Genetic causes and neonatal diagnosis of congenital isolated central hypothyroidism

Heinen, C.A.

Publication date
2018

Document Version
Other version

License
Other

2

Mutations in *TBL1X* are associated with central hypothyroidism

Charlotte A. Heinen, Monique Losekoot, Yu Sun, Peter J. Watson, Louise Fairall, Sjoerd D. Joustra, Nitash Zwaveling-Soonawala, Wilma Oostdijk, Erica L.T. van den Akker, Mariëlle Alders, Gijs W.E. Santen, Rick R. van Rijn, Wouter A. Dreschler, Olga V. Surovtseva, Nienke R. Biermasz, Raoul C. Hennekam, Jan M. Wit, John W.R. Schwabe, Anita Boelen, Eric Fliers, A.S. Paul van Trotsenburg

The Journal of Clinical Endocrinology and Metabolism (2016) 101:4564-4573

Abstract

Context: Isolated congenital central hypothyroidism (CeH) can result from mutations in *TRHR*, *TSHB*, and *IGSF1*, but its etiology often remains unexplained. We identified a missense mutation in the transducin β-like protein 1, X-linked (*TBL1X*) gene in three relatives diagnosed with isolated CeH. TBL1X is part of the thyroid hormone receptor-corepressor complex.

Objective: The objectives of the study were the identification of *TBL1X* mutations in patients with unexplained isolated CeH, Sanger sequencing of relatives of affected individuals, and clinical and biochemical characterization; in vitro investigation of functional consequences of mutations; and mRNA expression in, and immunostaining of, human hypothalami and pituitary glands.

Design: This was an observational study.

Setting: The study was conducted at university medical centers.

Patients: Nineteen individuals with and seven without a mutation participated in the study.

Main Outcome Measures: Outcome measures included sequencing results, clinical and biochemical characteristics of mutation carriers, and results of in vitro functional and expression studies.

Results: Sanger sequencing yielded five additional mutations. All patients (n = 8; six males) were previously diagnosed with CeH (free T4 [FT4] concentration below the reference interval, normal thyrotropin). Eleven relatives (two males) also carried mutations. One female had CeH, whereas 10 others had low-normal FT4 concentrations. As a group, adult mutation carriers had 20%–25% lower FT4 concentrations than controls. Twelve of 19 evaluated carriers had hearing loss. Mutations are located in the highly conserved WD40-repeat domain of the protein, influencing its expression and thermal stability. TBL1X mRNA and protein are expressed in the human hypothalamus and pituitary.

Conclusions: *TBL1X* mutations are associated with CeH and hearing loss. FT4 concentrations in mutation carriers vary from low-normal to values compatible with CeH.

Introduction

Central hypothyroidism (CeH) is characterized by suboptimal thyroid hormone (TH) secretion due to insufficient stimulation by TSH of an otherwise normal thyroid gland. CeH may be caused by congenital or acquired disorders of the pituitary gland or hypothalamus (1). The diagnosis is based on a plasma free T4 (FT4) concentration below the reference interval in combination with an inappropriately normal TSH. Congenital CeH has an estimated incidence of 1 in 18 000 and is isolated in 25% of cases (2). Until now, three genetic causes of isolated CeH have been discovered: mutations in *TRHR*, *TSHB*, and *IGSF1* (3-5). Yet the etiology of most cases of isolated disease has remained unexplained. In our ongoing search for other genetic causes, we studied three patients from one family with isolated CeH who tested negative for mutations in *TRHR*, *TSHB*, and *IGSF1*. In these patients, we identified a missense mutation in the gene encoding transducin β-like protein 1, X-linked (TBL1X). Sanger sequencing of TBL1X in 50 other patients with unexplained isolated CeH yielded five other missense mutations in five families.

TBL1X is an essential subunit of the nuclear receptor corepressor (NCoR)-silencing mediator for retinoid and thyroid hormone receptors (SMRT) complex, the major TH receptor (TR) corepressor (CoR) involved in T3-regulated gene expression. Disruption of NCoR in mice was found to result in decreased TH synthesis while possibly increasing peripheral sensitivity to TH (6). In humans, *TBL1X* deletions have been associated with hearing loss (7, 8), but not with CeH.

Here we report the phenotype of the probands and relatives with a mutation in *TBL1X* and the results of structural and functional studies of the mutated TBL1X protein

Methods

Acquisition of patients

In ongoing studies on X-linked CeH, we performed X-exome sequencing in three patients with CeH and two relatives from one family (family A, Figure 1A). The 25-year-old proband (A.III.8) and his sister's 1.5-year-old son (A.IV.1) were diagnosed with CeH after detection by the Dutch T4-based neonatal congenital hypothyroidism (CH) screening (2). They were treated with levothyroxine (LT4) from the ages of 6 months and 16 days, respectively. The proband's sister (A.III.6) was diagnosed with CeH when she was 27 years old and was subsequently treated with LT4. An overview of the X-exome sequencing results is summarized in Supplemental Table 1. After identification of a potentially pathogenic TBL1X variant in these patients, Sanger sequencing was performed on DNA samples from 50 unrelated patients with idiopathic CeH, resulting in the discovery of five other mutations in five patients. Through family studies, 11 other individuals with a mutation were detected. Written informed consent was obtained in all cases.

Phenotyping

All individuals with a mutation were phenotyped in detail, including assessment of growth and development, biochemical evaluation of endocrine axes (see Supplemental Material), brain magnetic resonance imaging, thyroid and testicular ultrasound, and pure tone audiometry (PTA) or otoacoustic emission testing. Because mice with a loss of hepatic TBL1X show hepatic hypertriglyceridemia and steatosis (9), plasma liver enzymes and lipids

were measured. PTA was performed in a soundproof booth, using a manual audiometer (Madsen Electronics) with TDH-39 headphones, calibrated according to International Organization for Standardization-389-1, with adequate masking (10). To compare hearing thresholds between groups, we used the air conduction thresholds, corrected for gender and age, according to the International Organization for Standardization 1999 (11).

Seven relatives without a mutation were invited for evaluation of the hypothalamus-pituitary-thyroid (HPT) axis, thyroid ultrasound, and PTA to serve as controls. A two-way, repeated-measures ANOVA analysis was used to compare the corrected thresholds of individuals with a mutation with those of relatives without a mutation. A value of $P < 0.05$ was considered to be significant. SPSS version 22 for Windows (SPSS, Inc) was used. The Medical Ethics Committee of the Academic Medical Centre (Amsterdam, The Netherlands) approved the study protocol (NL47462.018.13).

Genetic analyses

Genomic DNA isolation and X-exome enrichment were performed as described previously (5). The KAPA library preparation kit (Illumina) was used to prepare the DNA for X-exome sequencing on the Illumina MiSeq generating 250-bp paired-end reads. Read mapping, variant calling, annotation and filtering strategy were essentially as described earlier (12). Candidate variants were confirmed by Sanger sequencing, and Sanger sequencing of the complete coding region of *TBL1X* in unrelated individuals with CeH was performed using standard procedures (conditions and primer sequences are available upon request). All available first- and second-degree relatives of the probands were invited for molecular analysis. X-chromosome inactivation analysis was performed in females with a mutation as described previously (13).

Genes known or expected to cause isolated CeH (*TSHB, TRHR, IGSF1*) were sequenced using Sanger sequencing in the probands of all families. Whole exome sequencing (WES) and variant calling was performed by Beijing Genomics Institute using the Complete Genetics platform in all individuals with a mutation and CeH from families A-D to evaluate the presence of potentially pathogenic variants in other genes. Rare variants were identified by focusing on protein-altering and splice-site changing mutations that were present at a frequency of less than 1% in the general population (based on dbSNP (dbSNP build 141 GRCh37.p13), ESP6500 (http://evs.gs.washington.edu/EVS/), 1000 Genomes project (1000 Genomes phase 3 release version 5.20130502), GoNL (http://www.nlgenome.nl/) and more than 900 in-house reference samples. Missense mutations that were not likely to be pathogenic based on in silico prediction (Sorting Intolerant From Tolerant-score >0.1, and Polymorphism Phenotyping prediction <0.90) were discarded.

All variants were additionally checked against a list of genes that are known or presumed to be involved in HPT axis functioning based on their position in biological pathways, expression or animal models (Supplemental Table 2C). In family A, displaying vertical transmission, all variants present in each of the three affected members were checked against variants in a gene panel consisting of genes defined to be medically relevant by

the Medical Exome Project (14) and Clinical Research Exome (Agilent Technologies) or listed as disease causing in the online inheritance in man database.

Protein structural and functional studies

To express the TBL1/histone deacetylase 3 (HDAC3)/G protein pathway suppressor 2 (GPS2)/SMRT chimera complex, full length TBL1X and HDAC3 were cloned into the pcDNA3 vector, a chimera between GPS2 and SMRT was cloned into the pcDNA3 vector with a N-terminal 10xHis-3xFlag tag and a TEV protease cleavage site. To express TBL1X in isolation, full length TBL1X and the TBL1X WD40 domain (amino acids 100–526) were cloned into the pcDNA3 vector with a N-terminal 10xHis-3xFlag tag and a TEV protease cleavage site. Transient transfections in mammalian cells and protein purifications were performed as described previously (see Supplemental Material) (15).

mRNA expression and immunostaining

We studied eight human hypothalami and five pituitary glands obtained from the Netherlands Brain Bank, in accordance with the formal permission for the use of human brain material for research purposes. Three unfixed, frozen (-80 ˚C) hypothalami and three pituitary glands were used for mRNA expression, and three paraformaldehyde-fixed hypothalami were used for immunocytochemistry. In addition, we used frozen hypothalamus and pituitary glands from two patients for Western blot analysis (see Supplemental Material).

Results

Genetic analyses

X-exome sequencing and subsequent filtering steps yielded a single missense variant in *TBL1X* (transcript accession number NM_001139466.1) in the three patients with CeH from family A, subsequently confirmed by Sanger sequencing. Sanger sequencing of 50 other individuals with idiopathic CeH yielded mutations in five unrelated patients. None of the detected mutations were present in available databases (dbSNP, 1000 Genomes Project, Human Gene Mutation Database, National Heart, Lung, and Blood Institute Exome Sequencing Project, GoNL) or previously reported. All mutations were located in the highly conserved WD40-repeat domains of the TBL1X protein (Figure 2; NCBI Reference Sequence: NP_001132940.1). All available first and second-degree relatives of the probands were tested for mutations (Figure 1), and assessed clinically (Table 1). The variant identified in family A (Figure 1A) was also found in three relatives.

A second mutation was found in a 2.5-year old boy with CeH (Figure 1A; B.III.4) detected through the Dutch neonatal CH screening, and in his mother. A third mutation was found in a 15-year old girl (Figure 1C; C.II.1) diagnosed with CeH because of fatigue, weight gain and secondary amenorrhea. The mutation was also found in her father and two sisters. The fourth mutation was found in a 16-year old boy (Figure 1D; D.III.1), diagnosed with CeH after presenting with obesity, concentration difficulties and macrocephaly. His mother had the same mutation. The fifth mutation was found in a 17-year old boy (Figure 1E; E.II.1) diagnosed with CeH after presenting with short stature. The mutation was also found in his mother and sister. The sixth mutation was found in a 6-month old boy

(Figure 1F; F.II.1), detected through the Dutch neonatal CH screening, and in his mother. X-inactivation analysis showed absence of skewing in A.III.6, C.II.1 and E.II.2 (diagnosed with CeH), and A.II.6, B.II.4 and C.II.2 (low normal FT4 concentrations).

Sanger sequencing failed to show a variant in any of the genes known to cause CeH. Using WES, the three affected members of family A shared variants in eight genes (Table S2A). Mutations in four genes are not known to be associated with a specific phenotype. The phenotypes associated with mutations in the other four genes were not present in our patients and are likely not causative. WES in the six patients of families A-D yielded variants in six genes, two of which cause phenotypes with dominant inheritance not present in our patients (Supplemental Table 2B). Three of the remaining four genes (*BRD8, NCOA6, MED15*) may play a role in mediating TH-dependent activation of gene transcription (16-18). Sanger sequencing showed that two of the five relatives with a TBL1X mutation and a low normal FT4 had the same variant as the proband in their family, and three did not (Supplemental Table 2B). There was no relation between FT4 concentrations and the presence or absence of variants, suggesting absence of synergy of *TBL1X* mutations and variants. Mutations in the fourth gene (*THRB*) have been associated with resistance to thyroid hormone (RTH), accompanied by elevated TH levels. This was not present in our patients.

Clinical phenotyping

Endocrine and anthropometric findings

The six probands and two members from family A had previously shown biochemical evidence of CeH, with FT4 concentrations between 56% and 93% of the lower limit of the reference interval (Table 1 and Figure 3). All were treated with LT4 during phenotyping. Ten individuals with a mutation (two males) had FT4 concentrations within the lower half of the reference interval (Tables 1 and Figure 3). E.II.2 had a FT4 concentration below the lower limit of the reference interval, without complaints. She is currently being monitored. Compared with a large adult control group, the FT4 concentrations of the 16 adults with a mutation were clearly and significantly lower (same FT4 assay, same laboratory; 11.2 vs. 14.6 pmol/L, $p < 0.001$) (Figure 3). The seven relatives without a mutation had FT4 concentrations within the reference interval, similar to the controls (14.1 vs 14.6 pmol/L; $p = 0.616$) (Supplemental Table 3 and Figure 3). T3 concentrations were normal in all individuals with a mutation (Supplemental Table 4). TRH stimulation testing before LT4 treatment performed in six individuals with a mutation and CeH showed normal timing and peak concentration of TSH (Supplemental Table 5) (19), indicating intact responsiveness of the pituitary gland to TRH.

