

## GENETIC DEFECTS IN THYROID HORMONE SUPPLY

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### ABSTRACT

Congenital hypothyroidism (CH) is the most frequent endocrine-metabolic disease in infancy, with an incidence of about 1/2500 newborns [1, 2]. In the last 20-30 years the incidence of congenital hypothyroidism in newborns has increased from 1:4000 to 1:2000 [3, 4]. This phenomenon could be explained by using a lower b-TSH cutoff, that allowed the detection of an unsuspected number of children with neonatal hypothyroidism [5]. With the exception of rare cases due to hypothalamic or pituitary defects, CH is characterized by elevated TSH in response to reduced thyroid hormone levels. In absence of an adequate treatment, CH determines growth retardation, delays in motor development, and permanent intellectual disability.

Primary CH is determined by alterations occurring during the thyroid gland development (thyroid dysgenesis, TD [6]) or alterations in the thyroid hormone biosynthesis pathways (thyroid dyshormonogenesis). Less common causes of CH are secondary or peripheral defects in TSH synthesis and/or action, defects in thyroid hormone transport, metabolism, or action [7]. Table 1 shows a summary of the forms of CH with a genetic cause.

In the majority of cases (80-85%), primary permanent CH is associated with TD. These forms include developmental disorders such as athyreosis, ectopy, hemiagenesis or hypoplasia.

TD occurs mostly as sporadic disease, however a genetic cause has been demonstrated in about 2-5% of the reported cases [8]. Genes associated with TD include several thyroid transcription factors expressed in the early phases of thyroid organogenesis (*NKX2.1/TITF1*, *FOXE1/TITF2*, *PAX8*, *NKX2.5*) as well as genes, like the thyrotropin receptor gene (*TSHR*) expressed later during gland morphogenesis. In the remaining 15-20% of cases, CH is caused by inborn errors in the molecular steps required for the biosynthesis of thyroid hormones, and generally it is characterized by enlargement of the gland (goiter), presumably due to elevated TSH levels [9]. Generally, thyroid dyshormonogenesis shows classical Mendelian recessive inheritance.

Rarely CH has a central origin, as consequence of hypothalamic and/or pituitary diseases, with reduced production or function of thyrotropin releasing hormone (TRH) or thyrotropin hormone (TSH) [10]. For complete coverage of this and all related areas of Endocrinology, please visit our FREE on-line web-textbook, [www.endotext.org](http://www.endotext.org).

## **EPIDEMIOLOGY**

CH is usually a sporadic disease with a frequency of about two girls for each boy [11]. Familial cases occur with a frequency that is 15-fold higher than by chance alone [12]. The genetic basis of these familial cases has been established in some, but not all pedigrees [13].

An increased prevalence of the disease is reported in twins [14], with approximately 12 fold increased incidence compared to singletons, even if a discordance rate of 92% between monozygotic (MZ) twins has been observed [15].

The incidence of CH differs significantly among different ethnicities and regions, ranging from 1 in 30,000 in the African-American population in the United States [16, 17] to 1 in 900 in Asian populations in the United Kingdom [18].

## **CLINICAL MANIFESTATIONS**

In absence of an adequate treatment, severe CH results in serious mental retardation, in motor handicaps as well as in the signs and symptoms of impaired metabolism. Before the introduction of a neonatal screening program, congenital hypothyroidism was one of the most frequent causes of mental retardation.

The clinically detectable consequences of CH strongly depend on severity and duration of thyroid hormone deprivation, but there is also a large individual variability in treatment response.

In the first four-six months after birth, only untreated patients with severe CH have clinical manifestations. Milder cases can remain undiscovered for years. Clinical features of CH are subtle and non-specific during the neonatal period due in part to the passage of maternal thyroid hormone across the placenta; however, early symptoms may include:

- Decreased activity
- Wide posterior fontanel
- Poor feeding and weight gain
- Small stature or poor growth
- Long-term jaundice
- Decreased stooling or constipation
- Hypotonia
- Hoarse cry
- Coarse facial features
- Macroglossia
- Umbilical hernia
- Developmental delay
- Pallor
- Myxedema

- Goiter

Infants with congenital hypothyroidism are usually born at term or after term. Infants with obvious findings of hypothyroidism (eg, macroglossia, enlarged fontanelle, hypotonia) at the time of diagnosis have intelligence quotients (IQs) 10-20 points lower than infants without such findings. Often, they are described as "good babies" because they rarely cry and sleep most of the time.

Anemia may occur, due to decreased oxygen carrying requirement. The accumulation of subcutaneous fluid (intracellularly and extracellularly) is usually more pronounced in patients with primary (thyroid) hypothyroidism than in those with pituitary hypothyroidism. Thickening of the lips and macroglossia is due to increased accumulation of subcutaneous mucopolysaccharides (i.e., glycosaminoglycans). Alteration of the mandibular second molars may be the consequence of long-term effects of severe hypothyroidism on craniofacial growth and dental development [19]. In addition, histological changes in the vocal cords (VCs) have also been described [20]. A recent study demonstrated that CH children diagnosed during neonatal screening and adequately early treated, showed similar vocal and laryngeal characteristics compared to children without CH [21].

A small but significant number (3-7%) of infants with CH have other birth defects, mainly atrial and ventricular septal defects or other cardiac malformations (approximately 10% of infants with CH, compared with 3% in the general population) [22].

### **NEONATAL SCREENING**

Most newborn babies with CH have few or no clinical manifestations of thyroid hormone deficiency, and in the majority of cases the disease is sporadic. Indeed, it is not possible to predict which infants are likely to be affected by CH. For these reasons, newborn screening programs were developed in the mid-1970s to detect this condition as early as possible. The screening consisted in the measurement of thyrotropin (TSH) on heel-stick blood specimens.

Congenital Hypothyroidism was one of the first diseases screened in neonatal screening programs (NS) [23, 24]. Screening programs for CH were initially developed in Quebec, Canada, and Pittsburgh, Pennsylvania, in 1974 [25], and have now been established in almost all over the World [26].

Since the introduction of the screening, prevalence of CH significantly changed ranging from 1:6500 (estimated before of NS program) [27], to 1:3000 live births in recent years [4]. This fact is probably associated with an increase in the survival of preterm newborns [4, 5], with environmental [14], and ethnic factors, as well as with the reduction in the cutoff values [3, 5] used for neonatal TSH.

Neonatal screening programs allow for early detection and treatment of CH, and have proven to be successful in preventing brain damage. Worldwide, most neonatal screening programs are TSH based in the first 3 days of life and effectively detect only thyroidal congenital hypothyroidism (CHT), missing the central CH (CCH). This is characterized by an impairment of TSH production, with low circulating thyroid hormones and low, improperly normal, or slightly high TSH levels [28].

