REVIEW

Genetic hyperthyroidism: hyperthyroidism due to activating TSHR mutations

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Abstract

Three syndromes affecting the thyroid gland are described in the literature separately: familial nonautoimmune hyperthyroidism, sporadic congenital nonautoimmune hyperthyroidism, and autonomous adenomas. Recent studies have shown that these three syndromes are caused by similar activating mutations of the TSH receptor gene (TSHR), and that the consequences of these mutations on the physiology and gene expression of the thyroid are qualitatively, but not quantitatively, similar. The three syndromes and two suggested unrecognized variants are in fact facets of the same disease, genetic hyperthyroidism due to TSHR mutations, the expression of which depends on the intensity of activation, its timing, and on the number of affected cells.

Introduction

The first description of familial nonautoimmune hyperthyroidism (FNAH) was published in 1982 (1, 2). FNAH is also called hereditary toxic thyroid hyperplasia or autosomal dominant autoimmune hyperthyroidism. It is hereditary through dominant activating mutation of the TSH receptor (TSHR) affecting all thyroid cells. A similar pathophysiology between autonomous adenomas (AAs; benign hyperfunctioning tumor). Owing to mutations (mostly TSHR), activating the TSH–cAMP cascade affects one cell clonally expanded in an adenoma or nonencapsulated nodule. Inactivating mutations of genes of signal transduction negatively controlling the TSH receptor cAMP pathway (e.g. phosphodiesterases) would give the same result and FNAH was suggested by two features: the tissues of the two diseases have the same histological aspect (1) and contrary to those provided by Graves’ cases, these tissues remain hyperfunctioning after being grafted into nude mice (3). This hypothesis was confirmed in 1994 by the identification of the mechanism of this particular form of thyrotoxicosis, an activating mutation of TSH receptor (TSHR) (4). The first case of a sporadic neomutation causing congenital hyperthyroidism was described in 1995 leading to the so-called ‘sporadic’ or ‘sporadic congenital nonautoimmune hyperthyroidism (SCNAH; these are germline neomutations affecting all thyroid cells.)’ form of the disease (5). A large fraction of AAs are also caused by activating mutations of the TSHR (6). Thus, activating mutations of the TSHR are the cause of the three syndromes: FNAH, SCNAH, and AA. While in the first two syndromes the mutation affects all thyroid cells, in AA it affects only one cell at the clonal origin of the benign tumor (7). The three syndromes, resulting from activating mutations of the TSHR, are the facets of one disease, genetic hyperthyroidism due to TSHR mutations (Fig. 1) (8). In this review, we describe the clinical and physiopathological aspects of this disease. The clinical hallmarks of FNAH and SCNAH have been analyzed in more detail in a recent review (9).

FNAH and SCNAH: clinical and biological characteristics

Based on the first five families comprising 49 patients suffering from FNAH, the clinical profile corresponding to the activating germline mutations of the TSHR was described in 1997 (10). The knowledge of the different clinical aspects of this disease is of crucial importance because they lead to suspect the diagnosis. The description may now be revisited, thanks to the increasing number of newly reported FNAH and SCNAH cases (2–4, 11, 12). In this review, we analyze the medical history of 152 FNAH coming from 27 families and 15 SCNAH caused by germline neomutations of the TSHR (Supplementary Tables 1 and 2, see section on supplementary data given at the end of this article).
Prevalence of the disease

The demographic prevalence is difficult to evaluate and is likely to be underestimated. This underestimation will be progressively reduced, thanks to a better knowledge of the characteristics of the disease. This is attested by the increasing number of cases by years: 2 families with FNAH between 1982 and 1993, 6 families with FNAH and 8 isolated cases of SCNAH between 1994 and 1999, and 19 families with FNAH and 7 isolated cases of SCNAH since 2000. The majority of cases have been reported in Europe, whereas only two FNAH and three SCNAH have been reported in the United States. This discrepancy could be due to the different approaches in the treatment of hyperthyroidism, which is more radical in the United States (surgery and radiiodine) than in Europe, where antithyroid drugs are widely used. Under the former treatment, the typical tendency to recur is more easily masked, as such recurrences are precluded by the removal or destruction of responsible thyroid cells.