All individuals with a mutation had a normal height (20) and normal age at onset and progression of puberty. Body mass index (BMI) was greater than 30 kg/m^2 in three of the nine adults, and +2 or greater standard deviation score (SDS) in three of the 10 children (20). Biochemical evaluation of the endocrine axes other than the HPT axis was normal (Supplemental Table 4).

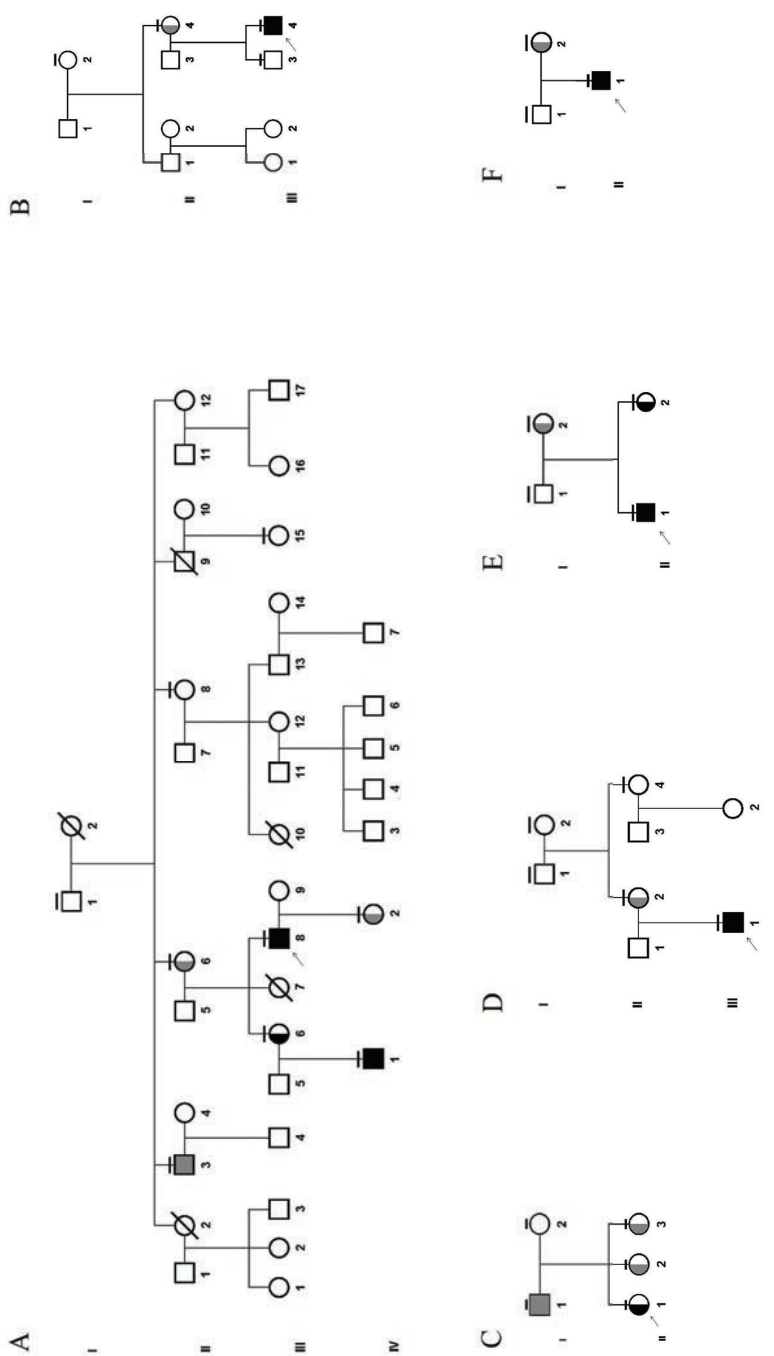

Figure 1. Pedigrees of the six families in which *TBL1X* mutations were found. Probands are indicated by an arrow, small horizontal lines indicate that mutation analysis was performed. Black and grey filled symbols represent mutation carrying individuals with CeH and euthyroidism, respectively. (A) Pedigree of family A, (B) family B, (C) family C, (D) family D, (E) family E, and (F) family F.

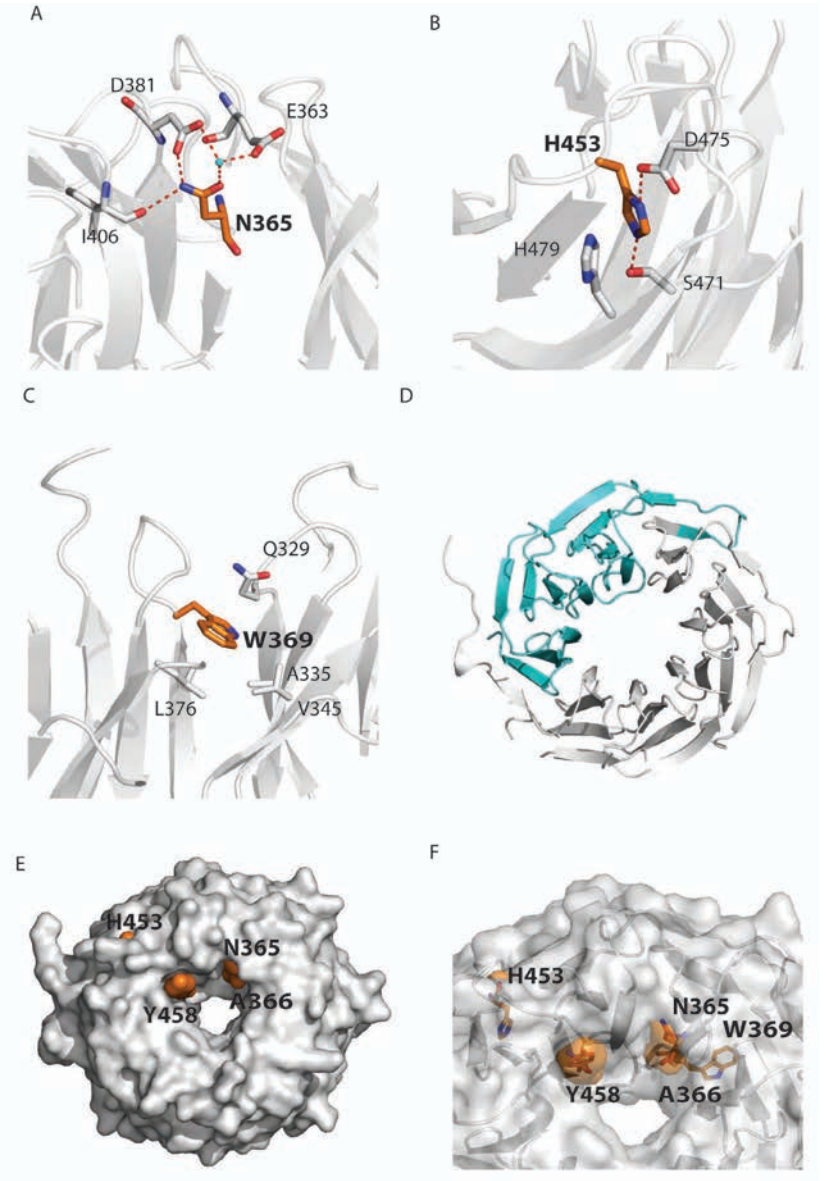

Figure 2. Schematic representations of the mutated amino-acids. The mutated amino-acids are shown on the crystal structure of the TBL1XR1 WD40 domain (PDB ID 4LG9). In each case the mutated residues are shown in orange, TBL1XR1 in grey, and water molecules in the crystal structure in cyan. The numbering of the amino-acids is as for TBL1X; (A) N365; (B) H453; (C) W369; (D) c.1312-1G>A splice mutation with the missing amino-acids in cyan (starting at asterisk). (E) Surface representation of the WD40 domain to show the mutations that are on the surface. (F) Transparent representation of the WD40 domain to show the buried and surface mutations.

Imaging
Brain magnetic resonance imaging performed in six individuals with a mutation and CeH showed normal hypothalamic and pituitary morphology. Adolescent and adult males had a normal testicular size (21). Thyroid ultrasonography of seven out of eight individuals with a mutation who were treated with LT4 showed thyroid volumes below the 2.5th percentile of age-specific reference intervals. Nine of 11 individuals with a mutation who were not treated with LT4 had thyroid volumes below the mean of age-specific reference intervals (22).

Audiometry
Individuals with a mutation had poorer hearing thresholds at high frequencies in PTA than their relatives without a mutation (Figure 4 and Supplemental Table 5), but the difference did not reach statistical significance. In total, 11 of the 15 individuals with mutations evaluated with PTA had hearing thresholds poorer than the age-specific reference interval (23). There was no correlation between the severity of the hearing loss and FT4 concentrations.

Of the three children evaluated with otoacoustic emission testing, one proband had poorer hearing thresholds relative to reference ranges, while two relatives with a mutation (one with CeH) had hearing thresholds at the lower limit of normal (24). Transiently evoked otoacoustic emissions and distortion product otoacoustic emissions were analysed in 6-month-old F.II.1, which showed no abnormalities. The main clinical manifestation of hearing loss was having difficulties in following a conversation in a noisy environment.

Table 1. Characteristics of the probands and relatives with a *TBL1X* mutation identified by Sanger sequencing.

Case	M/F	Nucleotide alteration	Amino-acid altera-tion	Age at diagnosis of CeH (years)	Age at confirma-tion TBL1X mutation (years)	FT4 concen-tration (pmol/L) without treatment (RI, 10-23)	TSH concen-tration (mU/L) without treatment (RI, 0.5-5.0)	BMI	Hearing loss (mHL R/L in dB) (RI)	Thyroid volume on ultra-sound (RI)
A.II.3	M	c.1246A>T	N365Y	-	53	10.2	1.50	21.4 kg/m^2	65/100 (p90: 50.9)[c]	8.1 ml (4.9-19.1)
A.II.6	F	c.1246A>T	N365Y	-	51	10.4	4.30	25.4 kg/m^2	80/95 (p90: 29.5)	8.3 ml (4.9-19.1)
A.III.6	F	c.1246A>T	N365Y	27	29	8.7	2.50	25.5 kg/m^2	55/35 (p90: 13.2)	4.4 ml (4.9-19.1)
A.III.8	M	c.1246A>T	N365Y	2 weeks[a]	25	6.8[b]	1.8	24.3 kg/m^2	60/35 (p90: 16.9)	3 ml (4.9-19.1)
A.IV.1	M	c.1246A>T	N365Y	2 weeks[a]	1.5	6.7 (12-30)	4.0 (1.7-7.9)	-1.2 SDS	15/15 (<16)	0 ml[e] (p2.5: 1)
A.IV.2	F	c.1246A>T	N365Y	-	1.5	15.4	1.90	+0.4 SDS	20-30 (<30)[d]	1.8 ml (p50: 2)
B.II.4	F	c.1510C>T	H453Y	-	42	16.3	3.0	44.4 kg/m^2	35/45 (p90: 19.5)	x
B.III.4	M	c.1510C>T	H453Y	2 weeks[a]	2	11.2 (12-30)	6.8(1.7-7.9)	+0.6 SDS	35/35 (<16)	0.9 ml (p2.5: 1)
C.I.1	M	c.1249G>A	A366T	-	50	13.5	1.70	24.8 kg/m^2	35/35 (p90: 46.4)	9.3 ml (4.9-19.1)
C.II.1	F	c.1249G>A	A366T	14	15	6.8	2.4	+2.1 SDS	35/25 (<16)	4.9 ml (4.9-19.1)
C.II.2	F	c.1249G>A	A366T	-	13	11.9	2.7	+1.5 SDS	20/20 (<16)	5.2 ml (p50: 7.4)
C.II.3	F	c.1249G>A	A366T	-	10	12.5	1.9	-0.1 SDS	20/25 (<16)	3.9 ml (p50: 5.2)
D.II.2	F	c.1526A>G	Y458C	-	47	14.3	0.44	33.1 kg/m^2	x	x
D.III.1	M	c.1526A>G	Y458C	6	16	7.8	1.2	+4.1 SDS	15/12 (<16)	3.3 ml (4.9-19.1)

[a], detected by neonatal screening; [b], determined at the age of 3 months; [c], hearing loss on left side partly caused by acoustic neuroma; [d], evaluated in free-field situation; [e], thyroid tissue too small to measure reliably; -, central hypothyroidism not present. Abbreviations: BMI, body mass index; CeH, central hypothyroidism; dB, decibel F, female; FT4, free thyroxine; kg/m^2, kilogram per square meter; L, left; M, male; mHL, maximum hearing loss; ml, milliliter; R, right; SDS, standard deviation score; TSH, thyroid stimulating hormone; T4, thyroxine; x, missing value; -, not applicable. Reference interval for FT4: 10-23 pmol/L, in neonates: 12-30 pmol/L, for TSH: 0.5-5 mU/L. Reference interval for TSH in neonates 1.7-7.9 mU/L.(45) BMI is expressed as kg/m^2 or SDS calculated with Dutch reference data.(20) Reference intervals for hearing loss for age.(24, 46) Reference intervals for thyroid size for age (17-19).