Recently, some countries have developed screening methods measuring both T4 and TSH on the same blood spot simultaneously or stepwise ("T4+TSH-method"). These methods allowed also the identification of CCH [29-31], however it should be noted that low T4 and normal TSH can be also associated with thyroxine-binding globulin (TBG) deficiency, a laboratory condition that requires no treatment. Discriminate between these two conditions is crucial [32] and measurements of circulating TBG or other tests may be necessary [33].

In the past years, the diagnosis of primary CH was made when serum TSH was  $\geq 10 \mu\text{IU/mL}$ , regardless of the T4 concentration. A recent

retrospective study including children screened from 2003 to 2010, showed that 9.13% of the children with b-TSH levels between 5 and 10  $\mu\text{IU/mL}$  also developed hypothyroidism [34]. Indeed, the authors suggested to reduce the cut-off for b-TSH to 5  $\mu\text{IU/mL}$ . The lower cut-off levels allowed the identification of undiagnosed CH cases, however determined significant increases in the number of children to recall, led to higher costs of the screening and generated anxiety in parents and relatives of healthy babies [35]. Despite these problems, the usage of lower TSH cut-off has also been proposed in several other studies [36-38].

### **ADDITIONAL TESTS FOR DIAGNOSIS**

When the TSH concentration on a dried blood spot exceeds the established threshold, additional studies can be performed to obtain diagnostic confirmation and etiological definition of CH. If these studies will determine a delay in the beginning of the treatment, they should be performed later during the babies life.

Tests commonly used to determine the underlying cause of congenital hypothyroidism are presented in Table 2.

- Thyroid scintigraphy, with  $^{99\text{m}}$ technetium or  $^{123}\text{I}$ , is the most informative diagnostic procedure in patients with thyroid dysgenesis [39, 40] providing etiologic diagnosis, as in alteration in the iodine transporter (NIS) [40]. If the radioisotope uptake has not been performed at birth, it is necessary performed this imaging screening after 3 years of age, when the T4 treatment interruption does not compromise the neurocognitive development of the child [31]. Recently it has been suggested that intramuscular injections of recombinant human TSH can be useful to perform  $^{123}\text{I}$  uptake studies during L-thyroxine treatment in CH patients [41, 42].

- Ultrasound represents the gold standard for measuring thyroid dimensions, but lacks sensitivity for detecting small glands and it is less accurate than scintigraphy in showing ectopic glands [43]. Moreover, visualization of neonatal thyroid on ultrasound may be challenging for unexperienced sonographers [44].

More than 80% of newborn infants with TSH elevation can be diagnosed correctly on initial imaging with combined radioisotope scan and ultrasound.

- Assay of serum thyroglobulin (Tg) will be useful in to establish the presence of some thyroid tissue.

- More specialized tests, such as perchlorate discharge, evaluation of serum, salivary, and urinary radioiodine [45], and measurement of serum T<sub>4</sub> precursors, may be necessary to delineate specific inborn errors of thyroid hormone biosynthesis [46].

- When both the maternal and fetal thyroid glands are compromised, significant cognitive delay can occur despite early and aggressive postnatal therapy. Maternal thyrotropin-stimulating hormone receptor (TSHR)-blocking antibodies (Abs) can be transmitted to the fetus and cause combined maternal-fetal hypothyroidism. Measurement of TSHR Abs is necessary to establish the diagnosis; the presence of other thyroid Abs is insufficiently sensitive and may miss some cases [47].

- The measurement of the total urinary iodine excretion differentiates inborn errors from acquired transient forms of hypothyroidism due to iodine deficiency or iodine excess.

- A small number of infants with abnormal screening values will have transient hypothyroidism as demonstrated by normal serum T<sub>4</sub> and TSH concentrations at the confirmatory laboratory tests. Transient hypothyroidism is more frequent in iodine-deficient areas and it is much more common in preterm infants. CH can also be the consequence of intrauterine exposure to maternal antithyroid drugs, maternal TSHR-blocking antibodies (TSHRBAb), as well as heterozygous DUOX1 and DUOX2 or TSHR germ-line mutations [48, 49]. Because the transient nature of the hypothyroidism will not be recognized clinically or through laboratory tests, initial treatment will be similar to that of the infant with permanent CH, however at a later age interruption of therapy allows to distinguish transient from permanent hypothyroidism [50].

## GENETIC CLASSIFICATION OF CONGENITAL THYROID DISEASES

### 1. Central hypothyroidism

Congenital central hypothyroidism (CCH) is a rare disease in which thyroid hormone deficiency is caused by insufficient thyrotropin (TSH) stimulation of a normally-located thyroid gland. Patients with this disorder cannot be identified by neonatal screening program based on the measurement of TSH alone, while combined assay of T<sub>4</sub> and TSH will allow the identification of patients with CCH [29, 32, 51].

Initially the incidence was estimated between 1:29.000 and 1:110.000 [52-54], while the more recent study from the Netherlands suggests that it may occur in 1:16.000 newborns, representing up to 13% of cases of permanent congenital hypothyroidism [55, 56].

So far, rare genetic defects have been identified in patients affected by CCH. The disorder can be caused by mutations in genes involved in pituitary development such as *POU1F1*, *PROP1*, *HESX1*, *LHX3*, *LHX4* and *SOX3*. In these cases, central hypothyroidism does not occur in isolation, but is one of the evolving pituitary hormone deficiencies [57].

In contrast, the isolated CCH is determined by mutations in genes specific to the hypothalamic-pituitary-thyroid axis such as: *TSHB* (encoding the B-subunit of the TSH glycoprotein hormone), *TRHR* (the specific 7-transmembrane domain receptor for hypothalamic thyrotropin-releasing hormone [58]), *IGSF1* (a protein regulating the expression of TRHR in pituitary thyrotropes) [59], and the recently identified *TBL1X* (a subunit of the NCoR-SMRT complex) [60].

#### 1.1 Developmental defects of the pituitary

The pituitary gland is formed from an invagination of the floor of the third ventricle and from Rathke's pouch, developing into the thyrotropic cell lineage and the four other neuroendocrine cell types, each defined by the hormone produced: TSH, growth hormone (GH), prolactin, gonadotropins (luteinizing hormone [57] and follicle-stimulating hormone [61]), and adrenocorticotrophic hormone (ACTH).

The ontogeny of the pituitary gland depends on numerous developmental genes that guide differentiation and proliferation. These genes are highly conserved among species, suggesting crucial evolutionary roles for the proteins (PIT1 and PRPO1, HESX1, LHX3, LHX4 and SOX3).

