First case of fetal goitrous hypothyroidism due to *SLC5A5/NIS* mutations

Athanasia Stoupa^{1,2,3}, Ghada Al Hage Chehade³, Dulanjalee Kariyawasam³, Celine Tohier¹, Christine Bole-Feysot⁴, Patrick Nitschke⁵, Helene Thibault⁶, Marie-Laure Jullie⁷, Michel Polak^{1,2,3,8,9} and Aurore Carré^{1,2}

¹INSERM U1016, Cochin Institute, Faculté de Médecine, Université Paris Descartes, Sorbonne Paris Cité, Paris, France, ²IMAGINE Institute affiliate, Paris, France, ³Pediatric Endocrinology, Gynecology and Diabetology Unit, Hôpital Universitaire Necker-Enfants Malades, AP-HP, Paris, France, ⁴Genomics Platform, INSERM UMR 1163, Imagine Institute, Paris Descartes Sorbonne Paris Cité University, Paris, France, ⁵Bioinformatics Platform, IMAGINE Institute, Paris Descartes University, Paris, France, ⁶Pediatric Endocrinology, ⁷Department of Pathology, CHU de Bordeaux, Bordeaux, France, ⁸Centre de Référence des Maladies Endocriniennes Rares de la Croissance et du Développement, Necker-Enfants Malades University Hospital, Paris, France, and ⁹Centre régional de dépistage néonatal (CRDN) Ile de France, Paris, France

Correspondence should be addressed to M Polak or A Carré
Email
michel.polak@aphp.fr or
aurore.carre@inserm.fr

Abstract

Background: Among patients with congenital hypothyroidism, 35% have dysmorphogenesis (DH) with thyroid gland *in situ* with or without goiter. The majority of DH cases are due to mutations in genes involved in thyroid hormone production as *TG*, *TPO*, *SLC5A5/NIS*, *SLC26A4/PDS*, *IYD/DEHAL1*, *DUOX2*, and *DUOX2A2*, and are usually inherited on an autosomal recessive basis. Most previously reported cases of fetal hypothyroidism and goiter were related to *TG* or *TPO* mutations and recently *DUOX2A2*.

Patient: In a male patient with antenatal goiter treated with intraamniotic levothyroxine injections, whose long-term follow-up is described in detail, two novel *NIS* mutations were detected. Mutations of *NIS* were located in exon 1 (c.52G>A, p.G18R) and exon 13 (c.1546C>T, p.R516X), each mutation was inherited from parents, who are healthy carriers. The p.G18R mutation affecting the first transmembrane domain of the protein can be responsible for deficient iodide uptake. However, the second is a nonsense mutation leading probably to mRNA degradation. In addition, the patient has undergone a thyroidectomy and we have studied the thyroid tissue. The thyroid histology showed heterogeneity with large follicles, epithelial hyperplasia and many areas of fibrosis. Immunohistochemistry with *NIS* specific antibody showed *NIS* staining at the basolateral plasma membrane of the thyrocytes.

Conclusions: We report the first case of fetal goitrous hypothyroidism due to two novel *NIS* mutations with access to thyroid tissue of the patient, specific histology studies and long-term follow-up. This case expands our knowledge and provides further insights on molecular causes of fetal goiter in humans.

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Introduction

Biosynthesis of thyroid hormone requires iodide that enters in thyrocytes by sodium iodide symporter (*NIS/SLC5A5*) at the basolateral membrane. Mutations of *NIS* are responsible for congenital hypothyroidism due to dysmorphogenesis (DH) inherited on an autosomal

recessive basis (1). In a patient with antenatal goiter treated with intraamniotic levothyroxine injections who was found to have two novel *NIS* mutations, the thyroidectomy specimen was assessed by histology and immunohistochemistry.

Methods

This study was approved by French Biomedecine Agency and written informed consent was obtained from the patient and/or his family.

Detection of mutations

We designed a TNGS panel (HypothySeq NGS) of 78 genes including genes known to be associated with CH (thyroid dysgenesis; dyshormonogenesis; thyroid hormone transport protein defects; and inborn errors in thyroid hormone membrane transport, metabolism, or action) and candidate genes that have been identified in animal models (mouse and zebrafish knock-out models) or by microarray assays but not yet validated in humans. Sensitivity (false-negative rate) of the panel was assessed in positive controls with known thyroid disease-causing mutations, including mutations in dyshormonogenesis genes, and specificity (false-positive rate) in healthy individuals previously screened using whole exome sequencing for another research project. TNGS on HiSeq 2500 system (Illumina, San Diego, CA) and bioinformatics analyses were already described (2).

