

## Molecular genetic defects in congenital hypothyroidism

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### Abstract

Recently molecular genetic defects in some cases of congenital hypothyroidism (CH) as well as of rare cases of central hypothyroidism have been identified. These studies have led to the description of so far unexplained forms of these disorders. In some patients with CH early diagnosis by newborn screening and early treatment was not able to lead to a normal mental development. This could subsequently be explained by molecular defects of transcription factors (FOXE-1/FKHL15, NKX2.1) which are important not only for the embryonic development of the thyroid gland but also for other organs including the central nervous system (CNS). These findings will help in understanding the critical role of thyroid hormones in the pre- and postnatal CNS development. However, many questions regarding the molecular defects and their consequences in the majority of patients with CH still remain open and will be addressed in this article.

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### Epidemiology of congenital hypothyroidism (CH)

Screening programmes report a rather constant incidence of permanent primary CH of 1:3000 to 1:4000 newborns with the exception of a decreased prevalence in the African-American and an increased prevalence in the Hispanic population (1). Girls are more frequently affected than boys (female to male ratios ranging from 2:1 to 4:1) (2, 3). Table 1 shows the classification and diagnosis of the different types of permanent CH.

The most common aetiology of primary CH is a spectrum of defective thyroid gland development (thyroid dysgenesis) including so called 'athyrosis' (without visible thyroid tissue in imaging studies), thyroid ectopy (frequently located in a sublingual position), hypoplasia (remnants of thyroid tissue in the normal position) and also hemithyroidoea. These forms represent 75–85% of all cases of permanent CH (3–5), while defects of thyroid hormone biosynthesis characterized by a normal sized or enlarged thyroid gland in the normal position are present in only 15–20% of the patients. A classification which is based solely on the results of imaging studies is in many instances not conclusive, because the available studies have limitations. For example, thyroid scintigraphy, which is based on the presence of functional thyroid tissue, fails to detect remnants of hypo- or non-functioning thyroid tissue. Therefore, the term 'apparent athyrosis' (6) has been created to describe patients with no visible thyroid tissue in thyroid scans but demonstration of a thyroid gland remnant in ultrasound studies as well as measurable serum L-thyroxine and thyroglobulin concentrations.

Therefore, the results of the serum measurements of thyroid hormones and thyroglobulin should be taken into account when a classification of CH is performed.

Reports on associated birth defects in patients with permanent CH vary in their frequencies ranging from a slight increase of 2.4% (7) in comparison with the normal population up to significantly increased frequencies of 20–25% (8, 9). More recent studies have shown that the increased risk of associated malformations, especially of congenital heart defects in patients with permanent CH, is limited to patients with thyroid dysgenesis (3). However, so far no patient with a known molecular defect of a transcription factor responsible for the embryonic development of the heart has been described who also suffered from severe CH. Therefore, it remains a challenge to identify the molecular cause in patients with thyroid dysgenesis and congenital heart defects. It is also not explained why female patients are more frequently affected by thyroid dysgenesis than males and it is discussed controversially if a female preponderance is present only in certain forms of dysgenesis (3, 6), suggesting different molecular causes. On the other hand, if a female preponderance is observed in all forms of thyroid dysgenesis, they might represent a spectrum of different degrees of severity of the same underlying molecular defect.

### Central hypothyroidism

Central hypothyroidism is estimated to occur in 1 in 50 000 newborns. The most prevalent cause of central hypothyroidism is a defective development of the

**Table 1** Epidemiology and diagnosis of the different types of CH.

Type of CH	Ultrasound appearance	Thyroid scan	Serum TSH	Serum T4	Serum TG
Thyroid dysgenesis					
Athyrosis	No thyroid tissue	No uptake	Increased	Undetectable to very low	Undetectable to very low
Ectopy	No thyroid tissue	Ectopic uptake	Increased	Low to low normal	Low to normal
Hypoplasia	Hypoplastic remnant	No uptake	Increased	Undetectable to low normal	Undetectable to normal
Defects of hormone biosynthesis	Normal or enlarged thyroid	No uptake or normal uptake; normal or enlarged volume	Increased	Undetectable to low normal	Undetectable to increased
Central hypothyroidism	Hypoplastic gland	Decreased uptake; decreased volume	Undetectable or low	Undetectable to low normal	Undetectable to normal

