

Chapter 16b. Genetic Defects in Thyroid Hormone Supply

INTRODUCTION

The synthesis, storage and secretion of thyroid hormone require a sequence of precisely tuned reactions, in which a large number of proteins and factors are involved^[1]. Aberrations in any of these reactions may lead to aberrant thyroid development and/or metabolic defects in thyroid hormone synthesis. Thyroid metabolism is under close surveillance of the hypothalamus and pituitary gland and it is evident that disorders affecting the thyrotropic axis also belong to the panel of genetic thyroid diseases^[2]. The majority of patients with congenital thyroid diseases are mildly to severely hypothyroid, some have goiter without hormonal administration. Genetic aberrations resulting in hyperthyroidism are relatively rare. The absence of thyroid tissue leading to sporadic congenital hypothyroidism (CH) and the occurrence of endemic cretinism due to iodine deficiency were already described in the middle of the 19th century. The first descriptions of inherited defects causing hypothyroidism and goiter are from Pendred^[3] and Osler^[4] published more than a century ago. The major problems, both in CH and iodine deficiency, are life-long cognitive and motor problems as a consequence of impaired brain development. The extent of these features depends on the severity and duration of the hypothyroid state.

Table 1. DISORDERS OF THE THYROID SYSTEM CONSIDERED IN THIS SECTION

A.	<i>Central congenital hypothyroidism</i>		<i>Related Gene</i>
	1.	Disorders in development of the hypothalamus and/or pituitary gland	(LHX3, HESX1, PROP1, POU1F1)
	2.	Disorders in thyrotropic cell function	(TRH-R, TSH- β)
B.	<i>Thyroidal congenital hypothyroidism</i>		
	1.	Disorders in thyroid development	(NKX2.1, FOXE1, PAX8)
	2.	Disorders in thyroid hormone synthesis	
		- hyporesponsiveness to TSH	(TSH-R)
		-defects in iodide transport	(NIS, PDS)
		-defects in iodide organification	(TPO, THOX2)
		-defects in the processes involved in thyroglobulin synthesis	(TG)
		-defects in iodide recycling	

C.	<i>Thyroidal congenital hyperthyroidism</i>		
	1.	Disorders in thyroid hormone synthesis	
		-activating mutations in TSH signaling	(TSH-R, G _s α)

Since the initial descriptions of CH, our understanding of thyroidal inborn errors has grown tremendously. At the moment several genetic factors of thyroid and pituitary genesis have been elucidated and many players in thyroid hormonogenesis have been identified. With the clarification of the (almost) complete nucleotide sequence of the human genome^{[5] [6]} and the application of sensitive and rapid immunological and molecular biological techniques, our knowledge in this field is increasing rapidly^[7]. Genetic evaluation of aberrations in the ontogeny of the pituitary gland (Table 16-1A) and the thyroid (Table 16-1B) is accessible since mutated transcription factors have been identified in some patients with pituitary or thyroid malformation. On describing a disease, one has to bear in mind that genes do not operate on themselves. Each gene interacts either directly or through its protein product(s) with many other genes and gene products. This may result in marked variations in the symptoms of patients with the same disorder, even in monogenic diseases. In this chapter the spectrum of currently identified genetic defects in thyroid hormone synthesis is described and a general approach to the clinical and biochemical investigations of congenital disorders of pituitary gland and thyroid gland is presented. The clinical-pathological entities, and as far as known the molecular backgrounds, are listed in Table 16-2.

CONGENITAL HYPOTHYROIDISM: DIAGNOSTICS AND TREATMENT

CLINICAL MANIFESTATIONS

Untreated severe CH results in serious mental and motor handicaps, sometimes so outspoken that the patient is unable to function independently and needs institutionalization.

Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy demonstrating that maternal plasma thyroxine concentrations are important for optimal development of the fetal central nervous system^[8]. Because of the protective effects of a substantial maternal-fetal transfer of T₄^[9], the delay in cerebral development is for the greater part caused by lack of thyroid hormone after birth. The largest beneficial effect of T₄-supplementation is obtained when diagnosis is made and treatment is initiated immediately after birth. It is not clear whether the delayed prenatal brain development is completely remediable by early postnatal treatment.

The clinically detectable consequences of CH are strongly dependent on extent and duration of the hypothyroid period, but there is also a large inter-individual variability. During the first years of life only patients with severe CH show clinical manifestations such as an open posterior fontanelle, prolonged jaundice, feeding problems, enlarged tongue and muscular hypotonia. Milder forms of CH can remain unnoticed for years. In most CH patients the thyroid gland has not developed properly and hypothyroidism does not lead to goiter, the most characteristic sign of CH. The development of goiter is exclusively attributed to CH resulting from dys-hormonogenesis where persistent stimulation by increased plasma TSH levels leads to proliferation of the thyroid and nodule formation. In the first weeks after birth there is no appar-

ent correlation between the extent of the defect (represented by the plasma FT4 and TSH concentrations) and the degree of goitrogenesis. Rarely goiter causes airway obstruction. When T4 supplementation starts in the first weeks of life, and plasma TSH concentrations are kept within the lower half of the normal range, goiter development can be suppressed permanently.

NEONATAL SCREENING

In 1974 it became possible to measure T4 and TSH relatively easily and inexpensively in just a few drops of blood absorbed in filter paper. Many countries introduced mass neonatal screening procedures in order to detect CH as soon as possible after birth. Since the introduction of this screening the apparent overall incidence of CH has increased considerably, in fact it doubled, as a consequence of the detection of (very) mild or transiently expressed disorders that formerly either remained undetected, or were not recognized as congenital problems. Results from a number of international studies show that the incidence of permanent thyroïdal CH is approximately 1: 3,500 newborn children (in areas without iodine deficiency). There is considerable ethnic variation in incidence, ranging between the 1:30,000 among Afro-Americans in the United States^[10] and the 1:900 among Asian populations in the United Kingdom.^[11] It is noteworthy that with a few exceptions the international screening programs ignore patients with permanent CH due to pituitary and hypothalamic disorders, having an estimated incidence of roughly 1:20,000.^{[12] [13] [14] [15]} The reason that central hypothyroidism is not detected is explained by the almost worldwide decision to screen for elevated TSH levels, and not for decreased thyroxine concentrations, in order to avoid large numbers of false positives. However, high false positive rates can be avoided when screening is based on T4/TBG and TSH levels in the heel puncture blood spots with the lowest T4 values, as is done in the Netherlands. 14 15 Central CH includes various genetic defects, mostly resulting in multiple pituitary hormone deficiencies. (Table 16-2)

ETIOLOGIC CLASSIFICATION

With regard to the etiologic classification of CH, a clinical-pathological approach is followed. This is most appropriate for optimal patient care and aims to perform the diagnostic work up as efficient as possible and to realize optimal treatment as soon as possible after birth. This procedure also allows an adequate estimation of the risk of recurrence (taking into account the aspect of prenatal diagnosis and therapy) and the risk of other endocrine or non-endocrine complications. The final goal is to assign one particular etiology for every case of CH, but for each patient the clinical relevance of diagnostic evidence has to balance the burden of the necessary investigations. For obvious reasons only diagnostic tools adapted for the use in very young children are appropriate.

