Mini Review

HORMONE RESEARCH IN PÆDIATRICS

Horm Res Paediatr 2010;73:423–429
DOI: 10.1159/000281438

Received: September 16, 2009
Accepted: January 10, 2010
Published online: May 6, 2010

Spectrum of Human *Foxe1/TTF2* Mutations

Mireille Castanet Michel Polak

Faculty of Medicine René Descartes, Paris V, Site Necker, Institut National de la Santé et de la Recherche Médicale (INSERM) U845 and Pediatric Endocrine Unit, Assistance Publique – Hôpitaux de PARIS (AP-HP), Hôpital Necker Enfants-Malades, Paris, France

Key Words
Thyroid dysgenesis · Bamforth syndrome · Congenital hypothyroidism

Abstract
FOXE1 (or *TTF-2*) has been recognized as one of the thyroid dysgenesis (TD)-related genes based on its early expression at the thyroid bud stage and on the finding in *Foxe1* knockout mice of a sublingual or absent thyroid gland. In humans, three homozygous loss-of-function missense mutations located within the forkhead domain have been reported in 5 patients with Bamforth syndrome. This syndrome is a rare inherited condition whose main features are congenital hypothyroidism (CH) due to TD (usually athyreosis), cleft palate, and spiky hair, with or without choanal atresia and bifid epiglottis. These *FOXE1* mutations were typically inherited from heterozygous carrier parents who were usually consanguineous. Recently, a novel missense mutation was found in a patient with sporadic Bamforth syndrome, inherited via uniparental isodisomy. Altogether these observations strongly suggest that *FOXE1* is involved in both familial and sporadic syndromic CH due to TD in association with cleft palate. Nevertheless, despite intensive research, *FOXE1* mutations have been identified in only a minority of the affected patients. Recent data suggest that the transcription factor encoded by *FOXE1* may act as a susceptibility factor for TD via variations in *FOXE1* polyalanine tract length, which may modulate the risk of TD.

FOXE1/TTF2 and Its Role in Development

Thyroid transcription factor 2 (TTF2), also known as forkhead box E1 (FOXE1) and formerly as forkhead drosophila homolog-like 15 (FKHL15), is a member of the forkhead/winged-helix family. It was originally identified as a thyroid-specific nuclear protein capable of recognizing and binding to a DNA sequence present in both the thyroglobulin *(Tg)* and thyroperoxidase *(TPO)* promoters, two genes expressed exclusively in the thyroid follicular cells [1, 2]. In the adult thyroid gland, the FOXE1 protein acts mostly as a transcriptional activator [3, 4] but may also serve as a repressor [5] of these two specific thyroid genes. After thyroid-specific transcription was shown to contribute to hormonal control, Foxe1 was detected in embryonic mice from E8.5 onwards in all the endodermal cells of the floor of the foregut endoderm, including the thyroid anlage, and in the epithelium lining both the anterior pharynx and the pharyngeal arches [6]. Caudally, Foxe1 was found along the entire foregut, in-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1663–2818/10/0736–0423$26.00/0

Accessible online at:
www.karger.com/hrp

Mireille Castanet, MD, PhD
INSERM U845
Necker School of Medicine, 156, rue de Vaugirard
FR–75015 Paris (France)
Tel. +33 1 40 61 54 03, Fax +33 1 40 61 55 87, E-Mail mireille.castanet@inserm.fr

cluding the future esophagus. At later stages of development, *Foxe1* has been found expressed in the thyroid gland, as well as in the tongue, epiglottis, palate, esophagus, definitive choanae, whiskers, and hair follicles; whereas its expression is downregulated in the pituitary gland [3, 7]. All these observations suggested a role of Foxe1 in development. Contrary to earlier reports, *Foxe1* expression has been found maintained during subsequent developmental stages and persists in adulthood, except in the anterior pituitary, where *Foxe1* expression is not detectable after E11.5 [7].

In humans, the pattern of *FOXE1* expression is similar to that in mice [3, 8–10]. *FOXE1* expression was first detected at Carnegie stage (CS) 15 in the thyroid primordium and was shown to persist in the thyroid gland throughout development. *FOXE1* expression was found at CS19 in the thymus and, at a low level, in the oropharyngeal epithelium; and at 11 weeks of development in the tracheal and esophageal epithelium [10]. In adults, FOXE1 was detected in the exocrine cells of the seminiferous tubules of the testis, epidermis, and hair follicles [11, 12].

