A novel mutation in the TG gene (G2322S) causing congenital hypothyroidism in a Sudanese family: a case report

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Abstract

Background: Congenital hypothyroidism (CH) has an incidence of approximately 1:3000, but only 15% have mutations in the thyroid hormone synthesis pathways. Genetic analysis allows for the precise diagnosis.

Case presentation: A 3-week old girl presented with a large goiter, serum TSH > 100 mIU/L (reference range: 0.7–5.9 mIU/L); free T4 < 3.2 pmol/L (reference range: 8.7–16 pmol/L); thyroglobulin (TG) 101 μg/L. Thyroid Tc-99 m scan showed increased radiotracer uptake. One brother had CH and both affected siblings have been clinically and biochemically euthyroid on levothyroxine replacement. Another sibling had normal thyroid function. Both Sudanese parents reported non-consanguinity. Peripheral blood DNA from the proposita was subjected to whole exome sequencing (WES). WES identified a novel homozygous missense mutation of the TG gene: c.7021G > A, p.Gly2322Ser, which was subsequently confirmed by Sanger sequencing and present in one allele of both parents. DNA samples from 354 alleles in four Sudanese ethnic groups (Nilotes, Darfurians, Nuba, and Halfawien) failed to demonstrate the presence of the mutant allele. Haplotyping showed a 1.71 centiMorgans stretch of homozygosity in the TG locus suggesting that this mutation occurred identical by descent and the possibility of common ancestry of the parents. The mutation is located in the cholinesterase-like (ChEL) domain of TG.

Conclusions: A novel rare missense mutation in the TG gene was identified. The ChEL domain is critical for protein folding and patients with CH due to misfolded TG may present without low serum TG despite the TG gene mutations.

Keywords: Congenital hypothyroidism, Goiter, Novel mutations, Thyroglobulin, TG
obstruction and producing stridor. Her thyroid stimulating hormone (TSH) on NS was 14 mIU/L (cut-off range for notification: > 13 mIU/L). The result was communicated to the relevant clinicians but, unfortunately, appropriate follow-up had not occurred. At 3 weeks of age when she presented with goiter, serum analysis showed a TSH > 100 mIU/L (reference range: 0.7–5.9 mIU/L) with low free T4 of < 3.2 pmol/L (reference range: 8.7–16 pmol/L) and high TG of 101 μg/L (reference range for all age: 1.6–59.9 μg/L). The urinary iodine concentration was 0.63 μmol/L, suggesting that she had mild iodine deficiency. Thyroid ultrasound and MRI (Fig. 1a) showed both lobes of the thyroid gland to be significantly enlarged. Nuclear scan following injection of 42 MBq Tc-99 m pertechnetate showed a large goiter with homogeneous markedly increased radiotracer uptake (Fig. 1b). Levothyroxine replacement was started based on the diagnosis of CH as standard of care.

Her brother was also found to be hypothyroid based on NS, with an elevated TSH of 60 mIU/L. He had been on levothyroxine replacement since that time, with no concerns regarding growth or development. His thyroid ultrasound and nuclear scan at 2 years of age showed a eutopic thyroid gland with increased nuclear tracer uptake of 5.2% (normal range 1–4%) following withdrawal of levothyroxine replacement for 5 days. Both siblings had TSH and TH concentrations in the reference ranges while under levothyroxine replacement and with dose adjustment and they have been clinically euthyroid (Fig. 2). The proposita’s TG decreased to 2.2 μg/L and the affected brother had low TG of 0.3 μg/L. Other members of the family had no symptoms and their thyroid tests were normal (Fig. 2). Subsequent perchlorate discharge studies were performed on the affected brother following withdrawal of levothyroxine replacement for 3 weeks [TSH 180 mIU/L (reference range: 0.7–4.0 mIU/L), FT4 4.4 pmol/L (reference range: 7.5–17 pmol/L), TG 9.6 μg/L] and on both parents. The results demonstrated increased uptake of radiotracer in the brother and normal tracer uptake in the parents with no washout of I-123 following perchlorate administration (Fig. 3). This suggests preservation of the organification process [7]. Both maternal and paternal families are non-consanguineous and of Sudanese origin (maternal grandfather; Gogrial and maternal grandmother and paternal grandparents; Aweil East, of South Sudan, respectively). There was no history of thyroid disorders in either family.