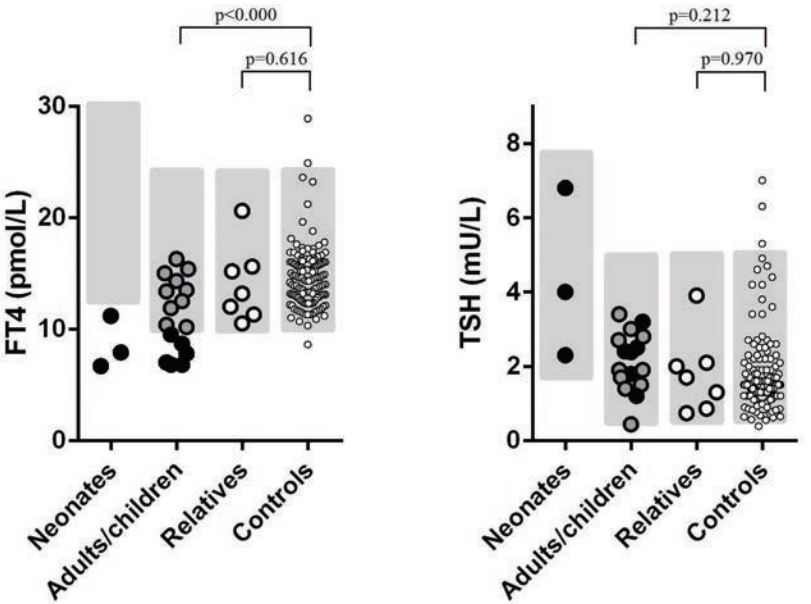

Figure 3. Graphic representation of plasma FT4 and TSH concentrations. Plasma FT4 (left panel) and TSH concentrations (right panel) in untreated condition. Neonates: neonates with a *TBL1X* mutation; Adults/children: adults and children with a *TBL1X* mutation (black filled symbols: diagnosed with CeH, grey filled symbols: biochemically euthyroid); Relatives: adult relatives without a *TBL1X* mutation; Controls: adult controls. The grey shaded areas denote the reference intervals. Reference interval for FT4: 10-23 pmol/L, in neonates: 12-30 pmol/L, for TSH: 0.5-5 mU/L, in neonates 1.7-7.9 mU/L. The adult controls (N = 136) were recruited for earlier studies for the express purpose of establishing reference intervals. They were all healthy subjects, not suspected of an endocrine disorder.

Liver enzymes and lipids

Liver enzymes were normal in all individuals with mutations except D.III.1, who showed slight elevation most likely caused by obesity (Supplemental Table 7). Low-density lipoprotein or total cholesterol concentrations were above the upper limit of age specific reference intervals in one of the eight evaluated individuals with a mutation and CeH, and in five of the nine evaluated individuals with a mutation without CeH (Supplemental Table 7). Since family C had a positive family history of early cardiovascular disease, C.II.2 was screened for familial hypercholesterolemia, which did not demonstrate mutations in the *LDLR* and the *APOB* genes.

Protein structural analyses

To investigate the functional consequences of the mutations in families A-D in the TBL1X WD40 domains, we expressed isolated WD40 domains and the full-length proteins. We also coexpressed full-length proteins with interaction partners GPS2/SMRT and HDAC3 (Figure 5).

TBL1X proteins containing the mutation N365Y or H453Y, were poorly expressed compared to wild-type protein, and were associated with elevated expression of the chaperone heat shock protein 70 kDa, suggesting that these mutations result in aberrant protein folding or stability. This fits well with their largely buried location in the structure of the WD40 domain, such that mutation to a larger tyrosine side chain cannot be tolerated. Due to the poor expression of the N365Y and H453Y proteins we were unable to further analyse their structure and functional characteristics. We note that the entirely

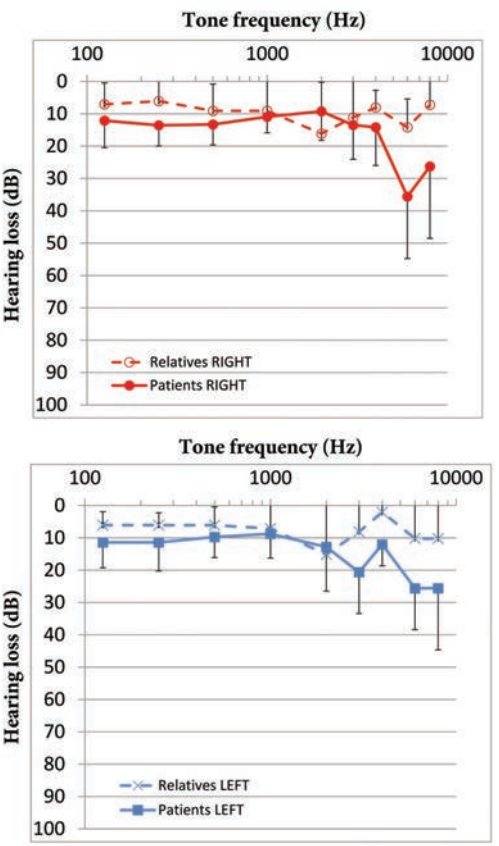

Figure 4. Mean and standard deviation of decibels hearing loss. Hearing loss per frequency in the right (left panel) and left ear (right panel) of individuals with a mutation (solid line), and relatives without a mutation (dashed line). Abbreviations: Hz, hertz; dB, decibel.

buried location of W369 would strongly suggest that mutation of this residue to an arginine would have a strongly deleterious effect on protein folding and stability.

In contrast, in all three contexts wild-type TBL1X and TBL1X bearing the A366T and Y458C mutations were readily expressed and purified, either in isolation or in complex with partner proteins.

To investigate whether the A366T and Y458C mutations result in an altered protein structure or stability, we performed circular dichroism (CD) monitored thermal denaturation studies of the WD40 domains (Figure 5). The wild-type, and A366T and Y458C mutant WD40 domains showed CD spectra characteristic of their largely β-sheet structure. Both wild-type and A366T mutant WD40 domains underwent a cooperative denaturation at 70°C. Together these data suggest that this protein is correctly folded. In contrast, the Y458C mutation appeared to undergo thermal denaturation at a lower temperature, suggesting reduced thermal stability that may in part be responsible for its impaired biological function. We also performed a proteomic analysis of proteins that copurified with the A366T and Y458C mutant proteins and compared this with wild-type protein. In all cases the proteins associated with endogenous proteins known to be part of the corepressor complex. The consistent difference between the A366T and Y458C mutants and wild-type proteins was that the mutants showed association with cytoplasmic cytoskeletal proteins suggesting that the relative nuclear versus cytoplasmic localization may be perturbed.

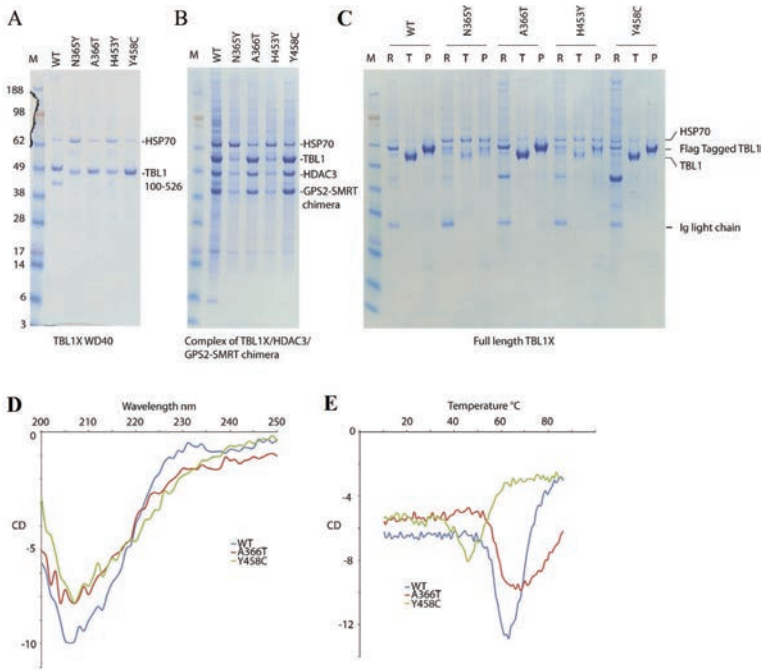

Figure 5. Transient expression in HEK293 cells and small scale purification of wild type and mutated TBL1X. (A) SDS-PAGE of the purification of the isolated wild type TBL1X and mutant WD40 domains. (B) SDS-PAGE of the purification of the wild type and mutant TBL1X/HDAC3/GPS2-SMRT chimera complex. (C) Transient expression in HEK293 cells and small scale purification of wild type and mutated full length TBL1X. SDS-PAGE of the purification of the TBL1X, R is the resin, T is the TEV eluted protein and P is the peptide-eluted protein. (D) CD spectra from 250 to 200 nm at 20°C of wild type, A366T and Y458C TBL1X WD40 domains. (E) Melting curves at 215 nm from 10 to 90°C of wild type, A366T and Y458C TBL1X WD40 domains

RT-PCR, Western blotting and immunocytochemistry

TBL1X mRNA expression was present in the pituitary and in hypothalamic nuclei (suprachiasmatic nucleus, supraoptic nucleus, paraventricular nucleus, infundibular nucleus and lateral hypothalamic area) of each hypothalamus studied (Figure 6A). A Western blot showed clear bands in the pituitary (n = 2) and hypothalamus (n = 2) at the expected height (55 kDa) for TBL1X (Figure 6B). TBL1X immunostaining was present throughout the hypothalamic gray. In particular, prominent neuronal staining was present in the paraventricular and supraoptic nucleus (Figure 6C).

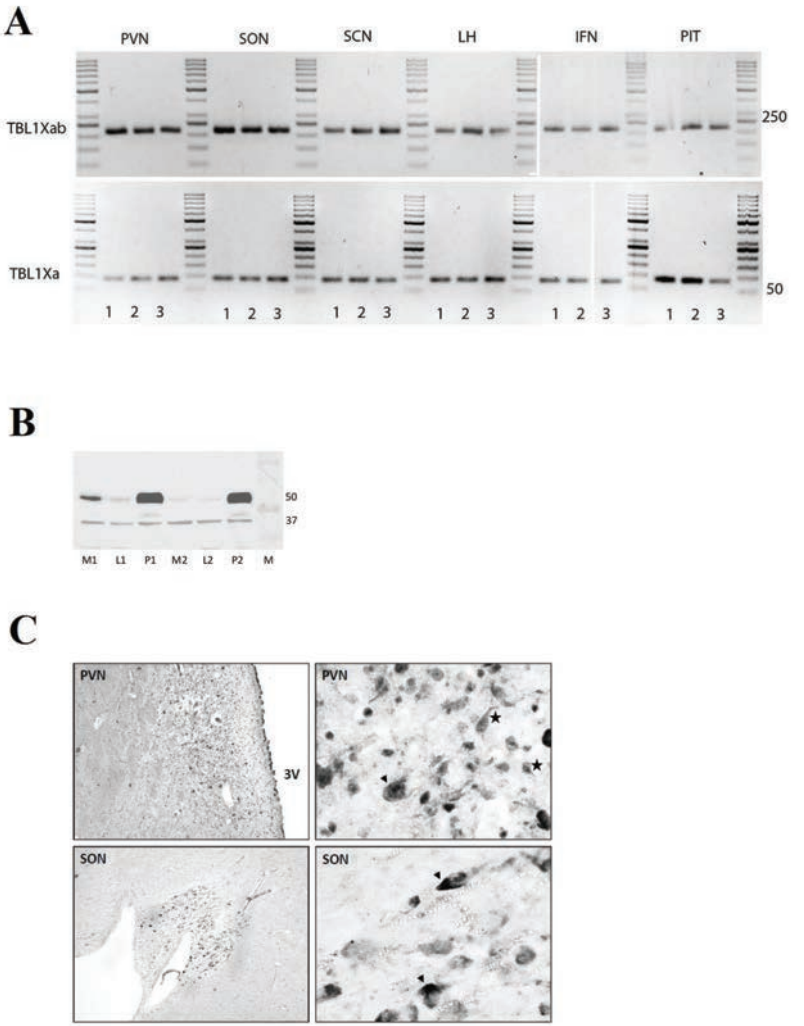

Figure 6. Expression of TBL1X in human brain tissue. (A) TBL1X variants TBL1Xa and TBL1Xab transcripts PCR product of three subjects (1, 2 and 3) on 2% agarose gel. The expected products are 83 bp (TBL1Xa) and 216 bp (TBL1Xab). Abbreviations: INF, infundibular nucleus; LH, lateral hypothalamus; PIT, pituitary; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; SON, supraoptic nucleus. (B) Western blot of TBL1X (55 kD) in the median hypothalamus (M), lateral hypothalamus (L) and pituitary (P) of two subjects (1 = 97-90 and 2 = 97-235; see Supplemental Table 8 for further details). Beta-actin is used as a housekeeping protein (37 kD), the blot contains 10 µg protein of each sample. (C) Representative TBL1X immunostaining in the hypothalamus of subject 2013-083. Arrowheads: magnocellular neurons, stars: parvocellular neurons. Abbreviations: PVN, paraventricular nucleus; SON, supraoptic nucleus; 3V, third ventricle. In the left upper panel, the PVN is visible just lateral to the third ventricle. The higher magnification shows darkly stained, mostly parvocellular and magnocellular neurons. The left lower panel shows an overview of the SON, the higher magnification in the right lower panel shows darkly staining, mostly magnocellular neurons.