FOXE1 and Knock-Out Mouse Model

The finding that *FOXE1* was expressed in the thyroid anlage at the very onset of thyroid morphogenesis immediately suggested a role for this gene in thyroid organogenesis. This role was confirmed by the generation of *Foxe1*-null mice, of which 50% had no thyroid gland (athyreosis) and 50% an ectopic thyroid gland [13]. Thus, *Foxe1* seemed crucial either to the proper migration of the thyroid follicular cell precursors or to the control of thyroid cell survival. This hypothesis was confirmed by a study of null-mutant mice showing that thyroid bud migration was a cell-autonomous event that required *Foxe1* expression in the migrating cells [14]. Note that all the *Foxe1* null mutants had thyroid anlages detectable at early developmental stages based on the expression of *Titf1/Nkx2–1* and *Pax8*, demonstrating that Foxe1 is not required in the first step of thyroid bud formation.

Migration of thyroid follicular cells from the foramen caecum of the tongue to the neck and terminal differentiation of these cells are believed to be two separate events. Therefore, data on thyroid function in *Foxe1* null mutants would be of interest. However, these animals die at birth. The development of an animal model characterized by thyroid-specific *Foxe1* knock-out is thus needed to allow studies on the role of Foxe1 in thyroid function.

In addition to thyroid dysgenesis, and in keeping with the role of *Foxe1* expression, the palatal shelves fail to fuse in *Foxe1* null mutants. The result is an extensive cleft or secondary palate, which probably explains that the animals die within 48 h after birth [13].

Taken together, these data support the view that both thyroid organogenesis and palate closure involve epithelial cell migration and that this event, in turn, requires Foxe1. Therefore, abnormalities in *FOXE1* may be involved in human disorders characterized by both thyroid dysgenesis and a cleft palate.

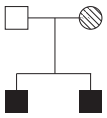
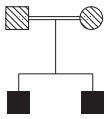
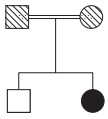
Human *FOXE1* Gene

In 1998, a database search showed more than 90% homology between the rat Ttf2 gene and the human FKHL15 gene isolated 1 year earlier from a cDNA library enriched for transcripts from 9q22 [8]. A probe specific of the 3-prime UTR of FKHL15 detected a 5.3-kb transcript that was highly expressed in thyroid tissues and a second 3.2-kb transcript found in both the thyroid and the testis. This gene, originally identified in Drosophila, was subsequently named TTF2 or FOXE1, as it belongs to the 'forkhead' gene family. It consists of a single exon encoding a 42-kDa protein that has 367 amino acids with a highly conserved 100-amino acid DNA-binding motif called the 'forkhead domain', a 19-residue polyalanine tract, and two putative nuclear localization signals flanking the forkhead domain.

FOXE1 Mutations (table 1)

A65V. The combination of thyroid dysgenesis and cleft palate was first reported in humans in 1989 by Bamforth et al. [15] who described two brothers born separately to healthy unrelated parents. Both brothers had CH due to athyreosis, a cleft palate, spiky hair, bilateral choanal atresia, and a bifid epiglottis. The similarities between this phenotype and the abnormalities seen in *Foxe1* knock-out mice prompted Clifton-Bligh et al. to screen the *FOXE1* gene in the patients, in 1998. They thus identified the first homozygous missense mutation, causing an alanine-to-valine substitution at codon 65 (A65V; 602617.0001) [12]. The mutation was inherited from the heterozygous mother, who had normal thyroid function both basally and in response to TRH. Paternal DNA was not available for testing. Functional studies showed marked impairment of the transcriptional activity of the mutant FOXE1 protein, strongly suggesting a causal relationship between the mutation and the abnormal phenotype.