Immunohistochemistry studies

After approval of the experimental design and protocols by our institutional review board, adult thyroid tissue samples were obtained from patients undergoing thyroid surgery. Thyroid tissues were fixed in 3.7% formaldehyde solution and embedded in paraffin. Then 4- μ m sections were cut, deparaffinized by serial passages in toluene and alcohol, heated for 12 min in three 4-min in microwave citrate buffer (0.1M, pH 6) solution cooled, permeabilized with TBS-Triton 1% for 17 min, blocked with TBS-BSA 3%-Tween 0.1% and, finally, incubated with Anti-Sodium-Iodide Symporter antibody (1:500, # ABC1454, Merck Millipore) overnight at 4°C. The NIS antibody targets an epitope within 13 amino acids from the C-terminal end. After three 5-min washes in TBS-Tween 0.1%, the sections were incubated with biotinylated secondary antibody for 1 h, after which immunostaining using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA) was performed according to the manufacturer's instructions. The sections were then incubated in 3,3'-diaminobenzidine tetrahydrochloride and counterstained with Hematoxylin-Eosin. Photographs were taken using a microscope (Leitz DMRB; Leica) and digitized using a Hamamatsu C5810 chilled 3CCD camera (Middlesex, NJ).

RNA extraction, RT-PCR and cDNA sequencing

Thyroid tissue removed during thyroidectomy from the patient was immediately snap-frozen and stored at -80°C . Total RNA was isolated using the Qiagen RNeasy Microkit or Minikit (Qiagen). The Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific) was used for RT of 250 ng of each RNA sample as previously described (3). Sanger sequencing was performed to test the cDNA.

Patient

The patient was a boy born at 38 weeks to healthy French Caucasian non-consanguineous parents. A routine antenatal sonogram at 25 gestational weeks (GW) showed enlarged thyroid. In the mother, thyroid hormone assays, including markers of thyroid autoimmunity, iodine status, and thyroid ultrasound were normal. Maternal iodide intake during pregnancy was at 200 μg daily. Assays on a fetal blood sample retrieved at 25 GW demonstrated hypothyroidism (TSH: 150 mIU/L (normal: 6.85 ± 2.85 mIU/L); and FT4, 3.3 pmol/L (normal: 13.1 ± 1.6 pmol/L)) (4). Four weekly intraamniotic L-thyroxine injections were given in dosages of 300 μg /injection starting at 31 gestational week (31, 32, 33, 34 GW) and two additional injections of 400 μg were administered at 35 and 36 GW. Regular fetal thyroid status monitoring showed that the hypothyroidism improved without resolving fully (TSH: 79 mIU/L; and FT4: 5.8 pmol/L at 36 GW). Ultrasound scan revealed that the fetal goiter diminished, without any evidence of tracheal compression and the amount of amniotic fluid was normal. At birth at 38 GW, body measurements were normal (weight: 3050 g; height: 50 cm; head circumference: 35 cm) and persistent goiter was noted. Cord blood assays showed high TSH (68 mIU/L; normal < 11.8 mIU/L) and low FT4 (5.6 pmol/L; normal > 10.4 pmol/L). At 12 h of postnatal age, TSH remained elevated (110 mIU/L) and FT4 low (8 pmol/L). Ultrasound on day 1 revealed that the thyroid gland was enlarged (right lobe, 30 mm $\times$ 12 mm; left lobe, 21 mm $\times$ 10 mm; normal: 19.4 mm (9–25) $\times$ 8.8 mm (0.5–1.4)) (5). Delayed bone maturation on a knee X-ray confirmed the severity of fetal hypothyroidism. No evidence of tracheal compression was visible on a chest and neck X-ray. Thyroid I^{123} scintigraphy demonstrated decreased radionuclide uptake and the perchlorate discharge test was normal (3.7%, $n < 10\%$). Thyroglobulin measurement was unfortunately not available. Levothyroxine supplementation was started on day 2

(6.5 µg/kg/day). On day 3, TSH had dropped to 21 mIU/L and FT4 increased to 23.6 pmol/L. The levothyroxine dosage was adjusted regularly based on thyroid function tests and growth. Thyroid function tests during infancy are shown at Supplementary Table (see section on [supplementary materials](#) given at the end of this article). Physical and neurological development were normal during follow-up and the patient completed a high school education. At 15 years and 9 months of age, because of poor compliance with periods of missing substitution and goiter growth, the patient reported episodes of dysphagia and dysphoria.

Thyroid gland size by sonography was increased (volume: 137.6 mL (normal: 8 ± 3 mL); right lobe, $88 \times 36 \times 32$ mm; left lobe, $100 \times 51 \times 32$ mm) and several nodules with a maximal length of 25 mm visible bilaterally. [Figure 1A](#) shows the goiter before total thyroidectomy, performed at 16 years of age. By gross examination, the total weight was 303 g and several thyroid nodules were found ([Fig. 1B](#)). Microscopically, the nodules were vesicular, with microfollicles. There was no cytological or architectural evidence of malignancy. The patient is currently 21 years of age under daily levothyroxine.