We have developed a set of diagnostic profiles for the currently known etiologies. 14 15 Each of the available determinants (plasma concentrations of FT4, TSH, Tg, urinary excretion of iodinated peptides, ultrasound and /or radioiodide imaging of thyroid tissue, and uptake studies with radioiodide and sodium perchlorate) have a low specificity, but by combining the test results it is usually possible to establish the proper clinical-pathological entity. Although DNA diagnostics, that can be performed in DNA isolated from white blood cells, has great advantages it is important to realize that the identification of a mutation in one of the genes involved in thyroid hormone synthesis does not automatically mean that this mutation is causally related to the disease. In case of large pedigrees with multiple affected subjects, cosegregation of the genotype with the CH phenotype may prove linkage. In other cases in vitro experiments are needed to establish that the gene mutation results in impaired production or activity of the protein in question. Moreover, the elucidation of diseases at the molecular level will be complicated if the disorders are multigenetically determined, as is suspected for thyroïdal or pituitary dysgenesis.

DIAGNOSIS

The great majority of patients with CH detected by neonatal screening have a thyroid malformation. Therefore, the first step in classification should be an imaging procedure, either by ultrasound or radioiodide. The presence of Tg in the circulation proves that at least some thyroid tissue exists. If the infant has a normally shaped and located thyroid gland, irrespective of its size, further studies with ^{123}I will provide information about the thyroidal uptake of iodide, the response to perchlorate, and the saliva/blood ratio of radioiodine. These aspects will give information about the clinical-pathological entity and will give direction to the molecular biological approach.^[16]

The measurement of the total urinary iodine excretion differentiates inborn errors from acquired, transient forms of hypothyroidism due to iodine deficiency or iodine excess. Because it is essential to treat the affected newborn infant without delay blood and urine samples must be obtained immediately after referral. In an infant with severe hypothyroidism, the radioiodide study can be done after T4 therapy is started, as long as the patient's plasma TSH concentration remains elevated. However, if the infant presents with slightly decreased free T4 plasma levels the ^{123}I uptake study should be performed either before the start of the treatment or several years later after interruption of T4 therapy for at least 4 weeks. Recently it has been reported that ^{123}I uptake studies can be performed during L-thyroxine treatment in adult CH patients after intramuscular injections with recombinant human TSH^[17].

A definite determination of the underlying cause depends on elucidation of the responsible mutation in the genetic code. When we consider the whole CH population, it is clear that still a large proportion of these patients wait for a molecular explanation, possibly even related to still unknown etiologies.

Patients with congenital hyperthyroidism are not detected by neonatal screening based on TSH determination or by screening based on low T4 levels. Newborns with overt signs of hyperthyroidism are mostly victims of maternal TSH receptor stimulating antibodies. This cause has to be excluded before taking genetic defects in the hypothalamic-pituitary-thyroid axis into consideration.

TREATMENT

Irrespective of the cause of CH, early treatment is mandatory to prevent cerebral damage. Initially, plasma FT4 concentrations have to be increased up till a level that TSH normalizes with minimal delay. In general, children with (thyroidal) CH need plasma FT4 concentrations around the upper limit of the age-dependent normal values in order to suppress plasma TSH well within the normal concentration range.^[18] They also persistently need higher plasma FT4 levels than children with acquired (thyroidal) hypothyroidism of the same age. These phenomena persist till adulthood. In most of the dyshormonogenic disorders the thyroid will eventually become hyperplastic and nodular if plasma TSH concentrations are even only slightly elevated for a longer period. However, when T4 therapy has been started promptly after birth and plasma TSH concentrations are maintained well within the normal range, goiter should not occur. The realization of plasma Tg levels within the normal range in patients who initially showed elevated plasma Tg-concentrations will reduce the risk of thyroid overgrowth. The optimal dose of T4 in patients with TSH deficiency must be based on determinations of the plasma (free) T4 concentration. In children with central CH the TSH secretion is completely suppressed from the very moment that plasma FT4 normalizes and remains suppressed in later years. Detectable TSH in patients with (permanent) central CH indicates under-treatment with T4. In exceptional cases, such as those with partial TSH hyporesponsiveness, hypothyroidism may be compensated completely and goitrogenesis will not occur. Newborns with congenital hyperthyroidism, caused by a mutated TSH receptor, have to be treated immediately with methimazole. In case of failure, and preferably at later age, the thyroid gland has to be kept inactive permanently, either by radioiodine treatment or by surgery.

Of course the patients must be kept euthyroid by administration of T4, as in children with CH.

Table 2. ETIOLOGIC CLASSIFICATION OF CONGENITAL HYPOTHYROIDISM IN NEONATES AND YOUNG CHILDREN

Clinical-pathological Entity	Plasma T4 Concentration	A. Plasma Thyroglobulin concentration B. Urinary Iodopeptide excretion	Ultra-sound and/ or Radioiodide Imaging of the Thyroid Gland	A. Thyroidal Radio-iodide uptake. B. NaClO4 Effect *	Miscellaneous Features	Gene(s) Involved (Mode of Inheritance)
<i>CENTRAL CONGENITAL HYPOTHYROIDISM **</i>						
<i>Hypothalamic/pituitary dysgenesis</i>	Low	A. Low B. NI	Normal to hypoplastic	NI	Decreased to mildly increased plasma TSH;MPHD; MRI: PPE; septo-optic dysplasia; pituitary hypoplasia	LHX3, HESX1, PROP1, POU1F1 (AR or AD): mutations only found in a small minority of this group of patients
<i>Hypothalamic/pituitary dysmorphogenesis</i>						
TRH receptor defect (TRH hyporesponsiveness)	Low	A. Low B. NI	Normal to hypoplastic	NI	Normal plasma TSH; no PRL-response after TRH	TRH-R (AR)
TSH deficiency	Low	A. Low B. NI	Normal to hypoplastic	NI	Decreased plasma TSH	TSH β (AR)
TSH hypoactivity	Low to normal	A. Low to normal B. NI	Unknown	A. Low to normal B. NI	Elevated plasma TSH in (radio-) immunoassays	TSH β , processing defect (AR)
<i>THYROIDAL CONGENITAL HYPOTHYROIDISM</i>						
<i>Thyroid dysgenesis</i>						