T4, thyroxine; TG, triglycerides.

hypothalamus or pituitary leading to multiple pituitary hormone deficiencies. It has been formerly suggested that an impaired mental development in patients with central hypothyroidism might be due to episodes of hypoglycaemia caused by adrenal insufficiency or growth hormone (GH) deficiency. In some patients with mutations of transcription factors involved in the development of the pituitary as well as of other structures of the nervous system, the loss of function of these transcription factors resulted in neurological symptoms independently of the lack of thyroid hormones or glucocorticoids. On the other hand, the identification of  $\beta$ -thyrotrophin (TSH) mutations in isolated central hypothyroidism has led to the new understanding that central hypothyroidism alone can lead to substantial and irreversible central nervous system (CNS) defects. However, the severity of clinical hypothyroidism varies in different genotypes and it remains to be elucidated why some mutations result in more severe defects than others.

### Inheritance of CH

CH in the era of newborn screening remains a sporadic disease and is not regarded as a hereditary disorder because familial occurrence is rare. Even in patients with putative autosomal recessive defects of thyroid hormone biosynthesis familial cases have been described rarely and systematic molecular genetic studies of candidate genes of thyroid hormone biosynthesis in patients with normally developed or enlarged thyroid glands are scarce. Therefore, no data on the epidemiology of the different defects of thyroid hormone biosynthesis or on the possible modes of inheritance are available so far.

Recent studies have described autosomal recessive inheritance (6) and familial dominant occurrence of thyroid dysgenesis (10). Moreover, an increased frequency of minor abnormalities of the development of the thyroid and pharyngeal derivatives has been described in first-degree relatives of patients with CH due to thyroid dysgenesis (11). But again epidemiological data on the prevalence of familial thyroid dysgenesis

are scarce, but it has become apparent that thyroid dysgenesis, at least in a subset of the patients (so far in less than 5%) is caused by molecular genetic defects, which in principle are inheritable. It is in the majority of patients, however, that a non-Mendelian genetic defect or somatic mutations have to be considered as possible mechanisms.

### Genetic defects in thyroid development

Studies of mouse models with targeted disruption of genes involved in the development of the thyroid gland have provided insight in the molecular mechanisms of organogenesis and thereby are the basis for molecular genetic studies in human patients affected by thyroid dysgenesis (12, 13). In mice, organogenesis and migration have been shown to be dependent on the normal expression and interplay of at least four different transcription factors: HEX, NKX2.1, PAX-8 and FOXE-1. Targeted mutagenesis of these transcription factors in mice furthermore has demonstrated associated developmental defects of other organs because none of these factors is exclusively expressed in the thyroid.

The PAX-8 gene belongs to a family of genes which is characterized by a highly conserved paired-box DNA binding domain, which encodes for proteins, with an important role in the entire embryonic development. PAX-8 is expressed in the thyroid primordium, the mid- and hindbrain region and in the developing kidney. Mice homozygous for disruption of the PAX-8 gene are characterized by severe hypothyroidism and a small hypoplastic thyroid remnant without follicular structures (14). If these mice are not rescued with thyroid hormone replacement they die shortly after birth, but although PAX-8 is expressed also in the kidney and the CNS no obvious abnormalities of these organs have been described in homozygous or heterozygous knock-out animals. Screening for mutations in the PAX-8 gene of patients with CH has led to the identification of several patients with heterozygous mutations which are inherited in a dominant fashion (15–17). Most patients do not present other developmental

defects, but in two unrelated male patients one hypoplastic kidney and one renal agenesis was observed. In the families of patients with CH due to PAX-8 mutations other carriers of heterozygous mutations had only mild adult-onset hypothyroidism or were euthyroid, indicating incomplete penetrance, which has been reported also in other disorders related to defects of PAX proteins (16). It remains to be elucidated if the presence of renal abnormalities in a few patients is just coincidental or can be explained by additional defects, e.g. in the PAX-2 gene, because PAX-8  $-/-$  mice present with renal abnormalities if they carry heterozygous or homozygous loss-of-function mutations of the PAX-2 gene (18).