*Lhx3* and *Lhx4* belong to the LIM family of homeobox genes that are expressed early in Rathke's pouch. In *Lhx3* knockout mice the thyrotropes, somatotropes, lactotropes, and gonadotropes cell lineages are depleted, whereas the adrenocorticotrophic cell lineage fails to proliferate. This murine knock out model shows that pituitary organ fate commitment depends on *Lhx3*. *Lhx4* null mutants show Rathke's pouch formation with expression of a glycoprotein subunit, TSH-beta, GH and Pit1 transcripts, although cell numbers are reduced.

In humans, homozygous or compound heterozygous carriers of *LHX3* mutations present with combined pituitary hormone deficiency diseases and cervical abnormalities with or without restricted neck rotation. Some patients also present with sensorineural hearing loss. Mutations can also be frameshift or splicing anomalies. In addition, the heterozygous carriers of a dominant negative *LHX3* mutation are characterized by limited rotation of the neck. Patients with heterozygous missense or frameshift mutations in *LHX4* have variable phenotypes, including GH disease and variable TSH, gonadotropin and ACTH deficiencies with a hypoplastic anterior pituitary, with or without an ectopic posterior pituitary [62, 63].

*Hesx1* (also called *Rpx*), a member of the paired-like class of homeobox genes, is one of the earliest markers of the pituitary primordium [64]. Extinction of *Hesx1* is important for activation of downstream genes such as *Prop1*, suggesting that the proteins act as opposing transcription factors [65]. Targeted disruption of *Hesx1* in the mouse revealed a reduction in the prospective forebrain tissue, absent optic vesicles, markedly decreased head size, and severe microphthalmia. A similar phenotype it has been observed in patients with the syndrome of septo-optic dysplasia (SOD). SOD is a complex and highly variable disorder, diagnosed in the presence of: 1) optic nerve hypoplasia, 2) midline neuroradiologic abnormalities and/or 3) anterior pituitary hypoplasia with consequent hypopituitarism [62]. The number of genetic factors implicated in this condition is increasing and currently includes *HESX1*, *OTX2*, *SOX2* and *SOX3*. These genes are expressed very early in forebrain and pituitary development and so it is not surprising that mutations affecting these genes can induce the SOD disorders. Very recently Sonic hedgehog (Shh) has been associated to SOD, since mouse embryos lacking in the gene exhibit key features of the disease, including pituitary hypoplasia and absence of the optic disc [66].

The human *HESX1* gene maps to chromosome 3p21.1–3p21.2, and its coding region spans 1.7 Kb, with a highly conserved genomic organization consisting of four coding exons. The first homozygous missense mutation (Arg160Cys) was found in the homeobox of *HESX1* in two siblings with SOD [64]. Subsequently several other homozygous and heterozygous mutations have been shown to present with different phenotypes characterized by pituitary hormone deficiency and SOD [65, 67].

### 1.2 Defects in the TRH and TRH receptor

The TRH receptor (TRHR) is a G-protein- coupled receptor located at pituitary thyrotropes and activated by hypothalamic TRH. The synthesis, secretion, and bioactivity of TSH necessary for following production of thyroid hormones, depend by TRH-TRHR signaling [59].

In mice, homozygous deletion of the *TRH* gene produced a phenotype characterized by hypothyroidism and hyperglycemia [68]. Only a few patients with reduced TRH production have been described in the literature [69, 70], but no human mutations have been identified so far. Mice lacking the TRH receptor appear almost normal, with some growth retardation, and decreased serum T<sub>3</sub>, T<sub>4</sub>, and prolactin (PRL) levels but normal serum TSH [71]. So far, four mutations in TRHR gene were identified in human. In the first case, the patient was a compound heterozygote for an early stop codon (p.R17X) and an in-frame deletion added to a missense change (p.S115- T117del + p.A118T) in the other allele [58]. The same p.R17X mutation was found also in the second patient in homozygous state [72], whereas the third exhibited a homozygous missense mutation (p.P81R) [73]. More recently has been identified in a consanguineous family a homozygous missense mutation (c.392T>C; p.I131T) located at a highly conserved hydrophobic position of G-protein-coupled receptor, which reduces the affinity for TRH, compromising the signal trasduction [74]. The same mutation, was present in the mother, two brothers and grandmother, but in heterozygous status leading to isolated hyperthyrotropinemia.

### 1.3 Defects in Thyroid-Stimulating Hormone (TSH) synthesis

The thyroid stimulating hormone (TSH) is produced and secreted by the thyrotrophic cells of the anterior pituitary gland and it is the classic ligand for the TSH receptor (TSHR) in the thyroid. TSH is a heterodimeric glycoprotein consisting of an  $\alpha$  subunit and  $\beta$  subunit, The  $\alpha$  subunit is shared with other glycoprotein hormones (i.e. follicle-stimulating hormone (FSH), luteinizing hormone (LH), and chorionic gonadotropin (CG)), whereas the TSH $\beta$  subunit is unique, determining the specificity of TSH. The beta-subunit (gene map locus 1p13) synthesis is under the control of several transcription factors, including POU1F1 and PROP1.

#### *Pit1/POU1F1*

Pit1 (called POU1F1 in humans) is a pituitary-specific transcription factor belonging to the POU homeodomain family. The human *POU1F1* maps to chromosome 3p11 and consists of six exons spanning 17 Kb encoding a 291 aminoacid protein. Identified mutations of the *POU1F1* gene in human result in combined pituitary hormone deficiency (CPHD) with an incidence between 38% and 77% in unselected cohorts, and between 25% and 52% in patients with a family history of CPHD. To date, several recessive and six dominant *POU1F1* gene mutations have been described in CPHD patients and include missense, nonsense, frameshift, whole gene deletion and two mutations that result in the mis-splicing of the pre-mRNA [75, 76]. Deficiency of GH, prolactin and TSH is generally severe in patients harbouring mutations in *POU1F1*. The patients are often affected by extreme short stature, learning difficulties, and anterior pituitary hypoplasia [76].

#### *PROP1*

Prop1 (Prophet of Pit1) is a pituitary-specific paired-like homeodomain transcription factor required for the expression of *Pit1*, and transcriptional activator to stimulate pituitary cell differentiation. Dwarf mice, harboring a homozygous missense mutation in *Prop1*, exhibit GH, TSH and prolactin deficiency, and an anterior pituitary gland reduced in size by about 50%. Additionally, these mice have reduced gonadotropin expression [77].

The human *PROP1* maps to chromosome 5q. The gene consists of three exons encoding for a 226 aminoacids protein. After the first report of mutations in PROP1 in four unrelated pedigrees with GH, TSH, prolactin, LH and FSH deficiencies [78], several distinct mutations have been identified in over 170 patients [65], suggesting that mutations in *PROP1* are the most prevalent cause of multiple pituitary hormone deficiency, accounting for up to 50% of familial cases, although the incidence of *PROP1* mutations is much lower in sporadic cases [62].