Clinical-pathological Entity	Plasma fT4 Concentration	A. Plasma Thyroglobulin concentration B. Urinary Iodopeptide excretion	Ultra-sound and/ or Radioiodide Imaging of the Thyroid Gland	A. Thyroidal Radio-iodide uptake. B. NaClO4 Effect *	Miscellaneous Features	Gene(s) Involved (Mode of Inheritance)
Thyroid agenesis	Absent ***	A. Absent B. Absent	Absent	A. Absent B. NI		NKX2-1 (syn: TTF-1), FOXE1, (syn:TTF-2; FKHL15), PAX8 (AR or AD): mutations only found in a minority of patients with thyroid dysgenesis
Cryptopic thyroid rudiment	Absent ***	A. Low to normal B. Absent	Absent	A. Absent B. NI		
Dystopic thyroid rudiment	Low to normal	A. Low to high B. Absent to low	(Sub-)lingual remnant	A. Low to normal B. NI		
Eutopic thyroid rudiment	Low to normal	A. Unknown B. Unknown	Hypoplastic eutopic remnant	A. Low to normal B. Unknown		
<i>Thyroid dysmorphogenesis</i>						
TSH receptor synthesis defect (TSH hyporesponsiveness)	Low to normal	A. Low to normal B. Unknown	Normal to hypoplastic	A. Low/ B. NI		TSH-R (AR)

Clinical-pathological Entity	Plasma FT4 Concentration	A. Plasma Thyroglobulin concentration B. Urinary Iodopeptide excretion	Ultra-sound and/ or Radioiodide Imaging of the Thyroid Gland	A. Thyroidal Radio-iodide uptake. B. NaClO4 Effect *	Miscellaneous Features	Gene(s) Involved (Mode of Inheritance)
Gsa synthesis defect (TSH hyporesponsiveness)	Normal to low	A. Low to normal B. Absent	Normal	A. Low B. NI	(P)PHP, AHO	GNAS (AD)
Total iodide transport defect	(Very) low	A. Very high B. Absent	Normal to hypoplastic	A. Absent B. NI	****	NIS (AR)
Pendrin synthesis defect (Pendred's syndrome)	Normal to low	A. Normal to high B. Absent	Normal to hypoplastic	A. Normal to high B. Partial release	Sen-sorineural deafness; vertigo in rare cases	PDS (AR)
Total iodide organification defect	Absent***	A. Very high B. Absent	Normal to hypoplastic	A. Rapid and high B. Total release		TPO (AR) THOX2(AR)
Partial iodide organification defect	Low to normal	A. (Very) high B. Absent to low	Normal to hypoplastic	A. High B. Partial release	Hypothyroidism seems transient in some cases	THOX2 (AD) TPO (AR)
Thyroglobulin synthesis defect	Low to normal	A. Absent to normal B. High	Normal to hypoplastic	A. Rapid and high B. Normal	Elevated plasma conc. of non-hormonal iodine	Tg (AR)
Iodide recycling defect (dehalogenase defect)	Low to normal	A. (Very) high B. Increased excretion of MIT & DIT	Normal to hypoplastic	A. High B. Normal	Hypothyroidism may not yet be manifest in the first weeks after birth	Possible candidate gene: DEHAL1.

Clinical-pathological Entity	Plasma T4 Concentration	A. Plasma Thyroglobulin concentration B. Urinary Iodopeptide excretion	Ultra-sound and/ or Radioiodide Imaging of the Thyroid Gland	A. Thyroidal Radio-iodide uptake. B. NaClO ₄ Effect *	Miscellaneous Features	Gene(s) Involved (Mode of Inheritance)
<p>Legend * In general, the radioiodide uptake is a function of the amount of thyroid tissue and the degree of stimulation by TSH. Na¹²³I (1 MBq or 27 mCi for infants younger than 1 year and 2 MBq or 54 mCi for older children) is administered i.v. and thyroidal uptake is monitored for 2 h; NaClO₄ (10 mg/kg body mass, max. 400 mg) is administered i.v. 2 h after Na¹²³I, and thyroidal radioiodide uptake is monitored for 1 h; NaClO₄ effect after 1 h: <10% decrease is normal; 10%-20% is borderline; >20% is abnormal. ** The most significant determinant for central congenital hypothyroidism is MRI of the cerebral midline structures; the basal plasma TSH concentration varies from decreased to mildly increased; the TSH response after i.v. administered TRH (10 mg/ kg body mass) may discriminate between defects of hypothalamic or pituitary origin. *** In case a fetus is unable to produce any T₄, maternal-fetal transfer is responsible for T₄ concentrations of 35-70 nmol/L or 2.7-5.4 mg/dL in cord plasma, which disappears with a half-life of 2.7-5.3 days. **** Most characteristic determinant for the diagnosis (total) iodide transport defect is the (very) low saliva/plasma ratio of radioiodide: for neonates >10 is normal, 3-10 is borderline, <3 is abnormal. The saliva/blood ratio is 1.17 times the saliva/plasma ratio (95% confidence interval, 1.15-1.19). Partial iodide transport defect is an ill-defined condition; if it exists, the diagnostic determinants entirely depend on the iodine intake. Abbrev.: CH, congenital hypothyroidism; MPHD, multiple pituitary hormone deficiency; PPE, posterior pituitary ectopy; (P)PHP, (pseudo-)pseudohypoparathyroidism; AHO, Albright's hereditary osteodystrophy; MIT, monoiodotyrosine; DIT, diiodotyrosine; AD, autosomal dominant; AR, autosomal recessive; NI, no indication for test.</p>						

GENETIC CLASSIFICATION OF CONGENITAL THYROID DISEASES

Reported mutations are accessible at the Human Gene Mutation Database at the Cardiff Institute of Medical Genetics. (<http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>)

A: CENTRAL CONGENITAL HYPOTHYROIDISM

Central CH might be caused by a dysfunctioning hypothalamus, pituitary gland or both. Knowledge about the ontogeny of the hypothalamus and the mechanism of TRH production and secretion is limited. Central CH may be caused by a distorted development of the thyroid regulatory system or a failure in TSH synthesis due to structural or regulatory gene defects. It has an estimated incidence of 1:20,000 births (0.005%) in the general population 13 14 15. Most patients with central CH are mildly to moderately hypothyroid due to insufficient secretion of biologically active TSH. (Table 16-2) On the other hand, the accompanying hormonal deficiencies, especially lack of cortisol, may be responsible for high morbidity and even mortality. About half of the children with central CH have a sporadic disorder characterized by multiple pituitary hormone deficiency (MPHD) and posterior pituitary ectopy (PPE) which is

the rudiment of a failed fusion between the invagination of the third cerebral ventricle and Rathke's pouch.

