO* variants: *c.2749-2A>C* (*g.IVS16-2A>C*) and *c.2752_2753delAG* (p.Ser918Cysfs*62). Bioinformatic analysis and structural modeling were performed to predict the pathogenic potential of the identified variants. Potentially pathogenic biallelic variants of *TPO* and *DUOX2* were detected in seven and two patients, respectively, while potentially pathogenic monoallelic *TPO* variants were identified in seven patients. Overall, 22 variants were found to be associated with hypothyroidism. All newly described mutations occur in the *TPO* regions encoding domains critical for protein structure and function and were predicted to result in a hypothyroid phenotype [68].

A novel homozygous 10-bp deletion at position 2812 in exon 16 was identified in a patient from Turkey. This frameshift mutation causes a profound alteration of the protein intracellular domain and represents the first reported mutation encoding inactive TPO molecule with a severely disrupted intracellular region. Histopathological analysis confirmed the presence of dyshormonogenic goiter with multiple follicular adenomas. The same deletion identified in the DNA of leukocytes was also detected in the thyroid tissue cDNA, thus excluding transcript instability or aberrant splicing as contributing mechanisms. Both clinically unaffected parents were heterozygous carriers of the mutation [69].

In conclusion, mutations in the intracellular domain of TPO are rare and typically occur as single variants. Eleven reported mutations included one homozygous 10-bp deletion (*del2812*) identified in Turkey and three nonsense mutations: Gly889X (China), Arg908fsX, and Gln912fsX (USA). Five of the identified mutations occurred in a compound heterozygous state. Notably, the *c.2647C>T* (p.Pro883Ser) mutation was detected in both heterozygous and compound heterozygous states in seven cases, across patients presenting with different hypothyroidism phenotypes.

CONCLUSION

Defects in dyshormonogenesis genes are the predominant cause of hereditary forms of CH. For instance, molecular genetic analysis of patients with CH

in Russia showed that such defects account for 84.0% of cases. Among the identified monogenic variants, the highest number of mutations were found in the *TPO* gene (35.0%), including in patients with thyroid hypoplasia [33].

In this study, 147 previously reported mutations in the *TPO* gene were used as a reference to identify the most frequently occurring mutations in the genomes of patients with CH across different regions of the world. Twenty-four mutations were identified, 23 of which are located in the extracellular domain of TPO: two in the 1-141 a.a. region, thirteen in the MPO-like region, four in the CCP-like region, and four in the EGF-like region. One mutation was found in the transmembrane domain. Notably, the five mutations observed in the largest number of carriers (Ala397ProfsX76, Arg540X, Glu799Lys, Cys808AlafsX24, and Gly860Arg) were located outside the IDR sequences identified using reference anti-TPO antibodies.

Regarding mutations within the IDR, particular attention has been given to mutations in the regions coding for two spatially proximate linear epitopes, namely residues 606-617 and 706-720. These indicated linear epitopes, one located in within IDR/A region (a.a. 599-617) responsible for the interaction with IDR/A-specific mAbs, and the other located within a fragment (a.a. 713-717) of the discontinuous IDR/B, are recognized by mAb1 from our panel of 36 mAbs and contribute to the formation of conformational epitope 3 on the TPO molecule. Autoantibodies targeting the IDR/A fragment 606-617 a.a, recognized by mAb1 are present in 82% of serum samples from individuals with AITD. Autoantibodies against conformational epitope 3 are detected in 96% of serum samples from patients with the Graves' disease and in 100% of samples from patients with AIT [45]. According to the literature, the *c.1851delC* (p.Ser617Argfs23) mutation in the a.a. 606-617, fragment was reported in a heterozygous and a compound heterozygous states in two patients with CH without goiter in Russia (2018) [33]. In the 706-722 region, only one nonsense mutation, Phe718X, was reported in a heterozygous state in a patient with TIOD in the Netherlands (2000) [8].

Abbreviations

AIT	autoimmune thyroiditis
AITD	autoimmune thyroid disease
CH	congenital hypothyroidism
IDR	immunodominant region
MNG	multinodular goiter
mAb	monoclonal antibody
NGS	next-generation sequencing
PH	persistent hypothyroidism
PIOD	partial iodide organification defect
SSCP	single-strand conformation polymorphism
TIOD	total iodide organification defect
TPO	thyroid peroxidase

Contributions

A.V.Z. developed the concept, analyzed published and research data; L.G.B. conducted literature search and review.

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Ethics approval and consent to participate

This work does not contain studies involving human or animal subjects.

Conflict of interest

The authors of this work declare that they have no conflicts of interest.

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