Consistent with the autosomal dominant inheritance, there is no sexual predominance: 69 males and 83 females in the familial form, as in the congenital form (7 males, 7 females, and sex is not indicated in 1 case).

Onset of thyrotoxicosis

The time of recognition of the clinical signs is highly variable, depending in part on the intensity of the activating mutant allele.

Thyroid volume

The thyroid size or morphology is highly variable as indicated in 97 cases: a normal thyroid volume is present in only 25 cases, and a goiter is present in the 72 others. The goiter is either homogeneous (57/72) or multinodular (15/72). By comparing with the natural history of goitrogenesis due to chronic TSH stimulation, it would be logical to consider that the thyroid

Figure 1 Activating mutations on TSHR gene in the various genetic hyperthyroidism syndromes. Comparison of amino acid structure of the TSHR and locations of gain-of-function mutations found in FNAH (green), in SCNAH (red), or in FNAH and SCNAH (yellow). TSHR mutations also found in AA are encircled in blue. TSHR mutations found in AA only are not indicated.
structure will depend on the age of diagnosis. In the youngest patients, the thyroid is normal or slightly increased, thereafter a homogenous goiter appears and tends to develop multinodularity later. In fact, the situation is more complex since a goiter was already observed at birth in 5 out of 14 of SCNAH (the morphology was not indicated in one case) (5, 13–16). This discrepancy could be due to the type of TSHR gene mutation leading to a variable intensity of activation. However, this assumption does not explain the different aspects of thyroids encountered independently of age in a same family with the same mutation (normal thyroid volume, diffuse, or multinodular goiter) (Supplementary Table 1, see section on supplementary data given at the end of this article). Insulin-like growth factor 1 (IGF1) supply and other genetic, epigenetic, and environmental factors are probably the modifying factors (6, 17).

Pathology

By light microscopy, a diffuse hyperplasia is encountered when the thyroid volume is normal or in case of a homogenous goiter (1, 11, 18–21). The general aspect is completely different from Graves’ tissue due to the absence of any lymphocytic infiltrate or inflammatory features and the diagnosis must be suspected already from this routine examination when histological evaluation is possible (1, 2). The thyroid tissue of FNAH and SCNAH consists in clusters of small hyperactive follicles alternating with the areas of large follicles constituted by flat cells. This aspect is similar to toxic AA tissue, which led us to suggest the term ‘toxic thyroid hyperplasia’ (1). Immunohistology is not usually performed and is not necessary for the diagnosis. The absence of Graves’ tissue characteristics, the absence of activated T and B lymphocytes, the absence of aberrant expression of HLA II antigens by the thyroid cells, the absence of deposits of immunoglobulins, exclude Graves’ disease diagnosis, but the presence of autoimmune reactions does not exclude SCNAH (1). The pathologic aspects are less characteristic in patients harboring multinodular goiters. In these cases, necrotic or hemorrhagic events could lead to the development of inflammation and of areas invaded by macrophages and a few inflammatory cells.

The early, constant, and unregulated stimulation of thyroid cells by the activated mutated TSHR raises the question of the risk of thyroid cancer. Sixty-seven thyroid ablations have been realized, and only three cancers were found: two of micropapillary structure (2) and one oncocyctic carcinoma (19). It is impossible to assert any relevance concerning the association of the two diseases. The small number of thyroid cancers is consistent with their low frequency in other cases of chronic TSHR activation, i.e. in AAs and in Graves’ disease (8).