Affected individuals exhibit recessive inheritance [67]. The timing of initiation and the severity of hormonal deficiency in patients with *PROP1* mutations is highly variable: diagnosis of GH deficiency preceded that of TSH deficiency in 80%. Following the deficiencies in GH and TSH, there is a reduced fertility due to gonadotropin insufficiency. Although most patients fail to enter puberty spontaneously, some start puberty before deficiencies in LH and FSH evolve. ACTH deficiency is a relatively late manifestation of *PROP1* mutation, often evolving several decades after birth. The degree of prolactin deficiency and pituitary morphological alterations are variable [65].

### 1.4 Structural Thyroid-Stimulating Hormone defects

Mutation in the TSH-beta gene are a rare cause of congenital hypothyroidism. Available data have been reviewed by Miyai [79, 80].

Several mutations in TSHB gene were identified in the last years, including missense, non-sense, frameshift and splice-site. The most commonly reported mutation is the C105Vfs114X mutation, located on exon 3 of the *TSHB* gene, and firstly described in 1996 [81]. In all the reported cases, the mutations were homozygous or compound heterozygous. So far, no genotype-phenotype correlation has been reported. The patients present all clinical sign of hypothyroidism, and the severity of the pathology depend by start of treatment. Very recently [82], direct sequencing of the coding region of the TSHB gene revealed two homozygous nucleotide changes. The first C.40A>G (rs10776792) is a recurrent alteration that can also be found in healthy individuals. The other variation was c.94G>A at codon 32 of exon 2, which results in a change of glutamic acid to lysine (p.E32K). For both variations, both patients were homozygous and the parents were heterozygous.

### 1.5 Deficiency of immunoglobulin superfamily member 1 (*IGSF1*)

*IGSF1* is a plasma membrane immunoglobulin superfamily glycoprotein [83, 84]. Human *IGSF1* and murine *Igsf1* mRNAs are highly expressed in Rathke's pouch and in adult pituitary gland and testis. Moreover, *IGSF1* protein is expressed in murine thyrotropes, somatotropes, and lactotropes, but not in gonadotropes or in the testis [85]. *Igsf1* knockout mice showed no alternation of follicle stimulating hormone synthesis or secretion, and normal fertility [61].

The physiological role of *IGSF1* is unknown, but it's lack is responsible for a variety of symptoms such as hypothyroidism, prolactin deficiency, macroorchidism and delayed puberty. *IGSF1* is important for the pituitary-thyroid axis and the development puberty and thus represents a new player controlling growth and puberty in childhood and adolescence. So far, 10 distinct *IGSF1* mutations have been described in 26 patients [85], one deletion in male patient [86], and other six mutations have been identified in Japanese subjects [87-90]. Recently, a novel insertion mutation, c.2284\_2285insA [91], has been discovered by whole-exome sequencing in three siblings affected by mild neurological phenotype. The mutations included in-frame deletions, single nucleotide deletions, nonsense mutations, missense mutations and one single-base duplication. *In vitro* expression studies of several mutations done to analyze the functional consequences demonstrated that the encoded proteins migrated predominantly as immature glycoforms and were largely retained in the endoplasmic reticulum, resulting in decreased membrane expression [85]. It is likely that there is no clear genotype-phenotype correlation. Even in familial cases sharing the same *IGSF1* defects, a variable degree of hypothyroidism was observed [85, 92]. Other genetic or environmental factors may influence the phenotypic expression of *IGSF1* deficiency.

### 1.6 TBLX1

TBL1X, transducin  $\beta$ -like protein 1 X-linked, is a part of the nuclear receptor corepressor (NCoR)-silencing mediator for retinoid and thyroid hormone receptors (SMRT) complex. In mice, the reduction of TH synthesis can be caused by disruption of NCoR, while the peripheral sensitivity to TH increases [93]. Initially, *TBL1X* gene mutations in humans were associated to hearing loss [94], but not to CCH, but Heinen & co recently identified six novel missense mutations in eight patients diagnosed with isolated CCH and hearing defects [60]. Functional studies demonstrated that the mutations cause an aberrant protein folding and stability, altering the structural and functional properties of TBLX1.

## 2. Alterations of thyroid morphogenesis (thyroid dysgenesis)

Thyroid dysgenesis (TD) is the most frequent form (~ 75%) of primary permanent congenital hypothyroidism (CH). TD includes several disorders caused by errors during thyroid development, such as athyreosis (absent gland), hypoplasia (reduced gland) or ectopy (gland located in aberrant position) [46].

The most critical events in thyroid organogenesis occur during the first 60 days of gestation in man and the first 15 days in mice. It is likely that alterations in the molecular events occurring during this period can be associated to TD. Studies on thyroid development in normal and mutated mouse embryos indicate that the simultaneous presence of *Pax8*, *Nkx2-1*, *Foxe1*, and *Hhex* is required for thyroid morphogenesis. Indeed, thyroid dysgenesis is present in animal models with mutations in these genes, and mutations in the same genes have been identified in patients with congenital hypothyroidism associated with TD.

### 2.1 Athyreosis

Athyreosis is the absence of thyroid follicular cells (TFC) in orthotopic or ectopic location. This condition can either be the consequence of lack of formation of the thyroid bud or results from alterations in any of the step following the specification of the thyroid bud and determining a defective survival and/or proliferation of the precursors of the TFC. In athyreotic patients, the presence of cystic structures resulting from the persistence of remnants of the thyroglossal duct is frequently reported. This finding indicates that in these subjects some of the early events of thyroid morphogenesis have taken place but the cells fated to form the TFCs either did not survive or switched to a different fate. In many cases, scintigraphy failed to demonstrate the presence of thyroid tissue, but thyroid scanning by ultrasound reveals a very hypoplastic thyroid gland.

So far, the absence of thyroid was reported in 3 patients with CH associated to *FOXE1* gene defects (Bamforth-Lazarus syndrome) (p.S57N, p.A65V, and p.N132D), in four subjects carrying a mutation in *PAX8*, in two patients with *NKX2-1* mutation, in two patients with *NKX2-5* mutation [8, 95] and in one patient with both a heterozygous *NKX2-5* mutation and a heterozygous mutation in the *PAX8* promoter region [96]. Recently, mutational screening in *TSHR*, *NKX2.1*, in *FOXE1*, in *NKX2.5* and in *PAX8* was performed in 100 Chinese subjects affected by thyroid athyreosis [97]. Several mutations have been identified, but the most of them were previously reported and the bioinformatics analysis suggested they were benign with no clinical relevance. Only the *TSHR* variants have been suggested to have deleterious effects by *in silico* analysis.