Table 1. Mutations in the human *FOXE1* gene published so far: pedigree, phenotype, and results of functional studies

FOXE1/TTF2 mutation	Pedigree	Phenotype of affected members	Functional studies	Reference
A65V		congenital hypothyroidism athyreosis + cleft palate + choanal atresia + bifid epiglottis	total abolition of DNA binding	Bamforth et al. [15], 1989; Clifton-Brigh et al. [12],1998
S57N		congenital hypothyroidism athyreosis + cleft palate	partial abolition of DNA binding	Castanet et al. [16], 2002
R102C		congenital hypothyroidism severe thyroid hypoplasia? + cleft palate + choanal atresia	total abolition of DNA binding	Baris et al. [17], 2006

Interestingly, at the time of the report describing the mutation, the boys were aged 16 and 13 years and were receiving thyroxine replacement. They had normal physical growth, pubertal development, and anterior pituitary function. These data suggest that *FOXE1* may not be a key factor in the regulation of puberty, despite its expression in the testis.

S57N. Subsequently, we studied two brothers who had some of the features of Bamforth syndrome (i.e. CH, athyreosis, cleft palate, and spiky hair; fig. 1) but without choanal atresia or epiglottic bifidity. In these patients, we identified the second homozygous loss-of-function missense mutation, causing a serine-to-asparagine substitution at codon 57 (S57N; 602617.0002). Direct sequencing of the two DNA's healthy parents allowed to shown that the mutation was inherited from the two heterozygous consanguineous parents [16].

R102C. Later on, a third homozygous missense mutation, changing an arginine to a cysteine at codon 102 (R102C) and inherited from both consanguineous parents, was identified in a female child with CH due to TD, cleft palate, choanal atresia, and spiky hair [17]. Ultrasonography showed some hypoechoic tissue in the paratracheal region suggesting severe thyroid hypoplasia rather than athyreosis. However, hormone assays and radioisotope scanning showed that the tissue was non-functional, and no thyroglobulin was detectable, indicating possible athyreosis. This observation highlights the difficulty of distinguishing between severe thyroid hypoplasia and athyreosis. It has been previously established that paratracheal hyperechoic tissues could indicate either persistence of the ultimobranchial bodies as a cystic structure or part of the thyroid-forming material [18]. Thus, the diagnosis requires both high-definition ultrasonography to detect any thyroid tissue and high-quality scintigraphy with either iodine (^{123}I) or sodium technetium-99m pertechnetate (^{99m}Tc) to detect any functional tissue. In addition, serum thyroglobulin measurement may assist in the diagnosis [19].

Other FOXE1 Mutations That Have Been Identified but Not Yet Published

Recently, we identified a novel homozygous missense mutation (F137S) in a patient with CH, severe thyroid hypoplasia, cleft palate, and spiky hair, who was born to nonconsanguineous parents. Surprisingly, although the patient's mother was an unaffected heterozygous carrier, the father's DNA contained only the wild-type sequence. Using microsatellite markers and MLPA studies, we demonstrated that the mode of inheritance was consistent with complete maternal uniparental disomy (UPD) for chromosome 9, an as yet unreported mode of inheritance for *FOXE1* mutations [unpubl. data]. This observation shows that *FOXE1* mutation must be investigate in both familial and sporadic cases even without consanguineous parents.

The other *FOXE1* mutation (W97R) has been reported in abstract form but further details on the phenotype and molecular study results in the affected patient are needed [20].

