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Case report

A Novel mutation in the Thyroglobulin Gene resulting in Neonatal Goiter and Congenital Hypothyroidism in an Eritrean infant

Short Title: Novel Mutation in the Thyroglobulin Gene

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What is already known on this topic:
Congenital hypothyroidism may be caused by thyroid dysgenesis or thyroid dyshormonogenesis.
Thyroid dyshormonogenesis is cause by mutations in genes involved in hormonogenesis and is usually inherited in an autosomal recessive manner
Mutations in the thyroglobulin gene are a recognized cause of dyshormonogenic congenital hypothyroidism, which may cause fetal or neonatal goiter

What this study adds:
The ***TG*** c.5686+1delG **pathogenic variant** has not been previously described as causing congenital goitrous hypothyroidism.

Abstract
Congenital Hypothyroidism (CH) due to dyshormonogenesis may occur due to mutations in any of the key genes involved in thyroid hormone biosynthesis (***TG, TPO, DUOX2, DUOXA2, SLC5A5, IYD, SLC26A4*** **and** ***SLC26A7***). Mutations in the Thyroglobulin gene (*TG*) are frequently associated with goiter, which may present fetally or neonatally, although a spectrum of phenotypes is reported.
We present the case of a woman of Eritrean origin who presented in the third trimester of pregnancy in the early stages of labor. Ultrasound at presentation revealed a fetal neck swelling consistent with a goiter. Following delivery by caesarian section with minimal respiratory support, the infant was found to be hypothyroid with undetectable serum levels of thyroglobulin. Sequencing of the thyroglobulin gene revealed a homozygous donor splice site **pathogenic variant** (c.5686+1delG) not previously described in the literature. Levothyroxine treatment resulted in normal **growth and psychomotor** development.
Goitrous CH with inappropriately low thyroglobulin has previously been reported in patients harbouring homozygous single nucleotide substitutions at the same *TG* donor splice site which result in exon skipping and Endoplasmin Reticulum retention of *TG*. We conclude that the *TG* c.5686+1delG pathogenic variant is the likely basis for our patient's fetal goiter and congenital hypothyroidism, and that the clinical phenotype associated with *TG* c.5686+1delG is comparable to that seen with single nucleotide substitutions at the same site.
Keywords: Congenital Goiter, Hypothyroidism, Thyroglobulin, Novel Mutation, Case report

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Introduction

Congenital hypothyroidism (CH) is the commonest neonatal endocrine condition with recent reports demonstrating an incidence of around 1 in 1500 live births [1]. Untreated CH can result in profound neurodevelopmental delay, however, since the introduction of newborn screening for CH in most western countries, there has been a dramatic improvement in neurodevelopmental outcomes associated with the condition [2]. CH is traditionally subdivided into thyroid dysgenesis (TD), and dyshormonogenesis where TD refers to structural abnormalities of the thyroid including thyroid agenesis or an ectopic/sublingual thyroid gland and dyshormonogenesis describes inadequate thyroid hormone biosynthesis by a normally-located, often goitrous gland, due to molecular defects in the thyroid hormone biosynthesis machinery [3]. Dyshormonogenesis usually has an identifiable monogenic basis, involving mutations in thyroglobulin (***TG***) or genes involved in iodine transport, organification and recycling (***SLC5A5, SLC26A4, DUOX2, DUOXA2, TPO, SLC26A7, IYD***), although oligogenic mutations may also contribute [3,4]. Inheritance of dyshormonogenesis is usually autosomal recessive although CH due to ***DUOX2, DUOXA2*** and ***IYD*** mutations may also be dominantly inherited.

Dyshormonogenesis may present with goiter in the neonatal period or later on, and may rarely be associated with fetal goiter. The mechanical consequences of fetal dyshormonogenic goiter may be associated with significant morbidity. Polyhydramnios may occur due to esophageal compression, and at delivery, neck hyperextension may result in malpresentation; additionally, neonatal tracheal compression may cause fatal respiratory compromise. Complications can also arise from the underlying foetal thyroid dysfunction that can negatively affect development in utero and subsequent neurodevelopmental outcomes [5].

