GENETIC DEFECTS IN THYROID HORMONE SUPPLY

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REVISED 16 DEC 2014

INTRODUCTION

Congenital hypothyroidism (CH) is the most frequent endocrine-metabolic disease in infancy, with an incidence of about 1/2000-4000 newborns (1,2). 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 due to alterations occurring during the gland development (thyroid dysgenesis, TD) or distruptions in thyroid hormone biosynthesis (thyroid dysormonogenesis). Less common causes of CH are secondary or peripheral defects in TSH synthesis and/or action, defects in thyroid hormone transport, metabolism, or action (3).
In the majority of cases (80-85%), primary permanent CH is due to alterations occurring during the gland organogenesis, resulting in a thyroid that is absent (athyreosis), hypoplastic (thyroid hypoplasia) or located in an unusual position (thyroid ectopy). All these entities are grouped under the term "thyroid dysgenesis" (TD)(4). TD occurs mostly as a sporadic disease, however a genetic cause of the disease has been demonstrated in about 5% of the reported cases (5). 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(6). Thyroid dyshormonogenesis shows classical Mendelian recessive inheritance.
Rarely CH has a central origin, as consequence of hypothalamic and/or pituitary diseases, with reduced production and/or effect of the thyrotropin releasing hormone (TRH) or of the thyrotropin hormone (TSH)(7).

CONGENITAL HYPOTHYROIDISM: DIAGNOSIS AND TREATMENT

Clinical manifestations

CH is usually a sporadic disease with a 2:1 female to male ratio. Familial cases occur with a frequency that is 15-fold higher than by chance alone (8). The genetic basis of these familial cases has been established in some, but not all pedigrees (9). Most newborn babies with congenital hypothyroidism have few or no clinical manifestations of thyroid hormone deficiency, and the majority of cases are sporadic. As a result, it is not possible to predict which infants are likely to be affected. For these reasons, newborn screening programs in which either thyroxine (T4) or thyrotropin (TSH) are measured in heel-stick blood specimens were developed in the mid-1970s to detect this condition as early as possible. These screening efforts have been largely successful, but more severely affected infants may still

have a slightly reduced IQ and other neurologic deficits despite prompt diagnosis and initiation of therapy.
International studies show that the incidence of permanent primary CH is approximately 1 in 3000 newborns (in iodine sufficient areas). There is considerable ethnic variation in incidence, ranging from 1 in 30,000 in the African-American population in the United States (10,11) to 1 in 900 in Asian populations in the United Kingdom (12).
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 (Table 1). 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. 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) because of decrease in the degradation of these substances. The long-term effects of severe hypothyroidism on craniofacial growth and dental development have also included impaction of the mandibular second molars (13).
Family history should be carefully reviewed for information about similarly affected infants or family members with unexplained mental retardation. Maternal history of a thyroid disorder and mode of treatment, whether before or during pregnancy, can occasionally provide the etiology of the infant's problem.

A small but significant number (3-7%) of infants with congenital hypothyroidism 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) (14).

NEONATAL SCREENING

Screening programs for CH were initially developed in Quebec, Canada, and Pittsburgh, Pennsylvania, in 1974 (15), and have now been established in Western Europe, North America, Japan, Australia, and parts of Eastern Europe, Asia, South America, and Central America (16). Since the introduction of the screening, the apparent overall incidence of CH has increased considerably as a consequence of the detection of mild disorders that previously remained undetected or were not recognized as congenital problems.

Neonatal screening programs allow for early detection and treatment of CH, and have proven to be successful in preventing brain damage.

The population-based newborn screening measures TSH or TSH and total T4 in dried blood spots obtained in the first 3 days of life. In newborns with a screening result suspicious for hypothyroidism, the diagnosis of primary CH is confirmed when serum TSH levels are above and free T_4 levels are below the age-related reference ranges. Hypothalamic-pituitary hypothyroidism is more difficult to diagnose. Most infants with this diagnosis are missed in screening programs unless T_4 and TSH or TSH, T_4 and thyroxine binding globulin (TBG) are simultaneously measured.

Worldwide, most neonatal screening programs are TSH based in the first 3 days of life and effectively detect only thyroidal congenital hypothyroidism (CHT). Only a few screening programs in some countries employ a method in which total T4 or free T4 and TSH are measured simultaneously or stepwise ("T4+TSH-method") enabling detection of CHT as well as CHC (central congenital hypothyroidism) (17-19).