FKHL15/FOXE-1 belongs to a family of transcription factors characterized by a forkhead DNA binding domain. In mice with homozygosity for targeted disruption of the FOXE-1 gene both thyroid agenesis and thyroid ectopy was identified, indicating that also in humans athyrosis and ectopy may be regarded as different degrees of severity of the same molecular defect. Furthermore, these mice have a cleft palate, which makes their feeding impossible and therefore early neonatal death is unavoidable (12). While screening of the FOXE-1 gene of patients with CH without associated problems failed to demonstrate any mutation (19), the study of two siblings with the so-called 'Bamforth syndrome' (20) including athyrosis and CH, developmental delay, cleft palate, choanal atresia, bifid epiglottis and spiky hair demonstrated homozygosity of a loss-of-function mutation of the FOXE-1 gene in both siblings (21). Up to now only three different families with mutations have been described due to the autosomal recessive inheritance of severe loss-of-function mutations (22). In other patients with cleft palate and CH, no mutations could be identified, therefore mutations, e.g. affecting FOXE-1 gene expression, might still be present in patients not presenting the complete syndromic picture.

The NKX2.1 (TTF-1, TITF-1 or T/ebp) gene encodes for a transcription factor of the homeobox domain containing genes of the NKX2 family. NKX2.1 is expressed in the thyroid, forebrain, basal ganglia, pituitary and the lung. Targeted disruption of both NKX2.1 alleles led to a complex phenotype of newborn mice (13). These mice die shortly after birth because of respiratory distress resulting from a defective lung development with insufficient surfactant production. While homozygous newborn mice were lacking any thyroid tissue at birth (athyrosis), heterozygous mice exhibited no abnormalities of thyroid development. In addition, in the homozygous mice the pituitary was completely absent and the development of the hypothalamic region was abnormal. Due to the early death of homozygous mice a study of hypothalamic-pituitary function or neurological testing could not be performed. The search for mutations in the NKX2.1 gene in patients with CH did not reveal abnormalities (23).

However, two studies were published on patients with deletions of chromosome 14q13 and 14q12-13.3 encompassing the NKX2.1 locus, who presented with mild hyperthyrotrophinaemia, neonatal respiratory distress and pulmonary problems as well as unexplained ataxia and muscular hypotonia (24, 25). However, in these reports it was suggested that the neurological problems observed in the patients might be due to the deletion of other genes located in the deleted chromosomal regions. These descriptions encouraged the investigation of the NKX2.1 gene of patients with CH, in whom the long-term outcome, despite an early onset of treatment and adequate dosage, was unfavourable due to pulmonary complications, severe muscular hypotonia and neurological symptoms, which were defined as choreoathetosis and in some patients due to mental retardation. So far in six of such patients heterozygous mutations of the NKX2.1 gene have been identified (26). The phenotype of thyroid and pulmonary manifestations covers a wide spectrum ranging from hyperthyrotrophinaemia, to severe CH due to thyroid agenesis, and severe neonatal respiratory distress syndrome requiring ventilation, to a slight increase in pulmonary infections, while choreoathetosis presents with less phenotypical variation. Recently, familial benign choreoathetosis without accompanying pulmonary or thyroid disorders has been attributed to NKX2.1 mutations as well (27). In respect of the severe phenotype of the NKX2.1 knock-out mice, homozygosity for NKX2.1 mutations in humans is probably not viable. The mechanism by which heterozygous mutations cause the phenotype is most likely haploinsufficiency. Although the heterozygous NKX2.1 mice previously have been reported to be unaffected, a more recent study describes abnormalities of thyroid function and neurological development in heterozygous mice (28). The phenotypical variation observed in patients with heterozygous NKX2.1 mutations can be due to modifier genes with a different expression in different genetic backgrounds. Thus a new syndrome of CH, pulmonary problems and the predominant symptom of choreoathetosis could be attributed to mutations of the NKX2.1 gene. These recent results have implications for the prognosis of patients with CH detected by newborn screening because the identification of defects of molecular mechanisms resulting in a defective development of the thyroid gland as well as of other organ systems will probably explain the less favourable outcome of some patients with CH.

### **Molecular defects in CH due to hypothalamic and pituitary defects**

Table 2 summarizes molecular mechanisms causing thyroid dysgenesis and Table 3 summarizes molecular mechanisms of central hypothyroidism.