The management of hypothyroid fetal goiter remains controversial. Since fetal goiter is a marker of serious thyroid dysfunction many recommend the determination of thyroid function and the consideration of in-utero treatment. Although fetal ultrasound, **Magnetic Resonance Imaging** and amniotic fluid TSH levels have all been methods suggested for determining fetal thyroid status, these have been shown to have variable levels of accuracy and cordocentesis has been recommended as the gold standard for determining fetal thyroid status. However, it must be taken into account that this may be associated with complications including cord bleeding, chorioamnionitis and preterm delivery among others [6,7].

When considering the decision to treat fetal goiter with associated hypothyroidism, one needs to take into account the size of the goiter, the effect on surrounding structures and associated features such as polyhydramnios. Treatment with both intra-amniotic levothyroxine and administration via the umbilical vein have been described with varying degrees of success, both with regards to reduction in goiter size, reduction in perinatal complications and improvement of fetal thyroid hormone levels [8,9]. However, conservative management, with radiological surveillance and elective delivery with respiratory support where necessary have also achieved favorable outcomes in some cases [10].

Genetic evaluation has been infrequently undertaken in cases with dyshormonogenic fetal goiter although underlying mutations in ***TG, TPO, DUOX2***, and ***DUOXA2*** have been reported in this context [10,11,12,13,14].

Case Presentation

A 35-year-old woman of Eritrean origin was referred to a tertiary obstetric centre due to the finding of a neck mass on fetal ultrasound on presentation at term. Exact gestation was unknown as she had had no previous antenatal follow up or ultrasound scans.

Maternal history was notable for multi parity, with four previous healthy live births with no history of any congenital anomalies or congenital hypothyroidism. She had no significant past medical history. Her non-consanguineous partner was also Eritrean with no significant past medical history.

On fetal ultrasound exam, a large mass was visualized in the neck and upper chest consisting of two lobes consistent with an enlarged thyroid gland (Figure 1). The mass including both lobes measured 55mm X 63mm. The trachea was noted to pass through the two lobes with no narrowing noted. Both carotids were displaced laterally by the mass. In addition, the **Superior Vena Cava** was significantly enlarged, the heart was enlarged and significant **Tricuspid Regurgitation** was visualized

In anticipation of difficulties in airway management following delivery, a cesarean section was planned with pediatric **Eear Nose and Throat** and anesthetic staff present. A live female infant was delivered by cesarean in good condition with Apgar's of 8 and 9 at 1 and 5 minutes respectively. Oxygen saturation was low and the infant was treated with high flow nasal cannula oxygen. Initial examination was notable for a large diffuse neck swelling (Figure 2) and mild respiratory distress with no other abnormal examination findings. The infant was transferred to the neonatal unit for further investigation and management.

In light of prenatal ultrasound findings and clinical examination, initial studies were carried out to investigate thyroid structure and function. Initial thyroid function **in the first 24 hours of life**, showed primary hypothyroidism although free T3 levels were preserved: TSH 272.4 mIU/l (0.4-20) FT4 6.3 pmol/l (10-30) FT3 5.5 pmol/l (2.5-9.8). Thyroglobulin was inappropriately low 0.7 picg/l (0-55), without detectable levels of thyroglobulin antibodies < 20U/ml).

A Thyroid ultrasound showed enlargement of both lobes of the thyroid gland including the isthmus. The gland was reportedly of normal texture and was hyperemic. A thyroid technetium scan was also performed (Tc-99m) which demonstrated a diffusely enlarged thyroid gland with diffusely increased uptake.

In view of the laboratory findings of primary hypothyroidism, treatment was commenced on day 1 of life with high dose levothyroxine (18 mcg/kg) with rapid normalization of thyroid function tests. The dose was gradually tapered down accordingly. The child continue**s** endocrine follow up and **at the age of 3 years** is currently well managed with medical therapy, with a **very small goiter which increases in size in accordance with increasing TSH levels.** She has no additional medical problems and shows normal **growth and** psychomotor development.