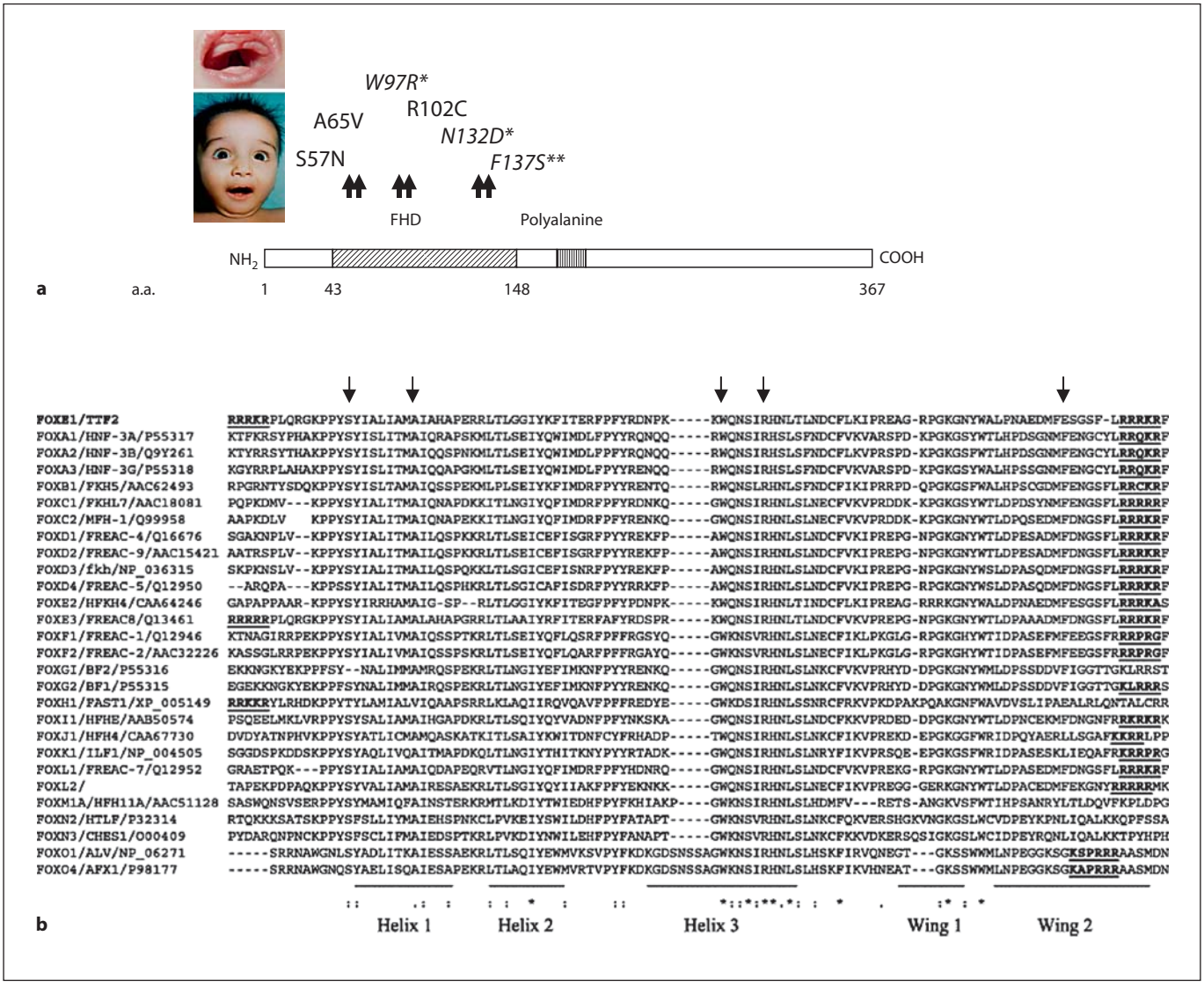


Fig. 1. Human *FOXE1* mutations. Schematic representation of the *FOXE1* gene and location of all the mutations, identified to date within the forkhead domain of the protein (box). The mutations in italic type were reported only in abstract form (*). Photographs of 1 of the 2 children with the S57N mutation at age 8 months: note the dysmorphic features included hypertelorism, low-set posteriorly rotated ears, low posterior hair line, spiky hair, and extensive cleft palate [16].

Functional Studies

All the missense mutations (except W97R) identified so far affect conserved amino acids and are located within the *FOXE1* forkhead domain (fig. 1). Functional studies have shown that mutant proteins exhibit decreases in both DNA binding and transcriptional activity. The first reported A65V mutation was highly deleterious, with the mutant protein being unable to bind to DNA or to activate transcription, whereas the S57N mutation seemed less harmful, allowing some DNA binding and transcriptional activity [12, 16]. These data suggested a possible genotype-phenotype correlation, with residual FOXE1 function accounting for the incomplete clinical phenotype in the second kindred. However, this hypothesis was refuted by functional studies showing complete absence of DNA binding and transcriptional activation in patients with the R102C and F137S mutations, some of whom exhibited an intermediate phenotype with the

