Autonomous Adenomas Caused by Somatic Mutations of the Thyroid-Stimulating Hormone Receptor in Children

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Key Words

Hyperthyroidism · Hot nodule · Somatic mutation ·
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Abstract

In adults, autonomous adenomas of the thyroid causing hyperthyroidism are relatively common and are most often due to somatic mutations that increase the constitutive activity of the thyroid-stimulating hormone receptor (TSHR). By contrast, autonomous adenomas in hyperthyroid children are exceptional and reports of their clinical and molecular characteristics are few. We reviewed papers describing 16 autonomous adenomas due to a somatic mutation activating the TSHR and diagnosed in patients younger than 18 years, to which we added two of our own unpublished observations in a 4- and 8-year-old with the same TSHR mutation (c.CAG>CAC; p.Asp633His). This revealed that (a) autonomous adenomas occur more often in the right lobe (11 of 14 with available information) and the associated hyperthyroidism tends to be more severe, possibly reflecting the richer vascular supply of the right thyroid lobe, and (b) mutations found in benign adenomas in children have been associated with cancer in adults, suggesting that malignancy requires a second 'hit' at a later age.

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Introduction

The first description of somatic mutations increasing the constitutive activity of the thyroid-stimulating hormone receptor (TSHR) as a potential cause of autonomous adenomas of the thyroid in hyperthyroid adults was published 20 years ago [1]. Since then, a number of reviews have established that this is the principal cause of autonomous adenomas in adults [2, 3]. Similar reviews of autonomous adenomas caused by TSHR mutations focusing on pediatric patients have not been published, probably because thyroid nodules, including autonomous adenomas, are encountered much less frequently in patients younger than 18 years than in adulthood [4, 5]. This review addresses the clinical and molecular features of autonomous adenomas occurring in children bearing a mutation in TSHR.

Autonomous adenomas are well defined, encapsulated and nearly always benign tumors characterized by autonomous secretion of thyroid hormones [6]. In addition to the somatic activating mutations of TSHR mentioned above, autonomous adenomas can also, albeit more rarely [7], be due to mutations activating *GNAS*, the gene encoding the Gs α protein. Both TSHR and Gs α activation result in increased cAMP production in thyroid follicular cells. The affected cells display unregulated functional ac-

Fig. 1. a *TSHR* is located on chromosome 14 and is composed of 10 exons. It encodes a member of the glycoprotein hormone receptor family of G-protein-coupled receptors whose activity is mediated by adenylyl cyclase. **b** TSHR activation stimulates the growth and differentiation of thyrocytes and the secretion of thyroid hormones.

tivity and clonally expand to form a lesion that progressively takes over the function of the normal tissue [3].

Activating TSHR mutations also underlie two other syndromes: familial non-autoimmune hyperthyroidism [8, 9] and sporadic congenital non-autoimmune hyperthyroidism [10]. These result from germline mutations leading to the whole thyroid gland being affected and have been extensively reviewed elsewhere [11].

Methods

We searched PubMed and the TSHR mutation database (<http://endokrinologie.uniklinikum-leipzig.de/tsh/>) with the following criteria: toxic or autonomous adenoma and children. This yielded 8 publications [2, 12–18] describing 16 autonomous adenomas in which a somatic TSHR mutation had been documented in 14 patients younger than 18 years (1 patient had 3 autonomous adenomas). To these, we added 2 of our own unpublished patients, who are described in detail below. Data collected included age at diagnosis, sex, affected thyroid lobe, nodule size and thyroid function when available.

In our 2 patients, informed consent for molecular genetic analysis was obtained from the parents. DNA was extracted from the thyroid nodule, from adjacent thyroid tissue and from leukocytes, and amplified by PCR followed by direct sequencing of exon 9 and part of exon 10 (from residue 430) of *TSHR*.

The Thyroid-Stimulating Hormone Receptor (fig. 1)

The TSHR gene is located on the long arm of chromosome 14 and encodes a 764-amino-acid protein with a molecular weight of 86,830 daltons [19]. TSHR is a mem-

ber of the glycoprotein hormone receptor family of G-protein-coupled receptors (GPCRs), and is characterized by a large N-terminal ectodomain, encoded by the first 9 exons of the gene; seven transmembrane segments and an intracytoplasmic domain with a carboxyl-terminal segment encoded by exon 10. Its long amino-terminal segment is responsible for high affinity binding of TSH. Figure 1 shows how the TSHR initiates intracellular signaling by activating G proteins that regulate the activity of effector molecules: the Gs protein, leading to the stimulation of the cyclic cAMP cascade, and, at higher TSH concentrations, the Gq protein, activating the phospholipase C (PLC) cascade. cAMP binds to protein kinase A (PKA), permitting enhanced activity of its catalytic subunit that phosphorylates different effectors. Activation of PLC leads to the generation of inositol 1,4,5-triphosphate (IP₃) and diacylglycerol (DAG), whose functions are related to promoting the release of Ca²⁺ into the cytoplasm and activation of the protein kinase C (PKC) pathway. Somatic mutations of the TSHR or Gsα proteins constitutively activate the cAMP pathway and induce the clonal expansion and hyperfunction of the thyroid follicular cells. Cells harboring the mutation also have an increased expression of the sodium iodide symporter, which results in a high uptake or 'hot nodule' image on scintigraphy. As synthesis and secretion of thyroid hormone increases by the increasing number of adenoma cells, the negative feedback at the hypothalamic-pituitary level results in suppressed TSH secretion and decreased or absent uptake in the normal thyroid tissue.

