REVIEW

Genetics of congenital hypothyroidism

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Congenital hypothyroidism is the most common neonatal metabolic disorder and results in severe neurodevelopmental impairment and infertility if untreated. Congenital hypothyroidism is usually sporadic but up to 2% of thyroid dysgenesis is familial, and congenital hypothyroidism caused by organification defects is often recessively inherited. The candidate genes associated with this genetically heterogeneous disorder form two main groups: those causing thyroid gland dysgenesis and those causing dyshormonogenesis. Genes associated with thyroid gland dysgenesis include the TSH receptor in non-syndromic congenital hypothyroidism, and $G_{\alpha s}$ and the thyroid transcription factors (TTF-1, TTF-2, and Pax-8), associated with different complex syndromes that include congenital hypothyroidism. Among those causing dyshormonogenesis, the thyroid peroxidase and thyroglobulin genes were initially described, and more recently *PDS* (Pendred syndrome), *NIS* (sodium iodide symporter), and *THOX2* (thyroid oxidase 2) gene defects. There is also early evidence for a third group of congenital hypothyroid conditions associated with iodothyronine transporter defects associated with severe neurological sequelae. This review focuses on the genetic aspects of primary congenital hypothyroidism.

In mammals the thyroid gland is the first glandular tissue to appear in development, arising from two regions of the endodermal pharynx which migrate and associate to become the characteristic bi-lobed structure.⁴ The median anlage, which eventually forms the follicular cells, arises from the midline of the anterior pharyngeal floor between the first and second branchial arches, being visible from days 16 to 17 of human gestation. At the same time two lateral anlagen (ultimobranchial bodies), which mainly become the parafollicular calcitonin secreting C cells but also contribute to a significant number of follicular cells,⁵ develop as caudal projections from the fourth or fifth pharyngeal pouches. The thyroid gland begins to trap iodide and therefore to secrete thyroid hormones only at 10 to 12 weeks of human gestation,⁴ with the trans-placental passage of maternal thyroid hormones before this being important for fetal development.

The essential role of thyroid hormones in central nervous system (CNS) maturation has been clearly demonstrated,⁶ and congenital hypothyroidism is eminently treatable by thyroxine replacement. Screening for neonatal hypothyroidism has therefore been established on a worldwide basis, beginning in Canada in 1974 and in the United Kingdom in 1982. The development of infants with congenital hypothyroidism has been revolutionised with the institution of early and adequate treatment afforded by screening, thereby preventing intellectual impairment and infertility. This is witnessed by the normal growth and neurological development in affected children, and by a dramatic increase in IQ, from an average of less than 80 to the mean of the general population.⁷

Most cases of congenital hypothyroidism occur sporadically. However, dyshormonogenetic cases are often recessively inherited, and recent cohort analyses estimate that approximately 2% of cases with thyroid dysgenesis are familial.⁸ It is also noteworthy that congenital hypothyroidism is associated with an increased incidence of birth defects, with surveys in the United Kingdom reporting a non-thyroidal congenital anomaly rate of 7% and an increased prevalence of chromosomal anomalies (1.5%).^{9–10}

The candidate genes associated with primary congenital hypothyroidism can be divided into

Congenital hypothyroidism is detected at a rate of 1 in 3000 to 4000 live births, making it the most common congenital endocrine disorder.¹ On a worldwide basis, hypothyroidism, including congenital forms, results most commonly from iodine deficiency. Otherwise congenital thyroid gland insufficiency results from developmental abnormalities at any level of the hypothalamic-pituitary-thyroid axis. Congenital hypothyroidism is most commonly caused by defects in thyroid development leading to thyroid dysgenesis (85%), which in turn consists of either thyroid agenesis (40% of all cases) or failure of the gland to descend normally during embryological development with or without ectopy (40%), or hypoplasia of a eutopic gland.² The remaining cases are associated with either a goitre or a normal thyroid gland.³ Rarely, central (secondary) hypothyroidism may be caused by pituitary or hypothalamic disease leading to deficiency of thyrotropin (TSH) or thyrotropin releasing hormone (TRH), respectively, which will not be covered by this review.

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Abbreviations: ERSD, endoplasmic reticulum storage disease; NIS, sodium iodide symporter; PHP, pseudohypoparathyroidism; PPHP, pseudopseudo-hypoparathyroidism; PTH, parathyroid hormone; TPO, thyroid peroxidase; TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone (thyrotropin)

mutation may contribute to thyroid dysfunction in that population, though this is very unlikely in the homozygous state.

There have also been reports of mildly raised TSH levels in individuals who are heterozygous for loss of function germline mutations in the *TSHR*.^{19 27 30–32} All have been reported to have normal sized eutopic thyroid glands on ultrasound scanning, except one who had thyroid hypoplasia.³² The clinical phenotype of these individuals is not readily distinguishable from those with compensated TSH resistance who are homozygous or compound heterozygous for mutations in the *TSHR* gene. Thus in families where non-autoimmune subclinical hypothyroidism appears to be inherited in an autosomal dominant manner, the possibility of a heterozygous mutation in the *TSHR* should be considered.

THYROID DYSGENESIS AND SYNDROMIC CONGENITAL HYPOTHYROIDISM

Evidence to date suggests that the development of the embryonic thyroid gland and its normal migration is dependent on the interplay between several transcription factors. In the thyroid gland, the target genes of these transcription factors include the thyroglobulin and thyroid peroxidase (TPO) genes. Transcription factors that have been studied in human congenital hypothyroidism include TTF-1, TTF-2, and the paired homeodomain factor Pax-8. In mice, the expression of TTF-1, TTF-2, and Pax-8 begins at the onset of thyroid migration on day 9.5 of gestation and these factors continue to be expressed throughout embryonic development.^{15 36 37} The onset of thyroid differentiation is heralded by the expression of *TSHR*, *TPO*, and *TG* on day 14.5 of mouse gestation.

TTF-2 is a transcription factor that is a member of the forkhead/winged helix domain protein family, many of which are key regulators of embryonic pattern formation and regional specification.³⁶ It is a phosphoprotein that consists of an N terminal region, a highly conserved forkhead domain, an α helical polyalanine tract, and unique C terminal residues.³⁸ Unlike some other transcription factors, such as HOX D13, polymorphism in the size of the polyalanine tract in TTF-2 has been shown not to affect its transcriptional function.³⁸ The human gene is located in chromosome 9q22 and consists of a single exon.³⁹ Animal studies have demonstrated the critical role of TTF-2 in thyroid embryonic development. Heterozygous *Ttf-2* knockout mice are euthyroid, with no visible phenotype, whereas homozygous null mice have cleft palate and thyroid dysgenesis, consisting of either thyroid agenesis or an ectopic sublingual gland, which is often lethal in the neonatal period.⁴⁰ In mouse embryos, *Ttf-2* is known to be expressed not only in the thyroid gland but also in the craniopharyngeal ectoderm involved in palate formation and in Rathke's pouch.³⁶ More recently, its expression has also been demonstrated in pharyngeal endoderm derivatives, such as tongue, palate, epiglottis, and oesophagus, and in choanae and whisker follicle in the mouse and in human thyroid, hair follicle, and prepubertal testis.^{41 42}

Two Welsh male siblings with a constellation of defects similar to those in the homozygous knockout mice (congenital hypothyroidism associated with thyroid agenesis and cleft palate)—with added features of spiky hair, bilateral choanal atresia, and hypoplastic bifid epiglottis⁴³—were investigated for defects in human *TITF-2* (also known as *FKHL15* or *FOXE1*) and found to be homozygous for a missense mutation (A65V) within its highly conserved forkhead DNA binding domain.³⁹ The mutant TTF-2 protein showed impaired DNA binding and loss of transcriptional function. This report represented the first description of a

genetic cause for thyroid agenesis in humans. Subsequently, two siblings were studied from an unrelated consanguineous family, with a milder phenotype consisting of thyroid agenesis, cleft palate, and spiky hair, but with absence of choanal atresia and bifid epiglottis (fig 2).⁴⁴ They were homozygous for a different missense mutation (S57N) in the forkhead domain of TTF-2, and the S57N mutant protein showed only partial loss of DNA binding and transcriptional activity in vitro, possibly explaining the incomplete clinical phenotype.

TTF-1 is a homeobox transcription factor of the NK-2 gene family which is related to *Drosophila* NK-2/vnd (ventral nervous system defective).⁴⁵ The homeobox gene superfamily encodes transcription regulatory proteins that act at critical points in development and ontogeny by sequence specific DNA binding, mediated by a structurally conserved homeodomain.⁴⁶ TTF-1 has two independent transcriptional activation domains located at the amino terminal (N domain) and the carboxy terminal (C domain) regions with respect to the DNA binding homeodomain.⁴⁷ The human locus is found on chromosome 14q13, and the gene (also known as *NKX-2.1*) comprises three exons encoding a 42 kDa protein.⁴⁸

Initial evidence of a role for TTF-1 (also known as thyroid specific enhancer binding protein, T/EBP) in the aetiology of congenital hypothyroidism also came from a report of a mouse model in which the *Ttf-1* gene had been disrupted by homologous recombination.⁴⁹ Heterozygous animals were initially described as having a normal euthyroid phenotype but more recently were found to have reduced motor coordination skills when compared with wild type mice.⁵⁰ Mice homozygous for the *Ttf-1* gene knockout were born dead, lacking lung parenchyma, with absent thyroid and entire pituitary glands, and with extensive defects in the ventral forebrain.