Several defects of the hypothalamic-pituitary axis can be expected in patients with central hypothyroidism.

**Table 2** Molecular defects leading to thyroid dysgenesis.

	<b>PAX-8</b>	<b>FOXE-1</b>	<b>NKX2.1</b>
Protein family	Paired domain transcription factor	Forkhead domain transcription factor	Homeodomain transcription factor
Expression pattern	Thyroid; mid- and hindbrain; kidney	Thyroid; anterior pituitary	Thyroid; forebrain; pituitary; lung
Phenotype in knock-out mice	Thyroid hypoplasia; early death	Thyroid agenesis or thyroid hypoplasia; cleft palate; early death	Thyroid agenesis; pituitary aplasia; forebrain defects; disturbed lung development; neonatal death
Human thyroid phenotype	Thyroid hypoplasia; cystic rudiments; ectopy; severe to mild CH	Thyroid agenesis; severe CH	Thyroid agenesis; thyroid hypoplasia; normal thyroid; severe to moderate CH; hyperthyrotrophinemia
Manifestation in other human organs	Developmental defect of the kidneys	Cleft palate; bifid epiglottis; choanal atresia; spiky hair	Choreoathetosis; respiratory distress; pulmonary infections; mental retardation
Inheritance	Autosomal dominant	Autosomal recessive	Autosomal dominant

**Table 3** Molecular defects causing central hypothyroidism.

	<b><math>\beta</math>-TSH</b>	<b>POU1F1</b>	<b>Prop-1</b>	<b>LHX3</b>
Chromosome	1p13	3p11	5q	9q34
Inheritance	Autosomal recessive	Autosomal recessive or autosomal dominant	Autosomal recessive	Autosomal recessive
Pituitary hormone deficiency	TSH	TSH; GH; prolactin	TSH; GH; prolactin; gonadotrophin (corticotropin?)	TSH; GH; prolactin; gonadotrophin
Other manifestations	Mental retardation? (sensorineural deafness?)	Mental retardation?	Pituitary mass	Rigid cervical spine; limited head rotation; mental retardation? Pituitary mass

It can be assumed that loss-of-function mutations of several genes would lead to selective central hypothyroidism: (pre-pro)-thyrotrophin-releasing hormone (TRH), TRH receptor and  $\beta$ -TSH gene.

### **TRH and TRH receptor**

The human TRH gene has been mapped to chromosome 3q13.3-q21, but no human mutations have been described so far. It is possible that humans with a TRH defect probably will present with a rather complex phenotype due to the unpredictable symptoms of central TRH deficiency and deficiencies of other cleavage peptides of the pre-pro-TRH molecule. Targeted disruption of the TRH gene in mice led to an unexpected mild phenotype with central hypothyroidism and hyperglycaemia (29).

So far only one family with compound heterozygosity for loss-of-function mutations of the TRH receptor gene with central hypothyroidism has been described. The affected patient presented with severe CH, short stature and mental retardation, but initiation of treatment was delayed (30).

### **TSH**

CH caused by mutations in the TSH  $\beta$ -chain is rare. Loss-of-function mutations of the  $\beta$ -TSH gene present

with a variable phenotype. Some patients only have mild CH (31, 32), while others present with severe symptoms already in the neonatal period and are mentally retarded if thyroid hormone substitution is delayed. TSH is very low or immeasurable and cannot be stimulated by TRH. The most prevalent mutation identified in different populations, which results in severe CH, is a 1 bp deletion (T) from codon 105 (TGT) of the  $\beta$ -TSH gene, converting a cysteine to a valine residue (C105V) and yielding an additional eight amino acid non-homologous peptide extension on the mutant protein (33).

### **Developmental defects of the pituitary**

Defects of pituitary development result in various forms of impaired secretion of pituitary hormones. Usually the clinical picture is characterized by multiple hormone deficiencies including TSH; however, the time course of manifestations of the different hormone deficiencies is highly variable and therefore congenital hypothyroidism may be the first and leading symptom of a defective hypothalamic-pituitary development.

