Activating mutations of the thyrotropin receptor: a short review with emphasis on some pediatric aspects

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Introduction: the thyrotropin receptor

The thyrotropin (TSH) receptor belongs to a subfamily of G protein coupled receptors. The primary structure of this protein predicts the existence of seven segments with hydropathy compatible with a transmembrane location (1). The binding specificity and the effector properties of the TSH receptor are encoded in separate domains of the proteins; the extracellular part is involved in the binding of TSH and the transmembrane domain has the effector properties triggering G protein activation (2). The gene organization reflects these dual functions, as a single exon encodes the transmembrane domain whereas the rest of the extracellular domain is encoded for by nine exons (3).

Recently, gain of function mutations have been described in the TSH receptor, which explain some pathological conditions in humans (4, 5). This article discusses the responsibility of such mutations of the TSH receptor in hyperthyroidism; in the first part is described the phenotypes of the patient with special emphasis on familial and sporadic congenital hyperthyroidism caused by germline mutations, as well as the distinction between them and the somatic mutations which have been found in thyroid adenomas. The available in vitro or in vivo studies which confirm that the mutations are responsible for the phenotype are then reviewed. This review will focus on the clinical consequences of TSH receptor mutations, particularly in the setting of congenital hyperthyroidism, and provide a follow-up on the case previously described (6), rather than discuss the implications for the basic understanding of the structure and function of the TSH receptor. Other aspects of diseases linked to TSH receptor mutations, among which is the controversy linking thyroid neoplasms and such mutations, have been reviewed very recently (7).

The transducing system of the TSH receptor

Upon binding of TSH to its receptor, the main pathway of the signal transduction involves the activation of the adenylate cyclase through G protein coupling and the intracellular production of cAMP (8–11). The phospholipase C-diacylglycerol regulatory pathway is also activated, although at hormone concentrations five to ten times higher than the cAMP pathway (9–11). The cAMP pathway has been shown to be important for the positive control of thyroid hormone secretion and growth of the thyrocytes, whereas the inositol phosphate pathway seems mostly involved in the control of iodination and hormone synthesis (2, 12).

Since the TSH receptor has a central role in the control of human thyroid follicular cell proliferation and function, it is therefore expected that its deregulation would lead to pathological processes.

Activating mutations of the TSH receptor and hyperthyroidism phenotypes caused by somatic mutations

Autonomously functioning nodules may occur as solitary or multiple nodules leading to hyperthyroidism. The coexistence of autonomous and quiescent tissue in the same organ suggests an inherent defect in the pathological tissue. The observation of hyperthyroidism and independent growth of hot nodules suggests a chronic activation of the cAMP cascade which controls growth and function of thyroid follicular cells. The first mutations of the TSH receptor leading to toxic adenomas were described in the third intracellular loop of the receptor (13). Further mutations were subsequently identified (14, 15). The frequency of TSH receptor mutations in toxic nodules may be variable for technical reasons, direct sequencing being more sensitive than single strand conformation polymorphism analysis. In addition, most studies analyzed only a small fragment of the receptor and mutations in other parts of the receptor may have been missed (16, 17). With the entire sequencing of the transmembrane domain, 9 out of 11 toxic nodules were found to harbor TSH receptor mutations (18). The exact frequency of these mutations remains to be established, as a recent study, using direct sequencing, found only 20% of the nodules (9 out of 44) harboring TSH receptor mutations, whereas another confirmed the author’s previous publication with 82% of nodules (27 out of 33) harboring TSH receptor mutations (19, 20).

Phenotypes in congenital mutations

Following the discovery of TSH receptor gene mutations in toxic thyroid adenomas (13), such mutations were
searched for in sporadic congenital and familial non-autoimmune hyperthyroidism.

Familial congenital non-autoimmune hyperthyroidism with thyroid hyperplasia (autosomal dominant toxic thyroid hyperplasia (ADTTH)) due to germline mutations of the TSH receptor gene have been reported (4, 21–24). The characteristics of the cases of ADTTH described up to 1997 have been reviewed by Leclère (24). In the five analyzed kindreds, 49 out of 120 examined patients were found to have hyperthyroidism. Remarkably, women were affected more frequently in these series (32 women versus 17 men). The onset of thyrotoxicosis was early, but varied from 1 year of age to 10, 14, 17 and 23 years depending on the family. Interestingly, a systematic screening of the families revealed two unsuspected children with decreased TSH and no signs of hyperthyroidism, emphasizing the potential of detecting affected children before the appearance of the disease and its potential deleterious consequences (24). The goiters were homogenously diffuse in children, but tended to become multinodular later in life. No ophthalmopathy was detected and this was an important diagnostic criterion, even though our case (see below) illustrates that exophthalmia should not be an exclusion criterion for the diagnosis of non-autoimmune hyperthyroidism (6, 24). No circulating antithyroid antibodies, including anti-TSH receptor antibodies as searched for by binding or functional assays, were detected in those patients. Recurrence after antithyroid drug therapy, non-ablative radioiodine treatment or partial thyroidectomy was frequent. Pathological studies of thyroid glands from patients with ADTTH were distinct from those of autoimmune Graves’ disease as no mononuclear cells were infiltrating the thyroid and as none of the usual autoimmune markers of Graves’ immunohistology were present (24). In conclusion the diagnosis of non-autoimmune hyperthyroidism with thyroid hyperplasia should be considered in the presence of: a history of familial thyrotoxicosis; a high incidence and early occurrence of the disease; moderate and diffuse goiter (only in young people and not in the case of recurrence); absence of extrathyroidal signs of Graves’ disease (proptosis should not be an exclusion criterion); absence of circulating thyroid antibodies; and recurrence after medical treatment, non-ablative surgery or destruction of the thyroid with radioisotopes (24).