A1: Ontogeny of hypothalamus and pituitary gland

The pituitary gland develops from the fusion of two invaginations, one originating from the floor of the third ventricle and the other from the ceiling of the oral ectoderm (Rathke's pouch). In mice a number of homeodomain containing genes coding for *Lhx3*, *Lhx4*, *Hesx1* and *Ttf1*^[19] are essential for normal development of Rathke's pouch. For the differentiation of the progenitor cells into differentiated cell lineages, other transcription factors are required. For instance *Prop1* (human: *PROP1*), a paired-like homeobox gene, detected in the developing pituitary gland, and *Pit1* (human: *POU1F1*). The two transcription factors function sequentially in the same pathway to stimulate differentiation and proliferation of the thyrotrophic, lactotrophic and somatotrophic cells. When more genes involved in the development of the pituitary gland and hypothalamus become known, many exciting developments in ontogeny of these glands and the background of defects in these processes will be elucidated.

Defects in LHX3

The transcription factor *Lhx3* contains a DNA binding homeodomain and LIM domain that mediate protein/protein interaction and trans-activation functions. *Lhx3* is expressed in the embryonic rodent brain and spinal cord and later is restricted to the developing and adult pituitary gland. In *Lhx3* *-/-* knockout mice, four of the six anterior cell lineages - thyrotrophs, somatotrophs, gonadotrophs and lactotrophs - are specifically depleted, while corticotrophs fail to proliferate, which demonstrates that pituitary organ fate commitment is a function of *Lhx3*

The human homologue gene *LHX3* spans 7.2 kb and contains 7 exons, including two alternatively spliced first exons. The gene is located at chromosome 9q34.2-34.3. The alternative splicing results in two protein isoforms *LHX3a* and *LHX3b*.^[20]

Homozygous *LHX3* mutations have been found in two unrelated consanguineous families. The patients showed a combined complete deficit in all but one (the adrenocorticotropin) anterior pituitary hormones, retarded growth and a rigid cervical spine leading to limited head rotation. Both a homozygous missense mutation resulting in an amino acid change in the LIM domain (Y116C) and an intragenic deletion that results in a truncated protein lacking the DNA-binding homeodomain.^[21] The tyrosine missense mutation inhibits the ability of *LHX3* to induce transcription from selected target genes but does not prevent DNA binding and interaction of *LHX3* with partner proteins. Mutant *LHX3* missing a homeodomain does not bind DNA and is not able to induce transcription from pituitary genes.^[22]

Defects in HESX1

In mice, *Hesx1* is necessary for normal development of forebrain, eyes, and other anterior CNS structures, such as the hypothalamus, the pituitary gland and the olfactory bulbs. Defects in the gene coding for the homeobox gene *Hesx1* cause disorders that are comparable with septo-optic dysplasia in man. This entity is characterized by hypoplasia of the optic nerve, various types of forebrain defects and pituitary hormone deficiencies, including hypothyroidism.

A study in 38 patients with septo-optic dysplasia showed that 2 siblings from a consanguineous family, both with agenesis of the corpus callosum, optic nerve hypoplasia and panhypopituitarism, harbor a homozygous Arg53Cys missense mutation within the *HESX1* homeodomain, that destroys the ability of the protein to bind DNA.^[23] Heterozygous family members were phenotypically normal.

In 228 patients with a broad spectrum of congenital pituitary defects, ranging in severity from isolated growth hormone to septo-optic dysplasia with panhypopitu-

itarism three heterozygous HESX1 missense mutations were detected which display incomplete penetrance. The mutation Ser170 Leu is associated with GH deficiency in two brothers while only one of them has optic nerve hypoplasia. The Thr181Ala mutation is associated with isolated GH deficiency while the Gln6His occurs in a patient with multiple anterior pituitary hormone deficiencies.^[24]

A homozygous missense mutation that results in the single amino acid substitution Arg160Cys is associated with panhypopituitarism, ectopic/undescended posterior pituitary associated with a hypoplastic anterior lobe, abnormalities of the corpus callosum and septum pellucidum and optic nerve hypoplasia.^[25]

In a Japanese patient multiple pituitary hormone deficiencies, a hypoplastic anterior pituitary, ectopic posterior lobe and left optic nerve hypoplasia was ascribed to a de novo heterozygous frameshift mutation (306/307insAG) in exon 2 of the HESX1 gene. The frameshift introduces a premature stopcodon and the corresponding translation product lacks the DNA-binding domain.^[26]

Defects in PROP1

In mice Prop1 is a tissue specific paired like homeodomain transcription factor responsible for the initial determination of the pituitary Pit-1 dependent cell lineages (somatotropes, thyrotropes and lactotropes). Additionally it is necessary for the differentiation of the gonadotropes.

The human PROP1 gene is located on the distal end of chromosome 5q.^[27] Typically patients with homozygous or compound heterozygous inactivating mutations in the PROP1 gene have GH, TSH, PRL and LH-FSH deficiencies. Some cases with additional ACTH/cortisol deficiencies have been reported and it is evident that an impaired pituitary-adrenal axis does not exclude an underlying PROP1 defect, although the molecular explanation of the phenomenon is currently incomplete.

A 2-bp AG (A301G302del) deletion in the AG-rich region of PROP1 exon 2 appears to be a mutational hot spot. This frameshift mutation results in a premature stopcodon at amino residue 109 within the DNA binding domain. The phenotype observed in these patients is compatible with the complete loss of PROP1 activity. All patients (none received hormonal therapy) had extremely short stature and were sexually immature; their intelligence was normal probably reflecting the relatively mild hypothyroid state. Some patients developed partial cortisol deficiency.^{[28] [29] [30] [31] [32] [33] [34] [35]}

The mutation 149delGA results in the same Ser109X premature stopcodon³¹.