Ophthalmopathy

Thyroid ophthalmopathy is still considered as a specific sign of Graves’ disease (with the exception of a few cases of Hashimoto’s disease) and mainly linked with the presence of activating TSHR antibodies (thyroid stimulating immunoglobulins (TSI; that cause autoimmune hyperthyroidism (Graves’ disease))). A positive correlation between the levels of TSI and the severity of ophthalmopathy has been demonstrated long back and has been recently reemphasized (22). For this reason, the absence of any sign of ophthalmopathy in the two first families (1, 11) was not surprising and led us to consider this absence as a major marker of FNAH (10). Later on, this assertion has not been confirmed as 20 cases of 83 FNAH patients presented ocular symptoms. The ocular symptoms are differently described: proptosis (23), exophthalmia (11), prominence (3), and staring eyes (2). These ocular signs always affect young patients and are particularly frequent (8/10) in SCNAH patients. It is important to note the absence of any inflammatory features in all these cases. The ocular signs, in particular proptosis, could be due to the specific anatomy of the infant orbit where the external wall is less prominent than in the adult. The horizontal bicanthal line, the reference to appreciate the proptosis in adults, might not be adequate in the neonate or in very young children. It is noteworthy that when proptosis is present, a computer tomographic examination does not show any enlargement of ocular muscles (24–26). Another hypothetical explanation for proptosis could be the activation of the TSHR mimicking the effects of TSI on orbital structures. The reported presence of TSHR on orbital fibroblasts (27) and adipocytes (28), as well as the production of hyaluronan by orbital preadipocytes transfected with an activating mutant TSHR (29), suggest the possibility that ophthalmopathy may develop in the absence of TSI by a constitutive activation of these receptors. The case reported by Lavard bears against this hypothesis, because inspite of three relapses and the administration of four successive doses of radiodine, the exophthalmia decreased progressively and disappeared at the age of 18 years (30). The ocular symptoms are always relatively weak and without any of the inflammatory components of Graves’ ophthalmopathy.

Circulating thyroid antibodies

By definition, TSI are not relevant to the pathophysiology of the disease, but their presence remains conceivable in patients genetically predisposed to autoimmunity. Antithyroidperoxidase and/or antithyroglobulin antibodies have been found in three families but TSHR antibodies were always absent (18, 21, 31). The association of a constitutive activating TSHR mutation and thyroid autoimmunity remains exceptional but is of particular interest because the presence
of such antibodies could lead to the erroneous diagnosis of Graves’ disease (18). The index patient of the family described by Vaidya et al. (21) had no eye signs but the TSH-binding inhibitory immunoglobulins (TBII) were ‘borderline’ positive. Consequently, the presence of low levels of thyroid antibodies does not exclude the diagnosis if it is presented with some other features. The absence of TSI detected by the new commercial assays measuring TBII is important as the prevalence of TBII negative in Graves’ patients is estimated to be around 5% with the new generation TBII kits (32). Thus, the risk to confuse the two diseases is low and is avoidable if the clinical context is considered but there are still borderline cases.

**Resistance to conventional treatments**

Owing to the frequent confusion with Graves’ disease, the majority of the patients have been treated by conventional treatments: antithyroid drugs, partial thyroidectomy, or limited doses of radioiodine. Sixty-seven patients were followed up after the withdrawal of these treatments: all had one or several relapses. This can be a clue but many Graves’ disease patients also have relapse. The only way to avoid these relapses is to destroy all the thyroid tissue by either total thyroidectomy alone or in combination with radioiodine. If a total thyroidectomy is considered as being too aggressive in the youngest children, then a sustained treatment with antithyroid drugs is necessary until a more appropriate age for surgery. In some cases, antithyroid drugs poorly control thyrotoxicosis and the patients remain in subclinical hyperthyroidism (12, 15, 31, 33, 34).

**Particular aspects of SCNAH**

The neonatal expression of the disease can be seen both in some FNAH and in most SCNAH. De novo mutations occurred in 15 SCNAH (5, 12, 13). 10 of them presented thyrotoxicosis at birth and 4 of them were diagnosed at 5th, 8th, 9th, and 11th months of age (the age was not indicated in 1 case). The SCNAH are referred to as ‘sporadic’ congenital nonautoimmune thyrotoxicosis. Obviously, this denomination is relatively inappropriate because these neomutants if properly treated would be able to transmit the disease to their descendants. This is clearly demonstrated in three families (23, 35, 36) where the less severe mutations led to a delayed diagnosis. The early age of clinical manifestation indicates a more severe phenotype, presumably related to a higher activation of the receptor.