Figure 1

Novel *SLC5A5*/*NIS* mutations found in a patient with prenatal goitrous hypothyroidism. (A) Photograph showing the enlarged thyroid in our patient before thyroidectomy at 16 years old. (B) Macroscopical image of the thyroid after thyroidectomy. (C) Familial pedigree. The index case is shown by the black box. This patient has two novel *SLC5A5*/*NIS* mutations, c.52G>A and c.1546C>T. The chromatograms show the novel *SLC5A5* mutations carried by the father (c.52G>A) and mother (c.1546C>T), respectively. The black dots show the mutated nucleotide. The heterozygous state for each mutation is demonstrated in the electropherograms of genomic blood DNA sequencing. The electropherograms of patient's thyroid tissue cDNA sequencing shows the apparently 'homozygous state' for the c.1546C>T mutation. (D) Immunohistochemistry of thyroid tissue with Hematoxylin-Eosin and NIS-specific antibody. NIS staining appears in brown: (a) control thyroid tissue; (b) thyroid tissue from a patient with Graves' disease showing marked NIS expression, as a positive control for NIS staining; (c and d) thyroid tissue from our patient showing NIS expression at the basolateral thyrocyte membrane; (e and f) thyroid tissue from our patient showing large heterogeneous follicles surrounding by flat epithelium. a', b' and c' were enlargement of a, b and c.

Targeted-NGS (TNGS), including genes involved in thyroid development and function, identified two novel *SLC5A5/NIS* mutations in this patient, in exon 1 (c.52G>A, p.G18R) and exon 13 (c.1546C>T, p.R516X). Each mutation was also found in one of the parents, as confirmed by Sanger sequencing. Mutations affecting the first transmembrane domain of the protein, as p.G18R, can be responsible for deficient iodide uptake, as previously reported in patients with nearby located mutations as the p.V59E NIS mutant, at the end of the transmembrane domain 2 (6, 7). The RNA of thyroid tissue of the patient was analyzed. The cDNA appeared to be homozygous for the WT allele and the mutated allele (c.1546C>T, p.R516X) was not detected, suggesting a mRNA degradation (Fig. 1C).

The thyroid histology showed large and dilated follicles full of colloid without peripheral scalloping but associated with papillary infoldings. Follicles were covered by a flattened epithelium. Papillary cores were often enlarged, edematous and hyalinized, covered with round thyrocytes with moderately abundant cytoplasm. However, few smaller papillae with thin cores were covered by taller follicular cells. No inflammation was seen. Few adenomatous lesions were also a feature. Immunohistochemical studies showed NIS specific staining of the basolateral plasma membrane of the thyrocytes, with no staining of the cytosol or apical membrane (Fig. 1D). Staining of control thyroid tissue identified NIS. These findings are consistent with iodide uptake due only to the mutant p.G18R NIS protein, which at its normal location in the basolateral thyrocyte membrane.

Novel insights

- In a patient with antenatal goiter treated with intraamniotic levothyroxine injections, we found, for the first time, two novel *SLC5A5/NIS* mutations thanks to targeted-NGS.
- The thyroid histology of mutated patient showed enlarged follicles with papillary infoldings featuring enlarged cores and surrounded by a flat epithelium.
- Our case adds valuable information about the molecular causes of fetal hypothyroidism and goiter not due to maternal autoimmune thyroid disease, with or without a requirement for intraamniotic levothyroxine treatment.

Discussion

TNGS allowed the molecular diagnosis and identification of two novel *SLC5A5/NIS* mutations in a patient with fetal goiter. Identification of novel mutations, characterizing them at the molecular level and determining the molecular requirements at the relevant positions enriches our knowledge about NIS function and targeting to the plasma membrane. This is the first report of *SLC5A5/NIS* mutations in a patient with severe fetal goitrous hypothyroidism. Study of the goitrous thyroid tissue identified a nonsense-mediated mRNA decay process characterized by the degradation of the mRNA produced by the second nonsense mutation. This mechanism has been described in a patient with a mutation at amino acid 531 (p.Y531X), that is, near the mutated amino acid in our patient (p.R516X) (8). Fetal goiter is a rare condition that mainly occurs in iodine-sufficient areas as maternal Graves' disease. Our case adds valuable information about the molecular causes of fetal hypothyroidism and goiter not due to maternal autoimmune thyroid disease, with or without a requirement for intraamniotic levothyroxine treatment. In our patient, intraamniotic levothyroxine therapy decreased the size of the goiter, thereby decreasing the risk to the fetus, notably during delivery. Furthermore, hormone replacement therapy was initiated on day 2 of life, that is, earlier than in patients with CH diagnosed by neonatal screening. Long-term follow-up showed normal development in this patient. In addition, as previously described, patients with *NIS* mutations may develop multinodular goiter later in life (9). A hypothesis is that low intrathyroidal iodide and high serum TSH level for a short period in early life may account for tumorigenesis. This effect could be in combination with the *NIS* pathogenic mutations or independent of molecular defect.

In conclusion, we report the first case of *SLC5A5/NIS* mutations in a patient with fetal goitrous hypothyroidism, for whom thyroid tissue investigations and long-term follow-up data are described.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-20-0255>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this article.

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Author contribution statement

Michel Polak and Aurore Carré: These two authors are co-last authors.

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