Homozygous missense mutations resulting in the single amino acid change Arg73His or Arg73 cys or compound heterozygous in combination with Arg99X have been reported in patients with combined GH, TSH and LH-FSH deficiency. A subset of this group showed delayed ACTH deficiency.³⁴ Additionally a few other sporadic mutations have been reported.^{[36] [37] [38] [39] [40] 28 31}

Defects in POU1F1

The protein POU1F1 (Pit-1 in mice) is a pituitary specific transcription factor that contains a POU specific and a POU homeo domain which are necessary for high affinity DNA-binding to the promoter of target genes. POU1F1 activates the expression of the GH, PRL and TSH β genes. As the expression of the TSH β subunit in the thyrotroph precedes that of POU1F1 in mice, it is unlikely that POU1F1 is a cell specific activator of TSH expression during development.^[41] From these considerations it might be suggested that this transcription factor is essential for the differentiation and proliferation of somatotropic, lactotropic and a subset of thyrotropic cells.^[42]

Both heterozygous, compound heterozygous and homozygous POU1F1 deletions have been linked to hereditary central hypothyroidism combined with GH and PRL deficiency. The phenotype varies, even between sibs affected by the same POU1F1

mutation^{[43] [44] [45] [46] [47] [48] [49] [50] [51] [52] [53] [54] [55]} As in all types of CH, brain development may be affected in patients with central CH due to POU1F1 inactivating mutations

Thyrotrope function

TSH is synthesized in the pituitary gland and is regulated by both TRH and the thyroid hormone negative feedback system. The transcription factor POU1F1, apart from its role in pituitary development, acts as the main stimulator for TSH synthesis. (see also the section on ontogeny of the pituitary gland).

Defects in TRH receptor

The TRH receptor belongs to the superfamily of G-protein-coupled receptors. It contains an extracellular N-terminal domain, seven transmembrane helices, 3 intra- and 3 extracellular loops and an intracellular C-terminal part.^{[56] [57]} The activated TRH receptor is coupled to the phospholipase C and phosphokinase C second messenger pathways mainly through regulatory G proteins. Central CH with complete absence of TSH and PRL responses to TRH has been described in a 9 year old boy.^[58] The boy showed mild symptoms of isolated central hypothyroidism. He was short of stature with markedly delayed bone maturation. The patient is compound heterozygous for two mutations in the 5' part of the gene. *In vitro* studies show that both mutations result in a TRH receptor protein unable to bind TRH. The parents and the eldest brother were heterozygous for the defect and phenotypically normal.

Defects in TSH β -subunit

TSH consists of two different (α and β) noncovalently linked subunits. Whereas the α -subunits of the glycoproteins LH, FSH and CG are identical, the β -subunits are unique for each of these hormones and carry specific information for receptor binding and hormonal action. The TSH β gene is located on human chromosome 1p22.^[59] For biological activity hetero-dimerization of the α and β is required.^[60] Studies on hCG predict that the C19 and C105 residues in the TSH β -subunit interact with the TSH α -subunit and are required to maintain the structural integrity of the TSH molecule that is essential for receptor binding and biological activity.^[61] TSH stimulates the function and growth of the thyroid gland via interaction with a specific plasma membrane receptor.

Both CH due to impaired TSH synthesis and affected biological activity have been reported.^[62] In general patients with congenital secondary hypothyroidism due to inactivating mutations in the thyrotropin-beta gene present with low TSH and thyroid hormone plasma levels in the presence of other pituitary glycoprotein hormones. Stimulation by TSH-releasing hormone will fail to stimulate serum TSH whereas prolactin does increase appropriately. Small deletions, missense, nonsense and splicing-affecting nucleotide substitutions have been described that either impair TSH production or result in the secretion of biologically inactive TSH fragments.^{[63] [64] [65] [66] [67] [68] [69] [70] [71] [72] [73]} The C105Vfs114X mutation due to a single base deletion in exon 3 of the TSH gene is relatively common and may be considered a mutational "hot spot".^{[74] [75]}

THYROIDAL CONGENITAL HYPOTHYROIDISM

Ontogeny of the Thyroid Gland

The thyroid gland is the first endocrine gland to appear during embryonic development. The gland develops from a median endodermal thickening in the floor of the primitive pharynx. This placode (median anlage) develops into a diverticulum that grows caudally. By seven weeks of gestation, the human thyroid gland has usually reached its final site in the neck. Experiments with knock-out mice show that the transcription factors NKX2.1, FOXE1 and PAX8 are crucial for thyroid development.^[76] Hypoplasia caused by inactivation of the TSH receptor is a later phenomenon.^[77]

Defects in NKX2.1

The transcription factor NKX2.1 (TTF-1) is a member of a protein family essential for developmental processes. The NKX2.1 gene is localized on chromosome 14q13 and is expressed in thyroid, lung and several structures of the forebrain. Mice missing the NKX2.1 gene are stillborn, lack the thyroid gland, the pituitary gland, lung parenchyma, and show extensive defects in brain development.^[78] Mutations in the NKX2.1-gene are not a frequent cause of CH but result in a syndrome combining a variable degree of congenital hypothyroidism, choreoathetosis, muscular hypotonia and pulmonary problems.^{[79] [80] [81] [82]} The unfavorable outcome of these patients probably does not reflect the hypothyroid state but is most likely due to impaired NKX2.1 expression in the central nervous system. In mice NKX2.1 haploinsufficiency results in hypothyroidism caused by the concomitant reduced expression of the TSH-receptor.^[83] Hypothyroidism can range from thyroid agenesis with severe hypothyroidism to a moderate hypoplastic gland with mild hypothyroidism to complete euthyroidism.

Defects in FOXE1

The transcription factor FOXE1, also referred to as FKHL15 or TITF2/TTF-2, is expressed in the thyroid gland, foregut endoderm, craniopharyngeal ectoderm (involved in palate formation) and in Rathke's pouch. Mice lacking the gene encoding FOXE1, (*Titf-2* - / -)^[84] die within 48 hours after birth, mostly because of the severe cleft palate. These mice do not have a normally localized thyroid gland. The pituitary gland responded adequately to the decreased FT4 plasma levels by elevating the plasma TSH concentrations.

The human gene is located on chromosome 9q22 and a homozygous FOXE1 missense mutation in the gene is associated with CH due to thyroid agenesis, cleft palate, hypoplastic bifid epiglottis and choanal atresia in two siblings.^[85] The resulting Ala65Val amino acid change is located within the forkhead domain of the protein and results in impaired DNA binding and loss of transcriptional function. A less severe biallelic mutation, resulting in the S57N substitution within the forkhead DNA binding domain, has been described in patients with an incomplete clinical phenotype (with athyreosis and cleft palate, but without choanal atresia or bifid epiglottis) suggesting partial preservation of FOXE1 function *in vivo*.^[86]

Defects in PAX8

The third transcription factor known to be essential for thyroid development is PAX8. PAX8 is expressed in thyroid and kidney and binds DNA via the conserved paired domain. Mice, in which the *Pax8* gene is knocked out (*Pax8* - / -)^[87] have a hypothyroid phenotype with a severely hypoplastic thyroid gland devoid of follicular structures. In addition to its role in thyroid development, PAX8 is involved in thyroid peroxidase (TPO) and TG gene expression.