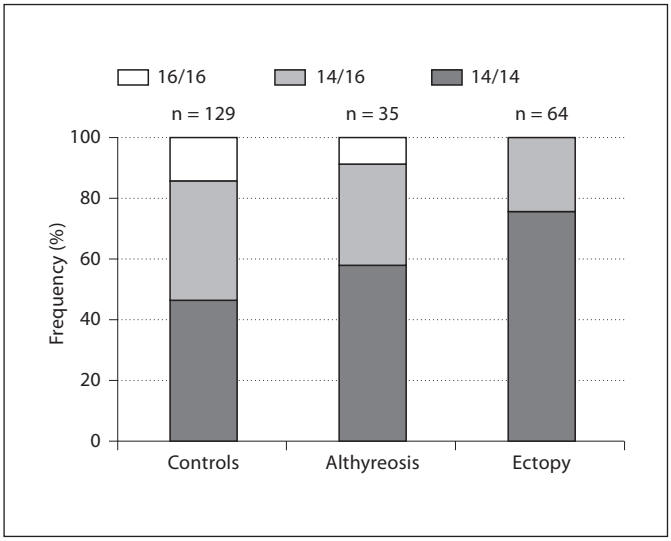


Fig. 2. Frequencies of the genotypes of the FOXE1 polyalanine tract (14/14, 14/16, and 16/16) in a control group and in two groups of patients with athyreosis and ectopic thyroid, respectively [25].

presence of residual thyroid tissue [17]. Data on additional kindred are needed to allow meaningful studies of genotype-phenotype correlations.

All these findings suggest that *FOXE1* mutations should be considered in patients with familial or sporadic syndromic CH, TD (athyreosis or severe thyroid hypoplasia), cleft palate, and spiky hair, a combination consistent with the *FOXE1* expression pattern. However, screening for *FOXE1* mutations in a large cohort of children with CH due to TD with or without cleft palate showed few mutations [22, personal data], suggesting that *FOXE1* may not be a key factor in thyroid development.

Polymorphisms Affecting FOXE1 Polyalanine Tract Length

The FOXE1 gene contains an expansion of trinucleotide repeats encoding polyalanine tracts. Such expansion has been previously shown to cause several diseases by causing abnormalities in alanine-containing transcription factors, which in turn lead to abnormal organ development [23, 24]. Recently, analysis of this polyalanine tract allowed is to obtain the first evidence suggesting that, instead of a disease-causing gene, *FOXE1* may be a TD susceptibility gene. Indeed, evaluation of *FOXE1* polyalanine tract length showed different haplotype distributions in 115 patients with TD (athyreosis or ectopic thyroid) and 129 controls (fig. 2). Furthermore, statistical analyses showed that the presence of 14 alanines in the homozygous state was associated with an increased risk of TD (OR, 2.59; p = 0.0005), whereas the presence of 16 alanines was inversely associated with TD (OR, 0.39; p = 0.0005), suggesting that the FOXE1 protein containing 16 alanines may protect against TD. This hypothesis was supported by the results of transmission disequilibrium testing in 39 parent-proband trios [25]. Furthermore, as previous studies have suggested involvement of the polyalanine tracts of some transcription factors in the ability to transactivate or repress the target gene expression [8, 26], we performed functional studies that allowed us to document differences in transcriptional activity between FOXE1 proteins with 14 versus 16 alanines. All these data strongly suggest a role for the polyalanine tract in the abnormal manifestations [25] and point to a new role of the *FOXE1* gene through its polyalanine tract that may affect thyroid development by modulating the genetic susceptibility to TD.

Role for FOXE1 in Thyroid Development

Although the available data strongly support a role for *FOXE1* in thyroid development, the underlying mechanisms remain poorly understood. *FOXE1* may be a component of one or more networks. During thyroid organogenesis, *FOXE1* expression by the future thyroid cells at the very early anlage stage (specification phase) occurs concomitantly with the expression of other transcription factors including *TITF1* (formerly called *TTF-1/NKX2.1*), *PAX8* [26], and *HHEX* (formerly called *HEX*) [28]. This combination of gene expression is a unique feature of thyroid precursor cells and their descendents, including the fully differentiated thyroid follicular cells. The hypothesis of a network has received support from a study in mice indicating precise spatial regulation of *Foxe1* expression, which depends on *Pax8* in the thyroid bud and on *Shh* in the neighboring pharyngeal cells [29]. Thus, regulatory interactions between Foxe1 and other transcription factors may act as a fail-safe mechanism that leads to the disappearance of thyroid cell precursors if any of the genes malfunction early during development, since each gene initiates a chain of events leading to the complete removal of thyroid cells [14]. Alternatively, *FOXE1* may affect thyroid development via some of the target genes, which would explain the variability of the *FOXE1*-altered phenotype. Several genes upregulated by *FOXE1* were identified recently [30] and some of them might play a role in thyroid development, while others might exert