Non-familial congenital non-autoimmune hyperthyroidism with thyroid hyperplasia due to sporadic mutation of the TSH receptor gene (Phe631Leu) was first described by Kopp et al. (25) as neonatal hyperthyroidism, diffuse goiter, markedly advanced bone age, and persistently low head circumference associated with psychomotor delay. After an 8 year course of antithyroid drug, thyroidectomy was performed due to recurrence of hyperthyroidism with an increase in goiter size and the appearance of multiple thyroid nodules. Recurrence of thyrotoxicosis after subtotal thyroidectomy led to radioiodine treatment which restored euthyroidism (25).

Subsequently, my colleagues and I reported a case of neonatal hyperthyroidism without stimulatory TSH receptor antibodies (TSab) (6). This patient was a small-for-date premature boy, born at 32.5 weeks of gestation from a normal mother. Pregnancy was uneventful except for fetal tachycardia noticed at 31 weeks of gestation. Hepatosplenomegaly, lymphadenopathy and petechiae, as well as thrombocytopenia and hepatic cholestasis, developed during the early days of life, and extensive investigations excluded viral or bacterial perinatal infection. At 6 days of life, thyromegaly and eyelid retraction were prominent and diagnosis of neonatal hyperthyroidism was made (free thyroxine >100 pmol/l. free tri-iodothyronine >35 pmol/l. TSH <0.1 IU/l. TSab negative). Advanced bone age at birth and elevated cord blood thyroid hormone levels indicated that hyperthyroidism was indeed present during fetal life. The absence of any known thyroid disease, normal thyroid hormone levels and the lack of TSab in the mother led us to suspect a non-immune etiology. Genomic sequencing of the last exon of the TSH receptor in the patient revealed a transversion resulting in a heterozygous substitution of one amino acid in the second transmembrane domain of the receptor (Met453Thr, Fig. 1). This substitution was shown to confer constitutive activity to the mutated receptor in in vitro studies (see below and Fig. 2). The child was treated with carbimazole from post-natal day 11: euthyroidism was achieved and regression of hepatosplenomegaly, polyadenopathy and cholestasis was noticed and a satisfactory weight gain obtained. L-Thyroxine was added at 7 months to prevent hypothyroidism. Thereafter euthyroidism was maintained.

Figure 1 Schematic representation of the localization of the amino acid change in the mutated TSH receptor, in the second transmembrane domain (X) leading to the constitutive activation of the TSH receptor and increased production of cAMP in the described case (see text and (6)).
with both drugs until the last follow-up visit at 3 years of age, but TSH remained suppressed until 12 months of post-natal age. Hyperthyroidism is not cured at present as intermittent removal of antithyroid drugs led to recurrence of the thyrotoxicosis.

No craniosynostosis was noticed in this child. No developmental impairments were noted during the first year. However, quantitative evaluation of the development, with the Brunet–Lezine test, conducted at 3 years of age revealed a developmental delay of a year, with a developmental age of 24 months. Motricity and behavior were normal. Interestingly, in the case described by Kopp et al. (25), or in a more recent case described by Holzapfel et al. (26) (where a child with a sporadic congenital hyperthyroidism due to a mutation resulting in a heterozygous substitution of one amino acid in the third transmembrane domain of the receptor (Ser505Asn) was found to have persistent speech disturbances), developmental impairments were found in association with microcephaly. Indeed our case emphasizes the potential deleterious effect of fetal hyperthyroidism, independently of the presence of craniosynostosis and/or microcephaly.

This child presented also with exophthalmia, documented by CT scan and NMR, even though no sign of thyroid autoimmunity was present. He was euthyroid at the time of these examinations and the parents did not have exophthalmia. Moreover, at 3 years of age, in a euthyroid state, exophthalmia is still present and documented at the NMR of the eyes, even though there is no enlargement of the eye muscles. The expression of the TSH receptor in retroorbital tissue might therefore be involved in the pathogenesis of exophthalmia in Graves’ disease (27).