Table 1. Somatic activating mutations of the *TSHR* in pediatric autonomous adenomas

TSHR mutation	Age, years	Sex	Side	Size, mm by echo	TSH, mU/l	FT ₄ , pmol/l	TT ₃ , nmol/l	FT ₃ , pmol/l	Ref.
Ser281Ile	0.1	M	R	31/22/20	<0.5	ND	10.0	NR	12
Ser281Ile	8	F	R	'large'	<0.01	29.6	NR	10.6	14
Ser281Ile	13	F	R	24/21/19	<0.01	15.4	NR	7.5	16
Met453Thr*	3	M	L	14/23/10	'Compensated hyperthyroidism'				17
Met453Thr*	11	F	R	45/37/28	0.03	>75.0	5.7	NR	13
Met453Thr	4	F	R	NR	0.01	NR	NR	NR	18
Ile486Phe*	15	F	R	NR	<0.1	NR	2.8	NR	2
Ile568Phe	12**	F	L	5/5/5	<0.01	10.3	NR	NR	16
Ile568Phe	14	F	L	NR	0.30	NR	NR	NR	18
Ile568Thr	16	F	NR	NR	<0.03	14.0	4.0	NR	2
Phe631Leu	14	F	NR	NR	NR	25.7	NR	NR	2
Thr632Ile*	9	F	R	24/7/12	0.01	18.0	NR	11.2	16
Asp633Ile	0.75	M	R	31/15/16	0.01	65.6	7.3	NR	15
Asp633Tyr*	12**	F	L	5/5/5	<0.01	10.3	NR	NR	16
Asp633Tyr*	18	F	R	50/40	NR	NR	NR	NR	14
Asp633His*	12**	F	R	20/15/15	<0.01	10.3	NR	NR	16
Asp633His*	4	F	R	20/20/35	0.03	58.6	8.7	ND	<i>This</i>
Asp633His*	8	F	L	26/20/17	0.01	22.2	4.0	ND	<i>study</i>

M = Male; F = female; R = right; L = left; NR = not reported; ND = not done. * Also found in carcinoma in adults: Met453Thr, papillary; Ile486Phe and Thr632Ile, follicular (<http://endokrinologie.uniklinikum-leipzig.de/tsh/>); Arg633His: insular [38]. ** This patient had 3 nodules, each bearing a different mutation.

Somatic Mutations Activating TSHR in Autonomous Adenomas

Autonomous adenomas occur as a consequence of an increase in the constitutive (i.e. in the absence of ligand) activity of the TSHR. Most of the mutations are located in the sixth transmembrane domain of the receptor, but they can also be found elsewhere in the serpentine and in a specific residue (Ser281) of the extracellular domain of the receptor [11], switching it from an inverse agonist to an agonist of the serpentine domain [20]. Some of the mutations found in autonomous adenomas are also present in the other two forms of non-immune hyperthyroidism, but are more frequently associated with its sporadic form. This suggests that familial transmission of TSHR activating mutations is more likely to occur for mutations that have a less pronounced effect and therefore do not interfere with reproductive fitness [21].

The somatic TSHR mutations in autonomous adenomas diagnosed in patients less than 18 years are listed on table 1. Whereas in adults the commonest TSH mutations found in autonomous adenomas are at residue 632 (Sanger Institute Catalogue of Somatic Mutations in Cancer: <http://www.sanger.ac.uk/cosmic>), mutations at the neighboring residue 633 account for 6/18 autonomous

adenomas in children. In addition, several of the mutations (noted by an asterisk in table 1) encountered in children with *benign* autonomous adenomas have also been reported in adults with *malignant* autonomous nodules.

Pathogenesis of Autonomous Adenomas

Once a somatic mutation has occurred, the thyroid cell has to divide successfully to form a clinically apparent autonomous adenoma. It has been estimated that at least 30 divisions would be required to reach a critical size [22]. This number is considerably higher than the number of divisions that are estimated to occur in an adult thyrocyte (5–7) [23]. In autonomous adenomas, telomere shortening [24] and the loss of negative feedback control [25] are consistent with rapid proliferation. However, the mitotic index observed in thyrocytes obtained from autonomous adenomas at the time of surgery is not consistent with rapid cell division [22]. The explanation is probably that, by the time of diagnosis, the lesions are no longer growing; supporting this explanation is the observation that, once an autonomous adenoma is diagnosed a further increase in size occurs in only a small percentage of cases [6].

