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Central congenital hypothyroidism caused by a novel mutation, C47W, in the cysteine knot region of TSHβ

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Abstract

Background: Isolated central congenital hypothyroidism (ICCH) is a rare form (1:50,000 newborn) of congenital hypothyroidism, which can present with growth and neuropsychological retardation. Unlike the more common primary CH (1:1,500–1:4,000), which presents on newborn screening with elevated serum thyroid stimulating hormone (TSH) and low thyroxine (T_4) and triiodothyronine (T_3), ICCH presents with low TSH and low thyroid hormone levels. ICCH therefore may be missed in most newborn screens that are based only on elevated TSH. Most cases of ICCH have been associated with mutations in the TSHβ gene.

Patient: We present a consanguineous Sudanese family where the proband was diagnosed with "atypical" CH (serum TSH was low, not high).

Intervention and Outcome: The propositus underwent whole exome sequencing and the C47W TSHβ mutation was identified. Sanger sequencing confirmed the proband to be homozygous for C47W and both parents were heterozygous for the same mutation. The mutation was predicted by several in silico methods to have a deleterious effect (SIFT 0.0, Damaging; Polyphen2_HDIV 0.973, probably damaging; MutationTaster 1, disease causing; and CADD 3.17,

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Author Contributions

RSE and MAA identified the patient and wrote the first draft; RJB helped in the writing of the manuscript and did the clinical chemistries; MKC and YW performed the initial sequencing analyses and YW evaluated all sequencing; AMD and SR confirmed the measurements in Chicago, provided intellectual input for the analyses and revised the manuscript; REW was overall supervisor of the data collection, provided funding and wrote the manuscript which was approved by all authors.

Disclosure Statement

The authors have no conflicts of interest to declare

16.62). C47W affects the first cysteine of the cysteine knot of the TSHβ subunit. The cysteine knot region of TSHβ is highly conserved across species and is critical for binding to the TSH receptor. Only two other mutations were previously reported along the cysteine knot and showed consistently low or undetectable serum TSH and low T_4 and T_3. Other TSHβ gene mutations causing ICCH have been reported in the “seatbelt” region, necessary for TSHβ dimerization with the alpha subunit.

Conclusions: Identification of a mutation in TSHβ gene reinforces the importance of identifying of ICCH that can occur in the absence of elevated serum TSH and demonstrates the functional significance of the TSHβ cysteine knot.

Keywords

Central congenital hypothyroidism; secondary hypothyroidism; TSH receptor

Introduction

Congenital hypothyroidism (CH) has an incidence worldwide of 1:1,500 to 1:4,000 newborns, depending on the thyroid stimulating hormone (TSH) cut-off value used in neonatal screening (1, 2). Most programs for the early detection of CH are based on the screening of neonates for high TSH followed by measurement of thyroxine (T_4). Detection of CH at birth allows for early diagnosis and initiation of treatment to prevent neuropsychologic and metabolic sequelae of hypothyroidism. Much less common (1:16,000 to 1:100,000 newborn) is central CH (CCH) where TSH is low or normal, thus in contrast with a low T_4 level (3, 4) and therefore missed on neonatal screening where only TSH is measured. Even less common is isolated CCH (ICCH) which is diagnosed in later childhood when children come to medical attention for growth disturbances or neurologic abnormalities. Endocrine investigation reveals low T_4 levels in the absence of an elevated TSH.

Mutations in TSHβ can result in ICCH (5). TSHβ is a relatively small gene of 2 coding and 1 noncoding exons. The central cysteine-knot of the TSHβ molecule is essential for the integrity of its structure and binding to the TSH receptor (6). In this study, we report a Sudanese family with a novel rare mutation, C47W, in a critical area of the TSHβ. This case illustrates the need for a full evaluation of TSH and thyroid hormone (TH) levels in screening programs for CH.