Discussion

In this study we identified six missense mutations in *TBL1X* in eight patients (six males) from six families previously diagnosed with CeH, and in 11 of their relatives (two males). Only one of these relatives met the biochemical criteria of CeH (E.II.2). The other 10 relatives had plasma FT4 concentrations in the lower half of the reference interval. As a group, the 16 adult mutation carriers had 20 to 25% lower plasma FT4 concentrations than controls, whereas relatives without a mutation had FT4 concentrations similar to controls. All individuals with a mutation and CeH had a very small thyroid gland, similar to patients with *TSHB* and *IGSF1* mutations (25, 26). In combination with these very small thyroid glands and relatively low thyroid volumes in individuals with a mutation without CeH, the lowered FT4 concentrations are suggestive of longstanding lower-than-normal TSH stimulation. *TBL1X* has not been associated with CeH or lower than average FT4 concentrations before. In addition, 12 of 19 evaluated individuals with a mutation had mild to profound hearing loss.

TBL1X consists of an N-terminal tetramerization domain and a WD40 domain. Whereas the tetramerization domain mediates assembly of the NCoR/SMRT corepressor complex (27), the WD40 domain is thought to be involved in mediating interaction of the complex with chromatin, enabling efficient histone deacetylation (28, 29). All mutations are in the WD40-repeat domains of TBL1X. Our protein studies suggest that the N365Y and H453Y mutations impair the folding or stability of the WD40 domain. Although the A366T and Y458C behaved very similar to the wild-type protein, the mutant's association with cytoplasmic cytoskeletal proteins suggests that the relative nuclear versus cytoplasmic localization may be perturbed. We speculate that the A366T and Y458C mutations impair interaction with partner proteins or chromatin. Taken together, these studies suggest that these four mutations alter the structural and functional properties of TBL1X.
We found *TBL1X* mRNA and TBL1X protein expression in the hypothalamic paraventricular nucleus (PVN) and pituitary gland. The prominent expression of TBL1X in the parvocellular neurons of the PVN suggests coexpression with TRH (30).

TBL1X is an essential subunit of the NCoR/SMRT complex, the major TH receptor (TR) corepressor (CoR) involved in T3-regulated gene expression. This complex mediates the ability of the TR to repress the transcription of positively regulated T3 target genes in the absence of T3 (6). CoRs are additionally known to enhance TR mediated basal activation of negatively regulated genes (such as TRH and TSHB) in the absence of T3, although the exact mechanism is only partly understood (7). In mice expressing a mutated NCoR protein resulting in a defective NCoR/SMRT complex, serum TH concentrations were decreased by 30%, whereas TSH was normal (6). This implies that the NCoR/SMRT complex is essential for adequate HPT axis regulation. Similar defective NCoR/SMRT complex functioning may very well be the underlying mechanism of the lowered FT4 concentrations in individuals with a mutation. We propose that a defective NCoR/SMRT complex is less able to activate transcription of negatively regulated genes in the absence of T3, resulting in decreased *TRH* and *TSHB* transcription, ultimately leading to decreased TH synthesis.

Because not all individuals with a mutation had CeH, one may hypothesize that mutations in genes other than *TBL1X* might be causally involved. We checked this by WES targeted for genes known or presumed to be involved in HPT axis sequencing (Supplemental Table 2C). In three of six patients with CeH and a mutation in *TBL1X,* WES demonstrated variants in such genes (Supplemental Table 2, A and B). However, these variants did not cosegregate with the CeH phenotype and were also present in individuals with a *TBL1X* mutation and a low normal FT4 concentration. This makes it unlikely that those variants are involved in the pathogenesis of the observed CeH. An alternative explanation for the wide range of FT4 concentrations of TBL1X mutation carriers may be that every healthy individual has his/her own optimal and stable FT4 concentration within the population reference interval (also known as the individual "set point" (31-33)), determined by both genetic and environmental factors. In the present series of patients the *TBL1X* mutations result on average in a 3-4 pmol/L lower FT4 concentration. One may hypothesize that without a mutation in TBL1X the CeH patients might not have had CeH, but a FT4 concentration in the lower tertile of the population reference interval. Their relatives would have had values dispersed normally within this reference interval. Finally, although a skewed X-inactivation was not present in peripheral mononuclear cells, it may be present in other tissues. A varying expression of TBL1X in the hypothalamus may also have caused the observed variation in phenotype.

Although the normal TSH concentrations in the CeH patients may seem unexpected at first sight, this is commonly seen in CeH (3-5) and may be explained by altered glycosylation of the TSH protein resulting in diminished bioactivity (1).
Hearing loss was previously reported in two unrelated patients with partial deletion of *TBL1X* (7, 8). However, the cause of the observed sensorineural hearing loss is unclear. Given the expression of TBL1X in mouse cochlea (7), a mutated TBL1X protein may have local detrimental effects on cochlear function or its development. Mutations in another subunit of the NCoR/SMRT complex, TBL1XR1, were found to cause hearing loss as well (34). Alternatively, because TH plays a crucial role in fetal inner ear maturation (35), the hearing loss may have resulted from the congenital hypothyroidism *per se.* However, the relatively mild hypothyroidism and lack of correlation of the severity of hearing loss with FT4 concentrations may argue against this.

In mice, loss of hepatic TBL1X was found to result in hepatic hypertriglyceridemia and steatosis (9). None of the present individuals with a mutation had hypertriglyceridemia or signs of hepatic steatosis. Because 6 of 17 had hypercholesterolemia, at this point we cannot exclude that hypercholesterolemia is part of the *TBL1X* mutation phenotype. In a genome-wide association study, *TBL1X* was identified as a candidate gene for male autism spectrum disorder (36), but none of present patients had been diagnosed with autism spectrum disorder.

An important question is whether the lowered plasma FT4 concentrations in individuals with *TBL1X* mutations, especially in those biochemically classified as having CeH, result in hypothyroidism at the level of TH target tissues. Earlier studies in mice expressing mutated NCoR suggested increased sensitivity to TH in peripheral tissues (6), and it is tempting to speculate that the same mechanism is present in individuals with a

TBL1X mutation. This might also explain why patients who were diagnosed at a later age developed well intellectually and reached normal adult heights. Another intriguing question is whether the defective NCoR/SMRT corepressor complex function has consequences for the intrinsic action of other nuclear receptors, such as retinoic acid receptor and retinoid X receptor.

In conclusion, we demonstrate that mutations in *TBL1X* are associated with CeH and hearing loss. At this point it remains unclear whether these patients display hypothyroidism at the tissue level. Further studies are clearly needed to address this issue.

Acknowledgements

The authors are grateful for the excellent help and advice of E. Endert, Laboratory of Endocrinology at the AMC. We thank dr. J. Kwakkel for excellent assistance with the RT-PCR. This work was financially supported by an AMC Foundation grant. JWRS is supported by a Senior Investigator Award WT100237 from the Wellcome Trust and a Biotechnology and Biological Sciences Research Council Project Grant BB/J009598/1. JWRS is a Royal Society Wolfson Research Merit Award Holder. We thank the patients and their families who kindly consented to participate.

Supplemental material

Phenotyping

Endocrine measurements

The patients and their relatives with and without a mutation were studied in detail in the Academic Medical Centre Amsterdam, the Erasmus University Medical Centre, and the Leiden University Medical Centre.

Free thyroxine (FT4), TSH and prolactin concentrations were measured by fluoroimmunoassay using the Delfia 1232 Fluorometer (PerkinElmer), thyroxine (T4), triiodothyronine (T3) and reverse T3 (rT3) by an in-house RIA. Adrenocorticotropic hormone (ACTH), cortisol and insulin like growth factor 1 (IGF-1) were measured by a chemoluminescence assay (Immulite 2000; Siemens). Plasma luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were measured with a Roche E170. Plasma testosterone was measured by liquid chromatography tandem mass spectrometry, while 17β-estradiol was measured by RIA (Siemens). Liver enzymes, cholesterol (including the fractions), triglycerides and glucose were measured with routine clinical chemical methods.

FT4 concentrations of the patients and relatives were compared with a large control group consisting of 136 adult men and women.

mRNA expression and immunostaining

mRNA expression

Serial, coronal 50 µm sections were cut from unfixed frozen hypothalami on a cryostat, and the areas of the paraventricular nucleus, suprachiasmatic nucleus, supraoptic nucleus, infundibular nucleus and lateral hypothalamic area were macroscopically dissected as previously described (37). From each area separately, the sections were collected and stored at -80 °C until processing. RNA was extracted from these nuclei and from the pituitary using TriReagent (Sigma) following the manufacturer's instructions and re-suspended in 10 microliter nuclease-free water. RNA concentration was measured on the Nanodrop (Nanodrop, Wilmington, Delaware USA), followed by DNAse treatment (Qiagen GmbH, Germany). cDNA was synthesized with an Applied Biosystem Kit and from every sample a –RT reaction was performed in order to check for genomic DNA contamination. The TBL1X gene has four splice variants which encode for protein isoform a (TBL1Xa) and b (TBL1Xb). Primers were designed to amplify isoform a [NM_005647.3 (splice variant 1) and NM_001139466.1 (splice variant 2), F: 5'-CTCTTCATCGTGCTGCCACC-3, R: 5'-CTCTCCCTCGCAAACGTTGA-3', product size 83 base pairs (bp), annealing temperature 65ºC], and both protein isoforms [NM_005647.3 (splice variant 1), NM_001139466.1 (splice variant 2), NM_001139467.1 (splice variant 3) and NM_001139468.1 (splice variant 4), F: 5'-AACAGGCTCTGTGATGGCTG-3', R: 5'-GGGATTACAAAGTTGCGCGT-3', product size 216 bp, annealing temperature 65ºC]. Real-time PCR was performed using the Lightcycler480 and Lightcycler480SybrGreen I Master mix (Roche Molecular Biochemicals, Mannheim, Germany). Melting curve analysis was performed and product

size was checked via DNA gel analysis. All samples contain mRNA as checked by HPRT expression (hypoxanthine phosphoribosyl transferase, a housekeeping gene) (37).

TBL1X immunocytochemistry
After paraformaldehyde (4%) fixation (2 weeks) and immersion in 30% sucrose [in PBS and 0,04% Na-azide] at 4°C, serial coronal sections (50 µm) were cut on a cryostate from the level of the lamina terminalis to the mammillary bodies. In order to be able to systematically map TBL1X expression throughout the hypothalamus, sections for TBL1X staining were selected at regular intervals (500 µm) over the entire rostro-caudal axis. TBL1X was stained free-floating according to the ABC method. Briefly, sections were rinsed in aqua dest and washed in Tris buffered saline (TBS; 0.05M Tris, 0.15M NaCl, pH 7.6; 2x10 min). Endogenous peroxidase was blocked by incubation for 10 min in 10% methanol and 3% H_2O_2. After rinsing in TBS (3x10 min), antigen was retrieved by incubation in TBS for 10 min at 90°C followed by cooling on ice for 15 min. Subsequently, sections were pre-incubated in 2% (w/v) milk-supermix (Elk Milk powder, Campina, The Netherlands, supermix - 0.05 M Tris, 0.15 M NaCl, 0.25% gelatine and 0.5% Triton X-100 at pH 7.6) for 1 h at room temperature (RT).

Sections were incubated with mouse monoclonal TBL1X antiserum (sc-365661, Santa Cruz Biotechnology) 1:100 in 2% milk-supermix overnight at 4°C. The next day, the sections were washed in TBS (3x10 min) and subsequently incubated with biotinylated horse anti-mouse IgG antibody (BA2000, Vector Laboratories, Peterborough, UK, 1:400 in supermix) for 1h at RT. After washing in TBS (2x10 min) the sections were incubated with ABC-elite (avidine-biotine complex, Vector Laboratories, Peterborough, UK, 1:800 in supermix) for 1h at RT and subsequently rinsed in TBS (2x10 min). Finally, sections were incubated in 0.5 mg/ml 3,3'-diaminobezidine (DAB, Sigma) in TBS containing 0.2%(w/v) ammonium nickel sulphate (BDH, Brunschwig, Amsterdam, The Netherlands) and 0.01%(v/v) H_2O_2 (Merck, Darmstadt, Germany) for approximately 7 min. The reaction was stopped in aqua dest. The sections were mounted on Menzel superfrost plus glass and cover-slipped using Entellan (Merck, Darmstadt, Germany). Antiserum specificity was tested using Western blotting.