The PAX8 gene maps to human chromosome 2q13-q14 (locus link zcgt q12-q14) and consists of at least 10 exons. All reported PAX8 inactivating mutations in man are monoallelic mutations and codon31 is considered a hot spot for recurrent mutations.^{[88], [89], [90], [91]}

The disorder has variable phenotypic expression, ranging from ectopy and hypoplasia of the thyroid associated with severe congenital hypothyroidism to eutopic thyroid tissue associated with mild hypothyroidism/ hyperthyrotropinemia. At the moment haploinsufficiency^[92] seems to be the most likely explanation why one mutated PAX8 allele in man leads to CH. This reduction in the amount of PAX8 protein could perturb the binding equilibrium with competitors or cofactors. In that case the phenotype would not only depend on the mutations in the PAX gene but also on mutations in competitor/cofactor genes, as demonstrated with Ref-1 nuclear protein^[93] a factor, controlling the DNA-binding capacity of PAX8. Also genomic imprinting or monoallelic expression may explain the pathogenic mechanism of PAX8 mutations. These findings are broadening the pathogenic mechanisms possibly involved in congenital hypothyroidism.

B2. Thyroid Hormone Synthesis

Responsiveness to TSH

TSH hypo-responsiveness results from lesions in the TSH stimulatory pathway. The TSH receptor molecule itself or proteins in the downstream signaling pathway, such as G proteins, adenylyl cyclase or the various kinases, may be involved.^[94] Activated Gs binds to and activates adenylyl cyclase, catalyzing ATP conversion to cAMP. Via an activated Gq protein, TSH activates a phospholipase C that specifically hydrolyzes PIP2 (phosphatidyl inositol 4,5 diphosphate), into IP3 and DAG (diacylglycerol). DAG stimulates specific protein kinases and IP3 causes release of Ca²⁺. Each intracellular signal molecule (cAMP, DAG, Ca²⁺) induces phosphorylation of specific proteins. Although the relation of phosphorylations to the physiologic effects of the hormone are still largely unknown, TSH exerts its effects via many metabolic steps in hormonogenesis, including iodide transport, iodination, endocytosis and lysosomal degradation of thyroglobulin, release and secretion of thyroid hormone.

Studies in a TSH receptor knock out mouse model demonstrate that TSH receptor expression is a prerequisite for expression of the sodium-iodide symporter, but is not essential for thyroglobulin expression. When normalized for body weight, thyroid size in the knock out mice model was 50% compared to control mice.^[95] In a different study, in the absence of either TSH or the TSH-R, a normal sized thyroid gland develops with normal expression of thyroglobulin, NKX2.1, FOXE1 and PAX8, whereas the expression of thyroidperoxidase and NIS are markedly reduced.^[96]

TSH binding to the TSH receptor (TSH R), located in the plasma membrane of the thyrocyte, leads to activation of thyroid metabolism via the Gs proteins. The TSH-R belongs to the superfamily of G-protein-coupled receptors. The human TSH-R maps to chromosome 14q31, comprises 10 exons, and contains a 1.8 kb coding sequence. The receptor contains an extracellular N-terminal domain with a repetitive Leu-rich motif, seven transmembrane helices, 3 intra- and 3 extracellular loops and an intracellular C-terminal part. 1 The activated TSH receptor is coupled to regulatory signal transducing proteins, Gs and Gq.

In 1995 compound heterozygous mutations in the TSH-R gene that result in amino acid changes in the extracellular domain of the TSH-R protein in a family with hypothyroidism compensated by high plasma TSH concentrations were reported for the first time.^[97] Many other de- or inactivating mutations have been published since then. They are listed on webpage <http://www.uni-leipzig.de/~innere/tsh/>. The diagnostic criteria for this type of disorder are CH and a eutopic non-enlarged thyroid gland. The impaired TSH signaling results in impaired thyroid hormone synthesis

that will be compensated by high TSH plasma concentrations. The high plasma TSH level does not result in exaggerated stimulation of thyroid metabolism and consequently goitrogenesis is not observed. Depending on the severity of the inactivating mutation hypothyroidism can range from mild to severe since the complete lack of TSH stimulation represses the metabolic activity of the thyroid gland.^[98]

A related type of TSH hyporesponsiveness is present in patients with pseudohypoparathyroidism type 1a (Albright's hereditary osteodystrophy),^[99] a variably expressed disorder with autosomal dominant inheritance. The disorder is caused by a defect in the expression of the α -subunit of the Gs protein. This protein functions in the cAMP second messenger system downstream of several transmembrane receptors, one of these is the TSH receptor. Several mutations have been reported^[100] and a list of known mutations is available online at <http://mammary.nih.gov/aho/>. Some patients tend to have mild manifestations of hypothyroidism with slightly decreased plasma FT4 levels. Detection of patients with pseudohypoparathyroidism type 1a by neonatal thyroid screening has been reported, but it is likely that most affected newborns will be missed, because their blood TSH and T4 concentrations will not reach the cut-off levels used in the screening programs. Otherwise, the mild hypothyroidism is just a minor component of this disease.

The cellular uptake of iodide from the extracellular fluid is the first step in thyroidal iodine metabolism. Iodide is imported against a chemical and electrical gradient. Under normal conditions the iodide uptake is rate limiting, followed by a rapid substitution reaction with tyrosine residues mainly in thyroglobulin (Tg), a process called iodide organification. Iodide organification occurs outside the cell at the apical border.^[101] Active iodide transport is not confined to the thyroid gland, it also occurs in the salivary glands, gastric mucosa, small intestinal mucosa, lacrimal gland, nasopharynx, thymus, skin, lung tissue, choroid plexus, ciliary body, uterus, lactating mammary tissue and placenta. In the thyroid gland iodide transport is regulated by TSH. Iodide transport over the plasma membrane is performed by a sodium iodide symporter [NIS].^[102] The efflux of iodide over the apical membrane is currently attributed to pendrin. In thyroid follicular cells, pendrin is inserted into the apical membrane and the protein is able to transport iodide when expressed in oocytes.^[103] [104] Another candidate is a putative human iodide transporter located at the apical membrane of thyrocytes that shares 70% similarity with human NIS.^[105]

NIS mRNA from rat^[106] and human^[107] were isolated in 1996. The cDNA was cloned, sequenced and expression in oocytes results in active iodide transport. The gene coding for NIS (SLC5A5) maps to human chromosome 19p13.2-p12 and has an open reading frame of 1929 nucleotides dispersed over 15 exons. The gene encodes a protein of 643 amino acids with an estimated molecular mass of 68,700. The current NIS secondary structure predicts 13 transmembrane segments. Four leucine residues in the transmembrane segment VI appear to comprise a conserved putative leucine zipper motif, which has been proposed to play a role in the oligomerization of subunits in the membrane.¹⁰²

For some time thyroidal iodide transport defects have been postulated for patients with goiters that could not be visualized with radio iodide. To date several cases with an autosomal recessive iodide transport defect characterized at the molecular level have been reported worldwide. Twenty seven cases from 13 families have been shown to have mutations in NIS. Nine mutations have been identified at the molecular level (for review see^[108]). Thyroid morphological findings are fairly heterogeneous in thyroids from patients with the same NIS mutation.¹⁰² In the neonatal period infants with an iodide transport defect have a normal-sized or somewhat enlarged thyroid gland by ultrasonography, elevated plasma Tg levels and no radio-iodide uptake.^[109] Measurement of saliva-to-plasma ¹²³I ratio is around unity. The degree of hypothyroidism is mild to severe, possibly dependent on the amount of iodide in the diet.

