

EUROPEAN THYROID ASSOCIATION HENNING PRIZE LECTURE, Goteborg, 10 September 2002

Primary congenital hypothyroidism: defects in iodine pathways

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Abstract

The thyroid gland is the only source of thyroid hormone production. Thyroid hormone is essential for growth and development, and is of special importance for the development of the central nervous system. It was for that reason that neonatal screening on congenital hypothyroidism was introduced and is now performed in many countries. Defects in thyroid hormone production are caused by several disorders in hormone synthesis and in the development of the thyroid gland (primary hypothyroidism) or of the pituitary gland and hypothalamus (central hypothyroidism).

This paper describes defects in the synthesis of thyroid hormone caused by disorders in the synthesis or iodination of thyroglobulin, leakage of iodinated proteins by a stimulated thyroid gland and the presence of abnormal iodoproteins, mainly iodinated albumin, in the thyroid gland and blood circulation. Circulating thyroglobulin and abnormal iodoproteins, as well as the breakdown products of these iodoproteins excreted in urine, are used for etiological diagnosis and classification. Moreover, our finding of an enzyme that catalyses the dehalogenation of iodotyrosines, which is important for iodine recycling and required for economical use of iodine, is also referred to.

European Journal of Endocrinology 149 247–256

Introduction

Thyroid hormone, exclusively produced by the thyroid gland, plays a key role in the growth and differentiation of many organs. It is of special importance for the development of the central nervous system in the pre- and postnatal period (1, 2). For thyroid development and for synthesis, storage and secretion of thyroid hormone a variety of transcription factors and a sequence of precisely tuned events (3) are required. Disorders in one of these factors or in the intermediate reaction steps are the molecular basis of abnormalities in thyroid development and/or in thyroid hormonogenesis that result in congenital hypothyroidism. Severe shortage of thyroid hormone after birth from several weeks on will result in serious mental and motor handicaps. The initial reports on the absence of thyroid tissue leading to sporadic cretinism and myxoedema, and the occurrence of endemic cretinism due to iodine deficiency are from the middle of the 19th century. The first descriptions of inborn errors in thyroid metabolism causing hypothyroidism with goitre are from Pendred (4) and Osler (5) at the end of the 19th century. Nevertheless, even in the middle of the twentieth century, many patients with congenital hypothyroidism were referred to paediatricians at school age (6).

Apart from the children with the classical type of congenital hypothyroidism, there are also special

groups of children who are in danger from a shortage of thyroid hormone after birth such as preterm born infants (7) and infants with Down's syndrome (8).

During pregnancy the mother provides substantial amounts of thyroid hormone to the foetus (9), so the delay in cerebral development caused by congenital hypothyroidism (CH) is mainly the result of postnatal thyroid hormone deficiency. The result of the maternal thyroid hormone provision to the foetus is that hypothyroid newborns do not show clear clinical signs at birth. The risk of mental retardation and the difficulty in recognising the disease were reasons for introducing neonatal mass screening programmes in many countries. The introduction was attended by a considerable increase in the apparent overall incidence of CH, most likely due to the recognition of mild disorders that formerly remained undetected, were detected later and/or were not recognised as congenital problems (10, 11).

Congenital hypothyroidism can be of thyroidal or of central (hypothalamic/pituitary) origin (Table 1), with a widely diverse molecular aetiology. Almost all screening programmes are directed towards the detection of thyroidal congenital hypothyroidism (incidence about 1:3100) (12) by measuring thyrotrophin (TSH) in filter blood spots, and they ignore central congenital hypothyroidism, which has an estimated incidence of about 1:20 000 births (12–14). In this

Table 1 Congenital hypothyroidism.**Thyroidal congenital hypothyroidism****Disorders in development of the thyroid gland**

- thyroid is absent
- under-development with migration failure
- under-development with normal migration

Disorders in thyroid hormone synthesis

- TSH hypo-responsiveness.
- defects in iodide transport from circulation into the thyroid cell
- defects in iodide transport from the thyroid cell to the follicular lumen, often combined with inner ear deafness (Pendred syndrome)
- defects in the synthesis of hydrogen peroxide
- defects in the oxidation of iodide, iodination and iodothyronine synthesis
- defects in processes involved in the synthesis or degradation of thyroglobulin
- defects in iodine recycling

Central congenital hypothyroidism**Disorders in development and/or function of the**

- hypothalamus
- pituitary gland
- both

paper, I mainly describe disorders in the mechanism of thyroid hormone synthesis taking place at the apical membrane of the thyroid gland.