Not unexpectedly, TTF-1 is expressed in the lungs and ventral forebrain, in addition to the thyroid gland.⁵¹ It is known to regulate the transcription of *TG* and *TPO* genes, the *TSHR* gene in thyroid follicular cells,^{52 53} and the surfactant protein B (*SPB*) gene in epithelial lung cells.⁵⁴ A role for TTF-1 in human respiratory development has been borne out by reports of heterozygous de novo *TITF-1* deletions (14q13–21 and 14q12–13.3, encompassing the *TITF-1* locus) and a mutation (at the 3' splice consensus of intron 2) inherited in an autosomal dominant manner being associated with compensated congenital hypothyroidism and unexplained respiratory distress in term babies in the neonatal period who had normal bronchial morphology.^{55–57} The probands had normal sized eutopic thyroid glands on radionuclide and ultrasound scanning, and asymmetrical ^{99m}Tc uptake was noted in one report.⁵⁷ The other prominent recurrent features associated with either de novo or dominantly inherited *TITF-1* mutations (including one interstitial deletion in chromosome 14q) are neurological and include hypotonia, persistent ataxia and dysarthria, microcephaly, choreoathetosis, and global developmental delay, suggesting a role for TTF-1 in human brain development.^{57–59} Major feeding difficulties were also noted. It is thought that the unfavourable neurological outcome was most probably related to TTF-1 deficiency in the thyroid and brain rather than to inadequately corrected congenital hypothyroidism or inadequate thyroxine replacement in an affected mother during pregnancy. Further data published recently suggest that *TITF-1* mutations, leading to haploinsufficiency, are also associated with isolated benign hereditary chorea, an autosomal dominant movement disorder.⁵⁰

Pax-8 is a transcription factor that is one of the nine members of the mammalian paired homeodomain family which recognise DNA through the conserved paired domain and are homologous to the *Drosophila* segmentation genes.⁶⁰

Figure 2 Patients with *TTF-2* mutations, showing spiky hair, micrognathia, and hypertelorism (A), and cleft palate (B). (From: Castanet M, *et al.* A novel loss-of-function mutation in *TTF-2* is associated with congenital hypothyroidism, thyroid agenesis, and cleft palate. *Hum Mol Genet* 2002;11:20521–9; reproduced by permission of Oxford University Press.)

PAX genes perform key functions in mammalian embryonic development, during which they show highly restricted temporal and spatial expression patterns.⁶¹ Pax-8 has a DNA binding domain at the amino terminal end, a carboxy terminal transcriptional activation domain, and a central homeodomain.⁶² The *PAX-8* gene maps to human chromosome 2q12-q14 and consists of 11 exons.⁶³ Studies have shown that Pax-8 plays a fundamental role not only in the initiation of thyroid cell differentiation but also in the maintenance of the differentiated state, and that it is essential for thyroid cell proliferation.⁶⁴ Once again, mouse models have been instrumental in demonstrating the critical role of Pax-8 in thyroid organogenesis and thyroid cell differentiation. *Pax-8* null mice have hypoplastic thyroid glands with absent median anlage derivatives (that is, follicular cells), whereas lateral anlagen derivatives (parafollicular calcitonin producing C cells) are present.⁶⁵

The survival of homozygous mutant mice depends on thyroxine replacement therapy, whereas heterozygous mice do not display an obvious thyroid phenotype. In contrast, heterozygous human *PAX-8* mutations have been described in patients with congenital hypothyroidism of varying severity in six families: four familial cases where congenital hypothyroidism appears to be inherited in an autosomal dominant manner and two sporadic cases.^{66–69} The thyroid glands in these patients are hypoplastic and sometimes ectopic in location. In one family, the thyroid gland was of normal size at birth but failed to develop normally postnatally, becoming hypoplastic.⁶⁹ In this family, the probands also showed decreased iodide trapping, with a positive perchlorate discharge test in one, initially suggestive of dyhormonogenesis as a basis for congenital hypothyroidism.

TPO is known to be particularly dependent on Pax-8 for efficient transcription, and therefore reduced *TPO* expression secondary to impaired Pax-8 function may be an explanation for the partial organification defect.⁶⁴ Renal hemiagenesis was also reported in two affected cases, one being associated with hypercalciuria.^{39–69} When present, renal agenesis was ipsilateral to thyroid hemiagenesis.⁵⁹ Thus congenital hypothyroidism caused by *PAX-8* mutations can occur as

non-syndromic or syndromic congenital hypothyroidism. Outside the thyroid gland, Pax-8 is also expressed in the kidney, where it is known to activate the Wilms' tumour gene (*WT1*) promoter, and in the developing brain.⁷⁰ Marked phenotypic variability has been found within affected families, suggesting varied penetrance and expressivity of *PAX-8* gene defects.

All mutations to date have been located in the conserved paired domain of *PAX-8*, and the mutant proteins have been shown to have markedly reduced DNA binding with subsequent loss of transcriptional activation function. At the structural level, these mutations are thought to disrupt the pronounced gain of α helical content following interaction of Pax-8 with DNA—that is, impairment of the unstructured to structured transition that occurs during DNA recognition (loss of “induced fit”).⁶⁸ Pax-8 activates transcription of *TPO*, *TG*, and *NIS*, and acts synergistically with TTF-1 to activate the promoter of human *TG* gene.^{70–72} Dominant negative properties of the mutant allele or haploinsufficiency are not considered likely underlying mechanisms for pathogenicity of Pax-8 mutations. The exact mechanism is still unclear but one possibility is that the human *PAX-8* locus is imprinted, with selective expression of the mutant allele leading to disease.

The stimulatory G protein α subunit gene (*GNAS1*) is located on chromosome 20q13 and contains over 13 exons that encode $G_s\alpha$, the α subunit of the heterotrimeric stimulatory G protein which has intrinsic GTPase activity.⁷³ G proteins mediate signal transduction across cell membranes, coupling extracellular receptors—including those binding TSH, TRH, parathyroid hormone (PTH), and luteinising hormone—to intracellular effector proteins such as ion channels and the adenylyl cyclase and phospholipase C second messenger systems.⁷⁴ Heterozygous inactivating mutations in *GNAS1* result in Albright hereditary osteodystrophy. This is an autosomal dominant disorder characterised by recognisable dysmorphic features including short stature, shortened fourth and fifth metacarpals and metatarsals, obesity, subcutaneous ossification, intracranial calcification, and variable mental retardation. Presumably owing to imprinting mechanisms there is an apparent parent of

origin effect whereby maternal transmission usually leads to a pseudohypoparathyroidism type Ia (PHP1a) phenotype, while mutations of paternal origin result in pseudopseudohypoparathyroidism (PPHP).⁷⁵ PHP1a consists of end organ resistance to multiple hormones including PTH and TSH, leading to low serum calcium and raised levels of phosphate, PTH, and TSH, whereas PPHP patients have Albright hereditary osteodystrophy with a normal biochemical profile. The degree of TSH resistance tends to be mild, and overt clinical hypothyroidism is not always present. The TSH response to TRH is exaggerated and the degree of TSH resistance may increase with age.⁷⁶

DYSHORMONOGENESIS AND CONGENITAL HYPOTHYROIDISM

The end product of a normally developed hypothalamic-pituitary-thyroid axis is the production of thyroid hormones. Iodide is actively transported and concentrated in the thyroid gland by the sodium iodide symporter present in the basolateral membrane of thyroid follicular cells (fig 3). Subsequently it is oxidised by hydrogen peroxide and bound to tyrosine residues in thyroglobulin to form iodotyrosine (iodide organification). Some of these hormonally inert iodotyrosine residues (monoiodotyrosine and diiodotyrosine) couple to form the hormonally active iodothyronines, T_4 and T_3 . Thyroid peroxidase (TPO) catalyses the oxidation, organification, and coupling reactions. Defects in any of these steps lead to dyshormonogenesis which typically manifests as congenital hypothyroidism and goitre,⁷⁷ and the responsible gene at each step has been cloned. Except in rare cases, all mutations in these genes appear to be inherited in an autosomal recessive fashion.

Figure 3 Schematic diagram of a follicular cell, illustrating the steps involved in thyroid hormone synthesis. TSH receptor (TSHR) bound to TSH stimulates iodide transport into the thyroid gland by the sodium iodide symporter (NIS). Subsequently, iodide is oxidised by hydrogen peroxide, generated by the recently discovered NADPH oxidase system (ThOX) and bound to tyrosine residues in thyroglobulin (TG) to form iodotyrosine (iodide organification). Some of these hormonally inactive iodotyrosine residues (monoiodotyrosine and diiodotyrosine) couple to form the hormonally active iodothyronines, T_4 and T_3 . Thyroid peroxidase (TPO) catalyses the oxidation, organification, and coupling reactions. The exact function of pendrin, a chloride-iodide transporter, in thyroid hormone synthesis is as yet unknown but it is thought to transport iodide into the colloid from the thyrocyte. Defects in any of these steps lead to dyshormonogenesis, which manifests clinically as congenital hypothyroidism with goitre.