Western blotting
Western blotting was performed on homogenates of human pituitary glands (n=2), the paraventricular region of the hypothalamus (median hypothalamus) (n=2) and the lateral region of the hypothalamus (lateral hypothalamus) (n=2). In short, frozen samples from pituitary, median hypothalamus and lateral hypothalamus were homogenized in RIPA buffer [50 mM Tris HCl pH 7.6, 150 mM NaCl, 1% Triton X100, 0.5% Sodium Desoxycholate, 0.1% SDS,2 mM EDTA, Phosphostop and protease inhibitor] and incubated for 30 min on ice. After centrifugation [20 min at 16000 rpm, 4°C], a sample was taken for protein measurement and the remaining was mixed with loading buffer [5% SDS, 2% 2-mercaptoethanol, 20% glycerol, 0.004% bromophenol blue, 0.125 M Tris-HCl,

1.4% DTT]. Samples were denatured before loading on 7.5% stacking/running gel. After running, the gels were blotted to a PVDF membrane.

The blots were washed in TBS-T and incubated with TBS-T 5% milk for 1h at RT, followed by TBL1X antibody in supermix (1:100) for 1h at RT and overnight at 4°C. The next day the blots were washed in TBS-T, incubated with the second antibody in TBS-T (polyclonal goat anti mouse HRP, Dako, Denemark) and washed. For detection we used Amersham ELC prime WB detection Reagent (GE healthcare). The blot was scanned on an ImageQuant™ LAS 4000. As a negative control the same protein samples were run, blotted in the same run, and incubated in a similar way, except that the incubation with the first antibody was omitted.

TBL1X wild type and mutant expression in transfected cells
HEK293F cells (Invitrogen) were co-transfected with mixtures of both tagged and untagged constructs using polyethylenimine (PEI) (Sigma). To transfect cells, 60 µg DNA total was diluted in 6 ml of PBS (Sigma) and vortexed briefly; 240 µl of 0.5 mg/ml PEI was added, and the suspension was vortexed briefly, incubated for 20 min. at room temperature, then added to 60 ml cells (final density was 1 x 10^6 cells per ml). Cells were harvested 48 h after transfection. For the interaction studies the cells were lysed by sonication in buffer containing 50 mM Tris/Cl pH 7.5, 100 mM potassium acetate, 5% v/v glycerol, 0.3% v/v Triton X-100, and Roche complete protease inhibitor (buffer A); the insoluble material was removed by centrifugation. The complex was then bound to Flag resin (Sigma), washed three times with buffer A, three times with buffer B (50 mM Tris/Cl pH 7.5, 300 mM potassium acetate, 5% v/v glycerol) and three times with buffer C (50 mM Tris/Cl pH 7.5, 50 mM potassium acetate, 5% v/v glycerol, 0.5 mM TCEP). The complex was eluted from the resin by overnight cleavage at 4 °C with TEV protease in buffer C.

Circular dichroism experiments
For the circular dichroism experiments large-scale transient transfections of the TBL1X WD40 domains were performed. The WD40 domains were further purified by gel filtration on a Superdex S-200 gel filtration column in 50 mM Tris/Cl pH 8, 150 mM NaCl, 0.5 mM TCEP after the Flag purification. Circular dichroism spectra were measured from 200-250 nm as the sample temperature was increased from 10-90°C (1°C per minute).

X-exome family A
An overview of the X-exome sequencing results is summarized in Table S1. 250bp paired end reads were generated in the Illumina MiSeq. More than 99.9% of the reads could be mapped back to the reference genome hg19. For each sample, the probe region with no coverage was less than 10%.

Supplemental Table 1. Overview of the X-exome sequencing results (family A).

	A.I.1	A.II.6	A.III.6	A.III.8	A.IV.1
	6-D709	3-D708	4-D712	2-D711	5-D710
Read length	250	250	250	250	250
Read number	1979652	2741064	2551444	2956518	2247606
Aligned reads	1978400	2739518	2549795	2954321	2245781
%aligned reads	0.999368	0.999436	0.999354	0.999257	0.999188
%duplication	0.019037	0.026781	0.018725	0.02625	0.028565
Mean bait coverage	13.224434	30.232479	28.578918	20.702005	15.876056
%zero coverage target	0.091229	0.063821	0.064605	0.072696	0.081049
%target bases 10X	0.615479	0.82638	0.826721	0.524983	0.70178

Supplemental Table 2A. Variants shared between the three affected members of family A

Gene	Coding effect	Protein alteration	OMIM reference	SIFT score	PolyPhen2	Inheritance	Gene-Phenotype relationship
HADHA	Nonsynonymous	p.E510Q	600890	0	Probably damaging	Recessive	Long-chain 3-hydroxylacyl-CoA dehydrogenase deficiency
SH3RF3	Nonsynonymous	p.K487N	**x**	0	Probably damaging	x	Unknown function
SYNPR	Nonsynonymous	p.L20F	**x**	0	Probably damaging	x	Unknown function
GLTSCR1L	Nonsynonymous	p.K1012R	**x**	0	Probably damaging	x	Unknown function
CYP39A1	Nonsynonymous	p.G410R	605994	0	Probably damaging	x	Involved in bile acid metabolism
RFPL4B	Nonsynonymous	p.G144S	**x**	0	Probably damaging	x	Unknown function
SLC6A5	Nonsynonymous	p.R769H	604159	0.01	Probably damaging	Recessive/ dominant	Hyperekplexia 3
CEP290	Nonsynonymous	p.R1746W	610142	0.01	Probably damaging	Recessive	Bardet-Biedl syndrome, Joubert syndrome, Leber congenital amaurosis, Meckel syndrome, Senior-Løken syndrome

x, not registered in OMIM

Supplemental Table 2B. Variants in a list of genes involved in HPT-axis functioning in any proband

Gene	Coding effect	Protein alteration	OMIM reference	SIFT score	PolyPhen2	Inheritance	Gene-Phenotype relationship	Proband	Individuals with the TBL1X mutation, with a second variant	Individuals with the TBL1X mutation, without a second variant
THRB	Non-synonymous	p.R120H	190160	0.033	Probably damaging	Recessive/ dominant	Thyroid hormone resistance	B.III.4	-	-
BRD8	Non-synonymous	p.K426Q	602848	0.001	Probably damaging	x	Enhances thyroid hormone-dependent activation of a reporter construct.	B.III.4	B.II.4	None
GLI2	Non-synonymous	p.D1520N	165230	0.001	Probably damaging	Dominant	Culler-Jones syndrome, Holoprosencephaly	A.III.8	-	-
GLI3	Non-synonymous	p.T319M	165240	0.002	Probably damaging/ Possibly damaging	Dominant	Greig cephalopolysyndactyly syndrome, Pallister-Hall syndrome, polydactyly postaxial types A1, B, preaxial type IV	B.III.4	-	-
NCOA6 (=TRBP)	Non-synonymous	p.G848S	605299	0	Possibly damaging	x	Enhances thyroid hormone-dependent activation of a reporter construct.	D.III.1	None	D.II.2
MED15	Non-synonymous	p.G86R	607372	x	x	x	Mediates thyroid hormone-dependent activation by the thyroid hormone receptor (TR) or a TR-associated protein	A.III.8	A.IV.2	A.II.3 A.II.6

x, not registered in OMIM; -, not applicable

Supplemental Table 2C. Genes that are known or presumed to be involved in HPT axis functioning based on their position in biological pathways, expression or animal models.

Gene	Location	Protein	Protein function	Evidence
IGSF1*	Xq26.1	Immunoglobulin superfamily memb. 1	Unknown	1
PAX6*	11p13	Paired box protein Pax-6	Development pituitary	1
OTX2*	14p22.3	Orthodenticle homolog 2	Development pituitary	1
HESX1*	3p14.3	Homeobox expressed in ES cells 1	Development pituitary	1
SOX2*	3q26.33	Sex determining region Y (SRY) box 2	Development pituitary	1
SOX3*	Xq27.1	Sex determining region Y (SRY) box 3	Development pituitary	1
GLI2*	12q13.3	Zinc finger protein GLI2	Development pituitary	1
LHX4*	1q25.2	LIM/Homeobox protein 4	Development pituitary	1
FGFR1*	8p11.22	Fibroblast growth factor receptor 1	Development pituitary	1
ARNT2*	15q24	Aryl hydrocarbon receptor nuclear translocator 2	Development pituitary	1
LEPR*	1p31.3	Leptin receptor	Development pituitary	1
LEP*	7q32.1	Leptin	Development pituitary	1
SLC16A2	Xq13.2	Monocarboxylase transporter 8 (MCT8)	T3 transporter	1
SECISBP2	9q22.2	SECIS-binding protein 2	Subunit deiodinases	1
DIO1	1p33-p32	Iodothyronine deiodinases type 1	TH (in)activation	2
DIO2	14q24.3	Iodothyronine deiodinases type 2	TH activation	2
DIO3	14q32	Iodothyronine deiodinases type 3	TH inactivation	2
WSB1	17q11.1	WSB1, SWiP-1	D2 inactivation	4
MARCH6	5p15.2	TEB4	D2 inactivation	4
USP33	1p31.1	VDU-1	D2 activation	4
THRA	17q21.1	Thyroid hormone receptors α1 and α2	TH receptor	2
THRB	3p24.2	Thyroid hormone receptors β1 and β2	TH receptor	2
NCoR1	17p11.2	Nuclear receptor corepressor 1	Corepressor (NCoR)	2
NCoR2	12q24.31	SMRT	Corepressor (NCoR)	3
TBL1X	Xp22.3	Transducin (beta)-like 1X-linked	Corepressor (NCoR)	1
TBL1XR1	3q26.32	Transducin (beta)-like related	Corepressor (NCoR)	4
GPS2	17p13	G-protein pathway suppressor 2	Corepressor (NCoR)	3
HDAC3	5q31.3	Histone deacetylase 3	Corepressor (NCoR)	3
CORO2A	9q22.33	Coronin actin binding protein 2A	Corepressor (NCoR)	3
ZBTB33	Xq23	Kaiso	Corepressor (NCoR)	3
Sin3A	15q24.2	Sin3A	Corepressor (Sin)	3
Sin3B	19p13.11	Sin3B	Corepressor (Sin)	3
HDAC1	1p35.1	Histone deacetylase 1	Corepressor (Sin)	3
HDAC2	6q21	Histone deacetylase 2	Corepressor (Sin)	3
RBBP4	1p35.1	Retinoblastoma binding protein 4	Corepressor (Sin)	5
RBBP7	Xp22.2	Retinoblastoma binding protein 7	Corepressor (Sin)	5
SAP18	13q12.11	Sin associated protein 18	Corepressor (Sin)	5
SUDS3	12q24.23	Suppressor of defective silencing 3	Corepressor (Sin)	5
RBP1	3q23	Retinol binding protein 1	Corepressor (Sin)	5
BRMS1	11q13.1	Breast cancer metastasis suppressor 1	Corepressor (Sin)	5
SAP130	2q14.3	Sin associated protein 130	Corepressor (Sin)	5
ARID4B	1q42.3	Sin associated protein 180 (SAP180)	Corepressor (Sin)	5
ING1	13q34	Inhibitor of growth family 1	Corepressor (Sin)	5
MTA1	14q32.33	Metastasis associated 1	Corepressor (NuRD)	3
MTA2	11q12-q13.1	Metastasis associated 2	Corepressor (NuRD)	3
MBD2	18q21.2	methyl-CpG binding domain protein 2	Corepressor (NuRD)	5
MBD3	19p13.3	methyl-CpG binding domain protein 3	Corepressor (NuRD)	5