Thyroid metabolism

Iodination takes place at the apical membrane (see oval in Fig. 1). The active transport of iodide into the follicular cells is mediated by the $\text{Na}^+ - \text{I}^-$ symporter (NIS) as the first crucial step in thyroid hormone

synthesis (for review see (15)). Iodide taken up by the thyrocytes is transported through the apical membrane by the anion transporter, pendrin (16). Recently, another apical iodide transporter protein (AIT) (17) has been described, which was identified as an NIS homologue.

The oxidation of iodide requires hydrogen peroxide that is synthesised outside the cell at the apical border catalysed by the thyroid-oxidase complex. Recently, two proteins of this complex ThOX1 and ThOX2 have been identified (18). The iodination of tyrosine residues, commonly referred to as iodide organification, is catalysed by the membrane-bound thyroperoxidase (TPO). Besides the binding of iodine to tyrosine, TPO also catalyses the coupling of iodotyrosines to iodothyronine residues in thyroglobulin (TG) under oxidative conditions. In order to deliver thyroid hormone to the blood circulation, TG is internalised by endocytosis that can take place by non-selective fluid phase uptake and by receptor-mediated processes. Two receptors playing a role in the endocytosis of TG have been identified: megalin (19) and the thyroid asialoglycoprotein (20) receptor. After endocytosis, thyroxine (T_4), triiodothyronine (T_3) and the remaining iodotyrosine residues in TG are released by lysosomal proteolysis. The iodothyronines are secreted into the circulation, non-covalently bound to plasma proteins and transported to target organs. The iodotyrosine molecules are deiodinated and the iodide can be reused as substrate for the iodinating processes in the thyroid.

In all these steps leading to the synthesis and release of thyroid hormone and the recycling of iodine, defects may occur (Table 2).

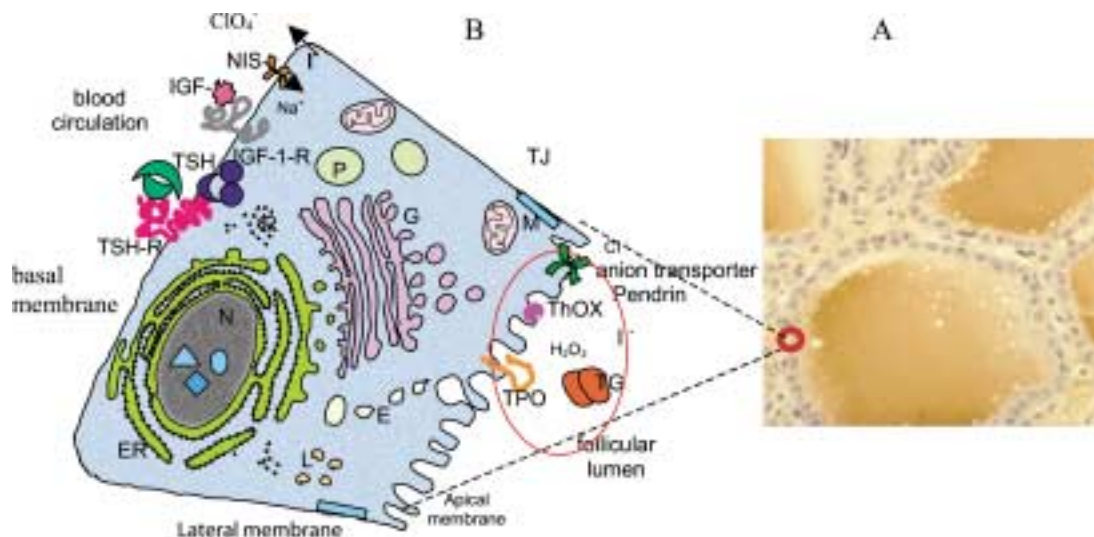


Figure 1 (A) Section of a normal thyroid gland stained with haematoxylin-eosin and immuno-stained with anti-thyroglobulin. (B) Schematic drawing of a thyroid cell with the main iodination pathways. Modified from Van de Graaf *et al.* (24). IGF-1, insulin-like growth factor I; IGF-1-R, IGF-1 receptor; TSH-R, thyrotrophin receptor; N, nucleus; M, mitochondria; L, lysosomes; E, endosomes; TJ, tight junction.