### 2.2 Ectopic thyroid

The ectopic thyroid is the consequence of a failure in the descent of the developing thyroid from the thyroid anlage region to its definitive location in front of the trachea. In the majority of cases, the ectopic thyroid appears as a mass in the back of the tongue (lingual thyroid, usually functioning). Sublingual ectopic tissues are less frequent; in this case, thyroid tissue is present in a midline position above, below or at the level of the hyoid bone. Ectopic thyroid tissues within the trachea or thyroid tissue in the submandibular region have also been reported.

The thyroid ectopy is the most common spectrum of thyroid dysgenesis, occurring in up 80% of CH caused by TD, but only the 3% of CH cases are explained by inherited mutation in the gene involved in thyroid development.

To date, mutational analysis performed in several countries, demonstrated the presence of mutation in patients with thyroid ectopy in *NKX2-5* gene (p.R25C, p.A119S, p.R161P), *FOXE1* (p.R102C) and *PAX8* (p.R108X, p. T225M, p.R31H) [8, 23].

Monozygotic (MZ) twins are usually discordant for CH due to thyroid dysgenesis, suggesting that most cases are not caused by transmitted genetic variation. One possible explanation could be the onset of somatic mutations in migrating genes after zygotic twinning. However, significant somatic methylation profile differences were not observed between ectopic and orthotopic thyroids [98], nor somatic mutations were found by exome sequencing of lymphocytic DNA from MZ twins discordant for CHTD [99]. Since the monoallelic genes are more vulnerable to other benign monoallelic genetic or epigenetic mutations, the autosomal monoallelic expression (AME) could explain discordance and the sporadic nature of CH [100]. The study showed that the AME is observed for some genes in ectopic and orthotopic thyroids. These genes are involved in epithelial–mesenchymal transition, cell migration, cancer, and immunity. Therefore, also in this case, no thyroid-specific mutations were observed in ectopic tissues in any of the genes normally involved in thyroid development and associated with thyroid dysgenesis.

Recently, several *DUOX2* mutations have been identified in a cohort of 268 children affected by TD (134 of whom were thyroid ectopy cases), by whole-exome sequencing (WES). Seven mutations were never reported before (G201E, L264CfsX57, P609S, M650T, E810X, and M822V, and E1017G) while eight (P138L, D506N, H678R, R701Q, A728T, S965SfsX29, P982A, and S1067L) have been previously described [101]. These findings suggest that also *DUOX2* could play a role in thyroid development.

### 2.3 Hypoplasia

Orthotopic and *hypoplastic thyroid* is reported in 5% of CH cases. Thyroid hypoplasia is a genetically heterogeneous form of thyroid dysgenesis, since mutations in *NKX2-1*, *PAX8* or *TSHR* gene have been reported in patients with thyroid hypoplasia.

*NKX2.1* mutations have been described in several patients with primary CH, respiratory distress and benign hereditary chorea, which are manifestations of the “Brain-Thyroid-Lung Syndrome” (BLTS). In the majority of cases haplo-insufficiency has been considered to be responsible for the phenotype. Only a few mutations produce a dominant negative effect on the wild type *NKX2-1*, and among those in two cases a promoter-specific dominant negative effect was reported [102]. So far, more than 96 mutations in the *NKX2.1* gene have been identified [103]. Interestingly, not all mutational carriers display the full phenotype of BLTS but have only involvement of two or even one part of the syndrome. Very recently, Hermanns & co [104] described a patient affected by TD with hypoplastic thyroid gland, respiratory disease and cerebral palsy who presented mutations in both *PAX8* (p.E234K) and *NKX2.1* (p.A329GfsX108) genes. Functional studies demonstrated no transcriptional activity or DNA-binding of *NKX2.1* mutant protein. Contrary the *PAX8* mutant protein was normally located into the nucleus, and has no functional impairment. These results confirm the role of *NKX2.1* mutant protein in the manifestation of the BLTS phenotype and suggest that other molecular mechanisms could be causative of the disease.

*NKX2.5* was recently found mutated in patients affected by thyroid hypoplasia and no cardiovascular defects [105]. Both these mutations (c.73C>T and c.63A>G) were previously described [106, 107]. The c.73C>T was found in patients affected by thyroid ectopy and without congenital heart defects [107] and showed a deficiency in dimer formation without effects on the DNA binding capacity. The c.63A>G did is a silent mutation that determines no changes in the aminoacid sequence. It has been reported in a patients with thyroid hypoplasia [108] but also in healthy controls [105].

The involvement of *PAX8* has been described in sporadic and familial cases of CH with thyroid hypoplasia [109-111]. All affected individuals are heterozygous for the mutations and autosomal dominant transmission with incomplete penetrance and variable expressivity has been described

for the familial cases. In vitro transfection assays demonstrated that the mutated proteins are unable to bind DNA and to drive transcription of the *TPO* promoter. By NGS analysis performed in a cohort of 11 families, a heterozygous PAX8 (p.R31C) was identified in two siblings with CH and hypoplastic thyroid [112]. One of the patients also presented unilateral kidney agenesis. The mutation completely inactivates the activity of the transcription factor, as previously reported for the p.R31H [113, 114]. The frequent observation of mutation occurring in this aminoacid suggested that position 31 in the PAX8 protein can be a mutational hot spot.

TSHR belongs to the G-protein coupled receptors superfamily. The gene encoding *TSHR* maps to chromosome 14q31 and to mouse chromosome 12. It consists in ten exons codify for a 764 aminoacid protein. The role of the TSHR in thyroid differentiation was first identified in *Tshr hyt/hyt* mice, affected by primary hypothyroidism with elevated TSH and hypoplastic thyroid, as a consequence of a loss of function mutation in the fourth transmembrane domain of TSHR (pro556Leu), which abolishes the cAMP response to TSH. Several patients with homozygous or compound heterozygous loss-of-function *TSHR* mutations have been reported. The disease, known as resistance to TSH (OMIM #275200) is inherited as an autosomal recessive trait, and patients are characterized by elevated serum TSH levels, absence of goiter with a normal or hypoplastic gland, and normal to very low serum levels of thyroid hormones. The clinical manifestations are very variable spanning from euthyroid hyperthyrotropinemia to severe hypothyroidism. A novel non-synonymous substitution was recently reported in the HinR of the large N-terminal extracellular domain of the TSHR gene in a patient with thyroid hypoplasia. Since this p.S304R TSHR variant does not affect the TSH binding nor the cAMP pathway activation, it was not possible to establish his role in the clinical phenotype [23].

