A Somatic Gain-of-Function Mutation in the Thyrotropin Receptor Gene Producing a Toxic Adenoma in an Infant

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Background: Activating mutations of the thyroid stimulating hormone receptor gene (*TSHR*) are rare in the neonate and in the pediatric population. They are usually present in the germline, and are either inherited or occur *de novo*. Somatic mutations in *TSHR* are unusual in the pediatric population.

Methods: We describe a nine-month-old infant with thyrotoxicosis who harbored an activating somatic mutation in *TSHR* that was not present in the germline.

Results: As genomic DNA analysis failed to show a *TSHR* gene mutation, a radioiodide scan was performed to reveal a unilateral localization of uptake suppressing the remaining thyroid tissue. Genomic and complementary DNA analyses of the active thyroid tissue, removed surgically, identified a missense mutation (D633Y) located in the sixth transmembrane domain of the *TSHR*. The absence of this *TSHR* mutation in circulating mononuclear cells and in unaffected thyroid tissue confirmed the somatic nature of this genetic alteration.

Conclusions: To the authors' knowledge, this is the youngest patient to receive definitive treatment for hyperthyroidism due to an activating mutation of *TSHR*.

Introduction

ACTIVATING MUTATIONS in the thyrotropin receptor gene (*TSHR*) have been shown to be a major cause of non-autoimmune hyperthyroidism and hyperfunctioning thyroid adenomas of the adult (1). The reported prevalence of *TSHR* mutations in toxic adenomas varies widely (reviewed by Gozu *et al.* [2]) but may be as high as 80% of patients with toxic nodules (3,4). Since the first description in 1993 of a *TSHR* mutation in a hyperfunctioning thyroid adenoma (5), approximately 50 different activating *TSHR* mutations have been reported (6) (see <http://gris.ulb.ac.be/>). Of these, more than one half are somatic mutations (7). Many are located within the sixth transmembrane domain and the third intracellular loop of the *TSHR* where the receptor interacts with G proteins. In contrast, the presence of a hyperfunctioning somatic mutation of *TSHR* is highly unusual in the neonate and infant and has been so far reported in a single case (8).

Patients and Methods

Case report and subjects

The patient was a boy born at 37 weeks, by cesarean section, to non-consanguineous African American parents. The

mother, a non-smoker and on no medications, followed a regular diet and had an uneventful pregnancy. Delivery was complicated by meconium with a transient decrease in fetal heart rate. Birth weight was 7 pounds 6 ounces (3.35 kg) and length 21 inches (53.3 cm). The infant was nursed with adequate weight gain until 3 months of age, at which time he developed eczema and recurrent episodes of wheezing. At 6 months, a pediatrician evaluated the patient for emesis, feeding difficulties, and a decrease in weight. Further evaluation by a gastroenterologist and an allergist lead to the institution of food supplementation in the form of a hypercaloric diet. This produced a catch-up weight gain by 9 months of age. Thyroid function tests performed at that visit revealed a TSH of <0.01 mU/L (normal range: 0.70–6.40 mU/L) and a free thyroxine (T₄) level of 5.1 ng/dL (normal range: 0.8–2.2). The patient was referred to the endocrine service for further evaluation. Family history is notable for autoimmune thyroid disease in the paternal grandmother who is on L-thyroxine. The parents and two paternal half-siblings are healthy.

Physical examination at 9 months revealed a head circumference of 48.5 cm (above the 95th percentile) with anterior fontanel barely palpable, length 75 cm (95th percentile), weight 8.0 kg (10th percentile). The patient was hyperkinetic

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with a pulse of 100 beats per minute and blood pressure of 90/60 mm Hg. Skin showed diffuse eczematous lesions. Eyes showed lid retraction and stare but full extraocular movements. The thyroid gland was not enlarged and no palpable masses were appreciated. The remainder of the exam was unremarkable with appropriate for age neurological development. TSH was <0.01 mU/L; free T_4 , 3.8–5.1 ng/dL; and total triiodothyronine (T_3), 377–609 ng/dL, on several determinations; and total T_4 , 17.0 μ g/dL. Thyroid stimulating IgG was 82% (normal 0–129%), and thyroperoxidase (TPO) and thyroglobulin (Tg) antibodies were negative. Bone age was 4 years at a chronologic age of 9 months. Skeletal survey showed increased ossification of the proximal and distal femoral and proximal tibial ossification centers for age but there was no craniosynostosis. There was normal sinus rhythm on electrocardiogram and normal pulmonary arterial pressures by echocardiography with normal left ventricular size and function. Thyroid ultrasound with color Doppler showed a focal, $3.1 \times 1.5 \times 1.6$ cm, hypoechoic and hypervascular area replacing the right lobe with normally appearing glandular tissue in the upper pole. The left lobe was normal in size and echotexture, measuring $1.7 \times 0.6 \times 0.7$ cm. Thyroidal ^{123}I scan revealed markedly increased uptake within the right lobe with suppression of the remainder of the gland (Fig. 1A).