The structural change brought about by a NIS mutation has only been established for a few mutations, for instance in case of the recurrent T354P mutation. The substituted threonine lies in the midst of a well-conserved putative transmembrane seg-

ment probably essential for Na⁺ binding and translocation, a function performed together with the cluster of hydroxyl groups originating in the serine and threonine residues present in the 9th transmembrane helix.^[110] The presence of an uncharged amino acid residue with a small side chain at position 395 appears to be required for NIS function, suggesting that glycine 395 is located in a tightly packed region of NIS. Substitutions of large amino acid residues at position 395, as in the reported G395R mutation, probably hamper the Na⁺/I⁻ coupling reaction.^[111] Furthermore flow cytometry experiments suggest that the Q267E mutation impairs NIS trafficking.^[112]

The PDS (Pendred's syndrome) gene encompassing 21 exons, is located at human chromosome 7q31 and codes for a 5kb mRNA with an open reading frame of 2343 nucleotides encoding a 780 amino acid protein called pendrin.

Pendrin is located exclusively at the apical membrane of the follicular cell. It is a member of a large family of anion transporters, predicted to contain 11 or 12 transmembrane domains. It transports iodide and chloride in a Na⁺-independent fashion.^[113] Mutation analysis has been performed in a great number of families. Frameshift mutations, mutations leading to aberrant splicing processes and missense and nonsense mutations have been reported. The Leu236Pro and the Thr416 Pro are recurrent mutations.^[114] Val138Phe is a founder mutation in German families with Pendred's syndrome.^[115] In most cases, mutations in the PDS gene cause (subclinical) hypothyroidism and gradual goitrogenesis combined with hearing loss. Isolated hearing defects in the absence of thyroid dysfunction, such as enlarged vestibular aqueduct and non-syndromic autosomal recessive deafness, are also associated with PDS mutations.^{[116],[117],[118]}

Iodination and coupling

Iodide transported into the thyroid gland is rapidly oxidized by hydrogen peroxide (H₂O₂) and bound to tyrosine residues in Tg. Subsequently, iodinated tyrosine residues couple to form iodothyronine residues, mainly T₄.^[119] Both iodination and coupling are catalyzed extracellularly by thyroid peroxidase (TPO) and require H₂O₂ at the apical border of the thyrocyte.

The concentration of iodide in the thyroid gland reaches a steady state between active influx, protein binding and efflux, resulting in a relatively low intracellular iodide concentration under normal conditions. Iodide uptake is completely inhibited by anions of similar molecular size and charge, such as perchlorate or thiocyanate. On administration in sufficient concentration, iodide excess still present in the thyroid will be released into the circulation. The kinetics of iodide uptake and release can be traced by administration of ¹²³I when the thyroidal iodide concentration in relation to that in the circulation and the degree of iodine bound to protein can be determined. Partial iodide organification defects are characterized by release of more than 10 % of the accumulated (radio)iodine after administration of sodium perchlorate (the radioiodide is usually given 2 hours before sodium perchlorate.). Total iodide organification defects are characterized by release of more than 90% of the (radio)iodide taken up by the gland within 1 hour after intravenous administration of sodium perchlorate and a complete loss of the thyroid image on the scintiscan is observed. For details see the legend to table 16-2.

The human TPO gene is located on chromosome 2p25, spans about 150 kb and the coding sequence of 3048 bp is divided over 17 exons.^[120] Inactivating mutations in both TPO alleles have been found in patients with CH, due to a total iodide organification defect (TIOD). Up to date about 20 different mutations have been described. The majority of the mutations are found in exons 8, 9 or 10, that encode the active center and heme binding site of the enzyme. The GGCC insertion in exon 8 at nucleotide 1277, leading to an early termination signal in exon 9, is the most frequently occurring mutation.^[121] In one patient with classical TIOD in exon 14 a homozygous deletion of a T nucleotide at cDNA position 2512 (codon 808) was observed. The transmission pattern was anomalous and in this case homozygosity is due to partial maternal isodisomy of the short arm of chromosome 2, carrying the defective TPO gene.^[122] The

patient, born small for gestational age, appears healthy while being treated with thyroxine. He has a normal phenotype, except for a unilateral preauricular skin tag. In another family TIOD is due to the monoallelic expression in thyroid of a single paternal mutant TPO allele.^[123] Recently inactivating TPO mutations have been reported in three siblings with mild hypothyroidism due to a partial iodide organification defect (PIOD).^[124] The patients were compound heterozygous for a missense mutation (G1687T) and a deletion in exon 10 (1808-13del). The protein with the missense mutation showed retention of the defective TPO molecules and the deleted protein showed diminished enzyme activity. The resulting impaired thyroid hormone production caused latent hypothyroidism after birth and compensatory thyroid gland enlargement later in life.

Recently, the H_2O_2 generation system of the thyroid has been partially unraveled at the molecular level, corresponding to a dual system composed of thyroid oxidase I (THOX1) and 2 (THOX2). THOX1 and THOX2 are two similar proteins highly homologous to other members of the NADPH oxidase family.^[125] Both proteins contain 7 transmembrane-spanning domains, 3 NADPH and 1 FAD binding site and 2 EF-hand motifs. THOX2 is expressed at a higher level than THOX1 in the thyroid, as determined by Serial Analysis of Gene Expression (SAGE). The ThOX genes are located in close proximity at human chromosome 15q14. Both genes contain 33 coding exons. Eight patients with PIOD and one patient with TIOD that could not be explained by inactivating TPO mutations, were screened for mutations in the THOX1 and THOX2 genes.^[126] The TIOD patient is permanently and severely hypothyroid and is homozygous for a premature stopcodon in THOX2. The resulting truncated protein does not contain any functional domains. In 3 of the 8 PIOD patients who were transiently and mildly hypothyroid at birth, monoallelic inactivating mutations in the THOX2 gene were detected. These findings establish the crucial role of THOX2 in thyroidal H_2O_2 production.