Gene	Location	Protein	Protein function	Evidence
CHD3	17p13.1	chromodomain helicase DNA binding protein 3	Corepressor (NuRD)	5
CHD4	12p13.31	chromodomain helicase DNA binding protein 4	Corepressor (NuRD)	3
SHC1	1q21	Src homology 2 domain containing	Corepressor	5
SUV39H1	Xp11.23	Suppressor of variegation 3-9 homolog	Corepressor	4
RPL7A	9q34.2	TRUP	Corepressor	3
C1D	2p14	SUN-CoR	Corepressor	3
NCOA1	2p23.3	SRC-1	Coactivator (SRC)	2
NCOA3	20q13.12	SRC-3	Coactivator (SRC)	3
MED1	17q12	TRAP220	Coactivator (TRAP/SMCC)	2
MED6	14q24.1	MED6; CRSP70	Coactivator (TRAP/SMCC)	5
MED7	5q33.3	MED7	Coactivator (TRAP/SMCC)	5
MED10	5p15.31	NUT10	Coactivator (TRAP/SMCC)	5
MED12	Xq13.1	TRAP230	Coactivator (TRAP/SMCC)	2
MED13	17q23.2	TRAP240	Coactivator (TRAP/SMCC)	3
MED14	Xp11.4-p11.2	TRAP170	Coactivator (TRAP/SMCC)	5
MED15	22q11.21	Positive cofactor 2 (PC2)	Coactivator (USA)	3
MED16	19p13.3	TRAP95	Coactivator (TRAP/SMCC)	5
MED17	11q21	TRAP80	Coactivator (TRAP/SMCC)	5
MED20	6p21.1	TRFP	Coactivator (TRAP/SMCC)	5
MED21	12p12.3-p11.23	SRB7	Coactivator (TRAP/SMCC)	4
MED23	6q23.2	TRAP150	Coactivator (TRAP/SMCC)	5
MED24	17q21.1	TRAP100	Coactivator (TRAP/SMCC)	2
MED27	9q34.1-q34.3	p37	Coactivator (TRAP/SMCC)	5
MED30	8q24.11	TRAP25	Coactivator (TRAP/SMCC)	3
MED31	17p13.2	SOH1	Coactivator (TRAP/SMCC)	3
CCNC	6q16.2	SRB11	Coactivator (TRAP/SMCC)	3
CDK8	13q12.13	SRB10	Coactivator (TRAP/SMCC)	3
SMARCA4	19p13.2	BRG1	Coactivator (SWI/SNF)	3
SMARCB1	22q11.23	BAF47	Coactivator (SWI/SNF)	3
SMARCC1	3p21.31	BAF155	Coactivator (SWI/SNF)	5
SMARCC2	12q13-q14	BAF170	Coactivator (SWI/SNF)	5
SMARCD1	12q13.1	BAF60a	Coactivator (SWI/SNF)	5
SMARCD2	17q23-q24	BAF60b	Coactivator (SWI/SNF)	5
SMARCD3	7q35-q36	BAF60c	Coactivator (SWI/SNF)	5
SMARCE1	17q21.2	BAF57	Coactivator (SWI/SNF)	4
ACTL6A	3q26.33	BAF53a	Coactivator (SWI/SNF)	5
ACTL6B	7q22	BAF53b	Coactivator (SWI/SNF)	5
ACTB	7p22.1	actin-beta	Coactivator (SWI/SNF)	5
ARID1A	1p36.11	BAF250a	Coactivator (SWI/SNF)	3
ARID1B	6q25.3	BAF250b	Coactivator (SWI/SNF)	5
ARID2	12q12	BAF200	Coactivator (SWI/SNF)	5
PBRM1	3p21.1	BAF180	Coactivator (SWI/SNF)	5
SUB1	5p13.3	PC4	Coactivator (USA)	3
PSMC3	11p11.2	TBP-1	Coactivator (TFIID)	3
TAF4	20q13.3	TAFII130/135	Coactivator (TFIID)	4
TAF7	5q31.3	TAFII55	Coactivator (TFIID)	4
KAT2B	3p24.3	PCAF	Coactivator	3
NCoA6	20q11	TRBP	Coactivator	4
TRIP11	14q32.12	TRIP230	Coactivator	4

Gene	Location	Protein	Protein function	Evidence
CARM1	19p13.2	CARM1	Coactivator	3
BRD7	16q12.1	BRD7	Coactivator	3
BRD8	5q31.2	p120	Coactivator	4
ITGB3BP	1p31.3	NRIF3	Coactivator	4
NCoA4	10q11.23	ARA70	Coactivator	5
EIF3I	1p34.1	Trip-1	Coactivator	3
FliI	17p11.2	Flightless-1	Coactivator	3
RBP2	3q23	RBP2	Coactivator	5
NRIP1	21q11.2	RIP140	Coactivator	4
TRH	3q13.3-q21	preproTRH	prohormone TRH	2
PCSK1	5q15-q21	Prohormone convertase 1/3	Prohormone convertase	2
PCSK2	20p11.2	Prohormone convertase 2	Prohormone convertase	2
CPE	4q32.3	Carboxypeptidase E	Prohormone convertase	2
PAM	5q14-q21	peptidylglycine alpha amidating monooxygenase	TRH amidase	2
QPCT	2p22.3	N-glutaminyl cyclase	TRH cyclase	2
TRHDE	12q15-q21	pyroglutamyl peptidase II (PPII)	TRH degradation	4
CGA*	6q12-q21	TSH alpha subunit	TSH alpha subunit	2
TSHB	1p13	TSH beta subunit	TSH beta subunit	1
TRHR	8q23.1	TRH receptor	TRH receptor	1
SSTR2*	17q25.1	Somatostatin receptor 2	Central receptor	5
SSTR5*	16p13.3	Somatostatin receptor 5	Central receptor	5
RXRG	1q22-q23	Retinoid X receptor gamma	TR heterodimer	2
LHX3*	9q34.3	LIM/Homeobox protein 3	aGSU regulator	1
POU1F1*	3p11.2	PIT-1	TSHb regulator	1
PROP1*	5q35.3	Homeobox protein prophet of PIT-1	PIT-1 regulator	1
GATA2*	3q21.3	GATA binding protein	TSHb regulator	2

*, phenotype involves loss of multiple pituitary hormones. Level of evidence: 1, endocrine phenotype described in humans; 2, endocrine phenotype described in animal models; 3, gene knockout, small interfering RNA, or protein antibodies have effect on thyroid hormone regulation in cell constructs; 4, protein associates with thyroid hormone receptors in cell constructs; 5, theory/no evidence

Supplemental Table 3. Characteristics in relatives (male and female) not carrying *TBL1X* mutations.

Case	Sex	Age (yr)	LT4 treatment	FT4 (pmol/L)	TSH (mU/L)	TC (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TG (mmol/L)	BMI	Thyroid volume on ultra-sound (RI)
A.III.13	M	26	-	12.0	1.70	4.52	2.00	0.66*[a]	4.13*[a]	21.9 kg/m^2	x
A.III.15	F	29	-	10.5	2.10	4.52	2.77	1.03	1.61	38.8 kg/m^2	6.0 ml (4.9 – 19.1)
C.I.2	F	48	-	11.3	2.00	5.23	2.81	2.15	0.60	21.8 kg/m^2	15.5 ml (4.9 – 19.1)
D.I.1	M	82	-	15.6	0.74	x	x	x	x	31.4kg/m^2	18.0 ml (4.9 – 19.1)
D.I.2	F	79	-	20.6	1.30	6.65*	4.42*	1.81	0.93	22.7 kg/m^2	5.3 ml (4.9 – 19.1)
D.II.4	F	48	-	15.2	0.86	5.69	3.48	1.17	2.32	36.1 kg/m^2	28.0 ml (4.9 – 19.1)
E.I.1	M	50	-	13.2	3.90	3.1	1.88	0.84	0.9	28.3 kg/m^2	16.6 ml (4.9 – 19.1)

[a]: likely non-fasting; [b]: reference range up to 79 years of age*: abnormal values. Abbreviations: BMI, body mass index; F, female; FT4, free thyroxine; HDL, high-density lipoprotein; LDL , low-density lipoprotein; M, male; mHL, maximum hearing loss; ml, millilitre; RI, reference interval; T4, thyroxin; T3, triiodothyronine; TC, total cholesterol; TG, triglyceride; TSH, thyroid stimulating hormone; yr, years; x, missing value. Reference ranges: FT4: 10-23 pmol/L, TSH: 0.5-5 mU/L, T4 70-150 nmol/L,T3: 1.3-2.7 nmol/L. Adult reference range lipids: total cholesterol 2.9-6.1 mmol/L, LDL 1.2-4.3 mmol/L, HDL 1.0-2.7 mmol/L, triglycerides 0.45-2.60 mmol/L (38). Reference intervals for thyroid size for age (22).

Supplemental Table 4. Endocrine findings in individuals (male and female) carrying *TBL1X* mutations.

Case	Sex	Age (yr)	LT4 treat-ment	Time of blood with-drawal	T4 (nmol/L) without treat-ment	T3 (nmol/L) without treat-ment	rT3 (nmol/L)	ACTH (ng/L)	Cortisol (nmol/L)
A.II.3	M	53	-	14:17	85	1.7	0.18	x	115
A.II.6	F	51	-	08:20	80	2.4	0.33	19	604
A.III.6	F	29	+	08:54	65	1.95	0.22	18	465
A.III.8	M	25	+	07:42	75	2.10	0.30	21	824[c]
A.IV.1	M	1.5	+	08:42	135	2.1[d]	0.28	32	264
A.IV.2	F	1.5	-	[e]	x	x	x	x	x
B.II.4	F	42	-	08:50	138	2.0	0.49	23	196
B.III.4	M	2	+	08:34	116	2.4[d]	0.37	8	298[c]
C.I.1	M	50	-	09:57	95	1.8	0.25	7	192
C.II.1	F	15	+	09:54	110	1.7	0.24	18	301
C.II.2	F	13	-	09:43	85	2.0	0.22	14	231
C.II.3	F	10	-	09:42	95	2.8	0.21	7	166
D.II.2	F	50	-	09:28	95	1.7	0.29	8	255
D.III.1	M	16	+	09:22	50	1.6	0.13	11	159
E.I.2	F	48	-	11:00	90	1.65	0.22	20	331
E.II.1	M	17	+	13:00	75	1.6	0.18	32	309
E.II.2	F	15	-	13:00	85	1.95	0.24	8	229
F.I.2	F	22	-	10:52	75	1.7	0.19	18	235
F.II.1	M	6m	+	15:06	50	2.7[d]	0.37	52	116

[a], analysis during treatment with testosterone (treatment indication unknown); [b], analysis during treatment with estrogens; [c], analysis during treatment with hydrocortisone; [d], measured while under treatment; [e], venous blood withdrawal unsuccessful. Abbreviations: ACTH, adrenocorticotropic hormone; Estr, 17β-estradiol; F, female; FSH, follicle stimulating hormone; GH, growth hormone; IGF-1, insulin-like growth factor-1; LH, luteinizing hormone; M, male; PRL, prolactin; Test, testosterone; T3, triiodothyronine; T4, thyroxin; rT3, reverse T3; x, missing value; yr, years; -, not applicable. Reference intervals for T4: 70-150 nmol/L, T3: 1.3-2.7 nmol/L, rT3: 0.11-0.44 nmol/L, ACTH: 0-55 ng/L, Cortisol: 100-650 nmol/L, PRL: 0-22 µg/L. Reference intervals (females and males, age specific) for LH, FSH, 17β-estradiol and testosterone: between parentheses (39-42). Reference interval (age specific) for IGF-1: between parentheses (43, 44).

PRL (µg/L)	LH (U/L)	FSH (U/L)	Test (nmol/L)	Estr (nmol/L)	IGF-1 (nmol/L)
13.5	4.6 (2.0-10.4)	10.1 (2.0-12.4)	13.0 (9.3-31.3)[a]	-	16 (11-31)
6.3	36 (2.0-26.2)	45.0 (2.2-17.0)	-	0.43 (0.1-1.1) [b]	13 (11-31)
4.8	3 (2.0-26.2)	7.0 (2.2-17.0)	-	0.18 (0.1-1.1)	22 (15-42)
7.8	7 (2.0-9.4)	8.0 (1.4-8.9)	16.1 (11.6-33.8)	-	25 (15-45)
7.4	<1 (<0.1-4.0)	1.6 (<0.1-7.1)	<0.7 (<1.3)	-	9 (4-12)
x	x	x	-	x	x
10.5	4.2 (2.0-26.2)	8.8 (2.2-17.0)		0.09 (0.1-1.1)	9.4 (13-35)
9.8	<0.1 (<0.1-4.0)	1.0 (<0.1-7.1)	<0.1 (<0.5)	-	11 (4-12)
7.0	2.7 (2.0-10.4)	5.5 (2.0-12.4)	17.0 (9.3-31.3)	-	35 (12-33)
17	5.1 (<0.1-16.4)	6.4 (<0.1-11.0)	-	<0.04 (<0.07-0.4)	36 (21-93)
9.0	5.8 (<0.1-13.4)	6.7 (<0.1-12.0)	-	0.04 (<0.07-0.3)	77 (19-119)
8.0	<0.1 (<0.1-5.0)	3.8 (<0.1-4.3)	-	<0.04 (<0.07-0.2)	28 (13-90)
10.5	5.7 (2.0-26.2)	6.07 (2.2-17.0)	-	<0.04 (0.1-1.1)	11 (12-33)
8.0	5.2 (<0.1-3.7)	10.2 (<0.1-8.6)	6.5 (5.5-28.7)	-	32 (17-97)
6.5	22.5 (2.0-26.2)	35.0 (2.2-17.0)	-	<0.02 (0.1-1.1)	28 (12-33)
25.0	3.7 (<0.1-3.7)	2.16 (<0.1-8.6)	14.9 (5.5-28.7)	-	46 (17-97)
10.5	<1.0 (<0.1-16.4)	1.10 (<0.1-11.0)	-	<0.04 (<0.07-0.4)	36 (21-93)
3.0	8.9 (2.0-26.2)	3.46 (2.2-17.0)	-	0.35 (0.1-1.1)	25 (15-42)
150.0	<1.0 (0.1-4.0)	<1.0 (<0.1-7.1)	0.3 (<1.3)	-	3 (4-13)

Supplemental Table 5. TRH stimulation testing before treatment of patients with *TBL1X* mutations.