If hypothyroidism is confirmed by laboratory analysis, imaging studies should be performed, but it is not acceptable to delay hormone replacement therapy if imaging studies are not readily available (20).

DIAGNOSIS

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

Imaging studies are useful to establish the presence of thyroid morphogenesis alterations, which are the most common cause of CH. Thyroid scintigraphy, with ^{99m}technetium or ^{123}I, is the most informative diagnostic procedure in patients with thyroid dysgenesis (21,22). Scintigraphy should be performed immediately at birth, if this will not delay the start of thyroxine (L-T4) treatment, or around the age of 4, when L-T4 therapy can be interrupted for 4 weeks without consequences for the child development.

More than 80% of newborn infants with TSH elevation can be diagnosed correctly on initial imaging with combined radioisotope scan and ultrasound. Ultrasound cannot reliably detect thyroid ectopia. Although thyroid ultrasonography is useful in demonstrating enlarged or absent glands, it is less accurate than scintigraphy in showing ectopic glands (23). Radioisotope scan, especially if performed late, may show no uptake despite the presence of a eutopic gland (22).

Recently it has been suggested that intramuscular injections of recombinant human TSH can be useful to perform $^{123}I^-$ uptake studies during L-thyroxine treatment in CH patients (24,25). Assay of serum thyroglobulin (Tg) will be useful in to establish the presence of some thyroid tissue, while $^{123}I^-$ will provide information about the thyroidal uptake of iodide.

More specialized tests, such as perchlorate discharge, evaluation of serum, salivary, and urinary radioiodine (26), and measurement of serum T_4 precursors, may be necessary to delineate specific inborn errors of thyroid hormone biosynthesis (27).
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 (28).
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_4 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 (29,30).
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 from transient to permanent hypothyroidism (31).
The standard approach for the treatment of hypothyroidism is replacement therapy with thyroxine (levothyroxine sodium). Thyroxin (T4) is converted to the more metabolically active triiodothyronine (T3) in the peripheral tissues. This returns patients to a biochemical euthyroid state, with normal levels of TSH concentration and serum-free T4 (FT4). Once the euthyroid state is achieved, the patient's TSH and FT4 levels are followed at periods of six months to one year (13).

GENETIC CLASSIFICATION OF CONGENITAL THYROID DISEASES

1. Central hypothyroidism

Congenital central hypothyroidism (C-CH) 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 TSH measurement. However, neonatal screening for CH on the basis of thyroxine (T4) or free T4 and TSH concentrations can be performed to diagnose this type of CH (32-34).
C-CH is more prevalent than previously thought, reaching 1 in 16,000 neonates in countries consistently identifying C-CH through T4-based CH screening strategies (35). Neonatal detection and early treatment of C-CH would prevent the risk of developing mental retardation secondary to late diagnosis of infantile hypothyroidism (36) and is generally associated with alterations in hypothalamus or pituitary development causing life-threatening situations. The accompanying pituitary hormonal deficiencies, especially the lack of cortisol, may be responsible for high morbidity and mortality.
The current knowledge on the genetic bases of C-CH is scarce. At the hypothalamic level no gene defects causing C-CH have yet been identified in humans, but pituitary (thyrotrope)-selective genes encoding the TSH-releasing hormone (TRH) receptor (TRHR), the TSH β-subunit (TSHB) and, recently, the immunoglobulin superfamily factor 1 (IGSF1) are genes involved in isolated central hypothyroidism (36).

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 [LH] and follicle-stimulating hormone [FSH]), and adrenocorticotropic 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 adrenocorticotropic 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 (37,38).

Hesx1 (also called *Rpx*), a member of the paired-like class of homeobox genes, is one of the earliest markers of the pituitary primordium (39). Extinction of *Hesx1* is important for activation of downstream genes such as *Prop1*, suggesting that the proteins act as opposing transcription factors (40). 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 (37). 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 (41).

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 (39). Subsequently several other homozygous and heterozygous mutations have been shown to present with different phenotypes characterized by pituitary hormone deficiency and SOD (40,42).

1.2 Defects in the TRH and TRH receptor

In mice, homozygous deletion of the *TRH* gene produced a phenotype characterized by hypothyroidism and hyperglycemia (43). Only a few patients with reduced TRH production have been described in the literature (44,45), 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_3, T_4, and prolactin (PRL) levels but normal serum TSH (46). So far only one family with a compound heterozygous (47) and one family with homozygous (48) loss of function mutation of TRH receptor have been described.