Table 2 Disorders in thyroid hormone synthesis.

Class of defect	Features	Plasma TSH conc.	Plasma T4 conc.	¹²³ I ⁻ uptake + wash-out****	Putative mol. defects
Thyroglobulin synthesis defects	Presence of iodoalbumin in plasma and/or iodopeptides in urine. Plasma TG* conc. Not related to TSH plasma conc.	Elevated	Low-normal	Elevated uptake. Partial wash-out possible. Rapid turnover dependent on amount of TG in follicular lumen	A: Defects in TG-synthesis pathway B: Mutations in TG gene
Iodide transport**	Through basal-lateral membrane. Saliva/blood ratio of iodide near unity Through apical membrane*** deafness	Elevated Normal or elevated	Low Low or normal	No uptake Partial wash-out	Sodium-iodide symporter (NIS) Pendrin (PDS gene) + apical iodide transporter (AIT) and/or possibly other transporters?
Iodide organification defect**	Partial** (PIOD) deafness Partial defect (PIOD) Total (TIOD)	Normal-elevated Normal-elevated Elevated	Low-normal Low-normal Low	Partial wash-out Partial wash-out Total wash-out	Thyroid oxidase (ThOX2), monoallelic mutation ThOX2 or TPO biallelic mutations
Iodine recycling defect	Presence of mono- and di-iodotyrosines in urine	Variable, dependent on iodine status	Variable, dependent on iodine status	High uptake, with rapid turnover	DEHALI

Conc., concentration; PIOD, partial organification defect; TIOD, total organification defect.

* In thyroid by dyshormonogenesis elevated plasma TSH concentrations, stimulating the thyroid gland, result in elevated plasma TG concentrations, except in the cases of TG synthesis defects and TSH receptor inactivation where elevated TSH concentrations do not give increased plasma TG concentrations.

** Pendred's syndrome is characterised by a combined thyroid defect and deafness.

*** Pendred's syndrome is probably caused by an iodide transport defect over the apical membrane, radio-iodine uptake studies reflect a partial organification defect.

**** ¹²³I taken up that remains as iodide in the thyroid gland will be released rapidly when the NIS transporter is blocked by e.g. perchlorate ions. When the release is between 10% and 90% or >90% in half an hour it is called a partial or total wash-out respectively.

Disorders in thyroid hormone synthesis

Thyroglobulin synthesis

In the process of thyroid hormone synthesis and storage, TG plays a prominent role. TG is encoded by a large gene, mapped to human chromosome 8q24, spanning more than 300 kb (21) containing 48 exons (22). The primary structure of human TG was deduced from the sequence of its 8448-base mRNA (23). The open reading frame for human TG consists of 8307 nucleotides coding for a protein of 2768 amino acid residues which has 66 tyrosine residues and a signal peptide of 19 amino acids (24). The TG sequence contains several repeats – in the N-terminal half eleven type-1 cysteine repeats were described (25). These repeats have been reported in other proteins and may play a role in the self-folding of proteins or may act as a proteinase inhibitor (26).

The structure of TG is important for optimal T₄ production. The protein-dimer (molecular mass 660 000) consists of two identical monomers containing 10% carbohydrates. Four to five tyrosine residues (acceptor residues) in the TG monomer, which can be preferentially iodinated (27), are considered to couple with other specific iodo-tyrosine residues (donor residues) to form iodothyronine. The sites in the TG molecule where iodothyronine residues are formed are the so-called hormonogenic sites. An important hormonogenic site is the N-terminal tyrosine residue (tyr 5; acceptor) that most likely couples with iodotyrosine 130 as donor. It can be expected that mutations causing small changes in structure already influence coupling efficiency and, as a consequence, thyroid hormone synthesis and release (28). Even amino acid changes, considered to be polymorphic, may influence the efficiency of thyroid hormone synthesis, changes that may become important under iodine deficient conditions.