The characteristics of the congenital form is well detailed in 14 of the 15 SCNAH children: prematurity (10/14), low birth weight (12/14), small goiter (5/14), craniosynostosis (7/14), and mental retardation (6/10). As mentioned above, the eye signs are frequent. However, these symptoms are not specific and can also be seen in neonatal autoimmune Graves’ thyrotoxicosis. The two key elements in the diagnosis of neonatal nonautoimmune thyrotoxicosis are the absence of TBI and the persistence of hyperthyroidism beyond 3 months after birth. Neonates with hyperthyroidism due to placental transmission of maternal TSI are usually treated with antithyroid drugs around 3 months until the disappearance of maternal TSI. Here, contrary to what happens in the descendants of Graves’ disease patients, there is a systematic recurrence of hyperthyroidism after this period, which must raise the diagnosis of SCNAH and requests a TSHR mutation research.

In summary, the clinical and biological profiles of FNAH present sufficient characteristics to easily identify this entity: autosomal dominant transmission, frequency of the disease around 50% in the families, early onset of the clinical hyperthyroidism in the new generations, ocular signs attenuated and only seen in youngest patients, exceptional presence of circulating thyroglobulin and thyroperoxidas antibodies, absence of TSI, and remarkable tendency to relapse after conventional treatments given to the children of Graves’ disease patients. In the SCNAH, the appearance of ophthalmopathy does not exclude the diagnosis, which will be quickly made by the absence of thyroid immunity in the mother and the persistence of thyroid hyperfunction after the first weeks of life.

**AAs: clinical and biological characteristics**

The clinical phenotype and biology of AAs have been extensively studied (6). To summarize, constitutive activation of the TSHR-cAMP cascade leads to hyperfunction and growth of the cells as a well encapsulated adenoma or nonencapsulated nodule (14). Owing to functional hyperactivation and higher sodium iodide symporter (NIS) expression, the lesions will take up more radioiodide or pertechnetate than the surrounding tissue generating the scintigraphic aspect of a ‘hot’ nodule. Depending on the size of the lesion and the dietary iodide supply, autonomous thyroid hormone secretion will decrease by the negative feedback of the TSH levels and thereby decrease the stimulation and activity of the noninvolved thyroid tissue (quiescent tissue), which will take up less radioiodide. With increasing mass and/or iodide supply, the nodule may secrete more hormone than normal and thus induce thyrotoxicosis (6, 37). Other mutations leading to the activation of the TSH–cAMP cascade in one cell will lead to the same phenotype, i.e. activating mutations of Gαs have been detected in 5–10% of the adenomas while the TSHR account for about 70–80% (37–39). The cause of 20–30% of these adenomas is still unknown. A toxic AA in neonate
presents of course the same phenotypes as SCNAH or FNAH except for the presence of a nodule rather than diffuse hyperplasia (40).

AA tissues in vitro, when compared with their control quiescent tissues, appear more stimulated with a higher radiiodide uptake, spontaneous thyroxine secretion, and NIS and thyroperoxidase expression than the extratumoral quiescent tissue (41). However, their cAMP level is barely, if at all, increased (42).

Comparative biology of FNAH, SCNAH, and AA

The gene expression characteristics of pathological tissues of FNAH and AA have been extensively studied. These characteristics are consistent with our knowledge of the role of the TSH–cAMP cascade in thyrocytes (Fig. 2) (8). They are similar in both types of tissues but much less marked in FNAH cells. More than 90% of regulated genes in FNAH were significantly regulated in the same direction in AA, which underlines their common molecular causes. However, some genes were only regulated only in AA in agreement with the fact that in this case only a fraction of the tissue causes hyperthyroidism. Among the genes downregulated in both thyroid lesions, 76% were more downregulated in AA than in FNAH. A majority of all these regulations are downregulations. In both tissues a large proportion of the downregulated genes concern lymphocytes and proteins involved in inflammation, which is consistent with pathological findings. Among the genes regulated only in AA, most are involved in intermediary metabolism (8). For many of these, similar, but not statistically significant, effects were observed in FNAH tissue.