**Molecular genetics**

Genomic DNA was extracted from peripheral blood leukocytes as previously described [8]. The proposita’s DNA was submitted to whole exome sequencing. A panel of 50 genes related to thyroid development, function, serum and cell TH transport and hormone synthesis was evaluated (Additional file 1: Table S1). This led to the identification of a novel homozygous missense mutation in exon 40 of the TG gene: c.7021G > A, p. Gly2322Ser (numbering excludes 19 amino acid signal peptide). All exonic variants in the TG gene identified in the proposita are summarized in Additional file 2: Table S2. Sanger sequencing confirmed that the affected brother (III-2) was homozygous for the same mutation while the parents (II-2 and II-3) and maternal grandmother (I-4) were heterozygous for this mutation, indicating that they were unaffected carriers (Fig. 4a). The other brother (III-1) and the maternal grandfather (I-3) had the wild-type genotype and normal thyroid function tests (Fig. 2 and 4a). This variant was not present in the Genome Aggregation Database (gnomAD) used for analysis. Since the database did not likely include a large number of Sudanese individuals, 354 alleles from 4 Sudanese ethnic groups (Nilotes, Darfurians, Nuba, and Halfawien) [9] were also used for analysis. The TG mutation identified in our family was not present in the 177 Sudanese individuals, suggesting that the variant is rare in the Sudanese as well. Single nucleotide polymorphisms (SNPs) markers with high allele frequencies in the TG locus and markers in the flanking genes were
genotyped to reconstruct the haplotype for the region (8q24.22) (Fig. 5). The haplotype associated with CH present in both parents was identical for a stretch of ~1.71 centiMorgans (cM). The G2322S TG mutation affects a highly conserved amino acid in various species (Fig. 4b) and functional in silico prediction algorithms suggest that this mutation is deleterious (SIFT; 0.001, deleterious and PolyPhen2; 1.0, probably damaging).

Discussion and Conclusions

A novel homozygous TG gene mutation was identified in two children with CH and goiter. The unrelated parents are heterozygous for this “rare” mutation and, surprisingly, report non-consanguinity. Haplotype analysis of the SNP makers revealed that the haplotype containing the mutated allele was homozygous over the entire TG locus, spanning 1.71 cM (Fig. 5). This is consistent with the autosomal recessive pattern of inheritance [10]. These results suggest that the mutation occurred identical by descent [11] and that the parents have distant common ancestry [12]. This is likely because Sudan is known for high prevalence of consanguineous marriage [13] and it has been reported that marriage between 1st cousins accounted for more than 40% marriages [14]. DNA obtained from a Sudanese cohort [9] was analyzed by Sanger sequencing to confirm the frequency of the mutation in the population. However, none of 177 individuals had the mutated allele, indicating the minor allele frequency (MAF) for this variant to be less than 0.3% in the Sudanese population.

The TG protein is composed of three consecutive cysteine (Cys) repeat domains (domains I, II, and III) forming many disulfide bonds. These Cys repeat-domains are followed by the cholinesterase-like (ChEL) domain [4]. Although the TG protein contains 120 Cys, the ChEL domain contains only six Cys. While the mutation in the present case does not directly substitute a Cys residue, it is located in the ChEL domain and is similar to six pathogenic missense mutations previously reported in the domain. Two mutations in the domain (p.A2215D and p.R2223H) occur before the first disulfide bond of ChEL, whereas the remaining four mutations (p.G2300D, p.R2317Q, p.G2355V, and p.G2356R) fall between the first and second disulfide bonds. The G2322S variant in the present case also resides between the first and second disulfide bonds of the ChEL domain (Fig. 6). We used JPred 4 server for prediction of the secondary structure of the TG [15]. Although the first loop created by the first and second disulfide bonds of the ChEL domain (Fig. 6).
ChEl folding [4]. Interestingly, all pathogenic missense mutations in the ChEl domain are not located in the α helix, however they have been shown to result in CH. Animal studies confirmed that a misfolded TG resulted from similar variants in the ChEl domain. Introduction of p.L2263P in a mouse produced congenital goiter demonstrating that the mutation permitted full-length synthesis of TG but impaired folding necessary for TG homodimerization and transport from the endoplasmic reticulum (ER) [16, 17]. Another rodent, the rdw rat, harboring the TG p.G2300R mutation exhibited abnormal folding of TG resulting in an extended α helix within the ChEl domain and retention of TG within the ER lumen [18]. The p.G2322S mutation in the present case is located near these mutations, suggesting that TG folding, transport and secretion might be similarly impaired.