TPO, the enzyme responsible for iodide oxidation, organification, and iodotyrosine coupling, is a 933 amino acid, membrane bound, glycosylated, haem containing protein, located on the apical membranes of the thyroid follicular cell.⁷⁷ The human *TPO* gene is located on chromosome 2p25 and covers approximately 150 kb of DNA.⁷⁸ The most prevalent cause of dyshormonogenesis is TPO deficiency.⁷⁹ Defects in the *TPO* gene have been reported to cause congenital hypothyroidism by a total iodide organification defect.⁸⁰ Increasing numbers of mutations—including maternal isodisomy for chromosome 2p—have been reported, occurring throughout the 17 exons of the thyroid peroxidase gene, each resulting in an inactive TPO protein.^{80–91} There is one report of metastatic thyroid carcinoma arising within a congenital goitre associated with a mutation in the *TPO* gene.⁸⁵

Thyroglobulin is a homodimer with subunits of 330 000 Da, which is synthesised exclusively in the thyroid gland. The human gene is greater than 300 000 bp in size and is located on chromosome 8q24.⁹² The coding sequence is divided into 42 exons, each of about 200 bp in size, except that exon 9 and 10 contain 1101 p and 588 bp, respectively.⁷⁷ Thyroglobulin defects arising from mutations in the gene are associated with moderate to severe congenital hypothyroidism, usually with low serum thyroglobulin concentrations.⁹³ Affected individuals often have abnormal iodoproteins in their serum, especially iodinated albumin, and they excrete iodopeptides of low molecular weight in the urine.⁷⁷ There is ineffective formation of T_4 and T_3 resulting from a coupling defect. Owing to its size (coding sequence of 8244 bp), sequencing of the *TG* gene has been difficult. However, increasing numbers of mutations are being reported.^{94–95} Thyroglobulin is normally exported through the secretory pathway into the lumen of thyroid follicles to undergo iodination. Probands with deficient thyroglobulin resulting from an altered *TG* coding sequence have shown defective trafficking of thyroglobulin from the endoplasmic reticulum to Golgi, leading to an endoplasmic reticulum storage disease (ERSD).⁹⁶ A particular feature of some *TG* mutations involving cysteine residues in the protein is disruption of the three dimensional structure of the molecule, causing it to be retained in the endoplasmic reticulum as aggregates.⁹⁶ The *cog/cog* mouse with a point mutation in the *tg* gene, in a region which is strictly conserved in the thyroglobulin proteins from all known species, is an example of an ERSD and a model for severe congenital hypothyroidism associated with colloid deficient goitre and abnormal growth and central nervous system development.⁹⁷ These mice have aberrant folding, dimerisation, and export of thyroglobulin, leading to an abnormally distended endoplasmic reticulum, comparable to that observed in thyroid biopsies from children with congenital goitre from defective thyroglobulin synthesis.^{98–99}

NIS is an integral membrane protein of approximately 65 kDa with 12 potential transmembrane domains,¹⁰⁰ with both carboxy and amino termini located inside the cell. Its expression has not only been detected in normal and neoplastic thyroid but also in salivary glands, gastric mucosa, breast, colon, ovaries (lower species), placenta, skin, and choroid plexus.¹⁰¹ The human gene (*NIS*) has been mapped to chromosome 19p, and the coding region contains 15 exons encoding a protein of 643 amino acids.¹⁰¹ Before the cloning of *NIS*, a clinical diagnosis of hereditary iodide transport defect had been made for several decades on the basis of goitrous hypothyroidism and absent thyroidal radioiodine uptake.¹⁰² The first demonstration of a loss of function mutation in the *NIS* gene was reported in 1997, following which several mutations inherited in an autosomal recessive manner have been described.¹⁰³ The hypothyroidism is of variable severity (ranging from fully compensated to severe) and goitre is not always present. Individuals with a higher

dietary iodine intake are less likely to have severe hypothyroidism than those with iodine deficient diets.¹⁰⁴ This condition is therefore preferably treated with iodine supplementation rather than thyroid hormone replacement. People who are heterozygous for a mutation are euthyroid.¹⁰³ The loss of function associated with some of these mutations (Q267E and S515X) was accounted for by failure of membrane targeting by the transporter.¹⁰⁵

Pendred syndrome is an autosomal recessive disorder characterised by sensorineural deafness and goitre that was first described by Vaughn Pendred in 1896. The incidence of the disease is estimated to be 7.5 to 10 in 100 000, and it is thought to account for as many as 10% of cases of hereditary deafness, making it the most common cause of syndromic deafness.¹⁰⁶ Deafness is classically associated with a Mondini cochlear defect, consisting of a reduced number of turns in the cochlea, together with an enlarged vestibular aqueduct; these features are typically present at birth.^{107–108} Thyroid disease usually presents as a multinodular or diffuse goitre of varying size in most affected individuals, and is typically not evident until the second decade of life. Despite the goitre, individuals are likely to be euthyroid and only rarely present with congenital hypothyroidism.¹⁰⁹ TSH levels, however, are often in the upper end of the normal range, and hypothyroidism of variable severity may eventually develop.¹⁰⁶ Intrafamilial phenotypic variation has also been noted.¹¹⁰ Affected subjects usually have a positive perchlorate discharge test, with more than 15%—but not complete—release of radiolabelled iodine following perchlorate administration, indicating a mild thyroid organification defect.¹⁰⁶ A normal discharge test, on the other hand, does not exclude the diagnosis.¹¹⁰ The *PDS* gene is on chromosome 7q, contains 21 exons, and is found to be expressed in the cochlea as well as in the thyroid.¹¹¹ It encodes pendrin, a 780 amino acid protein (molecular weight 86 kDa) with 11 transmembrane domains which functions as a chloride-iodide transporter.¹¹² In contrast to the basolateral cell surface expression of NIS, pendrin has been localised to the apical membrane of a subset of thyroid follicles.¹¹² It has been hypothesised that pendrin transports iodide across the apical membrane of the thyrocyte into the colloid space and that this process is disrupted in Pendred syndrome, such that iodide is taken up normally by the thyrocyte but is not efficiently bound to thyroglobulin in the colloid.¹¹³

NADPH oxidases—encoded by two recently cloned genes, *THOX1* and *THOX2*, located at the apical membrane of thyrocytes—are involved in H₂O₂ generation in the thyroid.¹¹⁴ Both genes are highly expressed in thyrocytes.¹¹⁴ Thyroid peroxidase (TPO) has no biological activity in the absence of H₂O₂, which is likely to be the limiting factor for thyroglobulin iodination when iodide supply is normal.¹¹⁵ The two human *THOX* genes are arranged in a head to head configuration and are separated by a 16 kb long region.¹¹⁶ They span 75 kb and are composed of 35 and 34 exons, respectively. How *THOX* participates in Ca²⁺/NADPH dependent H₂O₂ generation in thyroid tissue is still unknown. Defects in the human *THOX2* have recently been reported, with heterozygous truncation mutations being associated with mild transient congenital hypothyroidism and a partial iodide organification defect, whereas homozygosity for such defects was associated with severe congenital hypothyroidism and a complete iodide organification defect.¹¹⁷ Goitre was not detected in these patients.

IODOTHYRONINE TRANSPORTER DEFECTS AND SYNDROMIC CONGENITAL HYPOTHYROIDISM

TSH secreted by thyrotrophs in the anterior pituitary gland stimulates the gland to synthesise and secrete thyroid hormones, principally thyroxine (T₄). T₄ is essentially a

prohormone as it is converted to the biologically more active hormone triiodothyronine (T₃) by 5'-monodeiodination in all tissues. In a negative feedback loop, T₃ suppresses TSH secretion. Thyroid hormone exerts its effects by acting on nuclear receptors, necessitating transmembrane passage of the hormone. Several classes of membrane thyroid hormone transporters have been identified recently, including *MCT8*, which was mapped to Xq13.2 and contains five exons.¹¹⁸ There are recent reports of mutations in this gene found in male individuals in four families with abnormal thyroid function tests consisting of low FT₄ and raised levels of FT₃ and TSH, which were not found at the time of neonatal screening, but detected within the first two years of life.¹¹⁹ Stigmata of congenital hypothyroidism were not present at birth. In addition, neurological abnormalities from early infancy were described, consisting of central hypotonia, peripheral hypertonia, dystonia, rotary nystagmus, dysconjugate eye movements, feeding difficulties, vomiting, recurrent aspiration, irritability, and subsequent spastic quadriplegia resulting in severe delay in motor and mental development with absent speech reported in one proband. Brain MRI and electroencephalography were normal. Thyroid imaging has not been described. Female relatives harbouring mutations in *MCT8* displayed milder thyroid phenotypes without the neurological features. Expression of this gene has been found in all tissues examined, including brain, thyroid, pituitary, and placenta.¹¹⁹ This is the first example of X linked congenital hypothyroidism resulting from a defect in target tissue rather than in the pituitary-thyroid biosynthetic pathway.