Table 2. Patients' characteristics at diagnosis

	Patient 1	Patient 2
Sex	Female	Female
Age, years	4	8
Heart rate, bpm	150	121
Blood pressure, mm Hg	150/80	116/61
Symptoms/signs	Premature loss of a deciduous tooth; jitteriness; heart murmur (mitral valve prolapse); asymmetric goiter	Neck nodule
Height, SDS	2.68	0.05
Weight, SDS	1.51	0.3
Bone age	7 years	Not done
Technetium uptake	Increased by right lobe, none by left lobe	Increased by left lobe; none by right lobe
Ultrasound		
Nodule side and size	Right, 2.0×2.0×3.5 cm	Left, 2.6×2.0×1.7 cm
Contralateral lobe	Hypoplastic (see text)	Normal
TSH, mU/l (N)	<0.01 (0.9–5.7)	<0.03 (0.9–5.7)
FT ₄ , pmol/l (N)	58.6 (9.0–16.1)	22.2 (9.0–16.1)
Total T ₃ , nmol/l (N)	8.7 (1.4–3.7)	4.0 (1.4–3.7)
Size of nodule, cm	3.0×2.5×1.6	3.0×1.8×1.8
Nodule weight		
Absolute, g	7.0	6.2
% of patient weight	0.03	0.02
Histology	Follicular adenoma	Follicular adenoma, lymphocytic infiltrate
TSHR mutation	Asp633His	Asp633His

SDS = Standard deviation score; N = normal values.

Clinical Presentation of 16 Patients

Sixteen autonomous adenomas harboring an activating TSHR mutation in 14 patients under the age of 18 years have been reported in the literature (table 1). To these, we add 2 previously unreported girls from our own institution. These 2 girls, aged 4 and 8 years at diagnosis, presented with a benign autonomous adenoma that was due to the same somatic mutation in the TSHR gene but with very different clinical features and postoperative evolutions. These are described below.

Patient 1 was noted to have marked tachycardia, a heart murmur, hypertension and an asymmetric goiter at a routine visit, prompted by the premature loss of a deciduous tooth at the age of 4 years. The parents also reported increasing jitteriness over the last 6 months. Linear growth had accelerated with relative weight loss. Clinical, biochemical, imaging, histological and molecular data are depicted in table 2. A diagnosis of non-autoimmune hyperthyroidism due to an autonomous adenoma with associated mitral valve prolapse was made. The patient was put on β -blockers and a right hemithyroidectomy was performed 2 weeks later. A well-encapsulated nodule was found to occupy most of the right lobe; the left

lobe was hardly visible. The pathological diagnosis was benign follicular adenoma. Two weeks after surgery, the patient became profoundly hypothyroid [FT₄ 3.2 pmol/l (N 9.0–16.1)], although TSH was still suppressed at 0.03 mU/l (N 0.9–5.7), only rising to 29.94 mU/l one month later. The patient was then started on levothyroxine treatment (50 μ g daily). Consistent with partial catch-up growth of the left thyroid lobe from 0.45 ml (–2.8 SD) at diagnosis to 0.88 ml (–2.3 SD) 2 years later [26], serum TSH only rose to 6.64 mU/l after a 1-month withdrawal of levothyroxine 3 years after surgery. 'Catch-down' growth after surgery resulted in an adult height within the target range (fig. 2). Nine years after thyroidectomy, the mitral valve prolapse had disappeared and thyroid function had become normal off levothyroxine (serum TSH: 3.66 mU/l).

Patient 2 presented at 8 years of age with progressive enlargement of the left side of the neck. On physical examination, there was mild tachycardia and the thyroid was enlarged with a palpable, non-tender, mobile nodule of the left lobe. Clinical, biochemical, imaging, histological and molecular data are depicted in table 2. At surgery, a well-encapsulated nodule was found. The pathological diagnosis was benign follicular adenoma. The tissue sur-

Fig. 2. Growth chart of patient 1. An abrupt increase in height velocity was noted between 3 and 4 years of age and a significant advancement of bone age was documented at the time of surgery (triangle). After treatment, 'catch-down' growth was observed and adult height was within the target height range.

rounding the nodule showed a marked lymphocytic infiltrate. One month after surgery, serum TSH rose to 6.55 mU/l but normalized spontaneously 3 months later and remained normal over the subsequent 7 years.