Most cases with CH due to the TG gene mutations show decreased serum TG levels before TH replacement and it is a key factor for the diagnosis of TG defects [6, 19]. Interestingly, the present case had a TG of 101 μg/L at diagnosis. Reference range of serum TG for infants have not been established. Depending on the platform of analysis, the upper limit of normal for infants between 15 and 180 days was from 43.37 to 147.28 μg/L [20]. Due to lack of a specific TG reference range for infants using our method (IMMULITE 2000 ®; Siemens, Germany), the TG value of the present case may be high normal, but definitely above values reported in individuals with the TG gene mutations. Moreover, her TG decreased as TSH fell. The affected brother (III-2) had the same mutation in the TG gene, although his TG before levothyroxine replacement
therapy was unknown. Among 61 cases in which serum TG values without TH replacement were reported, only three cases, beside the present case, showed increased TG (Table 1) [21–23]. The TG mutations p.C1977S and p.A2215D have been reported in multiple cases, and levels of TG in the affected, not included in the list, were normal or low. As with the p.G2322S or p.A2215D in the ChEL domain, the p.C1977S located in the Cys repeat domain III was also associated with misfolding [21, 24]. In general, unfolded protein is blocked from being transported within a cell and then refolded or degraded by chaperone proteins through an intracellular signaling pathway called unfolded protein response (UPR) [24]. Interestingly, absence of the chaperone protein activation was observed in the p.C1977S case with hyperthyroglobulinemia [24]. Thus, incomplete UPR may be associated with secretion of some mutated TG without retention in the ER resulting in higher serum TG. The initial serum TG level of the present case was 101 μg/L. In contrast, CH due to TPO deficiency, in 63 published cases in whom the TG gene was normal had on the average serum TG of more than 1000 μg/L before TH replacement [25–27]. Therefore the TG mutation in the present case resulted in a lesser serum TG level than that observed in CH due to TPO mutations but higher than that of patients with TG gene mutations in the majority of whom serum TG is undetectable or less than 5 μg/L [19]. The higher level of serum TG in the present case suggests reduced secretion or rapid degradation of a misfolded molecule. As mentioned above, several other cases of TG gene mutations manifesting serum TG levels above 5 μg/L have been reported [21–23].

Fig. 5 Haplotypes present in the family members using single nucleotide polymorphic markers. Black filled, semi-round or semi-square and open symbols indicate affected individuals, unaffected carriers and wild type, respectively. The inherited portion of the disease associated haplotype is indicated in blackened bar. a) Marker location on the human genome reference sequence (NCBI Build 38); b) Marker location on the international HapMap project.
**Fig. 6** Schematic representation of ChEL domain in the TG gene. Amino acid numbering is indicated above TG structure. α helix motifs are indicated in gray boxes with diagonal lines. Cystein residues are indicated in black lines. Pathogenic missense mutations are indicated in gray lines. *Mutation in the present case