Thyroglobulin synthesis defects

The characterization of TG synthesis defects is not easy to establish. The physiological characteristics leading to this classification are shown in Table 2. The concentrations of TSH and thyroid hormones as well as the iodine uptake studies are indicative but one of the parameters important for the diagnosis is the relatively low plasma TG concentration in relation to the plasma TSH level. In practice, the definition 'TG synthesis defect' is a clinical-physiological one. It does not cover defects in the gene coding for TG, but includes defects in the whole process of TG synthesis (29).

Thyroglobulin synthesis defects in animals Before studies with recombinant DNA and transgenic mice became possible, the availability of animals with inherited congenital hypothyroidism and goitre proved very

useful as models for the study of disorders in TG synthesis.

Five animals with TG synthesis defects have been described, and four are elucidated at the molecular level. The Afrikaner cattle (30) in which affected animals were borderline euthyroid required special care for normal growth, development and reproduction. The molecular defect appeared to be caused by a non-sense mutation (Arg697Stop) in exon 9 in the gene coding for TG. This mutation gives rise to a thyroglobulin fragment with a molecular mass of 75 000 plus an alternatively spliced TG monomer (molecular mass 250 000), missing exon 9 (31, 32).

The Australian Merino sheep (33) were severely hypothyroid and most lambs were either stillborn or died soon after birth. In the thyroid gland and blood circulation, abnormal iodinated proteins were observed, among which was iodinated albumin. In the thyroid gland no TG (molecular mass: 660 000) could be found. The defect inherited in an autosomal recessive way (34).

In the *cog/cog* mouse, congenital goitre is linked to the TG locus and is caused by a Leu2366Pro transition in the acetylcholinesterase domain of the TG molecule (35). This mutation resulted in an endoplasmic reticulum (ER) storage disease.

Another mutation in the TG molecule that inherited in an autosomal recessive way has been found in severely hypothyroid Rdw rats (plasma T₄ concentration: 15 nmol/l; normal: 64 nmol/l, TSH concentration: 9.8 ng/ml; normal: 1.1 ng/l). DNA sequencing revealed a Gly2320Arg mutation in the acetylcholinesterase-like domain. The molecular chaperones GRP94, GRP78 and calreticulin were accumulated in the thyroid gland of these animals. TG was not secreted into the follicular lumina, but was retained in the dilated ER (36).

The Dutch goats, a mixed strain of Saanen and dwarf goats, were severely hypothyroid. Almost all affected goats required thyroid hormone treatment or extra iodide administration to survive (37). The defect is inherited in an autosomal recessive way (38). The molecular defect in the TG gene was localised in exon 8 caused by a TAC(Tyr)/296/TAG(Stop) mutation (39). The mutation gives rise to a cys-rich N-terminal TG peptide fragment with a molecular mass of 35 kDa, and about 40 kDa when glycosylated, as in the *in vivo* situation. These TG fragments are transported to the follicular lumen, conjugated by disulphide bridges to high molecular weight complexes from which the tyrosine residues can be iodinated and coupled to thyroid hormone residues when more than the normal amount of iodine was administered, rendering the animals euthyroid (40, 41). For the neonates, it was very beneficial to give the mothers iodide (37, 42).

Thyroglobulin synthesis defects in man Five mutations in the TG gene have been described in

human pathology. A homozygous C886T transition in exon 7 results in the replacement of an Arg residue into a premature stop codon at amino acid position 277 in TG upon translation (43). Clinically, hypothyroidism, observed in three related patients, was not severe indicating that the short TG fragment was still able to produce thyroid hormone, a phenomenon that was also observed in the goat model and in *in vitro* experiments (44). In these experiments it was shown that the preferential coupling of iodinated tyrosine residues 5 and 130 was still present, but less pronounced than in intact TG.

In a Japanese family a deletion of 68 amino acids from the N-terminal part of thyroglobulin, due to an in-frame mutation of exon 4 was observed (45). The resulting TG molecule lacks the donor tyrosine residue 130 and a cys-containing repeated sequence characteristic for the first half of the molecule. Consequently, this patient was severely hypothyroid.