Due to the association of neonatal goiter with laboratory evidence of hypothyroidism, low serum titre of thyroglobulin and diffuse uptake of technetium on nuclear scanning, a genetic defect in the thyroglobulin gene was suspected and genetic studies were undertaken with written informed parental consent .

Genetic Studies

Sanger sequencing of the thyroglobulin gene (***TG***, ENST00000220616.9) revealed a novel donor splice site **pathogenic variant** at the exon 30-intron 30-31 boundary; c.5686+1delG which is absent from the gnomAD database [15] (Figure3). The patient was homozygous for the **pathogenic variant**. **Due to parental reluctance to pursue further genetic testing,** DNA was not available from her parents or four siblings for genotyping.

Discussion/Conclusion

Thyroglobulin is a large secretory protein which is crucial for thyroid hormone biosynthesis and storage in the thyroid follicular lumen. The *TG* gene encodes a protein of 2768 amino acids in length including a 19 amino acid N-terminal signal peptide. The recently-solved protein structure of TG has defined five regions (N-terminal domain, core, flap, arm and C-terminal domain) containing domains of type-1 to type-3 cyteine rich TG repeats and a C-terminal choline-esterase-like domain (ChEL) as well as a probable 4 hormonogenic acceptor tyrosines and five donor tyrosines [17]. TG is synthesized in the endoplasmic reticulum and folds with the assistance of molecular chaperones before trafficking to the apical membrane. The complex protein folding and intracellular trafficking of TG are essential for its normal follicular secretion and require both endoplasmic reticulum (ER) chaperones and oxidoreductases, as well as specific intramolecular interactions [16]. The Chel domain has an important role in permitting TG intracellular trafficking and secretion and intradomain disulfide bonds between the many cysteine residues in TG are essential for the correct folding of newly synthesized TG [16,18,19].

TG mutations are a common cause of dyshormonogenesis with an estimated frequency of at least 1:100,000 and are usually inherited in an autosomal recessive manner although CH has rarely been associated with monoallelic mutations [19,20,21]. To the best of our knowledge, ***TG*** c.5686+1 delG has not previously been reported, however, three different point mutations resulting in single nucleotide substitutions at the same site have been reported in association with congenital goitrous hypothyroidism; ***TG* c.5686+1G>A, 5686+1G>C and 5686+1G>T** [20,21,22,23,24,25]. Analysis of patient-derived thyroidal tissue has confirmed that ***TG* c.5686+1G>T and c.5686+1G>C** mutations cause skipping of exon 30 with a resultant in-frame deletion of 46 amino acids in the TG type III repeat domain, causing the loss of 1- putative N-linked glycosylation site and modifying the TG protein structure [23,25]. In common with most pathogenic ***TG*** mutations, ***TG* c.5686+1G>T** results in TG misfolding and retention within the endoplasmic reticulum with decreased export to the colloid. [26]. The fact that ***TG*** c.5686+1 delG disrupts the same canonical donor splice site guanine residue, suggests that it is highly likely to be pathogenic, although functional studies to confirm this were not undertaken in our study. **Due to parental reluctance to pursue further genetic testing**, we were unable to obtain DNA to confirm segregation of homozygosity for the mutation with CH phenotype.

Individuals harbouring ***TG*** mutations exhibit a spectrum of thyroid dysfunction, ranging from biochemically severe CH to euthyroid goiter [23,27,28,29,30]. Goiter occurs frequently, commonly manifesting in the neonatal period (although onset may be delayed), and a small minority of cases exhibit fetal goiter [12,20,31,32]. The biochemical hallmark of CH due to a ***TG*** mutation comprises an inappropriately low or undetectable circulating thyroglobulin level despite elevated circulating TSH concentration or goiter, and failure of exogenous TSH to stimulate a rise in serum TG. Thyroidal iodide uptake is enhanced and organification of iodide is usually preserved [21,24]. In some cases, the FT3/FT4 ratio is elevated [25].