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 α subunit and β subunit, The α subunit is shared with other glycoprotein hormones (i.e. follicle-stimulating hormone (FSH), luteinizing hormone (LH), and chorionic gonadotropin (CG)), whereas the TSHβ 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 (49,50).

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 (50).

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 (51).

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 (52), several distinct mutations have been identified in over 170 patients (40), 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 (37).

Affected individuals exhibit recessive inheritance (42). 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 (40).

1.4 Structural Thyroid-Stimulating Hormone defects

Mutation in the TSH-beta gene are a rare cause of congenital hypothyroidism, and in all the reported cases, the mutations were homozygous or compound heterozygous. Available data have been recently reviewed by Miyai (53). The phenotype is very variable and it may range from a very mild hypothyroidism to severe forms associated with mental retardation in case of delayed treatment. Patients with mutation in the TSH-beta are characterized by the presence of low levels of circulating TSH that will not be stimulated by TRH (54). Finally, cases of immunologycally reactive but biologically inactive TSH have also been reported (53).

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

IGSF1 is a plasma membrane immunoglobulin superfamily glycoprotein (55,56). 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 (57). *Igsf1* knockout mice showed no alternation of follicle stimulating hormone synthesis or secretion, and normal fertility (58).

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, 8 distinct mutations and 2 deletions in *IGSF1* have been identified in 11 families (57), and three other mutations reported in Japanese patients (59). 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 (57). 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 (57,60). Other genetic or environmental factors may influence the phenotypic expression of IGSF1 deficiency.

2. Alterations of thyroid morphogenesis (thyroid dysgenesis)

In the majority of cases (80-85%), primary permanent CH is due to alterations occurring during the gland organogenesis, resulting either in a thyroid that is absent (thyroid agenesis or athyreosis) or hypoplastic (thyroid hypoplasia) or located in an unusual position (thyroid ectopy). All these entities are grouped under the term "thyroid dysgenesis" (TD)(4). TD occurs mostly as a sporadic disease, however a genetic cause of the disease has been demonstrated in about 5% of the reported cases.

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

The absence of thyroid follicular cells TFC in orthotopic or ectopic location is called *athyreosis*. 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.
So far, the absence of thyroid was reported in 3 patients with CH associated with *FOXE1* gene defects (Bamforth-Lazarus syndrome), in three subject carrying a mutation in *PAX8*, in two patients with *NKX2-1* mutation, in two patients with *NKX2-5* mutation and in one patient with both a heterozygous NKX2-5 mutation and a heterozygous mutation in the PAX8 promoter region (5) (Table 4).
Homozygous mutations in *FOXE1* gene have been reported in patients affected by Bamfort's syndrome. This syndrome is characterized by cleft palate, bilateral choanal atresia, spiky hair and athyreosis. All the affected members carry homozygous missense mutations in conserved amino acids of FOXE1, and the mutant proteins, when tested *in vitro*, show a reduction in both DNA binding and transcriptional activity. While in mice the absence of *FoxE1* causes either athyreosis or ectopy, in humans *FOXE1* mutations have never been associated to thyroid ectopy.

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. An ectopic thyroid can be found in any location along the path of migration from the foramen caecum to the mediastinum. 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.
In humans more than 50% of TD cases are associated with an ectopic thyroid; however, up to now, only three heterozygous mutations in the *NKX2-5* gene (p.R25C, p.A119S, p.R161P), one mutation in *FOXE1* and two mutations in *PAX8* (p.R108*, p. T225M) have been associated to the human ectopic thyroid (5) (Table 4). Thyroid ectopy was observed also in two cases of PAX8 (p.R31H) mutation, a finding that has not been reported previously (18). The functional studies of the mutant NKX2-5, demonstrated a significant functional impairment with reduction of transactivation properties and a dominant negative effect. The patients described were all heterozygous and the mutations were inherited from one of the

parents, suggesting that *NKX2-5* mutations have variable penetrance and clinical significance (61).
Surprisingly, 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 is somatic mutation in genes involved in thyroid migration occurring after zygotic twinning. To test the hypothesis of somatic mutation, Magne (62) performed whole exome sequencing of DNA from three pairs of MZ twins discordant for CH with ectopic glands, but no somatic mutations were identified.
Recently gene expression, genome-wide methylation, and structural genome variations have been compared between normal and ectopic thyroid tissue. Ectopic thyroids show a differential gene expression compared to normal thyroids, although molecular basis could not be properly defined probably as consequence of the small number of samples examined or of the different gene expression pattern between adult and embryonic gland (63).