### Table 1 Clinical characteristics of the CH cases due to the TG gene mutations with detectable TG levels

| Exon | Nucleotide | Amino Acid | TG domain | Age | Gender | Goiter | TSH (mIU/L) [normal] | TT4 (μg/dL) [normal] | FT4 (pmol/L) [normal] | TG (μg/L) [normal] | Antibodies | References |
|------|------------|------------|-----------|-----|--------|--------|----------------------|----------------------|----------------------|----------------------|------------|------------|
| 33   | c.5985 T > A | p.C1977S   | Repeat domain III | adult | F      | +      | 1.1 [0.1–4.0]        | N/A                  | 9.8 [8–28]           | 181 [15–50]         | negative   | [21]       |
| 33   | c.5985 T > A | p.C1977S   | Repeat domain III | adult | F      | +      | 1.2 [0.1–4.0]        | N/A                  | 9.1 [8–28]           | 117 [15–50]         | negative   | [21]       |
| 38   | c.6701C > A | p.A2215D   | ChEL      | 34 yrs. | M      | +      | 4 [0.5–4.5]           | 6 [4–12]             | N/A                  | 29.2 [1.5–15]       | N/A        | [22, 23]   |
| 40   | c.7021G > A | p.G2322S   | ChEL      | 3 wks  | F      | +      | > 100 [0.7–5.9]      | < 3.2 [8.7–16]       | N/A                  | 101 [N/A]           | negative   | III-3, this report |

All patients were without TH replacement at the thyroid function tests

Abbreviations: TG thyroglobulin, TSH thyroid-stimulating hormone, TT4 total thyroxine, FT4 free thyroxine, N/A not available, L-T4 levothyroxine, ChEL cholinesterase-like, F Female, M Male, yrs years, wks weeks

The International System of Units: TT4 μg/dL = 12.87 nmol/L
The delayed TSH rise in the proposita may be due to an unknown genetic cause or an environmental factor such as iodine exposure.

In conclusion, a novel homozygous mutation in the TG gene was identified. The mutation was not found in four Sudanese groups of different ethnic origin (n = 177). The mutation is located in the ChEL domain of the TG which is essential for protein folding. Patients with CH due to misfolded TG may present without low serum TG.

Additional files

**Additional file 1:** Table S1. Genes related to thyroid disorder. 50 genes related to thyroid development, function, serum and cell TH transport and hormone synthesis. (XLSX 30 kb)

**Additional file 2:** Table S2. Exonic variants in the TG gene identified in the proposita by whole exome sequencing. All exonic variants in the TG gene identified in the proposita. (XLSX 41 kb)

Abbreviations

CH: Congenital hypothyroidism; ChEL: Cholinesterase-like; cM: CentiMorgans; Cys: Cysteine; ER: Endoplasmic reticulum; gnomAD: Genome Aggregation Database; L-Tr: L-Levothyroxine; MAF: Minor allele frequency; NS: Neonatal screening; SNP: Single nucleotide polymorphism; T₃: Triiodothyronine; T₄: Thyroxine; TG: Thyroglobulin; TH: Thyroid hormone; TSH: Thyroid stimulating hormone; UPR: Unfolded protein response

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Availability of data and materials

The datasets used and analyzed during the current study, such as the raw data from WES sequencing and Sanger sequencing files, are available from the corresponding author on reasonable request.

Web Resources

Genome Aggregation Database (gnomAD): http://gnomad.broadinstitute.org/
SIFT: http://sift.jcvi.org/
PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/
Jpred 4: http://www.compbio.dundee.ac.uk/jpred/

Authors’ contributions

ES, BG and TH acquired the clinical data. HH, MN and MJ obtained the DNA samples of the Sudanese groups. YW and MC performed the molecular genetic studies and analyzed the data. YW, AO, SR and RW interpreted the data. YW wrote the initial draft of the manuscript which was edited by all authors. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Molecular genetic study which was performed in Miami has been approved by The Human Subject Research Office of The University of Miami (IRB number: 20140632). Written informed consent for participating in the study by The Human Subject Research Office of The University of Miami (IRB number: 20140632). Written informed consent for publishing all data of the study, such as medical data, images and genetic results has been obtained from all study participants or in the case of subjects under the age of 18 assent was obtained from the child and consent from their parents.

Consent for publication

Written informed consent for publishing all data of the study, such as medical data, images and genetic results has been obtained from all study participants or in the case of subjects under the age of 18 assent was obtained from the child and consent from their parent.

Competing interests

The Authors declare that they have no competing interests.

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