### **Pit-1**

Pit-1 (human homologue: POU1F1) is a transcription factor important for pituitary development and

pituitary hormone expression. Two animal models of Pit-1 defects, the Snell and Jackson mice, are characterized by deficiencies of GH, prolactin and TSH. Several reports on humans with loss-of-function mutations in the transcription factor Pit-1 have documented severe CH as the leading symptom in the newborn period with a delayed manifestation of GH deficiency (34, 35). The mode of inheritance is autosomal recessive if the mutation is located in the DNA binding domain, while other mutations with a dominant negative effect are inherited in an autosomal dominant manner.

### Prop-1

The expression of another pituitary specific transcription factor Prop-1 (Prophet of pit-1) identified in the Ames mice precedes the expression of Pit-1. Prop-1 deficiency results in impaired GH, prolactin and TSH but also gonadotrophin secretion. Autosomal recessive inheritance of loss-of-function mutations in patients with combined pituitary hormone deficiencies have been reported (36). However, the severity of the phenotype varies widely and the manifestation of the different hormone deficiencies follows an unpredictable time course. Severe CH as the leading symptom so far has not been described.

### LHX3

More recently, we were able to describe the first human mutations of the LHX3 gene encoding for a LIM homeodomain containing transcription factor involved in the development of the anterior pituitary (37). Previously, targeted disruption of the LHX3 gene in mice had resulted in a disturbed development of the anterior pituitary with conserved function of the corticotrophs. In these consanguineous pedigrees, the mutations were inherited homozygously. In contrast, in screening for mutations in sporadically occurring combined pituitary hormone deficiencies no abnormalities of the LHX3 gene were identified. Most importantly, as a peculiar finding, a short neck with limited ability to rotate the head was observed in all affected children but no abnormality of the spine could be visualized on X-ray or magnetic resonance imaging. In these patients CH was the first and leading symptom, which preceded the diagnosis of GH or gonadotrophin deficiency. Thus CH due to hypothalamic–pituitary defects can be caused by different molecular mechanisms. However, the symptoms can be as severe as in primary CH and a delayed treatment can result in irreversible mental retardation.

### Summary

These recent results have implications for the counselling of parents and patients with CH detected by newborn screening. With the rapid advancement of diagnostic tools for molecular genetic studies it should

**Table 4** Unsolved problems in the genetics of CH.

Which are the causes for the increased prevalence of thyroid dysgenesis in females?
Do the different types of CH represent different molecular defects or do they represent the phenotypical spectrum of the same defects?
Can the increased frequency of congenital heart defects in patients with congenital hypothyroidism be explained by mutations in a certain candidate gene?
Is there a genotype–phenotype correlation in patients with a mutation, e.g. the $\beta$ -TSH gene, causing central hypothyroidism?
Could epigenetic mechanisms or somatic mutations represent plausible explanations for the sporadic occurrence of CH caused by thyroid dysgenesis?

be possible in the near future to include the investigation of candidate genes in the diagnostic work-up to ensure a correct counselling of the parents and early focused support for affected patients. From the current knowledge it is a valid hypothesis that long-term consequences for some patients with CH despite early and adequate treatment might result from a common defect in thyroid and brain development rather than from fetal or perinatal hypothyroidism.

CH in the era of newborn screening so far has remained a sporadic disease and is in general not regarded as a hereditary disorder because familial occurrence has remained rare. It will be interesting to conduct systematic follow-up studies regarding the heredity of CH in the first generation detected by newborn screening, because they will presumably have a normal reproduction rate, while in the era before newborn screening late-diagnosed patients with severe CH had a significantly reduced reproduction. The prevalence of identifiable disease-causing mutations, however, is very low and, as mentioned before, many questions regarding the molecular pathogenesis remain unsolved.

Table 4 lists unsolved problems in the genetics of CH.

Since the majority of patients with CH due to thyroid dysgenesis are not affected by the syndromic types of CH caused by mutations in known transcription factors, still unidentified genes may be involved or a complex trait of inheritance has to be postulated for CH. Moreover, the presence of somatic mutations is another possible pathomechanism. Therefore, in general, CH due to thyroid dysgenesis can be considered as a hereditary disorder, but the mode of inheritance and the modifying genetic and environmental factors are far from being clarified. Further systematic studies are necessary to assess the prevalence of mutations in known candidate genes for thyroid organogenesis and hypothalamic–pituitary development in patients presenting with CH.

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