Except for the higher sensitivity of FNAH compared with normal tissue for the growth effects of TSH, there is little evidence at the functional level of a higher stimulation of the FNAH tissue (8). As in AA, their cAMP level is not measurably increased. This counter-intuitive finding is easily explainable by several facts. First, the high variability of results from different tissues would blur small effects. Second, higher levels of cAMP in only some cells at a given time would be masked in measurements on the whole cell population. Third, the enhancement of cAMP concentration necessary to achieve a maximal stimulation of a function (i.e. secretion) is only a factor of 2 (43). FNAH tissues respond similarly to normal tissue to TSH and forskolin with regard to cAMP levels, inositol phosphate generation, $H_2O_2$ generation, and iodide organification (8). In fact, apart from the enhanced iodide trapping and the consequent higher hormone synthesis and secretion in adenoma they show little evidence of functional activation. In this regard, the FNAH phenotype is much milder than the AA. The reason why the chronic stimulation by the TSHR would achieve rather small cAMP responses can be multiple. Negative feedbacks are induced by the stimulation: i) probable downregulation of the receptor levels as in COS7 cells transfected with cDNA of constitutively activated receptors (44); ii) demonstrated direct activation and induction of cAMP phosphodiesterases; iii) induction of G-protein-coupled receptor kinases (45); and iv) induction of RGS2 inhibiting adenylate cyclase in human thyroid cells (46).

The much earlier age of occurrence of SCNAH versus FNAH also indicates a much more severe disease in the former (i.e. suggesting stronger activating mutations). The pathology of the tissue is similar to an increased vascularization and absence of lymphocytes, i.e. obvious indirect effects of the TSH–cAMP cascade. With regard to the growth effect, one cell at the origin of the AA must have divided at least 30 times without loss to achieve a size of 1 g. This is consistent with a higher, although still low, proportion of Kit$^+$-labeled cells (i.e. cycling cells) in the AA than in normal tissue (41). On the other hand, the cells in FNAH achieve a size of 2–3 times of a normal thyroid, i.e. a few divisions more than a normal thyroid. Thus, the level of thyrocyte stimulation is much lower in FNAH than that in AA. This is also evident clinically as the lower levels of hyperthyroidism are achieved by the whole thyroid in the FNAH than by a nodule in the AA. There is not a strict correlation between what we
know of the strength activation of the TSHR and phenotype. This is explained by several confounding factors including dietary iodide intake, volume of the affected tissue, level of negative intracellular feedback signaling, or epigenetic effects of chronic stimulation (6, 46). This is strikingly illustrated by the very different ages of onset of the FNAH manifestation for subjects within the same family.

The possible role of the TSHR activation of the Gq–phospholipase C (PLC), Ca2+ diacylglycerol cascade, has been less investigated. For the mutated receptors of AAs investigated, only few of the several demonstrated mutations presented some constitutive activation of the cascade in transfect COS cells, which shows that activation of this pathway is not necessary (44, 47, 48). Moreover, the H2O2 generation and the dependent iodide organification is neither enhanced in the FNAH tissue nor is the iodide organification capacity in the AA tissue (41, 42). Hot nodules display a significant iodide leak (49). Finally, half of AA studied in vivo displayed a positive NaClO4 test, i.e. a relative defect in iodination (50). Thus, stimulated tissues in FNAH and AA produce more thyroid hormone because at normal iodide level (<1 μM) they take up more iodide, the limiting step in total iodide organification, but not because of a stimulation of the organization step itself, i.e. of H2O2 generation or its activating Gq–PLC cascade.