#### 2.4 Hemiagenesis

Thyroid hemiagenesis (THA) is a rare congenital abnormality, in which one thyroid lobe fails to develop. Thyroid hemiagenesis is often associated with mild and/or transient hypothyroidism but several patients were found to be euthyroid. The incidence of the disorder is estimated at 0.05–0.5% of the general population. THA occurs usually as an isolated feature, more frequently in women than in men. In the large majority of the cases, the left lobe is absent [115].

The molecular mechanisms leading to the formation of the two thyroid symmetrical lobes, which are impaired in the case of hemiagenesis, are still unclear and in humans. In contrast, *Shh*<sup>-/-</sup> mice embryos can display either a non-lobulated gland [116] or hemiagenesis of thyroid [117], and hemiagenesis of the thyroid is also frequent in mice double heterozygous *Titf1*<sup>+/-</sup>, *Pax8*<sup>+/-</sup> [118].

In the majority of patients with thyroid hemiagenesis, the genetic background remains unknown. Additionally, THA family members commonly present other thyroid developmental anomalies (i.e., thyroid agenesis, ectopy or thyroglossal duct cyst), suggesting a common genetic background for different thyroid developmental anomalies of the gland.

Mutations in *NKX2.1*, *PAX8* or *FOXE 1* are rarely associated with THA. novel single nucleotide substitution in exon 2 of the PAX8 gene (c.162 A>T; p.S54C) was recently identified 13/16 members of a family with hypothyroidism and variable phenotype (thyroid hemiagenesis to normal) [119].

*FOXE1* contains within its coding sequence a polyalanine tract of variable length, ranging from 11 to 19 alanines [120]. Several studies have pointed to the potential role of *FOXE1*-polyAla length polymorphism in determining the susceptibility to TD [121-123].

Avery recent study, demonstrate the potential association between proteasome-related genes and THA. In a cohort of 34 sporadic patients and three families with THA several mutations have been identified in proteasome genes PSMA1, PSMA3, PSMD2, and PSMD3. The functional studies indicate that the mutations can lead to accumulation of undegraded protein aggregates and exert a toxic effect on the thyroid cell [124].

## 2.5 Other genetics defects

Recently, several other genes have been suggested to play a role in the pathogenesis of thyroid dysgenesis, including JAG1, GLIS3, CDCA8 or SLC26A4.

#### 2.5.1 *GLIS3*

In a rare syndrome, CH can be associated to neonatal diabetes (NDH). These patients exhibit reduced T3 and T4 levels with elevated TSH and Tg. Patients additionally develop hyperglycemia and hypo-insulinemia. They often also presented polycystic kidney disease, hepatic fibrosis, glaucoma and mild mental retardation. Thyroid ultrasound and scintigraphy suggested athyreosis or hypoplasia. In most of the cases, the patients do not respond to conventional treatment and TSH remains elevated, despite normalization of serum T4 levels. This form has been associated to *GLIS3* mutations [125, 126]. *GLIS3* is a transcription factor containing five Krüppel-like zinc finger domains and sharing high homology with *GLI* zinc finger proteins. It has been postulated to have a critical role in the regulation of a variety of cellular processes during development [127]. *GLIS3* may act as a transcriptional activator or repressor, but its precise role in thyroid development and function remains to be determined [128]. So far, few patients with syndromic CH and *GLIS3* mutations have been identified [126]. Very recently, a novel *GLIS3* deletion has been published in a CH girl that also presented camptodactyly, syndactyly and polydactyly [129], and mutations have been reported in patients with CH and abnormalities in external genitalia, not previously described [130].

#### 2.5.2 *JAG1*

Studies in zebrafish suggested the involvement of Notch pathway in congenital hypothyroid phenotype [131]. In humans, heterozygous *JAG1* variants are known to account for Alagille syndrome type 1 (ALGS1), a rare multisystemic developmental disorder characterized by variable expressivity and incomplete penetrance, but a recent study on a cohort of 21 young Alagille patients revealed an increased risk of non-autoimmune hypothyroidism (28%) in the presence of *JAG1* heterozygous mutations [132, 133].

#### 2.5.3 *CDCA8*

Recently, genetic variants in *CDCA8* (also called *BOREALIN*) were identified in a study of three consanguineous families with thyroid dysgenesis [134]. The thyroid phenotypes observed in patients carrying *CDCA8* variants is extensive, ranging from thyroid agenesis or ectopy to euthyroid individuals with asymmetric thyroid lobes or thyroid nodules. This variability makes the role of *CDCA8* in thyroid dysgenesis still unclear and controversial.

#### 2.5.4 *SLC26A4*

Pendrin (*SLC26A4*, *PDS*) alterations have been initially associated to Pendred syndrome (see later). Recently, NGS techniques used in patients with TD, demonstrated the frequent presence of *SLC26A4* mutations also in patients with TD. The mutations were initially identified in a patient with hypoplastic thyroid tissue and severe hearing problems [135], but later the prevalence of *SLC26A4* mutation was calculated to be 4% among studied Chinese CH patients [136].

#### 2.5.5 *DNAJC17*

Studies on mouse models indicated that neither Pax8 or Nkx2.1 heterozygous null mice showed overt thyroid defects, while double heterozygous mice for both Nkx2.1 and Pax8 (DHTP) had a severe hypothyroidism characterized by thyroid hypoplasia or hemiagenesis [118]. The DHTP hypothyroid phenotype was strain specific, and the same authors identified in *Dnajc17* the strain-related modifier gene for hypothyroidism. *DNAJC17* belongs to the heat-shock-protein-40 type III family. *DNAJC17* proteins interact, via a highly-conserved domain (J domain) with Hsp70 chaperone proteins, regulating their activity and controlling the disassembly of transcriptional complexes [137, 138]. Very recently a *DNAJC17* mutational screening has been performed in a cohort of 89 CH patients. The analysis identified only one rare variant (c.610G>C) and one polymorphism (c.350A>C) in affected patients. Both variants were already reported in databases and the frequency of the alleles was not different between TD patients and controls [139].

### **3. Defects in thyroid hormone synthesis (dyshormonogenesis)**

In about 15% of cases, CH is due to hormonogenesis defects caused by mutations in genes involved in thyroid hormone synthesis, secretion or recycling. These cases are clinically characterized by the presence of goiter, and the molecular mechanisms have been well defined.

In thyroid follicular cells, iodide is actively transported and concentrated by the sodium iodide symporter present in the baso-lateral membrane. Subsequently it is oxidised by hydrogen peroxide generation system (thyroperoxidase, Pendrin) and bound to tyrosine residues in thyroglobulin to form iodotyrosine (iodide organification). Some of these iodotyrosine residues (monoiodotyrosine and diiodotyrosine) are coupled to form the hormonally active iodothyronines (T4) and triiodothyronine (T3). When needed, thyroglobulin is hydrolyzed and hormones are released in the blood. A small part of the iodothyronines is hydrolyzed in the gland, and iodine is recovered by the action of specific enzymes, namely the intrathyroidal dehalogenases (Figure 1).