Case Report

The proband, a Sudanese male born following normal pregnancy and delivery presented at the age of three months with abdominal distension and constipation, in the absence of a goiter or umbilical hernia. Growth was normal for height and weight at the 50th percentile. Written informed consent was obtained from the parents for blood and serum draws and analyses. Serum TSH and free thyroxine (FT_4) were initially measured in Khartoum. Serum thyroid function tests (TFTs) after starting treatment including TSH, total thyroxine (TT_4), total triiodothyronine (TT_3), FT_4, thyroxine-binding globulin (TBG), thyroglobulin (TG), thyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TGAb) were completed

(Immulite 1000; Siemens, Munich, Germany and by Elecsys; Roche, Switzerland). Reverse triiodothyronine (TrT_3) was measured by radioimmunoassay (Adaltis Italia S.p.A, Bologna, Italy). DNA for sequencing was extracted from peripheral white blood cells using a Qiagen DNA Blood Mini Kit (Hilden, Germany). DNA from the mother and proband were sent for whole exome sequencing (Novogene, Agilent SureSelect Human All Exon V6 Kit). Whole exome sequencing (WES) results were first scanned for mutations in genes known to be related to TH synthesis or function (Supplemental Table 1) (7–9). Functional prediction scores Sorting Intolerant from Tolerant (SIFT) (10), Polyphen2 HumanDiv (HDIV) (11) MutationTaster (12) and Combined Annotation Dependent Depletion (CADD) (13) were tested in silico, allele frequency, and zygosity were all considered in determining the likely cause of the CCH phenotype. The mutation was confirmed by sanger sequencing of DNA from all available family members in order to determine the mode of inheritance.

TFTs at 3 months of age revealed a TSH reported as 0.01 mIU/L (reference range 0.4–4.3 mIU/L) and low FT_4 of 1.0 pmol/L (reference range 4–10.6 pmol/L). The proband had no family history of thyroid disease, however, both parents reported being first-degree cousins. The proband was diagnosed with CCH and started on levothyroxine ($L\text{-}T_4$) treatment. Now, at eleven-year-old on subtherapeutic $L\text{-}T_4$ replacement the TFTs demonstrated undetectable TSH on the Elecsys (<0.01) and on Immulite (<0.03) with Low FT_4 (Individual II-1, Figure 1). The proband had normal neurological and psychological development on physical examination. WES found a novel missense mutation located on exon 2 of the TSHβ, c.T141G p.C47W (numbering includes the 20 amino acid signal peptide). Sanger sequencing confirmed that both parents were heterozygous for the C47W mutation, while the proband was homozygous. The mutation was predicted by several in silico methods to have a deleterious effect (SIFT 0.0, Damaging; Polyphen2_HDIV 0.973, probably damaging; MutationTaster 1, disease causing; and CADD 3.17, 16.62). Analysis of the allele frequency in the African and general populations was not available as this is a novel mutation not located in the Genome Aggregation Database.

Discussion

TSHβ C47W significantly disrupts the structure and function of the normal TSHβ. The area of mutation is of crucial importance due to its key location along the cysteine knot, a highly-conserved sequence in mammals (Figure 2A). TSHβ C47W occurs on the first cysteine within the loop knot. Previous studies have shown the importance of the TSHβ cysteine knot structure in maintaining the specific three-dimensional structure needed for binding to the TSH receptor (TSHR) (6). While most documented mutations in the TSHβ gene are located further down the gene including the seatbelt region where dimerization with the alpha subunit occurs, three reported mutations occur within two different places on the cysteine knot (Figure 2B). All three mutations cause CCH in affected members where TSH was found to be undetectable or very low (14–16). Other mutations both upstream and downstream of the C47W mutation were also consistent with CCH, however, the phenotype greatly varied even within affected families. Mutation E32K, for example, showed two family members both affected with delayed development, but varying TFTs with one having low TSH and the other normal TSH (17). Similarly, reported splicing mutations along the gene show a mixture of normal to abnormal neonatal testing along with varying bone and

mental development in affected individuals (18, 19). Thus, some cases with the mutant TSHβ showed discrepancy between bioactivity and immunoactivity of TSH because of its ability to form a heterodimer with alpha subunit (20). On the other hand, protein modeling of this novel mutation illustrates the change in protein structure where a change in configuration and folding would greatly hinder the ability to bind with the alpha subunit and TSH receptor (Supplemental Figure 1). This change is consistent with the reported phenotype of the proband with low serum TSH and FT_4.