Thyroglobulin synthesis

Thyroglobulin(Tg) synthesis occurs exclusively in the thyroid gland. The Tg gene (>300 kb) contains 8307 coding basepairs,^[127] divided over 48 exons.^[128] Excluding a signal peptide of 19 aminoacids, the polypeptide chain is composed of 2,750 amino acids. Two 330 kDa Tg subunits containing 10% carbohydrate residues form a dimer.^[129] To obtain a Tg molecule of intact tertiary and quaternary structure, extensive posttranslational processing has to take place. After entering the endoplasmic reticulum, Tg synthesis proceeds according to the general principles with proper carbohydrate attachment and folding, with the aid of chaperone molecules. In the Golgi complex carbohydrates are attached. Phosphorylation and sulfation have been described as part of the processing. Iodination and coupling of iodotyrosine residues are the last posttranslational processes. Of the 66 tyrosine residues present, specific tyrosine residues are involved in preferential iodination and thyroid hormone formation. Tyrosine residue 5 (acceptor) is the preferential site where thyroid hormone is formed after coupling with most likely the tyrosine (donor) residue at position 130. Other acceptor and donor residues have been described. The role of the acceptor and donor molecules are dependent on the thyroglobulin stereochemical structure and can be taken over by other tyrosine residues under special conditions.^[130] After formation of thyroid hormone residues in Tg, a process that occurs at the apical border of the cell, thyroid hormone is liberated from Tg through endocytosis and lysosomal proteolysis. Disorders in all these processes have to be integrated in the current description of the diagnosis "Tg synthesis defects". The term must not be restricted to defects in the coding, splicing or regulating part of the Tg gene itself. Up till the 1960's it was believed that leakage of Tg out of the thyroidal follicles was completely prevented by the tight junctions, connecting the thyrocytes. However with the introduction of radioimmunoassays, it became clear that besides thyroid hormone, small amounts of Tg and other (iodinated) proteins are also released by the thyroid into the circulation. Various mechanisms underlying the enhanced release of Tg and other

iodinated proteins under the influence of TSH or thyroid stimulating antibodies have been described.^[131]

Patients suspect of disorders in the synthesis of Tg are moderately to severely hypothyroid.¹⁴ The plasma Tg concentration is usually low, especially in relation to the TSH concentration, and does not increase upon intravenous injection of TSH.^[132] As an exception, a patient with a high plasma concentration of a Tg antigen of low molecular weight has been described.^[133] Patients classified in the category "Tg synthesis defects" often have abnormal circulating iodoproteins, mainly iodinated serum albumin^[134] and excrete iodopeptides of low molecular weight in the urine.^[135]

Several mutations in the Tg gene have been described for both animals and humans.^[136] Linkage analysis identifies the thyroglobulin gene region as a major locus for familial congenital hypothyroidism.^[137] Overall the amount of data is limited, probably largely due to the size of the coding region that has to be screened in search for mutations. Compound heterozygous mutations in the thyroglobulin gene are associated with fetal goitrous hypothyroidism.^[138] On the other hand, six patients with CH showing the clinical-pathological characteristics of a Tg synthesis defect did not show any major mutations in the Tg mRNA indicating that the clinical-pathological classification is not sufficiently accurate to point out a particular molecular defect.^[139]

Iodide recycling

Tg, internalized by (micro)pinocytosis from the follicular lumen, is present in early and late endosomes. In these proteolytic enzymes containing organelles thyroid hormone is liberated. The hydrolysate contains amino acids including monoiodotyrosine (MIT) and diiodotyrosine (DIT). MIT and DIT are subsequently deiodinated by specific dehalogenase(s).

Disorders in this deiodinating system lead to excessive renal loss of iodine, in the form of MIT and DIT, mimicking hypothyroidism due to iodine deficiency.^[140] ^[141] Patients with iodotyrosine dehalogenase deficiency show high to very high initial radioiodide uptake, followed by a relatively rapid decline of the radioiodine content not influenced by sodium perchlorate administration. Much of the radioiodine is found in the form of radiolabeled MIT and DIT. The wasting of tyrosine-bound iodine from the thyroid, enhanced by increased TSH secretion, may lead to extremely low thyroidal iodine content. Diagnosis can only be made based on the presence of iodotyrosines in the urine or enzyme activity if tissue is available. The inheritance of the dehalogenation defect most likely is autosomal recessive, although some features of the disorder are expressed in relatives that are expected to be heterozygous, for example, goiter, a relatively high radioiodide uptake and increased urinary DIT excretion. The clinical expression strongly depends on the iodine content of the diet, which might explain why autosomal dominant inheritance has been suggested^[142] in some families.

The molecular background for these defects has not been elucidated yet, but a candidate gene for thyroid dehalogenase has been reported recently. The gene encodes a protein with a conserved nitroreductase domain that is capable to dehalogenate iodotyrosines.^[143]

C: THYROIDAL CONGENITAL HYPERTHYROIDISM

Defects in the TSH receptor

Congenital hyperthyroidism not caused by transplacental passage of maternal anti-thyroid antibodies is very rare. Somatic^[144], germline^[145], and de novo mutations^[146] in the TSH receptor causing constitutive activation of the receptor or "gain of function",

resulting in toxic adenomas, non-autoimmune and congenital hyperthyroidism have been described. Several monoallelic activating mutations have been reported. They are listed on webpage <http://www.uni-leipzig.de/~innere/tsh/>. Infants with a 'de novo' mutation in the TSH receptor born with tachycardia, tachypnea and goiter, are difficult to treat. Treatment with carbimazole and thyroxine is not always effective, making removal of the goiter necessary. (for more information: Vassart section 16A).

Activating mutations in $Gs\alpha$

Defects in $Gs\alpha$

The most well-known disease due to activating mutations in the $Gs\alpha$ protein is McCune-Albright Syndrome. The affected tissues of these patients contain $Gs\alpha$ mutations, that prevent dephosphorylation of GTP, resulting in persistent stimulation of adenylyl cyclase. All manifestations of this congenital syndrome are caused by an activating $Gs\alpha$ mutation that develops early in the embryogenesis and the accompanying clinical spectrum depends on the site and time during development when the mutation occurs. The syndrome is characterized by scattered regions of hyperpigmented (café au lait) skin and polyostotic fibrous dysplasia. Constitutive adenylyl cyclase activity can affect endocrine tissues, leading to hyperthyroidism, gonadotropin-independent precocious puberty, Cushing syndrome, or acromegaly later in life.^[147]

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