INVESTIGATION AND MANAGEMENT OF CONGENITAL HYPOTHYROIDISM

In those cases of congenital hypothyroidism where an underlying genetic defect is suspected—on the basis of family history, evidence of dysmorphogenesis, or the presence of other congenital anomalies—to suggest a syndromic form of the disorder, further investigation can prove fruitful. While the outcome of such genetic analysis may not necessarily affect the management of the index patient, the results can aid genetic counselling regarding recurrence risk within the family and may suggest the best treatment option—for example, congenital hypothyroidism from *NIS* defects is more amenable to treatment with iodide supplementation than with thyroxine. We suggest below some investigations to aid in the search for an underlying genetic cause in a small number of cases with congenital hypothyroidism. They are not intended to be a comprehensive list of recommended investigations for physicians managing routinely diagnosed cases.

As congenital hypothyroidism is largely a sporadic disorder, a family history is not present in most cases. However, a detailed history should be taken, including that of parental consanguinity, other affected family members, and a history of any extrathyroidal congenital anomalies (for example, cleft palate, renal anomalies, neonatal respiratory distress, and movement disorders). A neonate born with severe congenital hypothyroidism may have the associated features of prolonged jaundice, macroglossia, hoarse cry, and umbilical hernia on examination. The neck should be examined for a goitre and its presence would suggest dysmorphogenesis as the basis of congenital hypothyroidism. Rarely, the goitre can be detected in utero by ultrasound scanning, and such cases can be treated antenatally by intra-amniotic injections of thyroxine or triiodothyronine, which can successfully shrink the gland.¹²⁰ If a significantly enlarged goitre escapes antenatal detection, there could be acute problems postnatally if the upper airway is compromised, and the expert opinion of a paediatric ENT specialist is vital in

Figure 4 A proposed algorithm for investigating the genetic basis of congenital hypothyroidism. AHO, Albright hereditary osteodystrophy; CH, congenital hypothyroidism; *GNAS*, stimulatory G protein α subunit gene; *NIS*, sodium-iodide symporter gene; *PAX-8*, human Pax-8 gene; *PDS*, Pendred syndrome gene; TFTs, thyroid function tests; *TG*, thyroglobulin gene; *THOX2*, thyroid oxidase 2; TIOD, total iodide organification defect; *TITF-1*, human TTF-1 gene; *TITF-2*, human TTF-2 gene; *TPO*, thyroid peroxidase gene; *TSHR*, TSH receptor gene; USS, ultrasound scan.

such cases. The presence of any other congenital malformations should also be sought on examination. Delayed maturation of bone on x ray is of limited value in infancy as the ossification centres are composed mainly of cartilage; it is of greater value after two years in severe forms of congenital hypothyroidism. The initial crucial steps in investigating congenital hypothyroidism are to establish the severity of the hormone defect, to investigate for the possibility of dysmorphogenesis, and determine the morphology and position of the thyroid gland (fig 4).

Biochemical investigations

The first of these steps is established by obtaining the original diagnostic thyroid function tests from the neonatal blood spot screening card and from the first venous serum measurements, before the onset of L-thyroxine treatment. In up to 10% of cases, the congenital hypothyroidism is transient and self limiting, lasting up to a few months.¹²¹ Some of these cases can be accounted for by the presence of anti-TSHR antibodies in the mother, with transplacental passage to the fetus. When detected, the function of these TSHR autoantibodies should be measured, as there are reports of hyperthyroid mothers with Graves' disease giving birth to hypothyroid infants.¹²²

In other infants with transient congenital hypothyroidism and an abnormal perchlorate discharge test suggestive of a defect in organification within the thyroid, the possibility of heterozygous mutations in *THOX2* should be considered. The

measurement of serum thyroglobulin is also useful as all patients with organification disorders, and cases with severe TSHR defects, have raised levels when their circulating TSH is increased, except in cases with thyroglobulin synthesis defects where the thyroglobulin level is usually low. Thyroglobulin measurements can provide useful additional information about the thyroid gland—for example, if serum thyroglobulin is measurable where there is apparent athyreosis on thyroid imaging, this suggests that radiologically undetectable hypoplastic thyroid tissue is likely to be present.³²

It is also worth checking thyroid function (circulating TSH, FT4, and FT3) and thyroglobulin levels not only in affected family members but also in all first degree relatives of probands, as variable penetrance associated with autosomal inheritance might lead to the identification of a mildly affected relative not detected previously. Furthermore, with *TSHR* mutations, heterozygotes may also display a mild phenotype. Underlying autoimmune thyroid disease, causing raised TSH with low or normal thyroid hormones, can be excluded by the absence of thyroid autoantibodies. A useful test to determine whether the thyroid gland is resistant to TSH involves the administration of TRH, with measurement of both the pituitary TSH response and the subsequent rise in circulating T3 following this increase in TSH.

Defective iodide trapping by the thyroid gland caused by *NIS* mutations can be tested for by measuring the saliva to plasma (S/P) ratio of iodide one hour after oral administration of a small dose of radiolabelled iodide (between 25 and

50 μCi of either ^{125}I or ^{131}I labelled NaI). The normal S/P ratio is 25 to 140.¹²³ Thyroxine treatment need not be stopped or reduced before this test, as it does not affect salivary gland iodide trapping.

Radiological investigations

Thyroid scintigraphy, using either technetium (Tc) or radioactive iodine, is usually the first line imaging in many centres and provides information about the size and location of the gland, but it can be misleading. For example, it can indicate no uptake of isotope, suggesting apparent athyreosis, although a thyroid gland is present. Ultrasound scanning of the neck by an experienced radiologist can provide further valuable information, with assessment of whether thyroid gland size is appropriate for age and sex, and its location along the embryological line of development between the base of the tongue and thorax. Ultrasound scanning in conjunction with isotope scanning can provide further useful information. For example, if there is discordance between thyroid isotope scanning and ultrasound scanning, with the former showing no tracer uptake yet the latter showing the presence of a normal or even an enlarged thyroid gland, there is strong evidence for the involvement of *NIS* causing congenital hypothyroidism. Based on history and initial investigations, further imaging can be undertaken, such as a renal ultrasound scan in a family with autosomal dominant congenital hypothyroidism associated with thyroid gland hypoplasia (?*Pax-8* defect), or MRI of the inner ear in cases of congenital hypothyroidism with goitre and hearing loss. The proportion of radioiodine discharged from the thyroid following the administration of potassium perchlorate is an index of the severity of organification defects. Discharge of about 20–45% indicates a partial and mild defect, but values greater than 60% and 90% are typical of severe and complete defects, respectively.⁸⁰

Treatment

Once the diagnosis of congenital hypothyroidism is made on neonatal screening, appropriate thyroxine replacement therapy is initiated by a paediatrician. Studies have shown that several variables influence eventual IQ in children with congenital hypothyroidism. These include the severity of congenital hypothyroidism (as determined by thyroid function tests and delayed skeletal maturation at birth), the dose of thyroxine treatment, the timing of treatment onset, and serum free thyroxine concentrations during the first year.^{124–127} Despite the establishment of neonatal screening programmes, clinicians and families should be aware of reports suggesting that about 10% of early treated infants with severe hypothyroidism are likely to require special education.¹²⁸ Therefore clinically significant intellectual impairment should be actively screened for and treated when detected in these children.

CONCLUSIONS

Congenital hypothyroidism represents a common neonatal problem that is easily detected and treated, and hence the success of the nationwide neonatal screening programmes. While the great majority of cases are considered sporadic, there have been recent advances in elucidating some of the molecular mechanisms behind certain forms of this common congenital metabolic disorder. Organification defects leading to goitrous congenital hypothyroidism are now well known to have an autosomal recessive genetic basis. More recently, there is growing evidence for the role of germline gene defects causing congenital hypothyroidism associated with thyroid dysgenesis. These include a role for the *TSHR* gene in non-syndromic congenital hypothyroidism, with evidence for

its involvement in recessive disease, and possibly also heterozygous mutations in this gene in congenital or even acquired non-autoimmune hypothyroidism. Downstream of *TSHR*, defects in *G α* result in TSH resistance in Albright's hereditary osteodystrophy. Defects in the transcription factors *TITF-2* (cleft palate, spiky hair), *TITF-1* (neonatal respiratory distress, involuntary movement), and *Pax-8* (renal hemiagenesis) provide the basis for multisystem involvement in syndromic forms of congenital hypothyroidism. Finally, there is early evidence emerging for a third group of congenital hypothyroid conditions associated with defects in target tissue iodothyronine transporters, with devastating neurological features.