Defects in any of these steps lead to reduced circulating thyroid hormone, resulting in congenital hypothyroidism and goiter. In most of the cases, the mutations in these genes appear to be inherited in autosomal recessive fashion [9].

#### 3.1 Sodium-iodide symporter

The sodium-iodide symporter (NIS) is a member of the sodium/solute symporter family that actively transports iodide across the membrane of the thyroid follicular cells. The human gene (*SLC5A5*) maps to chromosome 19p13.2-p12. It has 15 exons encoding for a 643-amino acid protein expressed primarily in thyroid, but also in salivary glands, gastric mucosa, small intestinal mucosa, lacrimal gland, nasopharynx, thymus, skin, lung tissue, choroid plexus, ciliary body, uterus, lactating mammary tissue and mammary carcinoma cells, and placenta. Only in thyroid cells iodide transport is regulated by TSH. It has been demonstrated that the δ-amino group at position 124 of NIS protein, is required for the transporter's maturation and cell surface targeting [140].

The inability of the thyroid gland to accumulate iodine was one of the early known causes of CH, and before the cloning of NIS, a clinical diagnosis of hereditary iodide transport defect (ITD) was made on the basis of goitrous hypothyroidism and absent thyroïdal radioiodine uptake. To date, 15 mutations in the *SLC5A5* gene have been identified in patients with ITD [141]. Some of these, including V59E, G93R, Δ439-443, R124H, Q267E, T354P, G395R, and G543E, have been studied in detail and have provided key mechanistic information on NIS function. Since *SLC5A5* mutations are inherited in an autosomal recessive manner, NIS gene defects can be detected only when both alleles are mutated and the clinical picture is characterized by hypothyroidism of variable severity (from severe to fully compensated) and goiter. Furthermore, the actual prevalence of NIS gene mutations may be higher than that reported [142].

### 3.2 Thyroperoxidase

The most frequent cause of dyshormonogenesis is thyroperoxidase (TPO) deficiency. TPO is the enzyme that catalyses the oxidation, organification, and coupling reactions. Accumulation of iodine in the thyroid gland reaches a steady state between active influx, protein binding, and efflux, resulting in a relatively low free intracellular iodide concentration in normal conditions, while increased in the presence of TPO defects. The kinetics of iodide uptake and release can be traced by administration of radioiodide. Radioiodide uptake and perchlorate inhibition gives an idea of the intrathyroidal iodide concentration in relation to the circulating iodine. Iodine organification defects can be quantified as total or partial: total iodide organification defects are characterized by discharge of more than 90% of the radioiodide taken up by the gland within 1 hour after administration of sodium perchlorate, usually given 2 hours after radioiodide. A total disappearance of the thyroid image is also observed. Partial iodide organification defects are characterized by discharge of 20% to 90% of the accumulated radioiodine [143]. Mutations in *TPO* gene (particularly nonsynonymous cSNPs) can lead to severe defects in thyroid hormone production, due to total or partial iodide organification defects. Based on the literature, exons 7–11 encoding the catalytic center of the TPO protein (heme binding region) are crucial for the enzymatic activity. Nonsense, splice-site, and frameshift mutations have been also described by several groups [141].

### 3.3 DUOX1 and DUOX2

The generation of  $H_2O_2$  is a crucial step in thyroid hormonogenesis. DUOX1 and DUOX2 are glycoproteins with seven putative transmembrane domains. These proteins, map on chromosome 15q15.3, and their function remained unclear until a factor, named DUOXA2, which allows the transition of DUOX2 from the endoplasmic reticulum to the Golgi, was identified [144]. The coexpression of this factor with DUOX2 in HeLa cells is able to reconstitute the  $H_2O_2$  production *in vitro*. A similar protein (DUOXA1) is necessary for the complete maturation of the DUOX1. In murine models, only DUOX2 loss of function mutation have been associated with hypothyroidism; thus, the role of DUOX1 in thyroid biology remains unclear [145].

DUOX2 mutations usually cause transient CH or permanent CH with partial iodide organification defect. Permanent and transient CH may result from both mono- and biallelic mutations, and phenotypic heterogeneity may occur with similar mutations [146].

To date, at least 41 patients belonging to 33 families have been reported to carry mutations in DUOX2 gene [147]. Recently, a case of CH with a homozygous loss-of-function mutation in DUOX1 (c.1823-1G>C) was reported. The mutation was inherited digenically with a homozygous DUOX2 nonsense mutation (c.1300 C>T, p. R434\*) [148]. Probably, the inability of DUOX1 to compensate for the DUOX2 deficiency in these kindred may underlie the severe CH phenotype.

### 3.4 Pendrin

The Pendred syndrome is characterized by congenital neurosensorial deafness and goiter. The disease is transmitted as autosomal recessive disorder. Patients have a moderately enlarged thyroid gland, are usually euthyroid and show only a partial discharge of iodide after the administration of thiocyanate or perchlorate. The impaired hearing is not constant. In 1997, the *PDS* gene was cloned and the predicted protein of 780 amino acids (86-kD) was called Pendrin. The *PDS* gene maps to human chromosome 7q31, contains 21 exons, and it is expressed both in the cochlea and in the thyroid. Pendrin has been localized in the apical membrane of thyroid follicular cell [149]. In thyroid follicular cells, and in transfected oocytes, Pendrin is able to transport iodide.

A number of mutations in the *PDS* gene have been described in patients with Pendred syndrome. Despite the goiter, individuals are likely to be euthyroid and only rarely present congenital hypothyroidism. However, TSH levels are often in the upper limit of the normal range, and hypothyroidism of variable severity may eventually develop.

In the last years, mutation in the *PDS* gene have also been associated with thyroid dysgenesis [135, 136].

### 3.5 Thyroglobulin

Thyroglobulin is a homodimer protein synthesized exclusively in the thyroid. The human gene is located on chromosome 8q24 and the coding sequence, containing 8307 bp, is divided into 42 exons [150]. Patients with disorders of thyroglobulin synthesis are moderately to severely hypothyroid and often present goiter. Usually, plasma thyroglobulin concentration is low, especially in relation to the TSH concentrations, and does not change after T4 treatment or injection of TSH. Patients classified in the category “thyroglobulin synthesis defects” often have other abnormal iodoproteins, mainly iodinated plasma albumin, and they excrete iodopeptides of low molecular weight in the urine. At least 70 distinct inactivation *TG* gene mutations have been described [150, 151]. Scintigraphy shows high uptake (due to induction of NIS expression by TSH stimulation) in a typically enlarged thyroid gland.