Very recently, constitutive activation of the TSH receptor caused by mutations of the extracellular and not the transmembrane domain were described in toxic adenomas by the Brussels group (28). In addition, following the description of non-autoimmune hyperthyroidism due to mutations in the transmembrane domain of the receptor, Kopp et al. (29) reported a case of an activating mutation in the extracellular TSH-binding domain in a male infant with congenital hyperthyroidism due to a toxic adenoma. This shows clearly that activating mutations are not limited to exon 10 of the TSH receptor gene (28–30).

Taken together, constitutive activation of the TSH receptor due to neomutations in the gene is a possible, although rare, etiology of persistent neonatal hyperthyroidism, which may develop during fetal life. It should be suspected in the absence of maternal signs of thyroid autoimmunity (6, 23, 25, 29, 30). These TSH receptor gene mutations are likely to be the explanation for some cases of familial persistent neonatal hyperthyroidism described more than 20 years ago (31, 32).

**In vitro and in vivo studies confirm the stimulatory nature of the TSH receptor mutation**

Most of the published articles have used sequencing of PCR-amplified fragments of exon 10 of the TSH receptor gene, because this single exon encodes the transmembrane domain. The rationale for this approach was that the likelihood of finding activating mutations in this domain was high because it is involved in signal transduction, as demonstrated by studies with the alpha1-adrenergic receptor (6, 13, 22, 25, 33). The wild type and the mutated receptor gene were then transiently transfected in eukaryotic cells to confirm that the mutation conferred constitutive activation (Fig. 2).

Indeed basal cAMP production was increased with transfection of the mutated receptor compared with transfection of the wild type (6, 13, 22, 25). The mutant receptors retained their ability to respond to TSH as indicated by the increased production of cAMP induced by TSH. A control to make sure that the expression levels of the transfected receptors were similar for the mutated and the wild type receptors, consisted of either quantifying the binding sites of radiolabeled TSH or of directly quantifying the receptors (6, 13, 22, 25). Basal inositol phosphate production was usually not found to be increased when the mutated receptor was transfected (6, 16, 18, 20, 25). Differential effects of individual mutations on stimulation by TSH of cAMP or inositol phosphate production suggest that individual mutant receptors may achieve different active conformations, with different abilities to couple and activate.

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Figure 2 Data demonstrating the increased cAMP production in the transfection experiments in the described case. Basal cAMP production was increased when the mutated cDNA coding for the TSH receptor was transfected (Mut) as compared with the wild type cDNA coding for the TSH receptor (Wild). Control (Cont) transfections were accomplished by transfecting the vector without the cDNA coding for the TSH receptor. Mut retained partial ability to respond to TSH stimulation, in comparison with Wild (see text and (6)).
one or both transduction pathways (6, 13, 16, 22, 25). So far the reported germline mutations are mainly (with the exception of the recent report by Kopp et al. (29)) located in the transmembrane segments, the majority of them affecting highly conserved segments with respect to the other glycoprotein hormone receptors (6, 13, 16, 22, 25). The somatic mutations of the TSH receptor gene detected in thyroid adenomas are more dispersed. The meaning of this fact may become clearer as more data are accumulated. A model has been proposed by which the TSH receptor isomerizes between an active and an inactive state; the naturally occurring somatic or germline mutations would act by shifting the equilibrium toward the active conformation of the receptor in the absence of the ligand (4, 18). This results in constitutive activation of the cAMP pathway in all cases, but only few mutations result in an additional increase of inositol phosphate accumulation (4, 18). Of interest is the fact that the same mutations have been observed, in at least three instances, as germline and somatic mutations at several residues, being responsible for a very aggressive phenotype in the case of its congenital expression, leading to the conclusion that some additional factors, apart from the type of mutation, may play a role in their expression, iodine intake being one potential such factor (25, 34).

An in vivo approach was also used to confirm that hyperfunction of the hyperplastic thyroid tissue of patients suffering from toxic hyperplasia due to mutations of the TSH receptor was TSH independent (35). Grafting thyroid tissue from toxic hyperplastic thyroid and from toxic nodules into nude mice led to an increase of thyroid hormone levels, to a decrease of TSH levels, and from toxic nodules into nude mice led to an increase of thyroid hormone levels, to a decrease of TSH levels, weight loss and signs of thyrotoxicosis in the mice and subsequently to death of the grafted mice, whereas grafting of thyroid tissue from patients with Graves’ disease led to quiescence of the grafted tissue (24, 35). This demonstrated convincingly the TSH-independent mechanism of this form of hyperthyroidism and is consistent with the constitutive activation of the TSH receptor found in these cases (13, 22).

**Conclusion**

Following the cloning of the TSH receptor gene, various forms of hyperthyroidism have been explained at the molecular level by detecting activating mutations in this gene. Germline mutations are found in hereditary hyperthyroidism, *de novo* germline mutations can cause sporadic congenital hyperthyroidism, and somatic mutations are found in the majority of toxic adenomas (5). The discovery of these mutations leading to constitutive activation of the TSH receptor not only help to elucidate some forms of thyroid autonomy, but they also deepen our understanding of the structure–function relationship of the TSH hormone receptor.

**References**

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