Novel and as yet unidentified target genes regulated by these transcription factors may also prove to be important candidate genes in other forms of congenital hypothyroidism. A role for the transcription factor *Hoxa3* (human locus 7p15.1), a member of the Hox family of homeobox genes involved in regulating the development of pharyngeal glandular organs, has hitherto only been shown in murine embryonic thyroid development, with null mice having thyroid hypoplasia resulting from defects in both follicular and parafollicular cell development.¹²⁹ Interestingly, these mice also have other abnormalities, including thymic and parathyroid aplasia, anomalies of the heart and great vessels, and malformations of the throat and jaw, which are remarkably similar to those observed in 22q11 deletion (velocardiofacial) syndrome in humans.¹³⁰ It is also known that the amount of thyroid tissue in these patients is variably reduced, as in the *Hoxa3* mutant mice,¹³¹ and it has therefore been suggested that patients with 22q11 deletion syndrome may be at increased risk of thyroid dysfunction. *Hoxa5* (–/–) mutant mice have been reported to have hypothyroidism associated with transient growth retardation, delayed eye opening, and ear elevation.¹³² Thyroid gland development begins normally, but follicle formation and thyroglobulin processing are abnormal in late gestation. The expression of several molecular markers essential for thyroid gland formation and function—namely TTF-1, Pax8, and TTF-2—is affected in the developing thyroid gland, with the consequence of altered expression of thyroid effector genes, including the thyroglobulin and TPO genes. Murine *Nkx-2.5*, a member of the homeobox gene superfamily that is related to the *TITF1* (*NKX-2.1*), is expressed early during embryogenesis of both thyroid and myocardium,¹³³ and accordingly represents a strong candidate in those 4% of cases of congenital hypothyroidism that are associated with cardiac defects.¹³⁴

With regard to identification of additional novel candidate genes, future directions include genome-wide linkage analyses in large families with multiple probands or parental consanguinity, identification of congenital hypothyroidism phenotypes in transgenic or randomly mutagenised mouse models, and linkage analysis and positional cloning in well characterised contiguous gene syndromes with congenital hypothyroidism as a prominent feature (for example, William syndrome (7q11 deletion) where congenital hypothyroidism occurs in 25% of affected individuals). Known candidate genes will have to be carefully ruled out in investigating for this genetically heterogeneous condition where phenocopies pose difficulties—for example, in the absence of a family history it may be difficult to distinguish between *TSHR* mutations and *Pax-8* defects affecting only the thyroid.

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REFERENCES

- Toublanc J. Comparison of epidemiological data on congenital hypothyroidism in Europe with those of other parts of the world. *Horm Res (Basel)* 1992;**38**:230–5.
- Grant DB, Smith I, Fuggle PW, Tokar S, Chapple J. Congenital hypothyroidism detected by neonatal screening: relationship between biochemical severity and early clinical features. *Arch Dis Child* 1992;**67**:87–90.
- Medeiros-Neto G, Stanbury JB, eds. *Inherited disorders of the thyroid system*. Boca Raton: CRC Press, 1994:1–221.
- Pintar JE. Normal development of the hypothalamic-pituitary-thyroid axis. In: Braverman LE, Utiger RD, eds. *Werner and Ingbar's the thyroid*, 7th ed. Philadelphia: Lippincott-Raven, 1996:6–18.
- Williams E, Toyn C, Harach H. The ultimobranchial gland and congenital thyroid abnormalities in man. *J Pathol* 1989;**159**:135–41.
- Cao XY, Jiang XM, Dou ZH, Rakeman MA, Zhang ML, O'Donnell K, Ma T, Amette K, DeLong N, DeLong GR. Timing of vulnerability of the brain to iodine deficiency in endemic cretinism. *N Engl J Med* 1994;**331**:1739–44.
- Klein RZ, Mitchell ML. Hypothyroidism in infants and children. In: Braverman LE, Utiger RD, eds. *Werner and Ingbar's the thyroid*, 7th ed. Philadelphia: Lippincott-Raven, 1996:984–1008.
- Castanet M, Polak M, Bonaiti-Pellie C, Lyonnet S, Czernickow P, Leger J. Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. *J Clin Endocrinol Metab* 2001;**86**:2009–14.
- Grant DB, Smith I. Survey of neonatal screening for primary hypothyroidism in England, Wales, and Northern Ireland 1982–4. *BJM* 1988;**296**:1355–8.
- Law WY, Bradley DM, Lazarus JH, John R, Gregory JW. Congenital hypothyroidism in Wales (1982–1993): demographic features, clinical presentation and effects on early neurodevelopment. *Clin Endocrinol (Oxf)* 1988;**48**:201–7.
- Strader CD, Fong TM, Tota MR, Underwood D. Structure and function of G protein-coupled receptors. *Annu Rev Biochem* 1994;**63**:101–32.
- Vassart G, Dumont JE. The thyrotropin receptor and the regulation of thyrocyte function and growth. *Endocrinol Rev* 1992;**13**:596–611.
- Libert F, Passage E, Lefort A, Vassar G, Mattei MG. Localisation of human thyrotropin receptor gene to chromosome region 14q31 by in situ hybridization. *Cytogenet Cell Genet* 1990;**54**:82–3.
- Gross B, Misrahi M, Sar S, Milgrom E. Composite structure of the human thyrotropin receptor gene. *Biochem Biophys Commun* 1991;**177**:679–87.
- Damante G, Di Lauro R. Thyroid-specific gene expression. *Biochem Biophys Acta* 1994;**1218**:255–66.
- Stein SA, Oates EL, Hall CR, Grumbles RM, Fernandez LM, Taylor NA, Puett D, Jin S. Identification of a point mutation in the thyrotropin receptor of the hyt/hyt hypothyroid mouse. *Mol Endocrinol* 1994;**8**:129–38.
- Postiglione MP, Parlato R, Rodriguez-Mallon A, Rosica A, Mithbaokar P, Maresca M, Mariani RC, Davies TF, Zannini MS, De Felice M, Di Lauro R. Role of the thyroid-stimulating hormone receptor signaling in development and differentiation of the thyroid gland. *Proc Natl Acad Sci USA* 2002;**99**:15462–7.
- Evans C, Jordan NJ, Owens G, Bradley D, Ludgate M, John R. Potent thyrotrophin receptor-blocking antibodies: a cause of transient congenital hypothyroidism and delayed thyroid development. *Eur J Endocrinol* 2004;**150**:265–8.
- Sunthornthepvarakul T, Gootschalk ME, Hayashi Y, Refetoff S. Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *N Engl J Med* 1995;**332**:155–60.
- De Roux N, Misrahi M, Brauner R, Houang M, Carel JC, Granier M, Le Bouc Y, Ghinea N, Boumedienne A, Toublanc JE, Milgrom E. Four families with loss of function mutations of the thyrotropin receptor. *J Clin Endocrinol Metab* 1996;**81**:4229–35.
- Clifton-Bligh RJ, Gregory JW, Ludgate M, John R, Persani L, Asteria C, Beck-Peccoz P, Chatterjee VKK. Two novel mutations in the thyrotropin (TSH) receptor gene in a child with resistance to TSH. *J Clin Endocrinol Metab* 1997;**82**:1094–100.
- Biebertmann H, Schoneberg T, Krude H, Schuktz G, Gudermann T, Gruters A. Mutations in the human thyrotropin receptor gene causing thyroid hypoplasia and persistent congenital hypothyroidism. *J Clin Endocrinol Metab* 1997;**82**:3471–80.
- Abramowicz MJ, Duprez L, Parma J, Vassart G, Heinrich C. Familial congenital hypothyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. *J Clin Invest* 1997;**99**:3018–24.
- Gagne N, Parma J, Deal C, Vassart G, Van Vliet G. Apparent congenital athyreosis contrasting with normal plasma thyroglobulin levels and associated with inactivating mutations in the thyrotropin receptor gene: are athyreosis and ectopic thyroid distinct entities? *J Clin Endocrinol Metab* 1998;**83**:1771–5.
- Tiosano D, Pannain S, Vassart G, Parma J, Gershoni-Baruch R, Mandel H, Lotan R, Zaharan Y, Pery M, Weiss R, Refetoff S, Hochberg Z. The hypothyroidism in an inbred kindred with congenital thyroid hormone and glucocorticoid deficiency is due to a mutation producing a truncated thyrotropin receptor. *Thyroid* 1999;**9**:887–94.
- Tonacchera M, Agretti P, Pinchera A, Rosellini V, Perri A, Collecchi P, Vitti P, Chiovato L. Congenital hypothyroidism with impaired thyroid response to thyrotropin (TSH) and absent circulating thyroglobulin: evidence for a new inactivating mutation of the TSH receptor gene. *J Clin Endocrinol Metab* 2000;**85**:1001–8.
- Russo D, Betterle C, Arturi F, Chiefari E, Girelli ME, Filetti S. A novel mutation in the thyrotropin (TSH) receptor gene causing loss of TSH binding but constitutive receptor activation in a family with resistance to TSH. *J Clin Endocrinol Metab* 2000;**85**:4238–42.
- Bretones P, Duprez L, Parma J, David M, Vassart G, Rodien P. A familial case of congenital hypothyroidism caused by a homozygous mutation of the thyrotropin receptor gene. *Thyroid* 2001;**11**:997–8.
- Nagashima T, Murakami M, Onigata K, Morimura T, Nagashima K, Mori M, Morikawa A. Novel inactivating missense mutations in the thyrotropin receptor gene in Japanese children with resistance to thyrotropin. *Thyroid* 2001;**11**:551–9.
- Tonacchera M, Agretti P, De Marco G, Perri A, Pinchera A, Vitti P, Chiovato L. Thyroid resistance to TSH complicated by autoimmune thyroiditis. *J Clin Endocrinol Metab* 2001;**86**:4543–6.
- Alberti L, Proverbio MC, Costagliola S, Romoli R, Boldrighini B, Vigone MC, Weber G, Chiumello G, Beck-Peccoz P, Persani L. Germ-line mutations of TSH receptor gene as cause of nonautoimmune subclinical hypothyroidism. *J Clin Endocrinol Metab* 2002;**87**:2549–55.
- Park SM, Clifton-Bligh RJ, Betts P, Chatterjee VK. Congenital hypothyroidism and apparent athyreosis with compound heterozygosity or compensated hypothyroidism with probable hemizygosity for inactivating mutations of the TSH receptor. *Clin Endocrinol (Oxf)* 2004;**60**:220–7.
- Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, Vassart G. Somatic mutation in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* 1993;**365**:649–51.
- Duprez L, Parma J, Van Sande J, Allgeier A, Leclerc J, Schvartz C, Delisle MJ, Decoux M, Orgiazzi J, Dumont J, et al. Germ-line mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nature Genet* 1994;**7**:396–401.
- Jordan N, Williams N, Gregory JW, Evans C, Owen M, Ludgate M. The W546X mutation of the thyrotropin receptor gene: potential major contributor to thyroid dysfunction in a Caucasian population. *J Clin Endocrinol Metab* 2003;**88**:1002–5.
- Zannini M, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, Taylor BA, Phillips SJ, Simeone A, Di Lauro R. TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation. *EMBO J* 1997;**16**:3185–97.
- Zannini M, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, Taylor BA, Phillips SJ, Simeone A, Di Lauro R. TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation [erratum]. *EMBO J* 2001;**20**:2108.
- Hishinuma A, Ohya Y, Kuribayashi T, Nagakubo N, Namatame T, Shibayama K, Arisaka O, Matsuura N, Ieiri T. Polymorphism of the polyaniline tract of thyroid transcription factor-2 gene in patients with thyroid dysgenesis. *Eur J Endocrinol* 2001;**145**:385–9.
- Clifton-Bligh RJ, Wentworth JM, Heinz P, Crisp M, John R, Lazarus JH, Ludgate M, Chatterjee VKK. Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. *Nat Genet* 1998;**19**:399–401.
- De Felice M, Ovitt C, Biffali E, Rodriguez-Mallon A, Arra C, Anastasiadis K, Macchia PE, Mattei M-G, Mariano A, Scholer H, Macchia V, Di Lauro R. A mouse model for hereditary thyroid dysgenesis and cleft palate. *Nat Genet* 1998;**19**:395–8.
- Dathan N, Parlato R, Rosica A, De Felice M, Di Lauro R. Distribution of the ttf2/foxe1 gene product is consistent with an important role in the development of foregut endoderm, palate, and hair. *Dev Dyn* 2002;**224**:450–6.
- Sequeira M, Al-Khafaji F, Park S, Lewis MD, Wheeler MH, Chatterjee VK, Jasani B, Ludgate M. Production and application of polyclonal antibody to human thyroid transcription factor 2 reveals thyroid transcription factor 2 protein expression in adult thyroid and hair follicles and prepubertal testis. *Thyroid* 2003;**13**:927–32.
- Bamforth JS, Hughes IA, Lazarus JH, Weaver CM, Harper PS. Congenital hypothyroidism, spiky hair, and cleft palate. *J Med Genet* 1989;**26**:49–60.
- Castanet M, Park SM, Smith A, Bost M, Leger J, Lyonnet S, Pelet A, Czernichow P, Chatterjee K, Polak M. A novel loss-of-function mutation in TTF-2 is associated with congenital hypothyroidism, thyroid agenesis, and cleft palate. *Hum Mol Genet* 2002;**11**:2051–9.
- Harvey RP. NK-2 homeobox genes and heart development. *Dev Biol* 1996;**187**:203–16.
- Scott MP, Tamkun JW, Hatzell GW. The structure and function of the homeodomain. *Biochim Biophys Acta* 1989;**989**:25–48.
- De Felice M, Damante G, Zannini M, Francis-Lang H, Di Lauro R. Redundant domains contribute to the transcriptional activity of thyroid transcription factor 1. *J Biol Chem* 1995;**270**:26649–56.
- Guazzi S, Price M, De Felice M, Damante G, Mattei MG, Di Lauro R. Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. *EMBO J* 1990;**9**:3631–9.
- Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ. The t/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain and pituitary. *Genes Dev* 1996;**10**:60–9.