In both patients, the same mutation in exon 10, codon 633, of *TSHR* (c.GAC>CAC), which results in the substitution of aspartic acid by histidine (p.Asp633His), was found in the heterozygous state in the thyroid nodule, but neither in the adjacent normal thyroid tissue nor in the lymphocytes, indicating a somatic mutation. This mutation is located in the sixth transmembrane segment of the *TSHR*. Table 2 summarizes the characteristics of our patients at diagnosis.

Children with autonomous adenomas of the thyroid are usually brought to medical attention because of a cervical mass or because of signs of hyperthyroidism (often restricted to tachycardia). In young children with Graves' disease, there may be acceleration of linear growth and of bone maturation [27], but our patient 1 shows that this can be observed in non-autoimmune hyperthyroidism as

well (table 2); furthermore, long-term follow-up shows that adult height was within the target range (fig. 2). Such linear growth acceleration is not as pronounced in older children with hyperthyroidism due to either Graves' disease [28, 29] or to an autonomous adenoma (our patient 2). A distinctive feature of autonomous adenomas is the absence of ophthalmopathy and the absence of antibodies to the TSHR, which are the hallmark of Graves' disease. A thyroid nodule in a child with clinical and biochemical hyperthyroidism has to be further assessed with a thyroid scintigraphy, to evaluate its autonomy [5]. The presence of a 'hot nodule' confirms the diagnosis and surgical excision is recommended to cure the hyperthyroidism and also because even autonomous nodules can be malignant [13].

Table 1 lists all reported pediatric cases of somatic gain of function mutations of the *TSHR*, including those described in the present report. Age at diagnosis and severity of hyperthyroidism was very variable, with some patients having normal FT_4 , but elevated total or FT_3 concentrations. Table 2 shows that the degree of hyperthyroidism of our 4-year-old patient was much more severe, and possibly more protracted, than that of the 8-year-old in spite of both patients having the same somatic mutation in *TSHR* and both autonomous adenomas having the same weight relative to body size. This may result from the fact that the autonomous adenoma in patient 1 was in the right thyroid lobe, which is more richly vascularized than the left [30]. Asymmetry of the arterial supply may also explain why autonomous adenomas occur much more often in the right lobe in both children [4] and adults [31] and in the patients reviewed here (11 out of 14 with available information, table 1). It is also possible that other genes or environmental factors such as iodine intake modify the expression of the *TSHR* mutation; indeed, phenotypic variability between family members with the same germline mutation activating *TSHR* has also been described [32]. The relationship between mitral valve prolapse and hyperthyroidism due to either Graves' disease [33, 34] or *TSHR*-activating mutations [35] is controversial but the progressive reversibility of the latter after correction of the former in our patient 1 strongly suggests a causal relationship. Lastly, the severe and slowly reversible atrophy of the contralateral lobe in patient 1 extends to the thyroid the concept of disuse atrophy, which is well established in the contralateral adrenal of patients with a cortisol-producing adenoma [36].

As shown in table 1, several mutations found in 9 pediatric autonomous adenomas, which are *benign* lesions, have also been reported in *malignant* hot nodules in adults

(Sanger Institute Catalogue of Somatic Mutations in Cancer: <http://www.sanger.ac.uk/cosmic>). This might suggest that these mutations are associated with relatively rapid growth of adenomas, thus facilitating the later occurrence of a second oncogenic 'hit' [13]. This interpretation is supported by the restricted spectrum of mutations in children, with mutation of Asp633 appearing to be particularly frequent and leading to relative large adenomas (table 1). Of note, in the LH receptor, only the Asp578His somatic mutation, which is homologous to Asp633 in TSHR, causes Leydig cell adenomas in children [37]. It is also worth stressing that the Asp633His mutation found in our patients has been found in a 60-year-old woman with the most aggressive type of differentiated thyroid cancer, insular carcinoma [38]. Taken together, these data suggest that mutations at residue 633 in TSHR cause more pronounced proliferation than the others, leading to their being recognized in the pediatric age group and being a higher risk factor for malignancy in adulthood.

Conclusions

We review 18 autonomous adenomas (including 2 previously unpublished cases) of the thyroid caused by a somatic mutation activating the TSHR and diagnosed in patients aged less than 18 years. In the 2 previously unpublished cases, who had the same TSHR mutation, hyperthyroidism was much more severe in the 4-year-old

than in the 8-year-old; this did not appear to be related to the relative weight of the autonomous adenoma and may be related to the fact that the autonomous adenoma of the 4-year-old was in the right lobe, which receives a richer vascular supply. The richer vascular supply of the right thyroid lobe may also account for the more frequent occurrence of autonomous adenomas, both with (table 1) and without a documented TSHR mutation, on that side. On the other hand, the overall review also suggests that TSHR mutations causing benign autonomous adenomas in children might be associated with cancer in adults, implying that malignancy requires a second hit at a later age.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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