Mutations along the TSHβ gene consistently showed a thyroid phenotype, of low serum T_4 and T_3 with variable concentrations of serum TSH from undetectable to normal. Whereas those mutations specifically along the cysteine knot and those that cause a deletion of most or all of the gene consistently have been found to have low or undetectable serum TSH and low levels of FT_4. The mutant TSHβ in this case is likely due to not being secreted as it failed detection using monoclonal antibodies against two different epitopes (20). However an important structural change affecting both epitopes cannot be excluded. Other mutations reported in different areas outside the knot show less consistently thyroid tests abnormalities, with some affected individuals having normal levels of TSH despite the CCH phenotype. Our findings reinforce the importance of the cysteine knot structure in the function of TSHβ and illustrate the diagnosis of CCH among children with CH requires measurement of both TSH and T_4.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Statement of Ethics

Subjects have given their written informed consent to publish their case. The study protocol was approved by the University of Miami's Institutional Review Board on human research.

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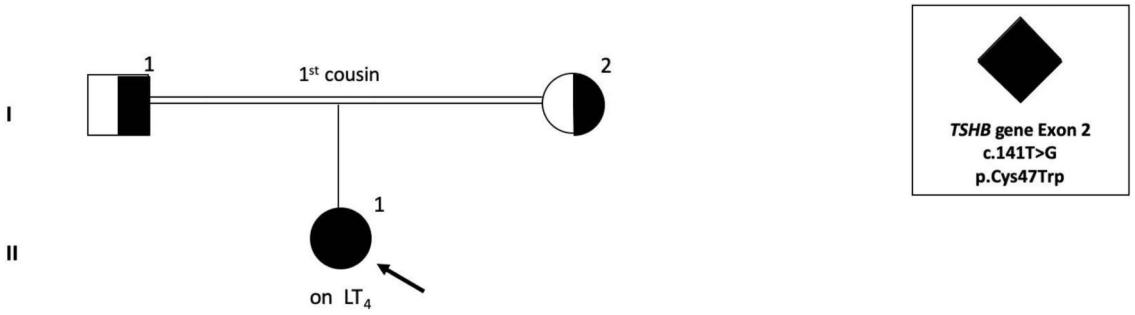
Established Facts

- Congenital hypothyroidism (CH) is common, however when neonatal screening only looks at elevated TSH, Central CH (CCH) can be missed.
- A cause of CCH can be mutations in the TSHβ gene.
- Treatment of CCH is with levothyroxine but monitoring of TSH is not sufficient for adequate dosing.

Key Insights

- We describe a case of CCH in a consanguineous newborn with low serum TSH and low thyroxine (T_4)
- The proband was homozygous for a mutation in the TSHβ gene, C47W, in the cysteine knot of the TSHβ protein.
- The location of the mutation illustrates the importance of the cysteine knot in the function of TSHβ.



	I-1		II-1		I-2		Normal Range	
Age (years)	47		11		36			
Platform	Immulite (MIA)	Elecsys (CHI)	Immulite (MIA)	Elecsys (CHI)	Immulite (MIA)	Elecsys (CHI)	Immulite (MIA)	Elecsys (CHI)
TT4 µg/dL	6.70	10.0	5.63	6.8	6.33	8.0	4.5-12.5	5.0-12.0
TT3 ng/dL	91.5	132	85.8	112	90.3	122	81-178	80-190
FT4 ng/dL	0.999		**0.659**		0.968		0.89-1.76	
TrT3 ng/dl		22.7 (RIA)		**9.7 (RIA)**		22.7 (RIA)		16-36 (RIA)
TSH µIU/mL	1.49	2.1	**0.027**	**<0.01**	1.01	1.0	0.4-4.0	0.4-2.6
TBG µg/mL	30.0		30.8		31.0		14.0-31.0	
TG ng/mL	4.98		19.6		3.53		1.7-55.6	
TPOAb IU/mL	24.2		12.9		<10.0		<35	
TGAb IU/mL	<20.0		<20.0		<20.0		<40	

Figure 1.