2.3 Hypoplasia

Orthotopic and *hypoplastic thyroid* is reported in 5% of CH cases. The relative proportions of the main TD phenotypes vary in different studies, depending on the methodology used to detect the presence of the gland. The most frequent form of TD is thyroid gland ectopy and only the combination of ultrasound sonography and ^{99}Tc scintigraphy might resolve a precise relative proportion of these phenotypes. 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 (Table 4).
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 (64). All the published *NKX2-1* mutations have variable functional effects, even if the mutations occur in similar regions of the protein. The phenotypes of patients affected by *NKX2-1* mutations are very variable and there is no correlation between the clinical manifestations and the molecular alterations. Usually, mutations in *NKX2-1* caused a Benign Hereditary Chorea (BHC), a rare autosomal dominant condition characterized by early onset of non progressive chorea. To date, 78 mutations associated with BHC have been reported in the *NKX2-1* gene (65). Thirty-four are de novo (43.6%), 29 (37.2%) showed an autosomal dominant transmission and, for 15, the transmission was not defined (19.2 %). Recently (66), it has been demonstrated that sudden unexpected falls may be an early clinical manifestation of BHC, predating the onset of abnormal movements. A heterozygous mutation of the NKX2-1 gene (14q13) was detected in these patients. The two novel mutations identified in both patients are predicted to cause NKX2-1 haplo-insufficiency, by truncating the protein before (c.437_438dupGC; p.N147Pfs*20) and inside (c.634 C>T:p.Q212*) the homeodomain, thus affecting its DNA binding properties and interfering with the transcriptional activity on target genes.

The involvement of *PAX8* has been described in sporadic and familial cases of CH with TD (67-73). 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.

TSHR mutations

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. Indeed, recently occurrence of a novel nonsynonymous substitution was reported in the HinR of the large N-terminal extracellular domain of the TSHR gene in a patient with thyroid hypoplasia. In contrast with four others in whom TSHR mutations of the hinge portion were previously identified, this p.S304R TSHR variation affected neither TSH binding nor cAMP pathway activation. This TSHR gene variant was documented in a CH patient, but the current data do not support its role in the clinical phenotype (74).

2.4 Hemiagenesis

Thyroid hemiagenesis 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 in the euthyroid state (both serum TSH and thyroid hormone levels are within the normal range). Thyroidal hemiagenesis is predominantly seen in females with an incidence to 0.2% in healthy children. In the large majority of the cases, the left lobe is absent (75).

The causes of thyroid hemiagenesis are still unclear and it is unknown whether the disturbance of the lobulation process is due to interference of environmental factors or to genetic abnormality. In addition, the occurrence of some cases of thyroid hemiagenesis among members of the same family suggests that genetic factors could be involved in this anomaly.

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, candidate genes responsible for the hemiagenesis of the thyroid have not yet been described. Indeed, *Shh*$^{-/-}$ mice embryos can display either a non lobulated gland (76) or hemiagenesis of thyroid (77), and hemiagenesis of the thyroid is also frequent in mice double heterozygous *Titf1*$^{+/-}$, *Pax8*$^{+/-}$ (78). To date, no genetic alterations have been found in patients with thyroid hemiagenesis.

FOXE1 within its coding sequence contains a polyalanine tract of variable length, ranging from 11 to 19 alanines. Several studies have pointed to the potential role of *FOXE1*-polyAla length polymorphism in determining the susceptibility to TD (79-81).

2.5 Other genetics defects

Congenital Hypothyroidism has also been associated with a rare syndrome, characterized by congenital hypothyroidism and neonatal diabetes (NDH). Patients with NDH exhibit diminished levels of T3 and T4 along with elevated TSH and Tg. Patients additionally