### 3.6 DEHAL1

In addition to the active transport from the blood due to NIS, iodine in the thyroid follicular cells derives also from the deiodination of monoiodotyrosine and diiodotyrosine. The gene encoding for this enzymatic activity was recently identified and named *IYD* (or *DEHAL1*) [152]. The human gene maps to chromosome 6q24-q25 and consists of six exons encoding a protein of 293 amino acids, a nitroreductase-related enzyme capable of deiodinating iodotyrosines. In the past it has been suggested that *IYD* mutations could be responsible for congenital hypothyroidism, but only in 2008 the first *IYD* mutations were described in three different consanguineous families. All the patients had homozygous *IYD* mutations, and presented goiter and hypothyroidism. The onset of symptoms was very variable, either at birth or later in infancy or childhood. A particular mutation of *IYD*, (c.658G>A, p.Ala220Thr), was reported in a heterozygous 14-yr-old boy affected by hypothyroidism and goiter, suggesting a possible dominant effect of the mutation. Very recently, a new *IYD* mutation was identified by genome-wide approach in a 20-yr-old patient with hypothyroidism and goiter and in his 4.5-yr-old apparently healthy sister in a consanguineous Moroccan family [153]. Since hypothyroidism is infrequent at birth, patients with biallelic *IYD* mutations are normally not identified as CH at the screening, but they subsequently came to medical attention between 1.5 and 8.0 years of age [141].

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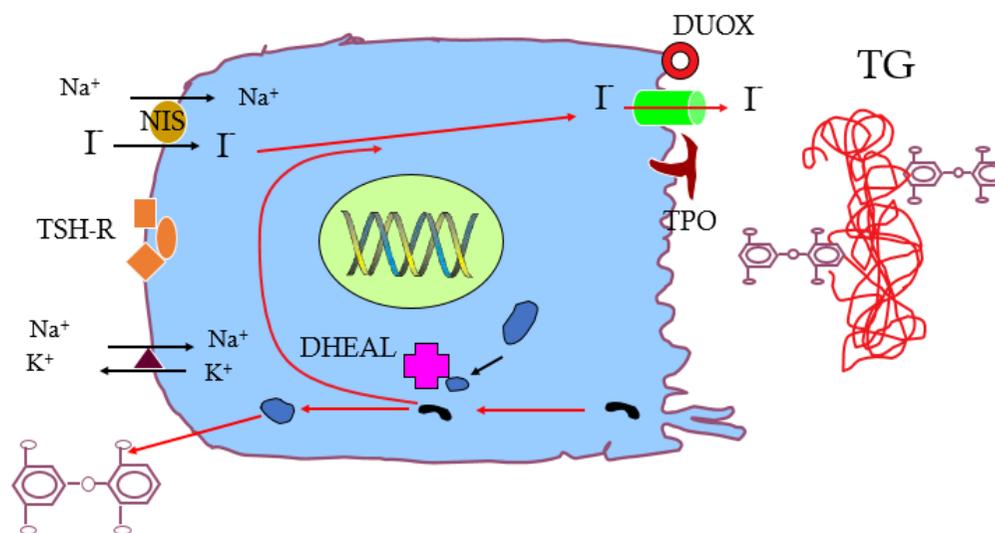
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Legend to figure.

## Main steps of thyroid hormone biosynthesis



**Figure 1. Main steps involved in the biosynthesis of thyroid hormones.** The picture schematizes the main enzymatic reactions involved in biosynthesis, production and release of thyroid hormones in the thyroid follicular cell. Congenital alteration in any of the reported steps can be associated to congenital hypothyroidism (dysormonogenesis).

**Table1-**  
**1. Clinical picture of the forms of congenital hypothyroidism with a genetic origin**

Thyroid alteration	Thyroid morphology	Gene	Clinical manifestations
Central hypothyroidism	No goiter	<i>LHX3</i> and <i>LHX4</i>	Hypothyroidism, combined pituitary hormone deficiency, short stature, metabolic disorders, reproductive system deficits, nervous system developmental abnormalities

		<i>HESX1</i>	Hypothyroidism, septo-optic dysplasia (SOD): hypoplasia of the optic nerves, various types of forebrain defects, multiple pituitary hormone deficiencies
		<i>TRH</i> and <i>TRHR</i>	Hypothyroidism, short stature
		<i>IGSF1</i>	Hypothyroidism, prolactin deficiency, macroorchidism, delayed puberty, neurological symptoms
		<i>TBLX1</i>	Congenital hypothyroidism and hearing defects
Thyroid dysgenesis	Athyreosis	<i>PAX8</i>	No goiter, severe hypothyroidism
		<i>NKX2-5</i>	No goiter, severe hypothyroidism, no cardiac alterations
		<i>FOXE1</i>	Severe hypothyroidism, Bamforth-Lazarus syndrome
	Thyroid ectopy	<i>NKX2-5</i>	No goiter, hypothyroidism, no cardiac alterations
		<i>FOXE1</i>	Hypothyroidism, Bamforth-Lazarus syndrome
		<i>PAX8</i>	Congenital hypothyroidism, non-syndromic
	Thyroid hypoplasia	<i>DUOX2</i>	Congenital hypothyroidism, non-syndromic
		<i>NKX2-1</i>	No goiter, variable hypothyroidism (mild to severe), choreoathetosis, pulmonary alterations
		<i>TSHR</i>	Resistance to TSH: no goiter, variable hypothyroidism (mild to severe)
Dysormonogenesis	Goiter	<i>PAX8</i>	No goiter, variable hypothyroidism (moderate to severe)
		<i>NIS</i>	Variable hypothyroidism (moderate to severe)
		<i>TPO</i>	Variable hypothyroidism (moderate to severe)
		<i>DUOX1</i> and <i>DUOX2</i>	Permanent hypothyroidism (mild to severe), transient and moderate hypothyroidism
		<i>DUOX2A2</i>	Variable hypothyroidism (mild to severe)
		<i>PDS</i>	Moderate hypothyroidism and deafness;
		<i>TG</i>	Variable hypothyroidism (from moderate to severe)
<i>DHEAL1</i>	Variable hypothyroidism (mild to severe)		

**Table 2. Tests used to complete the diagnosis of CH**

1. Imaging studies (to determine thyroid location and size)
  - a. Scintigraphy (99mTc or 123I)
  - b. Ultrasonography
2. Functional studies
  - a. 123I uptake
  - b. Serum thyroglobulin
3. Suspected inborn errors of thyroid hormone synthesis
  - a. 123I uptake and perchlorate discharge
  - b. Serum/salivary/urine iodine studies
4. Suspected autoimmune thyroid disease
  - a. Maternal and neonatal serum thyroid-antibodies determination
5. Suspected iodine exposure (or deficiency)
  - a. Urinary iodine measurement