The patient was referred for partial thyroidectomy with removal of the hyperfunctioning nodule. Thionamide therapy and supersaturated potassium iodide (SSKI) were instituted in preparation for surgery. At surgery, the right lobe of the thyroid gland appeared to be larger than the left and more vascular. The left lobe appeared normal. A right thyroid lobectomy was performed. Several small biopsies were taken from the contralateral lobe for histology and *TSHR* sequencing. Histological examination showed thyroid hyperplasia demarcated from the surrounding normal thyroid tissue by a thin rim of fibrous tissue (Fig. 1B and 1C). Subsequent to the surgery, the patient remains clinically and biochemically euthyroid on treatment with 37.5 μ g daily levothyroxine (LT_4). Head circumference, growth, and development are normal.

Studies were approved by the Institutional Review Board and informed consents were obtained to perform thyroid and

genetic evaluations of the patient and all available immediate family members.

Thyroid function tests

Total T_4 and T_3 were measured using commercial automated chemiluminescent immunometric methods and TSH by a third generation chemiluminescence assay (Elecsys 2010, Roche, Indianapolis, IN). 3,3',5'-L-triiodothyronine, or reverse T_3 (rT_3), was measured by radioimmunoassay (Adaltis, Italy) and serum Tg by an in-house assay as previously reported (9). The free T_4 index ($FT_4\text{I}$) was calculated as the product of the serum total T_4 and the normalized resin T_4 uptake ratio. TPO and T_G antibodies were measured by passive hemagglutination (Fujirebio, Inc., Tokyo, Japan).

DNA and RNA isolation, amplification, and sequencing

Genomic DNA was extracted from circulating mononuclear cells and all *TSHR* exons were amplified by the polymerase chain reaction (PCR) as described (10), and sequenced. PCR conditions will be provided upon request.

Thyroid tissue samples (hyperplastic and normal) excised at surgery were placed in a monophasic solution of phenol and guanidine isothiocyanate (TRIZOL[®] Reagent, Invitrogen Life Technologies, Carlsbad, CA) and shipped by FedEx from New York City to Chicago. Total RNA was extracted and the first strand cDNA was synthesized using the SuperScript[™] III First-Strand Synthesis System for reverse transcriptase (RT)-PCR protocol (Invitrogen Life Technologies). *TSHR* cDNA was then amplified by PCR using specific primer pairs (11) and sequenced.

Results

Thyroid function tests confirmed the diagnosis of hyperthyroidism in the proband. In addition to high T_4 and T_3 concentrations, rT_3 level was also high. The endogenous source of iodothyronines, accompanied by suppressed TSH, was confirmed by the presence of high serum Tg (III-1, Fig. 2). Thyroid function tests of the parents and paternal grand-

FIG. 1. Anatomical and histological findings. (A) Radioiodide scintillation scan showing increased uptake in the upper pole of the right lobe of the thyroid with suppression of the remainder of the gland. The locations of the chin (∇) and suprasternal notch (∇) are indicated. (B) Photomicrograph of affected thyroid tissue from the right lobe, showing hyperplasia. Epithelial cells lining the colloid are columnar with acidophilic cytoplasm and hyperchromatic basally located nuclei. The colloid is pale with prominent scalloping where it abuts the epithelium. (C) Photomicrograph of a biopsy from the left lobe of the thyroid gland, showing smaller follicles of fairly uniform size. The lining epithelium is cuboidal with pale acidophilic cytoplasm and uniform nuclei. The colloid is deeply eosinophilic.

FIG. 2. Family pedigree and results of thyroid function tests. Results are aligned with the individual's symbol and abnormal values are shown in bold numbers. Note that the proband (arrow) has increased serum concentration of all three iodothyronines and thyroglobulin but a suppressed thyrotropin. TT₄, total thyroxine; TT₃, total triiodothyronine; TrT₃, total 3,3',5'-L-triiodothyronine; FT₄I, free T₄ index; FT₄, free thyroxine; TSH, thyrotropin; Tg, thyroglobulin; TPOAb, thyroid peroxidase antibody; TgAb, thyroglobulin antibody.

mother were within the range of normal, except for the presence of TPO and Tg antibodies in the latter (I-1, Fig. 2), confirming the diagnosis of autoimmune thyroid disease for which she was taking LT₄.