- 50 **Breedveld GJ**, van Dongen JWF, Danesino C, Guala A, Percy AK, Dure LS, Harper P, Lazarou LP, *et al.* Mutations in *TTF-1* are associated with benign hereditary chorea. *Hum Mol Genet* 2002;11:971–9.
- 51 **Lazzaro D**, Price M, de Felice M, Di Lauro R. The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the fetal brain. *Development* 1991;113:1093–104.
- 52 **Civitreale D**, Lonigro R, Sinclair AJ, Di Lauro R. A thyroid-specific nuclear protein essential for tissue-specific expression of the thyroglobulin promoter. *EMBO J* 1989;8:2537–42.
- 53 **Francis-Lang H**, Price M, Polycarpou-Schwartz M, Di Lauro R. Cell-type-specific expression of the rat thyroperoxidase promoter indicates common mechanisms for thyroid-specific gene expression. *Mol Cell Biol* 1992;12:576–88.
- 54 **Bruno MD**, Bohinski RJ, Huelsman KM, Whitsett JA, Korfhagen TR. Lung cell-specific expression of the murine surfactant protein A (SP-A) gene is mediated by interactions between the SP-A promoter and thyroid transcription factor-1. *J Biol Chem* 1995;270:6531–6.
- 55 **Devriendt K**, Vanhale C, Matthijs G, de Zegher F. Deletion of the thyroid transcription factor-1 gene in an infant with neonatal thyroid dysfunction and respiratory failure. *N Engl J Med* 1998;338:1317–18.
- 56 **Iwatani N**, Mabe H, Devriendt K, Kodama M, Miike T. Deletion of NKX2.1 gene encoding thyroid transcription factor-1 in two siblings with hypothyroidism and respiratory failure. *J Pediatr* 2000;137:272–6.
- 57 **Doyle DA**, Gonzalez I, Thomas B, Scavina M. Autosomal dominant transmission of congenital hypothyroidism, neonatal respiratory distress, and ataxia caused by a mutation of NKX2-1. *J Pediatr* 2004;145:190–3.
- 58 **Pohlenz J**, Dumitrescu A, Zundel D, Martine U, Schonberger W, Koo E, Weiss RE, Cohen RN, Kimura S, Refetoff S. Partial deficiency of thyroid transcription factor 1 produces predominantly neurological defects in humans and mice. *J Clin Invest* 2002;109:469–73.
- 59 **Krude H**, Schutz, Biebermann H, von Moers A, Schnabel D, Neitzel H, Tonnies H, Weise D, Lafferty A, Schwarz S, DeFelice M, von Deimling A, van Landeghem F, DiLauro R, Gruters A. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. *J Clin Invest* 2002;109:475–80.
- 60 **Stuart ET**, Gruss P. PAX genes: what's new in developmental biology and cancer? *Hum Mol Genet* 1995;4:1717–20.
- 61 **Mansouri A**, Hallonet M, Gruss P. Pax genes and their role cell differentiation and development. *Curr Opin Cell Biol* 1996;8:851–7.
- 62 **Poleev A**, Fickenscher H, Mundlos S, Winterpacht A, Zabel B, Fidler A, Gruss P, Plachov D. PAX-8, a human paired box gene: isolation and expression in developing thyroid, kidney, and Wilms' tumours. *Development (Camb)* 1992;116:611–23.
- 63 **Poleev A**, Wendler F, Gickenscher H, Zannini S, Yaginuma K, Abbott C, Plachov D. Distinct functional properties of three human paired-box-proteins, PAX8, isoforms generated by alternative splicing in thyroid, kidney and Wilms' tumors. *Eur J Biochem* 1995;228:899–911.
- 64 **di Magliano MP**, Di Lauro R, Zannini M. Pax8 has a key role in thyroid cell differentiation. *Proc Natl Acad Sci USA* 2000;97:13144–9.
- 65 **Mansouri A**, Chowdhury K, Gruss P. Follicular cells of the thyroid gland require Pax8 gene function. *Nat Genet* 1998;19:87–90.
- 66 **Macchia PA**, Lapi P, Krude H, Pirro MT, Missero C, Chiovato L, Souabni A, Baserga M, Tassi V, Pinchera A, Fenzi G, Gruters A, Busslinger M, Di Lauro R. PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. *Nat Genet* 1998;19:83–6.
- 67 **Vilain C**, Rydlowski C, Duprez L, Heinrichs C, Abramowicz, Malvaux P, Renneboog B, Parma J, Costagliola S, Vassart G. Autosomal dominant transmission of congenital thyroid hypoplasia due to loss-of-function mutation of PAX8. *J Clin Endocrinol Metab* 2001;86:234–8.
- 68 **Congdon T**, Nguyen LQ, Nogueira CR, Habiby RL, Medeiros-Neto G, Kopp P. A novel mutation (Q40) in PAX8 associated with congenital hypothyroidism and thyroid hypoplasia: evidence for phenotypic variability in mother and child. *J Clin Endocrinol Metab* 2001;86:3962–7.
- 69 **Meus L**, Gilbert B, Rydlowski C, Parma J, Roussie A, Abramowicz M, Vilain C, Christophe D, Costagliola S, Vassart G. Characterization of a novel loss of function mutation of PAX8 in a familial case of congenital hypothyroidism with in-place, normal-sized thyroid. *J Clin Endocrinol Metab* 2004;89:4285–91.
- 70 **Fraizer GC**, Shimamura R, Zhang X, Saubers GF. PAX8 regulates human WT1 transcription through a novel DNA binding site. *J Biol Chem* 1997;272:30678–87.
- 71 **Ohno M**, Zannini M, Levy O, Carrasco N, Di Lauro R. The paired-domain transcription factor Pax8 binds to the upstream enhancer of the rat sodium/iodide symporter gene and participates in both thyroid-specific and cyclic-AMP-dependent transcription. *Mol Cell Biol* 1999;19:2051–60.
- 72 **Espinoza CR**, Schmitt TL, Loos U. Thyroid transcription factor 1 and Pax8 synergistically activate the promoter of human thyroglobulin gene. *J Mol Endocrinol* 2001;27:59–67.
- 73 **Kozasa T**, Itoh H, Tsukamoto T, Kaziro Y. Isolation and characterization of the human $G_{\alpha s}$ gene. *Proc Natl Acad Sci USA* 1988;85:2081–5.
- 74 **Aldred MA**, Trembath RC. Activating and inactivating mutations in the human GNAS1 gene. *Hum Mutation* 2000;16:183–9.
- 75 **Wilson LC**, Oude Luttikhuis MEM, Clayton PT, Fraser WD, Trembath RC. Parental origin of $G_{\alpha s}$ gene mutations in Albright's hereditary osteodystrophy. *J Med Genet* 1994;31:835–9.
- 76 **Yu D**, Yu S, Schuster V, Kruse K, Clericuzio CL, Weinstein LS. Identification of two novel deletion mutations within the $G_{\alpha s}$ gene (GNAS1) in Albright hereditary osteodystrophy. *J Clin Endocrinol Metab* 1999;84:3254–9.
- 77 **de Vijlder JJM**, Vulsma T. Hereditary Metabolic Disorders causing hypothyroidism. In: Braverman LE, Utiger RD, eds. *Werner and Ingbar's the thyroid*, 7th ed. Philadelphia: Lippincott-Raven, 1996:749–55.