Previously reported cases with homozygous disruption of the same donor splice site have shown variable biochemical phenotypes although goiter is a consistent feature. Two Brazilian siblings homozygous for the TG **c.5686+1G>T** mutation exhibited fetal or neonatal goiter and severe CH. [20]. An additional two Brazilian siblings with the same mutation initially presented with congenital goiter, however, although the eldest sibling exhibited severe hypothyroidism, his sister had a milder biochemical phenotype with low serum total T4 but normal total T3. It is hypothesized that the variable expressivity seen in this family may be partly explained by iodine status, since the elder sibling was raised predominantly in an iodine deficient area whereas the family's relocation to an iodine replete region when his sister was aged 2, may have ameliorated her thyroid dysfunction [23]. A homozyous ***TG* c.5686+1G>C** mutation was also identified in an iodine replete Pakistani girl, presenting aged 10 years with a massive goiter but normal TSH, subnormal FT4 and raised FT3 levels [25]. **It is likely that in this case and the mildly hypothyroid Brazilian sibling, small quantities of mutated thyroglobulin molecules reach the follicular lumen, permitting iodination and synthesis of thyroid hormones, which is facilitated by adequate iodine intake. The elevated FT3/FT4 ratio may be at least in part due to increased thyroidal type 2 deiodinase activity** [19,33]. ***TG* c 5686+1 G>A** was detected in compound heterozygosity with ***TG*** Q310P in two Japanese cases for whom detailed individual data was not presented although both were on treatment for screening-detected CH and had goiter in early childhood [24].

Here, we report a female patient of Eritrean origin with a large fetal goiter detected whilst in utero prior to delivery. Lack of ultrasound data from earlier in the pregnancy preclude definitive comments regarding the onset of goitrogenesis, however its large size and local compressive effects suggest it may have originated some weeks earlier. She had significant primary hypothyroidism at birth with preserved FT3 levels despite subnormal FT4 levels, and her thyroglobulin level was inappropriately low. These clinical and biochemical features are all recapitulated in previously reported patients harbouring mutations at the ***TG*** c.5686+1 donor splice site and we believe her homozygous TG c.5686+1 delG **pathogenic variant** to be the likely cause of her thyroid dysfunction although future functional studies will be required to confirm this.

Urinary iodine was not measured contemporaneously with presentation in this patient but since there is evidence to suggest that iodine intake during pregnancy may be inadequate in some areas of Israel suboptimal maternal iodine

status may have contributed to the fetal goitrogenesis although **the patient's normal FT3 suggests some iodination of mutant TG was occurring in the follicular lumen**. Although conservative management of her fetal goiter resulted in a relatively uncomplicated delivery, radiological monitoring for fetal goiter would be advisable during future pregnancies given the presumed 25% risk of having another affected child if both parents are heterozygotes for the ***TG*** c.5686+1 delG **pathogenic variant**. Additionally, maternal iodine status should be optimized.

Conclusion

We report a case of an infant presenting in late pregnancy with a large fetal goiter, congenital hypothyroidism determined on laboratory studies following delivery and an undetectable serum thyroglobulin. Sequencing of the thyroglobulin gene revealed an as yet unreported homozygous donor splice site **pathogenic variant** at the exon 30-intron 30-31 boundary; c.5686+1delG. Previous single nucleotide substitutions and this site have been described with variable phenotypes including both neonatal goiter, goitrous hypothyroidism, and goitrous euthyroidism. Genetic evaluation where carried out revealed skipping of exon 30 in the mRNA and subsequent generation of a shortened protein. Functional evaluation of single nucleotide substitutions at this site demonstrated retention of the TG protein within the endoplasmic reticulum and resulting decreased exportation to the follicular lumen. Due to **parental refusal further** genetic testing **on unaffected family members** was not carried out. However based on previous studies as described and the patient's clinical, biochemical and imaging phenotype we concluded that this **pathogenic variant** was the likely cause for the patient's clinical condition.