develop hyperglycemia and hypo-insulinemia often accompanied by polycystic kidney disease, hepatic fibrosis, glaucoma and mild mental retardation. Thyroid ultrasound and scintigraphy also suggested athyreosis or hypoplasia. In most cases, these patients do not respond to conventional treatment and TSH remains elevated, despite normalization of serum T4 levels. Several mutations in the GLI-similar 3 (GLIS3) gene have been identified in patients with NDH. *Glis3*-knockout mice die in one week after birth probably for severe neonatal diabetes. Hypothyroidism was also observed in *Glis3* knockout mice; however, histological examination of the thyroid gland suggested that Glis3 does not significantly affect thyroid gland development.
It has been suggested that Glis3 plays a critical role in the maintenance and/or proliferation of endocrine progenitor cells and in development and maintenance β-cells.
GLI-similar 3 (GLIS3) is a transcription factor containing five Kruppel-like zinc finger motifs. The human gene maps to chromosome 9p24.2 and encodes for a protein that is approximately 90 kD. *Glis3* is expressed in a tissue-specific manner with the highest levels of expression observed in the kidney, thyroid gland, endocrine pancreas, thymus, testis, and uterus. Low levels of expression have also been reported in the brain, lung, ovary and liver. Mutations in *GLIS3* have been identified in ten patients from seven families with nine of the ten born to consanguineous parents (82-84).

3. Defects in thyroid hormone synthesis (dyshormonogenesis)
As mentioned before, 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 in most of these forms have been well defined (6) (Table 5).
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 T_4 and triiodothyronine (T_3). When needed, thyroglobulin is hydrolyzed and hormones are released in the blood. A small part of the iodotyronines are hydrolyzed in the gland, and iodine is recovered by the action of specific enzymes, namely the intrathyroidal dehalogenases.
Defects in any of these steps lead to reduced circulating thyroid hormone, resulting in congenital hypothyroidism and goiter. With the exception of rare cases, all mutations in these genes appear to be inherited in autosomal recessive fashion (6).

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. In 1996, NIS mRNAs from rats (85) and humans (86) were isolated. 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 (87,88). Only in thyroid cells is iodide transport regulated by TSH. It was demonstrated that the δ-amino group at position 124, is required for the transporter's maturation and cell surface targeting (89).

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 thyroidal radioiodine uptake. To date, 15 mutations in the *SLC5A5* gene have been identified in patients with ITD. 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, heterozygous individuals, 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 (90,91).
Furthermore, under the conditions of high iodine intake, when full preservation of iodine concentrating function is not required to achieve normal thyroid hormone synthesis, mutations causing impairments of function may not be detected even in the homozygous patients. For these reasons, the actual prevalence of NIS gene mutations may be higher than that reported (92).
In the neonatal period, infants with iodide transport defects are found to have a normal-size or slightly enlarged thyroid gland by ultrasonography and elevated serum thyroglobulin levels (93). Radioactive iodide uptake is absent. Measurement of the saliva-to-plasma ^{123}I ratio is around one. The degree of hypothyroidism is variable and ranges from mild to severe, possibly depending on the amount of iodide in the diet. These children are severely hypothyroid if maintained with a normal iodine diet, while addition of high amount of iodide to the diet tends to compensate the iodide transport failure.

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 and iodide re-uptake can be inhibited by anions of similar molecular size and charge, such as perchlorate or thiocyanate. 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 (94).
The human TPO gene is located on chromosome 2p25 and spans approximately 150 kb; the coding sequence of 3048 bp is divided over 17 exons (95) and encodes for a 933 amino acid, membrane bound, glycated, heme-containing protein, located on the apical membranes of the thyroid follicular cell.

Mutations in *TPO* gene (particularly nonsynonymous cSNPs) can lead to severe defects in thyroid hormone production, due to total or partial iodide organification defects. *TPO* mutations are inherited as autosomal recessive traits. Based on the literature, Exons 7–11 encode the catalytic center of the TPO protein (heme binding region) which is crucial for the enzymatic activity, thus mutations in these regions are expected to have major effects on TPO activity and result in severe organification defect and hypothyroidism. Nonsense, splice-site, and frameshift mutations have been also described by several groups (91,96,97).
If untreated, patients with organification defects show variable degrees of mental retardation, very large goiter and hypothyroidism. In some cases with partial defects hypothyroidism appears compensated (97).