In a Brazilian family, two hypothyroid patients with large goitres were described. Investigation of these goitres revealed that in the TG gene a CGA(Arg)1510 TGA(stop) transition gave rise to a truncated TG polypeptide of 166 260 Da. Besides this mutation, an alternative splice product was present (46) that is also found in normal thyroid tissue, resulting in a TG lacking 57 amino acids.

Another family has been described in which the TG mRNA lacked 138 nt between position 5590 and 5727. The severely hypothyroid patients, homozygous for this deletion, presented multinodular goitres. Despite high TSH levels the serum TG level was below the detection level ($<1.0 \mu\text{g/l}$) and no increase could be observed after TSH stimulation (47). The disease in the affected patients was associated with massive induction of specific ER molecular chaperones including the hsp90 homologue, GRP94, and the hsp70 homologue, BiP. From thyroid tissues of the four patients, light microscopy demonstrated the presence of intracellular TG despite its absence in thyroid follicle lumina, while electron microscopy indicated abnormal distension of the endoplasmic reticulum. The data suggest that these patients synthesise a mutant thyroglobulin which is defective for folding/assembly, leading to a markedly reduced ability to export the protein from the ER to the follicular lumen (48). Thus, these kindreds suffer from a thyroid ER storage disease, a cell biological defect phenotypically indistinguishable from that found in *cog/cog* mice (35).

Patients containing a Cys1263Arg mutation (28) are described as having an arrest in the endoplasmic reticulum as well. These patients were mildly hypothyroid to euthyroid, indicating that in the follicular lumen sufficient TG had to be iodinated to deliver adequate amounts of thyroid hormone.

In Fig. 2, the locations of the various mutations in the TG protein molecule in man and animals are shown schematically.

From the localisation of the mutations, we get the impression that patients producing shorter TG fragments are less severely hypothyroid than the patients having mutations in the acetylcholinesterase domain of TG, indicating that shorter TG fragments are secreted more easily into the follicular lumen than TG molecules with more C-terminal mutations.

The patient where exon 4 was spliced out lacks a Cys-containing repeat and Tyr residue 130. This patient was also severely hypothyroid. Thyroid dysmorphogenesis might be caused because the Tyr130 donor residue was missing. Otherwise the lack of a Cys-containing repeat might cause misfolding of the protein resulting in accumulation of TG in the ER and proteolysis.

'Abnormal' iodinated proteins and breakdown products

It is known that besides TG other proteins such as albumin can be iodinated. Initial reports suggested that albumin was produced by the thyroid (49). In contrast with this, RT-PCR and labelling experiments showed that albumin in the thyroid gland originates from blood (50) and is iodinated. Some of the iodinated albumin rapidly leaks out, giving a high protein bound iodine value in the blood circulation (51). Some is broken down by lysosomal enzymes in the thyroid, giving rise to the formation of iodinated peptides and iodinated histidine which are excreted in urine, providing a new criterion in the diagnosis of TG synthesis defects, even prenatally (52).

Serum albumin may be taken up by the thyroid gland either by diffusion via the tight junctions or via basolateral endocytosis (53), and is iodinated in the follicular lumen. For the release of iodo-albumin into the circulation intra- or intercellular routes are postulated. The intracellular route follows the internalisation of iodinated albumin by pinocytosis and exocytosis at the basolateral membrane. The intercellular pathway is by leakage via the tight junctions.

The immuno-histological pictures show that the inflow and outflow of protein under these pathological conditions occur mainly intercellularly (Fig. 3) and is strongly TSH dependent. In accordance with these findings we have shown that the width and number of chains of the tight junctions are inversely related to the TSH level, most likely resulting in increased protein leakage at higher TSH levels (54) (Fig. 4A, B).

Iodide organification

The oxidation and binding of iodine to tyrosine residues in TG are commonly referred to as iodide organification. Under normal conditions, the iodide uptake is the rate-limiting step in the iodination process. Iodide in the thyroid is derived from two distinct sources: iodide transported into the cell via a transporter system (NIS) (15) and iodide obtained via deiodination of organic iodine compounds within the gland. How far both iodide pools are exchangeable is a subject of

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