Case	Sex	Baseline TSH (mU/L)	Baseline FT4 (pmol/L)	Baseline PRL (µg/L)	Peak TSH (mU/L)	Time peak TSH (minutes after TRH infusion)	Peak PRL (µg/L)	Normalised TSH (mU/L)	Time normalised TSH (minutes after TRH infusion)
A.III.6	F	1.50	8.1	x	17.90	30 min	x	5.50[a]	120 min
A.III.8	M	2.00	7.0	11.0	9.60	30 min	33.0	3.70	120 min
A.IV.1	M	4.00	7.8	91.0	11.20	30 min	170.0	3.70	180 min
D.III.1	M	0.64	x	7.5	5.60	30 min	35.4	1.88	120 min
E.II.1	M	1.50	7.0	13.8	10.07	30 min	37.5	3.92	120 min
F.II.1	M	2.20	6.7	150.0	11.30	20 min	265.0	2.60	180 min

[a]. lowest measured TSH concentration. Abbreviations: F, female; FT4, free thyroxine; M, male; min, minutes; PRL, prolactin; TSH, thyroid stimulating hormone; x, missing value

Supplemental Table 6. Hearing thresholds determined by PTA in individuals carrying *TBL1X* mutations and relatives.

Case	Sex	Age (yr)	Status	125 Hz (R/L)	250 Hz (R/L)	500 Hz (R/L)	1000 Hz (R/L)	2000 Hz (R/L)	3000 Hz (R/L)	4000 Hz (R/L)	6000 Hz (R/L)	8000 Hz (R/L)
A.II.3[a]	M	53	Carrier	66.3/66.3	81.3/81.3	80.7/90.7	80.1/80.1	96.4/101.4	105.9/ 105.9	95.4/95.4	x/x	73.1/73.1
A.II.6	F	52	Carrier	21.5/16.5	21.5/16.5	16.0/16.0	10.4/20.4	18.1/38.1	26.3/46.3	14.6/19.6	36.1/46.1	62.7/57.7
A.III.6	F	30	Carrier	19.6/14.6	19.6/19.6	19.5/4.5	9.4/-0.6	9.1/4.1	13.9/8.9	18.7/3.7	53.3/33.3	27.8/37.8
A.III.8	M	26	Carrier	9.8/9.8	14.8/4.8	19.8/9.8	14.7/4.7	14.6/14.6	19.3/24.3	19.0/19.0	58.8/33.8	43.6/28.6
B.II.4	F	42	Carrier	18.3/13.3	13.3/13.3	23.0/23.0	22.7/22.7	21.5/21.5	30.7/25.7	39.8/29.8	13.1/x	11.4/11.4
C.I.1	M	50	Carrier	6.9/1.9	11.9/6.9	16.4/6.4	5.9/-4.1	7.8/-2.2	13.2/23.2	18.6/18.6	x/x	12.5/2.5
C.II.1	F	15	Carrier	10.0/5.0	10.0/5.0	10.0/10.0	10.0/10.0	5.0/5.0	0.1/10.0	0.0/15.0	35.0/25.0	15.0/15.0
C.II.2	F	13	Carrier	10.0/5.0	10.0/10.0	15.0/5.0	10.0/-5.0	10.0/5.0	15.0/5.0	15.0/5.0	x/x	20.0/20.0
C.II.3	F	10	Carrier	5.0/5.0	5.0/0.0	10.0/10.0	10.0/5.0	10.0/5.0	10.0/5.0	10.0/0.0	x/15.0	20.0/25.0
D.II.2	F	51	Carrier	1.7/6.7	11.7/6.7	11.2/6.2	5.6/10.6	18.5/23.5	1.8/21.8	10.2/5.2	11.9/11.9	8.7/23.7
D.III.1	M	17	Carrier	10.0/5.0	5.0/0.0	5.0/5.0	10.0/5.0	10.0/0.0	10.0/0.1	10.0/-5.0	x/x	10.0/5.0
E.I.2	F	48	Carrier	2.3/2.3	2.3/2.3	1.9/1.9	6.4/1.4	-0.4/-0.4	8.3/13.3	1.9/6.9	9.2/14.2	-3.5/-3.5
E.II.1	M	17	Carrier	5.0/10.0	5.0/10.0	10.0/10.0	10.0/20.0	25.0/30.0	25.0/25.0	20.0/25.0	x/x	10.0/15.0
E.II.2	F	15	Carrier	5.0/10.0	5.0/0.0	5.0/0.0	10.0/0.0	5.0/0.0	10.0/5.0	0.0/5.0	10.0/x	20.0/0.0
F.I.2	F	22	Carrier	20.0/25.0	15.0/25.0	14.9/19.9	19.9/14.9	9.9/4.9	24.9/19.9	34.9/14.9	44.8/14.8	29.8/19.8
A.III.13	M	26	Relative	x	x	x	x	x	x	x	x	x
A.III.15	F	29	Relative	-0.4/4.6	-0.4/4.6	4.6/4.6	-0.5/4.5	-0.7/9.3	4.1/4.1	13.9/3.9	13.5/3.5	3.2/-6.8
C.I.2	F	48	Relative	12.3/2.3	12.3/-2.7	11.9/1.9	11.4/1.4	9.6/4.6	8.3/3.3	21.9/6.9	x/x	1.5/-3.5
D.I.1	M	82	Relative	2.7/2.7	7.7/7.7	15.7/15.7	23.6/23.6	41.3/41.3	32.9/17.9	9.5/-0.5	26.3/1.3	-10.1/-0.1
D.I.2	F	79	Relative	8.8/8.8	3.8/3.8	-3.0/2.0	0.1/-4.9	7.7/7.7	-2.9/-2.9	1.5/1.5	5.3/10.3	-0.8/9.2
D.II.4	F	48	Relative	7.3/2.3	2.3/2.3	16.9/1.9	6.4/6.4	19.6/19.6	13.3/18.3	11.9/11.9	19.2/34.2	41.5/51.5
E.I.1	M	50	Relative	16.9/11.9	16.9/11.9	11.4/6.4	15.9/5.9	12.8/-2.2	8.2/3.2	3.6/-6.4	6.6/1.6	2.5/-2.5

[a]: excluded from all calculations due to acoustic neuroma left. All hearing thresholds are corrected for age and gender. Abbreviations: Hz: Hertz; F: female, L: left; M: male; R: right; yr, years; x: missing data.

Supplemental Table 7. Lipid spectrum of individuals (male and female) with *TBL1X* mutations.

Case	Sex	Age	TC (mmol/L)	LDL (mmol/L)	HDL (mmol/L)	TG (mmol/L)	Albumin (g/L)	AST (U/L)	ALT (U/L)	GGT (U/L)
A.II.3	M	53 y	7.00*	5.30*	0.98*	1.60	46	15	10	17
A.II.6	F	51 y	6.73*	4.02	1.95	1.69	43	19	11	12
A.III.6	F	29 y	5.20	3.40	1.30	0.97	43	17	14	18
A.III.8	M	25 y	4.04	2.35	1.38	0.68	43	17	20	20
A.IV.1	M	1.5 y	3.90	2.40	1.30	0.69	42	45	21	8
A.IV.2	F	1.5 y	x	x	x	x	x	x	x	x
B.II.4	F	42 y	3.72	1.75	1.21	0.77	43	23	9	16
B.III.4	M	2 y	3.31	1.75	1.21	0.77	49	44	13	16
C.I.1	M	50 y	5.28	3.54	1.36	0.85	43	28	28	24
C.II.1	F	15 y	5.43	3.69*	1.11	1.39	47	20	12	14
C.II.2	F	13 y	6.09*	3.67*	1.86	1.25	46	18	11	12
C.II.3	F	10 y	4.11	2.25	1.55	0.69	46	33	14	11
D.II.2	F	47 y	6.14*	4.34*	1.40	0.90	43	20	18	12
D.III.1	M	16 y	4.69	2.90	1.18	1.35	50	83*	120*	81*
E.I.2	F	48 y	6.20*	4.03	1.75	1.00	45	23	19	11
E.II.1	M	17 y	2.70	1.37	1.13	0.40	51	29	17	14
E.II.2	F	15 y	4.50	2.30	1.77	1.00	40	20	8	7
F.I.2	F	22 y	2.81	1.38	1.01	0.93	44	17	8	9
F.II.1	M	6 mo	x	x	x	x	42	54*	24	7

*: abnormal values. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; F, female; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, male; mo, months; TC, total cholesterol; TG, triglyceride; x, missing value; y, years. Paediatric reference intervals for total cholesterol: 2.7-5.5 mmol/L, LDL: 1.1-3.4 mmol/L, HDL: 1.0-2.3 mmol/L, triglycerides: 0.34-1.95 mmol/L (38). Adult reference intervals for total cholesterol: 2.9-6.1 mmol/L, LDL: 1.2-4.3 mmol/L, HDL: 1.0-2.7 mmol/L, triglycerides: 0.45-2.60 mmol/L (38). Reference intervals for AST: <45 U/L, ALT: <40 U/L, albumin: 35-55 g/L, GGT: female <40 U/L, male <60 U/L.

Supplemental Table 8. Details of the brain material.

Subject NBB#	Brain area	Sex	Age (years)	pmd (h)	**Clinical diagnosis; cause of death**
Formalin fixed, paraffin embedded tissue					
2013-083	hypothalamus	M	78	6	Alzheimer's disease; aspiration pneumonia
2013-093	hypothalamus	F	91	6.5	Cerebrovascular accident
2014-017	hypothalamus	M	81	8	Alzheimer's disease, bladder carcinoma; dehydration
Unfixed, frozen tissue, used for quantitative polymerase chain reaction					
2002-050	Hypothalamus/pituitary	F	95	4	Alzheimer's disease; myocardial infarction
2005-022	Hypothalamus/pituitary	F	78	5	Alzheimer's disease; pneumonia
2002-055	Hypothalamus/pituitary	F	48	5	Multiple sclerosis; respiratory failure
Unfixed, frozen tissue, used for western blotting					
1997-026	Hypothalamus/pituitary	F	77	2	Alzheimer's disease; dehydration
1997-091	Hypothalamus/pituitary	F	85	2	Alzheimer's disease; cachexia and dehydration

Abbreviations: F, female; M, male; NBB#, Netherlands Brain Bank identification number; pmd, post-mortem delay (hours).

Reference list

1. Persani, L., *Clinical review: Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges.* J Clin Endocrinol Metab, 2012. 97(9): p. 3068-78.
2. van Tijn, D.A., J.J. de Vijlder, B. Verbeeten, Jr., P.H. Verkerk and T. Vulsma, *Neonatal detection of congenital hypothyroidism of central origin.* J Clin Endocrinol Metab, 2005. 90(6): p. 3350-9.
3. Collu, R., J. Tang, J. Castagne, G. Lagace, N. Masson, C. Huot, C. Deal, E. Delvin, E. Faccenda, K.A. Eidne and G. Van Vliet, *A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene.* J Clin Endocrinol Metab, 1997. 82(5): p. 1561-5.
4. Hayashizaki, Y., Y. Hiraoka, Y. Endo, K. Miyai and K. Matsubara, *Thyroid-stimulating hormone (TSH) deficiency caused by a single base substitution in the CAGYC region of the beta-subunit.* EMBO J, 1989. 8(8): p. 2291-6.
5. Sun, Y., B. Bak, N. Schoenmakers, A.S. van Trotsenburg, W. Oostdijk, P. Voshol, E. Cambridge, J.K. White, P. le Tissier, S.N. Gharavy, J.P. Martinez-Barbera, W.H. Stokvis-Brantsma, T. Vulsma, M.J. Kempers, L. Persani, I. Campi, M. Bonomi, P. Beck-Peccoz, H. Zhu, T.M. Davis, A.C. Hokken-Koelega, D.G. Del Blanco, J.J. Rangasami, C.A. Ruivenkamp, J.F. Laros, M. Kriek, S.G. Kant, C.A. Bosch, N.R. Biermasz, N.M. Appelman-Dijkstra, E.P. Corssmit, G.C. Hovens, A.M. Pereira, J.T. den Dunnen, M.G. Wade, M.H. Breuning, R.C. Hennekam, K. Chatterjee, M.T. Dattani, J.M. Wit and D.J. Bernard, *Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement.* Nat Genet, 2012. 44(12): p. 1375-81.
6. Astapova, I., K.R. Vella, P. Ramadoss, K.A. Holtz, B.A. Rodwin, X.H. Liao, R.E. Weiss, M.A. Rosenberg, A. Rosenzweig and A.N. Hollenberg, *The nuclear receptor corepressor (NCoR) controls thyroid hormone sensitivity and the set point of the hypothalamic-pituitary-thyroid axis.* Mol Endocrinol, 2011. 25(2): p. 212-24.
7. Bassi, M.T., R.S. Ramesar, B. Caciotti, I.M. Winship, A. De Grandi, M. Riboni, P.L. Townes, P. Beighton, A. Ballabio and G. Borsani, *X-Linked Late-Onset Sensorineural Deafness Caused by a Deletion Involving OA1 and a Novel Gene Containing WD-40 Repeats.* Am J Hum Genet., 1999. 64(6): p. 1604-16.
8. Somsen, D., L. Davis-Keppen, P. Crotwell, J. Flanagan, P. Munson and Q. Stein, *Congenital nasal pyriform aperture stenosis and ocular albinism co-occurring in a sibship with a maternally-inherited 97 kb Xp22.2 microdeletion.* Am J Med Genet A, 2014. 164A(5): p. 1268-71.
9. Kulozik, P., A. Jones, F. Mattijssen, A.J. Rose, A. Reimann, D. Strzoda, S. Kleinsorg, C. Raupp, J. Kleinschmidt, K. Muller-Decker, W. Wahli, C. Sticht, N. Gretz, C. von Loeffelholz, M. Stockmann, A. Pfeiffer, S. Stohr, G.M. Dallinga-Thie, P.P. Nawroth, M. Berriel Diaz and S. Herzig, *Hepatic deficiency in transcriptional cofactor TBL1 promotes liver steatosis and hypertriglyceridemia.* Cell Metab, 2011. 13(4): p. 389-400.
10. ISO, *389-1. Acoustics - Reference zero for the calibration of audiometric equipment.*, in *Part 1 - Reference equivalent threshold sound pressure levels for pure tones and supra-aural earphones.* 1998.
11. ISO, *1999. Acoustics – Determination of occupational noise exposure and estimation of noise-induced hearing impairment.* 1990.