- 78 **Endo Y**, Onogi S, Umeki K, Yamamoto I, Kotani T, Ohtaki S, Fujita T. Regional localization of the gene for thyroid peroxidase to human chromosome 2p25 and mouse chromosome 12C. *Genomics* 1995;25:760–1.
- 79 **Mangalbruks A**, Correa Billerbeck A-E, Wajchenberg B, Knobel M, Cox NJ, DeGroot LJ, Medeiros-Neto G. Genetic linkage studies of thyroid peroxidase (TPO) gene in families with TPO deficiency. *J Clin Endocrinol Metab* 1991;72:471–6.
- 80 **Bikker H**, Vulsma T, Baas F, de Vijlder JJM. Identification of five novel inactivating mutations in the human thyroid peroxidase gene by denaturing gradient gel electrophoresis. *Hum Mutat* 1995;6:9–16.
- 81 **Abramowicz MJ**, Targovnik HM, Varela V, Cochaux P, Krawiec L, Pisarev MA, Propato FV, Juvenal G, Chester HA, Vassart G. Identification of a mutation in the coding sequence of the human thyroid peroxidase gene causing congenital goiter. *J Clin Invest* 1992;90:1200–4.
- 82 **Bikker H**, den Hartog MT, Baas F, Gons MH, Vulsma T, de Vijlder JJ. A 20-basepair duplication in the human thyroid peroxidase gene results in a total iodide organification defect and congenital hypothyroidism. *J Clin Endocrinol Metab* 1994;79:248–52.
- 83 **Bikker H**, Waelkens JJJ, Bravenboer B, de Vijlder JJJ. Congenital hypothyroidism caused by a premature termination signal in exon 10 of the human thyroid peroxidase gene. *J Clin Endocrinol Metab* 1996;81:2076–9.
- 84 **de Vijlder JJM**, Bikker H, Ris-Stalpers C, Vulsma T. Structure, function, and relevance of thyroid peroxidase in inherited diseases of the thyroid. *Curr Opin Endocrinol Diabetes* 1997;4:328–32.
- 85 **Medeiros-Neto G**, Gil-da-Costa MJ, Santos CS, Medina AM, Silva JCE, Tsou RM, Sobrinho-Simoes M. Metastatic thyroid carcinoma arising from congenital goiter due to mutation in the thyroperoxidase gene. *J Clin Endocrinol Metab* 1998;83:4162–6.
- 86 **Kotani T**, Umeki K, Yamamoto I, Maesaka H, Tachibana K, Ohtaki S. A novel mutation in the human thyroid peroxidase gene resulting in a total iodide organification defect. *J Endocrinol* 1999;160:267–73.
- 87 **Kotani T**, Umeki K, Yamamoto I, Ohtaki S, Adachi M, Tachibana K. Iodide organification defects resulting from cosegregation of mutated and null thyroid peroxidase alleles. *Mol Cell Endocrinol* 2001;182:61–8.
- 88 **Pannain S**, Weiss RE, Kackson CE, Dian D, Beck JC, Sheffield VC, Cox N, Refetoff S. Two different mutations in the thyroid peroxidase gene of a large inbred Amish kindred: power and limits of homozygosity mapping. *J Clin Endocrinol Metab* 1999;84:1061–71.
- 89 **Santos CL**, Rego KG, Nascimento AC, Tambascia M, de Vijlder JJ, Medeiros-Neto G. A novel mutation in the TPO gene in goitrous hypothyroid patients with iodide organification defect. *Clin Endocrinol (Oxf)* 1999;51:165–72.
- 90 **Bakker B**, Bikker H, Vulsma T, de Randamie JSE, Wiedijk BM, de Vijlder JJM. Two decades of screening for congenital hypothyroidism in the Netherlands: TPO gene mutations in total iodide organification defects (an update). *J Clin Endocrinol Metab* 2000;85:3708–12.
- 91 **Ambrugger P**, Stoeva I, Biebermann H, Torresani T, Leitner C, Gruter A. Novel mutations of the thyroid peroxidase gene in patients with permanent congenital hypothyroidism. *Eur J Endocrinol* 2001;145:19–24.
- 92 **Berge-LeFranc JL**, Cartonon G, Mattei MG, Passage E, Malezet-Desmoulins C, Lissitzky S. Localisation of the thyroglobulin gene by *in situ* hybridization to human chromosomes. *Hum Genet* 1985;69:28–31.
- 93 **DeGroot LJ**. Congenital defects in thyroid hormone formation and action. In: DeGroot LJ, ed. *Endocrinology*, 3rd ed. Philadelphia: WB Saunders, 1995:871.
- 94 **Targovnik HM**, Frechtel GD, Mendive FM, Vono J, Cochaux P, Vassart G, Medeiros-Neto G. Evidence for the segregation of three different mutated alleles of the thyroglobulin gene in a Brazilian family with congenital goiter and hypothyroidism. *Thyroid* 1998;8:291–7.
- 95 **Van de Graff SAR**, Ris-Stalpers C, Veenboer GJM, Cammenga M, Santos C, Targovnik HM, de Vijlder JM, Medeiros-Neto G. A premature stop codon in thyroglobulin messenger RNA results in familial goiter and moderate hypothyroidism. *J Clin Endocrinol Metab* 1999;84:2537–42.
- 96 **Hishinuma A**, Takamatsu J, Ohyama Y, Yokozawa T, Kanno Y, Kuma K, Yoshida S, Matsuura N, Ieiri T. Two novel cysteine substitutions (C1263R and C1955S) of thyroglobulin cause a defect in intracellular transport of thyroglobulin in patients with congenital goiter and the variant type of adenomatous goiter. *J Clin Endocrinol Metab* 1999;84:1438–44.
- 97 **Kim PS**, Hossain SA, Park Y-N, Lee I, Yoo S-E, Arvan P. A single amino acid change in the acetylcholinesterase-like domain of thyroglobulin causes congenital goitre with hypothyroidism in the *cog/cog* mouse: a model of human endoplasmic reticulum storage diseases. *Proc Natl Acad Sci USA* 1998;95:9909–13.
- 98 **Medeiros-Neto G**, Kim PS, Yoo SE, Vono J, Targovnik H, Camargo R, Hossain SA, Arvan P. Congenital hypothyroid goiter with deficient thyroglobulin. Identification of an endoplasmic reticulum storage disease with induction of molecular chaperones. *J Clin Invest* 1996;98:2838–44.
- 99 **Kim PS**, Kwon O-Y, Arvan P. An endoplasmic reticulum storage disease causing congenital goiter with hypothyroidism. *J Cell Biol* 1996;133:517–27.
- 100 **Dai G**, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. *Nature* 1996;379:458–60.
- 101 **Smanik PA**, Ryu K-Y, Theil KS, Mazzaferri EL, Jhiang SN. Expression, exon-intron organization, and chromosome mapping of the human sodium iodide symporter. *Endocrinology* 1997;138:3555–8.
- 102 **Wolff J**. Congenital goiter with defective iodide transport. *Endocr Rev* 1983;4:240–54.
- 103 **Fujiwara H**, Tatsumi K, Miki K, Harada T, Miyai K, Takai S-I, Amino N. Congenital hypothyroidism caused by a mutation in the Na^+/I^- symporter. *Nat Genet* 1997;16:124–5.