Pedigree of the family with the results of thyroid function tests. Generations are denoted by their respective roman numeral. Each subject is identified by the number just above the corresponding symbol. Laboratory thyroid function tests are aligned below each symbol. Abnormal values are in bold. Abbreviations: L-T_4; levothyroxine, TT_4; total thyroxine, TT_3; total triiodothyronine, FT_4; free thyroxine, TSH; thyroid-stimulating hormone, TBG; thyroxine binding globulin, TG; thyroglobulin, TPO Ab; anti-TPO antibody, TG Ab; anti-thyroglobulin antibody, TrT_3; reverse triiodothyronine. Mia, Immulite assay done in Miami; Chi, Elecys assay done in Chicago.

[A]

Amino Acid Sequence Comparison								
	Amino Acid							
Mutation c.141T>G p.C47W	N	T	T	I	W	A	G	Y
Homo sapiens (human)	N	T	T	I	C	A	G	Y
Pan troglodytes (chimp)	N	T	T	I	C	A	G	Y
Pongo pygmaeus abelii (orangutan)	N	T	T	I	C	A	G	Y
Nomascus leucogenys (gibbon)	N	T	T	I	C	A	G	Y
Mus musculus (mouse)	N	T	T	I	C	A	G	Y
Sus scrofa (pig)	N	T	T	I	C	A	G	Y
Monodelphis domestica (opossum)	N	T	T	I	C	A	G	Y
Gallus gallus (chicken)	N	T	T	I	C	A	G	F
Anolis carolinensis (lizard)	N	T	T	I	C	E	G	F
Danio rerio (zebrafish)	-	-	-	-	-	-	-	-
Consensus	N	T	T	I	C	A	G	Y

[B]

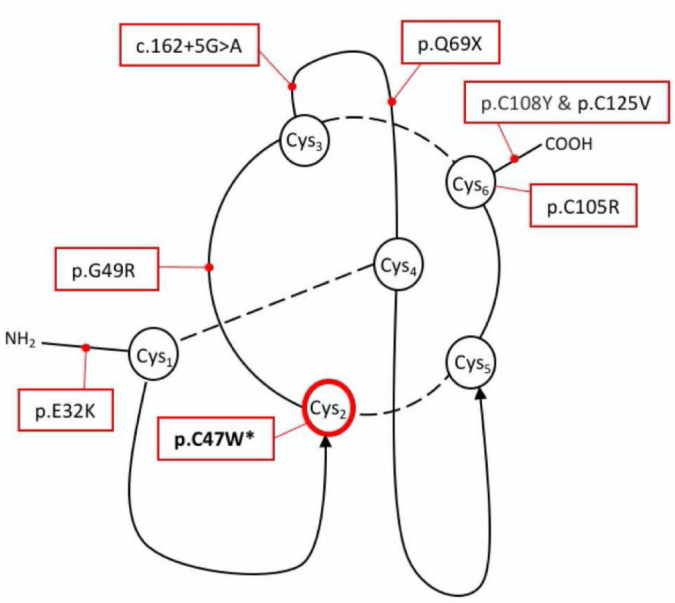


Figure 2.

[A] Amino acid sequence surrounding the mutants TSHβ. Highlighted area denotes major difference between consensus amino acid and mutant amino acid [B] Schematic representation of the TSHβ cysteine-knot and surrounding area with reported mutations. The size of the ring and loops are not drawn to actual scale. Cysteine components and approximate corresponding amino acid numbers are shown in boxes. The novel mutation reported herein is denoted by the red circle and asterisk (*). Previous reported mutations are shown with their approximate location. Loops are shown in different colors to distinguish approximate connections. A mutation resulting in a 6kb deletion of all exons of the TSHβ is not pictured (21). Schematic of the TSHβ cysteine knot figure is after Vitt et al (22).