global effects on the development of multiple organs. However, although the variable expression of the *FOXE1*-altered phenotype supports a role for additional genetic or stochastic events during organogenesis, the individual genetic background or sex-related factors may also be involved.

In conclusion, the currently available data provide strong evidence that genetic alterations of *FOXE1* lead to abnormal thyroid development. Mutations within the forkhead domain are responsible for syndromic CH with TD (ranging from severe hypoplasia to athyreosis), cleft palate, and spiky hair. The length of the FOXE1 polyalanine tract may affect the susceptibility to TD, most likely depending on additional genetic or stochastic events during organogenesis, as well as on individual genetic factors or sex-related factors. Thus, further studies are needed to enhance our understanding of the exact role for FOXE1 in thyroid development.

Acknowledgements

We thank Paul Czernichow for support and critical reading of the manuscript.

Dr. M. Castanet was supported by Leem (the French Pharmaceutical Companies Association) and was awarded the Young Investigator Award at the European Society for Paediatric Endocrinology meeting held in Rotterdam in 2006.

References

1 Civitareale 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;8:2537–2542.

2 Santisteban P, Acebron A, Polycarpou-Schwarz M, Di Lauro R: Insulin and insulin-like growth factor I regulate a thyroid-specific nuclear protein that binds to the thyroglobulin promoter. Mol Endocrinol 1992;6:1310–1317.

3 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 the differentiation. EMBO J 1997;16:3185–3197.

4 Ortiz L, Aza-Blanc P, Zannini M, Cato ACB, Santisteban P: The interaction between the forkhead thyroid transcription factor TTF-2 and the constitutive factor CTF/NF-1 is required for efficient hormonal regulation of the thyroperoxidase gene transcription. J Biol Chem 1999;274:15213–15221.

5 Perrone L, Di Magliano MP, Zannini M, Di Lauro R: The thyroid transcription factor 2 (TTF-2) is a promoter-specific DNA-binding independent transcriptional repressor. Biochem Biophys Res Commun 2000;275:203–208.

6 Damante G, Di Lauro R: Thyroid-specific gene expression. Biochim Biophys Acta 1994;1218:255–266.

7 Dathan N, Parlato R, Rosica A, De Felice M, Di Lauro R: Distribution of the *Titf2/Foxe1* gene product id consistent with an important role in the development of foregut endoderm, palate, and hair. Dev Dynamics 2002;224:450–456.

8 Chadwick BP, Obermayr F, Frischauf AM: FKHL15, a new human member of the forkhead gene family located on chromosome 9q22. Genomics 1997;41:390–396.

9 Trueba SS, Auge J, Mattei G, Etchevers H, Martinovic J, Czernichow P, Vekemans M, Polak M, Attie-Bitach T: PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. J Clin Endocrinol Metab 2005;90:455–462.

10 Trueba SS, Auge J, Mattei G, et al: PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. J Clin Endocrinol Metab 2005;90:455–462.

11 Sequeira M, Al-Khafaji F, Park S, et al: 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;13:927–932.

12 Clifton-Bligh RJ, Wentworth JM, Heinz P, Crisp MS, John R, Lazarus JH, Ludgate M, Chatterjee K: Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. Nat Genet 1998;19:399–401.

13 De Felice M, Ovitt C, Biffali E, Rodriguez-Mallon A, Arra C, Anastassiadis K, Macchia PE, Mattei MG, Mariano A, Schöler H, Macchia V, Di Lauro R: A mouse model for hereditary thyroid dysgenesis and cleft palate. Nat Genet 1998;19:399–401.