- 104 **Kosugi S**, Sato Y, Matsuda A, Ohyama Y, Fujieda K, Inomata H, Kameya T, Isozaki O, Jhiang SM. High prevalence of T354P sodium/iodide symporter gene mutation in Japanese patients with iodide transport defect who have heterogeneous clinical pictures. *J Clin Endocrinol Metab* 1998;**83**:4123-9.
- 105 **Pohlenz J**, Duprez L, Weiss RE, Vassart G, Refetoff S, Costagliola S. Failure of membrane targeting causes the functional defect of two mutant sodium iodide symporters. *J Clin Endocrinol Metab* 2000;**85**:2366-9.
- 106 **Reardon W**, Trembath RC. Pendred syndrome. *J Med Genet* 1996;**33**:1037-40.
- 107 **Mondini C**. Anatomia surdi nati sectio: de Boroniensi scientiarum et artium instituto atque aedemia commentarii. *Bononiae* 1791;**7**:419-31.
- 108 **Reardon W**, O'Mahoney CF, Trembath R, Jan H, Phelps PD. Enlarged vestibular aqueduct: a radiological marker of Pendred syndrome, and mutation of the PDS gene. *Q J Med* 2000;**93**:99-104.
- 109 **Batsakis JG**, Nishiyama RH. Deafness with sporadic goitre. *Arch Otolaryngol* 1962;**76**:401-6.
- 110 **Masmoudi S**, Charfedine L, Hmani M, Grati M, Ghorbel AM, Elgaied-Boulila A, Drira M, Hardelin J-P, Ayadi H. Pendred syndrome: phenotypic variability in two families carrying the same PDS missense mutation. *Am J Med Genet* 2000;**90**:38-44.
- 111 **Everett LA**, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxeianis AD, Sheffield VC, Green ED. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat Genet* 1997;**17**:411-22.
- 112 **Royaux IE**, Suzuki K, Mori A, Katoh R, Everett LA, Kohn LD, Green ED. Pendrin, the protein encoded by the Pendred syndrome gene (PDS), is an apical porter of iodide in the thyroid and is regulated by thyroglobulin in FRTL-5 cells. *Endocrinology* 2000;**14**:839-45.
- 113 **Scott DA**, Wang R, Kreman TM, Andrews M, McDonald JM, Bishop JR, Smith RJH, Karniski LP, Sheffield VC. Functional differences of the PDS gene products are associated with phenotypic variation in patients with Pendred syndrome and non-syndromic hearing loss (DFNB4). *Hum Mol Genet* 2000;**9**:1709-15.
- 114 **Caillou B**, Dupuy C, Lacroix L, Nocera M, Talbot M, Ohayon R, Deme D, Bidart J-M, Schlumberger M, Virion A. Expression of reduced nicotinamide adenine dinucleotide phosphate oxidase (*ThoX*, *LNOX*, *Duox*) genes and proteins in human thyroid tissues. *J Clin Endocrinol Metab* 2001;**86**:3351-8.
- 115 **Corvilain B**, Van Sande J, Laurent E, Dumont JE. The H₂O₂-generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid. *Endocrinology* 1991;**128**:779-85.
- 116 **Pachucki J**, Wang D, Christophe D, Miot F. Structural and functional characterization of two human *ThOX*/*Duox* genes and their 5' flanking regions. *Mol Cell Endocrinol* 2004;**214**:53-62.
- 117 **Moreno JC**, Bikker H, Kempers MJ, van Trotsenburg AS, Baas F, de Vijlder JJ, Vulsma T, Ris-Stalpers C. Inactivating mutations in the gene for thyroid oxidase 2 (*THOX2*) and congenital hypothyroidism. *N Engl J Med* 2002;**347**:95-102.
- 118 **Friesema EC**, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* 2003;**278**:40128-35.
- 119 **Dumitrescu AM**, Liao XH, Best TB, Brockmann K, Refetoff S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am J Hum Genet* 2004;**74**:168-75 (Erratum in: *Am J Hum Genet* 2004;**74**:598.)
- 120 **Agrawal P**, Ogilvy-Stuart A, Lees C. Intrauterine diagnosis and management of congenital hypothyroidism. *Ultrasound Obstet Gynecol* 2002;**19**:501-5.
- 121 **Becks GP**, Burrow GN. Diagnosis and treatment of thyroid disease during pregnancy. In: DeGroot LJ, Besser M, Burger HG, Jameson JL, Loriaux DL, Marshall JC, Odell WD Potts JT, Rubenstein AAAH, eds. *Endocrinology*, 3rd ed. Philadelphia: WB Saunders, 1995:799-820.
- 122 **Kohn LD**, Harii N. Thyrotropin receptor autoantibodies (TSHRabs): epitopes, origins and clinical significance. *Autoimmunity* 2003;**36**:331-7.
- 123 **Pohlenz J**, Rosenthal IM, Weiss RE, Jhiang SM, Burant C, Refetoff S. Congenital hypothyroidism due to mutations in the sodium/iodide symporter. *J Clin Invest* 1998;**101**:1028-35.
- 124 **Bongers-Schokking JJ**, Koot HM, Wiersma D, Verkerk PH, de Mulnick Keizer-Shrama SMPF. Influence of timing and dose of thyroid hormone replacement on development in infants with congenital hypothyroidism. *J Pediatr* 2000;**136**:292-7.
- 125 **Derksen-Lubsen G**, Verkerk PH. Neuropsychologic development in early treated congenital hypothyroidism: analysis of literature data. *Pediatr Res* 1996;**39**:561-6.
- 126 **Dubuis JM**, Glorieux J, Richer F, Deal CL, Dussault JH, Van Vliet G. Outcome of severe congenital hypothyroidism: closing the developmental gap with early high dose levothyroxine treatment. *J Clin Endocrinol Metab* 1996;**81**:222-7.
- 127 **Heyerdahl S**, Kase BF, Lie SO. Intellectual development in children with congenital hypothyroidism in relation to recommended thyroxine treatment. *J Pediatr* 1991;**18**:850-7.
- 128 **Tillotson SL**, Fuggle PW, Smith I, Ades AE, Grant DB. Relation between biochemical severity and intelligence in early treated congenital hypothyroidism: a threshold effect. *BMJ* 1994;**309**:440-5.
- 129 **Manley NR**, Capecci MR. The role of *Hoxa-3* in mouse thymus and thyroid development. *Development* 1995;**121**:1989-2003.
- 130 **de la Chapelle A**, Herva R, Koivisto M, Aula P. A deletion in chromosome 22 can cause DiGeorge syndrome. *Hum Genet* 1981;**57**:253-6.
- 131 **Pueblitz S**, Weinberg AG, Albores-Saavedra J. Thyroid C cells in the DiGeorge anomaly: a quantitative study. *Pediatr Pathol* 1993;**13**:463-73.
- 132 **Meunier D**, Aubin J, Jeannotte L. Perturbed thyroid morphology and transient hypothyroidism symptoms in *Hoxa5* mutant mice. *Dev Dyn* 2003;**227**:367-78.
- 133 **Lints TJ**, Parsons LM, Hartley L, Lyons I, Harvey RP. *Nkx-2.5*: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. *Development* 1993;**119**:419-31.
- 134 **Roberts HE**, Moore CA, Fernhoff PM, Brown AL, Khoury MJ. Population study of congenital hypothyroidism and associated birth defects, Atlanta, 1979-1992. *Am J Med Genet* 1997;**71**:29-32.