No mutation was found in genomic DNA from circulating mononuclear cells, or from genomic DNA or complementary DNA obtained from normal thyroid tissue (left lobe of the thyroid gland). On the other hand, both genomic and complementary DNAs obtained from two samples of the hyperplastic thyroid tissue from the right lobe revealed the same mutation in one of the two alleles of *TSHR*. A point mutation replacing the normal guanosine with thymidine at codon 633 (GAC→TAC) was detected, resulting in the replacement of the normal aspartic acid with a tyrosine (D633Y) located in the sixth transmembrane domain of the *TSHR*.

Discussion

Hyperthyroidism in the neonate and young infant is often described in the context of maternal autoimmune hyperthyroidism, irrespective of whether the mother is toxic during pregnancy. Approximately 1% of children born to women with a history of autoimmune thyrotoxicosis will develop neonatal thyrotoxicosis. Resolution usually occurs by 3–4 months of age, as the immunoglobulin levels of maternal origin fall. Non-autoimmune hyperthyroidism in neonates and infants is rare. In McCune–Albright syndrome, hyperthyroidism is caused by an activating mutation of the gene that encodes the α -subunit of stimulatory G proteins (1).

Constitutive activation of the *TSHR* resulting from germline mutations is responsible for most hereditary (familial) forms of non-autoimmune hyperthyroidism (cases reviewed by Chester *et al.* [12]). First reported in 1982 (13) and confirmed in 1994 (14), 35 families harboring 25 different activating germline mutation have been identified to date (6) (see <http://gris.ulb.ac.be/index.html?home.html>); 23 were familial and 12 sporadic. This autosomal dominant disorder is characterized by high variability in the phenotypic expression of

the disease with intrafamilial heterogeneity in age of onset and severity of disease among several generations (15–19). In these patients, thyrotoxicosis, in the presence or absence of goiter, may first become manifest at any time from birth to adulthood. The patients' course is prolonged with relapses occurring even two decades following subtotal thyroidectomy (19). Epigenetic factors modulating the impact of this heterozygote mutation and differences in iodine intake may alter the phenotypic expression of this inherited disorder.

Family studies were initially performed on our patient with the possibility of an inherited *TSHR* germline mutation. Sequencing of the entire coding region of *TSHR* and intron/exon junctions, using DNA extracted from circulating mononuclear cells, failed to show any abnormality. This finding prompted us to obtain a radioiodide scan, which indicated the presence of a hyperfunctioning nodule in the right thyroid lobe. Upon surgical excision, the lobe showed hyperplasia on histological examination (Fig. 1B). Genomic and complementary DNA analysis of this tissue revealed a missense *TSHR* mutation D633Y located in the sixth transmembrane domain of the *TSHR*. The absence of this *TSHR* mutation in circulating mononuclear cells and in the contralateral thyroid tissue, confirmed the somatic nature of this genetic alteration. An identical mutation has been reported previously in six hyperfunctioning adenomas of adults (20–23). In addition, the same codon has been found to undergo three different mutations producing hyperfunctioning adenomas in adult subjects: six D633E (3,5,20,24–26), two D633H (5,27), and one D633A (5).

The youngest patient with an activating somatic *TSHR* mutation reported to date demonstrated evidence of hyperthyroidism *in utero* as documented by fetal tachycardia at week 35 of gestation (8). However, definite treatment, by surgical removal of the hyperfunctioning thyroid adenoma, followed by molecular diagnosis, did not take place until the age of 2 years. Sequencing of *TSHR* revealed a heterozygous missense mutation, S281I, in the extracellular domain, which was restricted to the adenomatous tissue. Compared to the

wild-type TSHR, the mutant had an eightfold increase in the basal cAMP activity and a more modest increase in the affinity for TSH. This is comparable to 7.4-fold increase in the basal cAMP activity of the mutant TSHR D633Y reported herein (22).

We date the onset of clinically significant thyrotoxicosis in our patient sometime between 3 and 6 months of age. The earliest signs suggestive of thyrotoxicosis, in subsequently proven activating *TSHR* mutations, are in premature infants born at 32 and 33 weeks of fetal life (28–31). However, intra-uterine hyperthyroidism, manifesting as fetal tachycardia was observed in only two sporadic cases (8,29). As expression of *TSHR* in humans does not occur prior to the latter half of gestation (32), the presence of an activating *TSHR* mutation would theoretically not become apparent *in utero* prior to this time. Review of the prenatal data for our patient revealed no evidence for fetal tachycardia. Furthermore, blood obtained on the third day for neonatal screening of hypothyroidism had a T_4 of 19 $\mu\text{g}/\text{dL}$ (normal range 10–28 $\mu\text{g}/\text{dL}$) and a TSH <20 mU/L. Nevertheless, growth of the monoclonal cells expressing the mutant TSHR was sufficient to produce excess thyroid hormone before the age of 6 months, suggesting that the mutation in a single cell must have occurred *in utero*.

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Disclosure Statement

The authors report no conflicts of interest.

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