All the effects of the activating mutations of the TSHR can be accounted for by the stimulation of the cAMP cascade: increased iodide uptake by the induction of NIS, increased thyroid hormone secretion, and thyroid growth (http://www.thyroidmanager.org/). Indeed, similar effects result from constitutive activation of Gs in AAs (51), in the McCune–Albright syndrome, and in mice thyroid expressing the Gs-specific adenosine A2 receptor (52). Moreover, the growth effects of TSH on human thyroid cells in culture are fully reproduced by forskolin, a specific activator of adenylate cyclase (53). Nevertheless, the role of the Gq–PLC cascade should be investigated in a systematic study of the effects of all mutations of TSHR on this cascade. Indeed, some activity of this cascade is required for goitrogenesis in mice thyroids (54). In human thyroid, defects in Gq stimulation and consequently of iodination, an important compensatory TSH stimulation, but not goitrogenesis, is observed (55). Later, Winkler et al. (56) reported a TSHR-activated mutation with decreased stimulation of Gq but as evidenced by normal or elevated serum thyroid hormone levels no defect in iodination and no goiter were observed. In both cases, the basal IP generation is unaffected. Winkler et al. (56) suggested a necessary role for the Gq cascade in goitrogenesis. It is interesting, in this regard, that in dog, stimulation of this cascade may replace IGF1 as the necessary complement of cAMP in the stimulation of proliferation (57). However, this does not explain the AAs caused by Gs activation or by TSHR mutation activating Gs but not Gq (51).

### The concept of genetic hyperthyroidism due to TSHR mutations

Qualitatively, similar activating dominant TSHR mutations account for FNAH, SCNAH, and AA. Some AAs are caused also by activating mutations of Gq. Hyperthyroidism in the McCune–Albright syndrome, due to activating mutations of the Gq protein, i.e. downstream of TSHR, could be included in the definition of genetic hyperthyroidism. However, the McCune–Albright syndrome is a more complex multi-organ disease with a somatic mosaicism and hyperthyroidism is found only in a fraction of patients (58). Mutation of the TSHR conferring sensitivity to human chorionic gonadotrophin (hCG) and LH also causes a temporary hyperthyroidism pregnancy (59).

The differences between the pathologies are mostly related to the unicellular or whole organ expression of the mutation and on the intensity of the activation. To induce an AA from a mutation in one cell, the activation must be high; a low level of activation

![Figure 3](https://www.eje-online.org)
would only decrease the level of TSH or if lower may just lead to a not discernable small locus of hyperplasia. Congenital mutations affecting all thyroid cells cause FNAH and SCNAH (Fig. 3). The fact that SCNAH hyperthyroidism appears early in life, and that there are common mutations between SCNAH and AA but less between FNAH and AA suggests that SCNAH mutations are stronger than those of FNAH. One might presume that even stronger mutations would kill the fetus before birth. In addition, the mutations of SCNAH, although they could become familial now that we could cure the affected patients, would not have allowed the survival of the patients before, and therefore their transmission. Thus, one can postulate a continuum in the strength of the activation by the TSHR in genetic hyperthyroidism with different consequences when they affect one cell or the whole cell population (Fig. 3). Two, as not yet identified, syndromes should be looked for very active TSHR congenital neomutations leading to fetal death and abortion, and mild familial hereditary mutations leading to only a relative decrease in serum TSH levels with no hyperthyroidism. Increased sensitivity of TSHR to TSH would also lead to the latter phenotype. Relatively low serum TSH values could allow to detect such cases, just as a lower cut off has led to the detection of many previously undiagnosed cases of congenital hyperthyroidism (60). Similarly, mild activating mutations in single thyroid cells may give rise to small autonomous areas with high radioiodide uptake with no general consequences (61).

There are still outstanding questions. A systematic study of the strength of all TSHR activating mutations as well as their effects on the $G_\text{q}$–PLC cascade would constitute the best basis to correlate biology and phenotype (as done in (47, 55)). The hypothesis that a population of normal people with relatively low TSH level would correspond to defined genetic polymorphisms in TSHR sequences could be looked for as well as the possibility of mild FNAH in old patients developing hyperthyroidism with negative TSAb (62). Finally, the possibility of new TSHR strong activating neomutations in cases of spontaneous abortions may be examined. As discrete TSHR localization and role in different tissues are now discovered, it will be of interest for clinicians to look for anomalies in these tissues and functions in both FNAH and SCNAH (e.g. white adipose tissue, ovary, brain, etc.) (63–65).

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-10-0775.

Declaration of interest
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