14 Parlato R, Rosica A, Rodriguez-Mallon A, et al: An integrated regulatory network controlling survival and migration in thyroid organogenesis. Dev Biol 2004;276:464–475.

15 Bamforth JS, Hugues IA, Lazarus JH, Weaver CM, Harper PS: Congenital hypothyroidism, spiky hair, and cleft palate. J Med Genet 1989;26:49–51.

16 Castanet M, Park SM, Smith A, Bost M, Léger 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;11:2051–2059.

17 Baris I, Arisoy AE, Smith A, et al: A novel missense mutation in human TTF-2 (FKHL15) gene associated with congenital hypothyroidism but not athyreosis. J Clin Endocrinol Metab 2006;91:4183–4187.

18 Marinovic D, Garel C, Czernichow P, Leger J: Ultrasonographic assessment of the ectopic thyroid tissue in children with congenital hypothyroidism. Pediatr Radiol 2004;34:109–113.

19 Czernichow P, Schlumberger M, Pomarede R, Fragu P: Plasma thyroglobulin measurements help determine the type of thyroid defect in congenital hypothyroidism. J Clin Endocrinol Metab 1983;56:242–245.

20 Huebner A, Thorwarth A, Biebermann H, Birke I, Renault N, Aust D, Müller D, Grueters A, Kreuz F, Schwarze R, Krude H: Congenital hypothyroidism due to thyroid agenesis and cleft palate resulting from a novel homozygous mutation of FOXE1 (TTF2). Horm Res 2004;62(suppl 2):19.

21 Musa M, Tenang IE, Junit SM, Harun F: A novel asparagine to aspartate (N132D) mutation in the FOXE gene in congenital hypothyroidism. Horm Res 2005;64(suppl 1):98.

22 Tonacchera M, Banco M, Lapi P, Di Cosmo C, Perri A, Montanelli L, Moschini L, Gatti G, Gandini D, Massei A, Agretti P, De Marco G, Vitti P, Chiovato L, Pinchera A: Genetic analysis of TTF-2 gene in children with congenital hypothyroidism and cleft palate, congenital hypothyroidism, or isolated cleft palate. Thyroid 2004;14:584–588.
23 Brown LY, Brown SA: Alanine tracts: the expanding story of human illness and trinucleotide repeats. Trends Genet 2004;20:51–58.
24 Abu-Baker A, Rouleau GA: Oculopharyngeal muscular dystrophy: recent advances in the understanding of the molecular pathogenic mechanisms and treatment strategies. Biochim Biophys Acta 2007;1772:173–185.
25 Carre A, Castanet M, Sura-Trueba S, et al: Polymorphic length of FOXE1 alanine stretch: evidence for genetic susceptibility to thyroid dysgenesis. Hum Genet 2007;122:467–476.
26 Lavoie H, Debeane F, Trinh QD, et al: Polymorphism, shared functions and convergent evolution of genes with sequences coding for polyalanine domains. Hum Mol Genet 2003;12:2967–2979.
27 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 foetal brain. Development 1991;113:1093–1104.
28 Thomas PQ, Brown A, Beddington RSP: Hex: a homeobox gene revealing peri-implantation asymmetry in the mouse embryo and an early transient marker of endothelial cell precursors. Development 1998;125:85–94.
29 Parlato R, Rosica A, Rodriguez-Mallon A, Affuso A, Postiglione MP, Arra C, Mansouri A, Kimura S, Di Lauro R, De Felice M: An integrated regulatory network controlling survival and migration in thyroid organogenesis. Dev Biol 2004;276:464–475.
30 Hishinuma A, Ohmika N, Namatame T, Ieiri T: TTF-2 stimulates expression of 17 genes, including one novel thyroid-specific gene which might be involved in thyroid development. Mol Cell Endocrinol 2004;221:33–46.

Note Added in Proof

Recently, a new mutation N132D has been published but functional consequences to the human thyroperoxidase promoter were not convincing with only 5% loss of TPO transcriptional activity [Kang IN, Musa M, Harun F, Junit SM: Characterization of mutations in the FOXE1 gene in a cohort of unrelated Malaysian patients with congenital hypothyroidism and thyroid dysgenesis. Biochem Genet 2010;48:141–151].