3.3 DUOX1 and DUOX2

The generation of H_2O_2 is a crucial step in thyroid hormonogenesis. DUOX1 and DUOX2 (du al ox idase) are glycoproteins with seven putative transmembrane domains. These proteins, initially named THOX1 and THOX2 (for thyroid oxidase), map on chromosome 15q15.3, only 16kb apart from each other and in opposite transcriptional orientation. Their function remained unclear until a factor, named DUOXA2, which allows the transition of DUOX2 from the endoplasmic reticulum to the Golgi was identified (98). 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. Interestingly, both DUOXA genes maps in the 16kb that separates the *DUOX1* and *DUOX2* genes on chromosome 15.
Several mutations in DUOX genes have been reported in patients with congenital hypothyroidism showing very variable phenotype (99-101). In order to produce congenital permanent hypothyroidism a severe alteration of both alleles of DUOX2 gene is required. The presence of some residual activity in one of the alleles may produce a less severe phenotype, whereas monoallelic inactivation of the *DUOX2* gene is associated with transient CH. In addition, the phenotype of monoallelic inactivation seems to be modulated by other factors, including environmental conditions (such as iodine insufficiency), lifetime events (pregnancy, immediate postnatal life) or alterations in the dual oxidase maturation factor 2 (*DUOXA2*) (102,103).
DUOX2 is a transmembrane protein, which is expressed at the apical membrane of thyroid follicular cells. DUOX2 produces hydrogen peroxide (H2O2), which is required for iodination of thyroglobulin. After the first description of DUOX2 mutations in patients with CH, at least 41 patients belonging to 33 families have been reported to date. All previously reported DUOX2 mutation-carrying families have followed autosomal recessive inheritance, as is the case in other forms of thyroid dyshormonogenesis. Recently, a nonconsanguineous Japanese family harbouring biallelic DUOX2 mutations was reported, in which an apparently dominant inheritance (defined as pseudo-dominant inheritance) of non-autoimmune hypothyroidism presented. Although DUOX2 mutations are usually inherited recessively, the relatively high frequency of mutations (1:20000) could lead to pseudo-dominant inheritance in Japan (104).

3.4 Pendrin

In 1896, Vaughan Pendred described a syndrome characterized by congenital neurosensorial deafness and goiter (105). 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, and is due to a cochlear defect that corresponds to the Mondini's type of developmental abnormality of the cochlea.
In 1997, the *PDS* gene was cloned and the predicted protein of 780 amino acids (86-kD) was called Pendrin (106). 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 (107,108). In thyroid follicular cells, and in transfected oocytes, pendrin is able to transport iodide..Patients with Pendred's syndrome are subclinically hypothyroid with goiter, and show moderate-to-severe sensineural hearing impairment. Discharge of radioiodide after administration of sodium perchlorate is moderately increased (>20%). The prevalence varies between 1:15,000 and 1:100,000 (109).

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 (109-111).
Recently, a homozygous missense mutation (p.Leu597Ser) in the SLC26A4 gene was unexpectedly identified by exome-sequencing in a patient with hypoplastic thyroid tissue, otherwise healthy. In the same paper, by screening other patients with CH, a second case with a homozygous missense mutation (p.Gln413Arg) in the SLC26A4 gene was identified in a patient presenting severe hearing problems (112). Both mutations were previously described as loss-of-function mutations in patients with Pendred syndrome and non-syndromic EVA (enlarged vestibular aqueduct). The identification of these mutations in patients with thyroid dysgenesis suggested a possible role of SLC26A4 alterations in modulation of TD supporting the hypothesis of multigenic origin of congenital hypothyroidism (78).

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 (113), is divided into 42 exons (114). Following a signal peptide of 19 amino acids, the polypeptide chain is composed of 2750 amino acids containing 66 tyrosine residues. Thyroglobulin is a dimer with identical 330-kDa subunits containing 10% carbohydrate residues.
Patients with disorders of thyroglobulin synthesis are moderately to severely hypothyroid. Usually, plasma thyroglobulin concentration is low, especially in relation to the TSH concentrations, and do not change after T_4 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 (115).
Several mutations in the thyroglobulin gene have been reported in patients with CH (6,116,117) and in animals including Afrikander cattle (p.R697X) (118), Dutch goats (p.Y296X) (119), cog/cog mouse (p.L2263P) (120) and rdw rats (p.G2300R) (121).
Mutations in the human thyroglobulin gene are associated with congenital goiter and with moderate to severe hypothyroidism (6).

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 (122). The gene encoding for this enzymatic activity was recently identified and named *IYD* (or

DEHAL1) (123,124). 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 was suggested that *IYD* mutations could be responsible for congenital hypothyroidism, but only in 2008 were the first IYD mutations described in three different consanguineous families (125,126). 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 of a consanguineous Moroccan family (127).

The wide inter- and intrafamilial variability of the disease severity remains unclear, and can derive either form the molecular effects of the mutation (complete absence or partial activity of the protein), or due to environmental factors, such as iodine diet content.