12. Santen, G.W., E. Aten, Y. Sun, R. Almomani, C. Gilissen, M. Nielsen, S.G. Kant, I.N. Snoeck, E.A. Peeters, Y. Hilhorst-Hofstee, M.W. Wessels, N.S. den Hollander, C.A. Ruivenkamp, G.J. van Ommen, M.H. Breuning, J.T. den Dunnen, A. van Haeringen and M. Kriek, *Mutations in SWI/SNF chromatin remodeling complex gene ARID1B cause Coffin-Siris syndrome.* Nat Genet, 2012. 44(4): p. 379-80.
13. Joustra, S.D., N. Schoenmakers, L. Persani, I. Campi, M. Bonomi, G. Radetti, P. Beck-Peccoz, H. Zhu, T.M. Davis, Y. Sun, E.P. Corssmit, N.M. Appelman-Dijkstra, C.A. Heinen, A.M. Pereira, A.J. Varewijck, J.A. Janssen, E. Endert, R.C. Hennekam, M.P. Lombardi, M.M. Mannens, B. Bak, D.J. Bernard, M.H. Breuning, K. Chatterjee, M.T. Dattani, W. Oostdijk, N.R. Biermasz, J.M. Wit and A.S. van Trotsenburg, *The IGSF1 deficiency syndrome: characteristics of male and female patients.* J Clin Endocrinol Metab, 2013. 98(12): p. 4942-52.
14. Santani, A., S. Gowrishankar, C. da Silva, D. Mandelkar, A. Sasson, M. Sarmady, R. Shakhbatyan, S. Tinker, D. Church, B. Funke and M. Hegde, *THE MEDICAL EXOME PROJECT: From concept to implementation.* , in *American Society of Human Genetics Conference*. 2013.
15. Watson, P.J., L. Fairall, G.M. Santos and J.W. Schwabe, *Structure of HDAC3 bound to co-repressor and inositol tetraphosphate.* Nature, 2012. 481(7381): p. 335-40.
16. Ko, L., G.R. Cardona and W.W. Chin, *Thyroid hormone receptor-binding protein, an LXXLL motif-containing protein, functions as a general coactivator.* Proc Natl Acad Sci U S A, 2000. 97(11): p. 6212-7.
17. Monden, T., F.E. Wondisford and A.N. Hollenberg, *Isolation and characterization of a novel ligand-dependent thyroid hormone receptor-coactivating protein.* J Biol Chem, 1997. 272(47): p. 29834-41.
18. Fondell, J.D., M. Guermah, S. Malik and R.G. Roeder, *Thyroid hormone receptor-associated proteins and general positive cofactors mediate thyroid hormone receptor function in the absence of the TATA box-binding protein-associated factors of TFIID.* Proc Natl Acad Sci U S A, 1999. 96(5): p. 1959-64.
19. Wiersinga, W.M., *The peripheral conversion of thyroxine (T4) into triiodothyronine (T3) and reverse triiodothyronine (rT3).* in *Department of Endocrinology.* 1979, University of Amsterdam: Amsterdam. p. 205.
20. Fredriks, A.M., S. van Buuren, J.M. Wit and S.P. Verloove-Vanhorick, *Body index measurements in 1996-7 compared with 1980.* Arch Dis Child, 2000. 82(2): p. 107-12.
21. Spyropoulos, E., D. Borousas, S. Mavrikos, A. Dellis, M. Bourounis and S. Athanasiadis, *Size of external genital organs and somatometric parameters among physically normal men younger than 40 years old.* Urology, 2002. 60(3): p. 485-9; discussion 490-1.
22. van Rijn, R.R., *Skull, Intracranial Space, and Vertebral Column: Thyroid Gland Volume*, in *Differential Diagnosis in Pediatric Imaging*, R.R. van Rijn and J.G. Blickman, Editors. 2011, Thieme Verlagsgruppe: Stuttgart, New York, Delhi, Rio. p. 654.
23. Lawton, B., *Typical hearing thresholds: a baseline for the assessment of noise-induced hearing loss.* 1998, Southampton, GB: University of Southampton.
24. Clark, J.G., *Uses and abuses of hearing loss classification.* ASHA, 1981. 23(7): p. 493-500.
25. Bonomi, M., M.C. Proverbio, G. Weber, G. Chiumello, P. Beck-Peccoz and L. Persani, *Hyperplastic pituitary gland, high serum glycoprotein hormone alpha-subunit, and variable circulating thyrotropin (TSH) levels as hallmark of central hypothyroidism due to mutations of the TSH beta gene.* J Clin Endocrinol Metab, 2001. 86(4): p. 1600-4.

26. Joustra, S.D., C.A. Heinen, N. Schoenmakers, M. Bonomi, B.E. Ballieux, M.O. Turgeon, D.J. Bernard, E. Fliers, A.S. van Trotsenburg, M. Losekoot, L. Persani, J.M. Wit, N.R. Biermasz, A.M. Pereira, W. Oostdijk, J. Aisenberg, E.L. van den Akker, I. Bergada, G. Bocca, D. Braslavsky, B. Callewaert, E.A. Cummings, M.P. Cuppen, M. Dattani, H.M. Domene, J.C. van der Heyden, S. van Hulle, M.A. Jacobs, T.P. Links, L. Lunshof, D. Mul, F.S. Neijens, H.F. Pedro, M. Salerno, J. de Schepper, P.G. Voorhoeve, A.S. Zidell, P.A. van der Zwaag, N. Zwaveling-Soonawala and I.c.c. group, *IGSF1 deficiency: lessons from an extensive case series and recommendations for clinical management.* J Clin Endocrinol Metab, 2016: p. jc20153880.
27. Oberoi, J., L. Fairall, P.J. Watson, J.C. Yang, Z. Czimmerer, T. Kampmann, B.T. Goult, J.A. Greenwood, J.T. Gooch, B.C. Kallenberger, L. Nagy, D. Neuhaus and J.W. Schwabe, *Structural basis for the assembly of the SMRT/NCoR core transcriptional repression machinery.* Nat Struct Mol Biol, 2011. 18(2): p. 177-84.
28. Yoon, H.G., D.W. Chan, Z.Q. Huang, J. Li, J.D. Fondell, J. Qin and J. Wong, *Purification and functional characterization of the human N-CoR complex: the roles of HDAC3, TBL1 and TBLR1.* EMBO J, 2003. 22(6): p. 1336-46.
29. Makde, R.D., J.R. England, H.P. Yennawar and S. Tan, *Structure of RCC1 chromatin factor bound to the nucleosome core particle.* Nature, 2010. 467(7315): p. 562-6.
30. Fliers, E., N.W. Noppen, W.M. Wiersinga, T.J. Visser and D.F. Swaab, *Distribution of thyrotropin-releasing hormone (TRH)-containing cells and fibers in the human hypothalamus.* J Comp Neurol, 1994. 350(2): p. 311-23.
31. Medici, M., W.E. Visser, T.J. Visser and R.P. Peeters, *Genetic determination of the hypothalamic-pituitary-thyroid axis: where do we stand?* Endocr Rev, 2015. 36(2): p. 214-44.
32. Zwaveling-Soonawala, N., C.E. van Beijsterveldt, E.T. Mesfum, B. Wiedijk, P. Oomen, M.J. Finken, D.I. Boomsma and A.S. Paul van Trotsenburg, *Fetal Environment Is a Major Determinant of the Neonatal Blood Thyroxine Level: Results of a Large Dutch Twin Study.* J Clin Endocrinol Metab, 2015. 100(6): p. 2388-95.
33. Hansen, P.S., T.H. Brix, F.N. Bennedbaek, S.J. Bonnema, K.O. Kyvik and L. Hegedus, *Genetic and environmental causes of individual differences in thyroid size: a study of healthy Danish twins.* J Clin Endocrinol Metab, 2004. 89(5): p. 2071-7.
34. Heinen, C.A., A. Jongejan, P.J. Watson, B. Redeker, A. Boelen, O. Boudzovitch-Surovtseva, F. Forzano, R. Hordijk, R. Kelley, A.H. Olney, M.E. Pierpont, G.B. Schaefer, F. Stewart, A.S. van Trotsenburg, E. Fliers, J.W. Schwabe and R.C. Hennekam, *A specific mutation in TBL1XR1 causes Pierpont syndrome.* J Med Genet, 2016.
35. Ng, L., R.J. Goodyear, C.A. Woods, M.J. Schneider, E. Diamond, G.P. Richardson, M.W. Kelley, D.L. Germain, V.A. Galton and D. Forrest, *Hearing loss and retarded cochlear development in mice lacking type 2 iodothyronine deiodinase.* Proc Natl Acad Sci U S A, 2004. 101(10): p. 3474-9.
36. Chung, R.H., D. Ma, K. Wang, D.J. Hedges, J.M. Jaworski, J.R. Gilbert, M.L. Cuccaro, H.H. Wright, R.K. Abramson, I. Konidari, P.L. Whitehead, G.D. Schellenberg, H. Hakonarson, J.L. Haines, M.A. Pericak-Vance and E.R. Martin, *An X chromosome-wide association study in autism families identifies TBL1X as a novel autism spectrum disorder candidate gene in males.* Mol Autism, 2011. 2(1): p. 18.

37. Bisschop, P.H., M.J. Dekker, W. Osterthun, J. Kwakkel, J.J. Anink, A. Boelen, U.A. Unmehopa, J.W. Koper, S.W. Lamberts, P.M. Stewart, D.F. Swaab and E. Fliers, *Expression of 11beta-hydroxysteroid dehydrogenase type 1 in the human hypothalamus.* J Neuroendocrinol, 2013. 25(5): p. 425-32.
38. Hilsted, L., P. Rustad, L. Aksglaede, K. Sorensen and A. Juul, *Recommended Nordic paediatric reference intervals for 21 common biochemical properties.* Scand J Clin Lab Invest, 2013. 73(1): p. 1-9.
39. Bjerner, J., D. Biernat, S.D. Fossa and T. Bjoro, *Reference intervals for serum testosterone, SHBG, LH and FSH in males from the NORIP project.* Scand J Clin Lab Invest, 2009. 69(8): p. 873-9 e1-11.
40. Stricker, R., R. Eberhart, M.C. Chevailler, F.A. Quinn, P. Bischof and R. Stricker, *Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer.* Clin Chem Lab Med, 2006. 44(7): p. 883-7.
41. Soldin, O.P., E.G. Hoffman, M.A. Waring and S.J. Soldin, *Pediatric reference intervals for FSH, LH, estradiol, T3, free T3, cortisol, and growth hormone on the DPC IMMULITE 1000.* Clin Chim Acta, 2005. 355(1-2): p. 205-10.
42. Kushnir, M.M., T. Blamires, A.L. Rockwood, W.L. Roberts, B. Yue, E. Erdogan, A.M. Bunker and A.W. Meikle, *Liquid chromatography-tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals.* Clin Chem, 2010. 56(7): p. 1138-47.
43. Soldin, O.P., J.R. Dahlin, E.G. Gresham, J. King and S.J. Soldin, *IMMULITE 2000 age and sex-specific reference intervals for alpha fetoprotein, homocysteine, insulin, insulin-like growth factor-1, insulin-like growth factor binding protein-3, C-peptide, immunoglobulin E and intact parathyroid hormone.* Clin Biochem, 2008. 41(12): p. 937-42.
44. Rosario, P.W., *Normal values of serum IGF-1 in adults: results from a Brazilian population.* Arq Bras Endocrinol Metabol, 2010. 54(5): p. 477-81.
45. Mutlu, M., G. Karaguzel, Y. Aliyazicioglu, I. Eyupoglu, A. Okten and Y. Aslan, *Reference intervals for thyrotropin and thyroid hormones and ultrasonographic thyroid volume during the neonatal period.* J Matern Fetal Neonatal Med, 2012. 25(2): p. 120-4.
46. Johansson, M. and S. Arlinger, *Reference data for evaluation of occupationally noise-induced hearing loss.* Noise Health, 2004. 6(24): p. 35-41.