Author Contributions

ES, YN, EK and OH conceived of the presented idea. AKN performed Sanger sequencing. NS analyzed Sanger sequencing. ES wrote the manuscript with support from YN, NS and OH. All authors read and approved the final manuscript draft.

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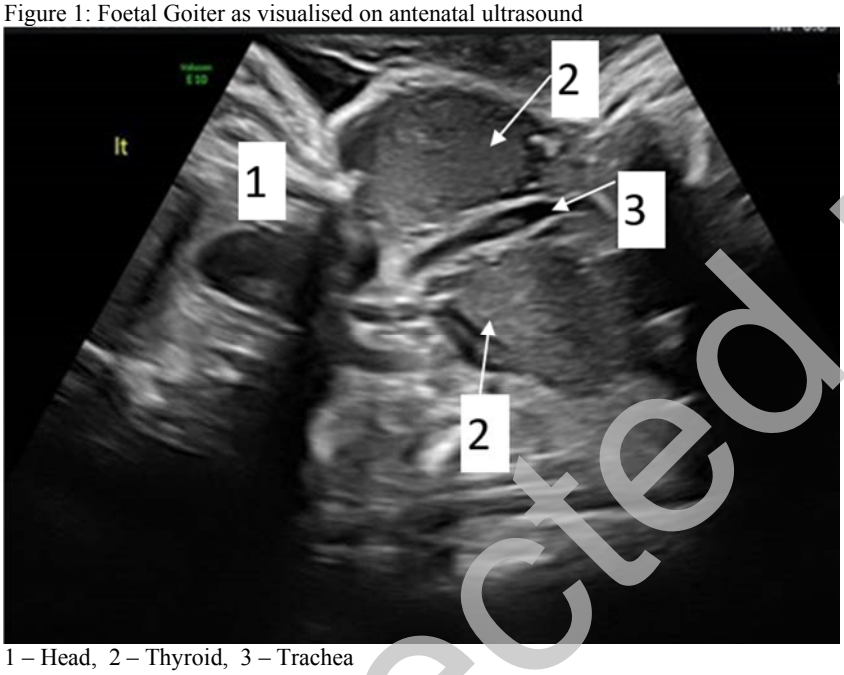
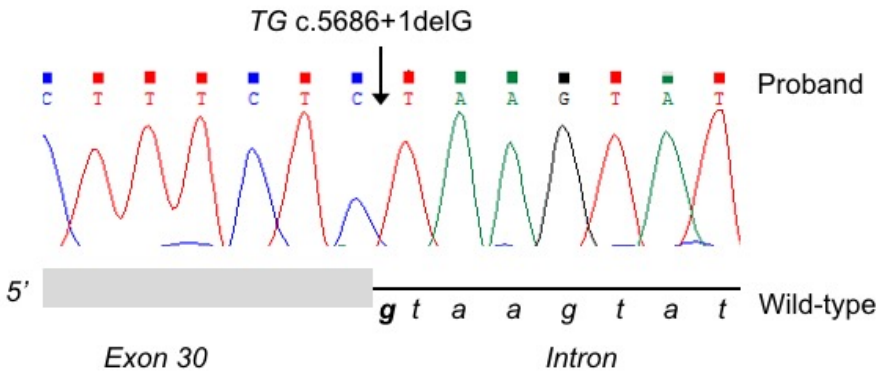
Figure 1: Foetal Goiter as visualised on antenatal ultrasound



1 – Head, 2 – Thyroid, 3 – Trachea

Figure 2: Neonatal Goiter

Figure 3.



Sanger sequencing chromatogram for the *TG* exon 30-intron boundary, demonstrating a single nucleotide deletion at the donor splice site (ENST00000220616.9, c.5686+1 delG).