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Table 1. Clinical picture of the forms of congenital hypothyroidism with a genetic origin

Thyroid alteration	**Thyroid morphology**	**Gene**	**Clinical manifestations**
Central hypothyroidism	No goiter	*LHX3* and *LHX4*	Hypothyroidism, combined pituitary hormone deficiency, short stature, metabolic disorders, reproductive system deficits, nervous system developmental abnormalities
		HESX1	Hypothyroidism, septo-optic dysplasia (SOD): hypoplasia of the optic nerves, various types of forebrain defects, multiple pituitary hormone deficiencies
		TRH and *TRHR*	Hypothyroidism, short stature
Thyroid dysgenesis	Athyreosis	*PAX8*	No goiter, severe hypthyroidism
		NKX2-5	No goiter, severe hypothyroidism, no cardiac alterations
		FOXE1	Severe hypothyroidism, Bamforth-Lazarus syndrome
	Thyroid ectopy	*NKX2-5*	No goiter, hypothyroidism, no cardiac alterations
		FOXE1	Hypothyroidism, Bamforth-Lazarus syndrome
	Thyroid hypoplasia	*NKX2-1*	No goter, variable hypothyroidism (mild to severe), choreoathetosis, pulmonary alterations
		TSHR	Reistance to TSH: no goiter, variable hypothyroidism (mild to severe)

		PAX8	No goiter, variable hypothyroidism (moderate to severe)
Dysormonogenesis	Goiter	*NIS*	Variable hypothyroidism (moderate to severe)
		TPO	Variable hypothyroidism (moderate to severe)
		DUOX1 and *DUOX2*	Permanent hypothyroidism (mild to severe), transient and moderate hypothyroidism
		DUOXA2	Variable hypothyroidism (mild to severe)
		PDS	Moderate hypothyroidism and deafness;
		TG	Variable hypothyroidism (from moderate to severe)
		DHEAL1	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

Table 3. Genes involved in thyroid development: chromosomal localization and molecular features of the gene product

Gene	Chromosome		Features of the gene product
	Mouse	Human	
Titf1/Nkx2-1	12 C1-C3	14q13	Homeodomain transcription factor
Pax8	2	2q12-14	Paired domain transcription factor
Foxe1	4	9q22	Forkhead domain transcription factor
Hhex	19	10	Homeodomain transcription factor
Nkx2-5	17	5q34	Homeodomain transcription factor
Tshr	12	14q31	G protein coupled receptor

Table 4. Genetic basis of thyroid dysgenesis

Thyroid alteration	Genes	Clinical manifestations	References
Athyreosis	*PAX8* *FOXE1* *NKX2-5*	Hypothyroidism Bamforth-Lazarus syndrome Athyreosis, no cardiac alterations	(67) (128,129) (61)
Thyroid Ectopy	*NKX2-5* *FOXE1*	Ectopy, no cardiac alterations Bamforth-Lazarus syndrome	(61) (130)
Thyroid hypoplasia	*NKX2-1* *TSHR* *PAX8*	Choreoathetosis, hypothyroidism, and pulmonary alterations Resistance to TSH Hypothyroidism	(131-148) (7,149) (67-73)

Table 5. Gene causing defects in thyroid hormone synthesis

Gene	Protein function	Inheritance	Human phenotype
Sodiun-Iodide symporter (*NIS*)	Transports iodine across basal membrane	AR	CH (moderate to severe); Euthyroid goiter
Thyroperoxidase (*TPO*)	Catalyses the oxidation, organification, and coupling reactions	AR	Goiter and CH due to a total iodide organification defect
Dual oxidases (*DUOX1* and *DUOX2*)	H_2O_2 generation in the follicle	AD and AR	Permanent hypo (from mild to severe); Transient and moderate hypo
Dual oxidase maturation factor 2 (*DUOXA2*)	Required to express DUOX2 enzymatic activity	AR	Goiter and CH due to partial iodide organification defect
Pendrin (*PDS*)	Transport iodine across apical membrane	AR	Goiter, moderate hypothyroidism and deafness;
Thyroglobulin (*TG*)	Support for thyroid hormone synthesis	AD and AR	Goiter and CH (from moderate to severe)
Iodotyrosine deiodinase (*DHEAL1*)	Nitroreductase-related enzyme capable of deiodinating iodotyrosines	AR	Hypothyroidism with variable age of diagnosis