CMLS, Cell. Mol. Life Sci. 58 (2001) 1301–1322
1420-682X/01/091301-22 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Human Genome and Diseases: Review

The TSH receptor and its role in thyroid disease

P. Kopp

Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University, Tarry 15, 303 East Chicago Avenue, Chicago, Illinois 60611 (USA), Fax +1 312 908 9032, e-mail: p-kopp@northwestern.edu

Received 31 January 2001; received after revision 3 April 2001; accepted 3 April 2001

Abstract. The thyrotropin (TSH) receptor plays a preeminent role in thyroid physiology and disease. TSH, acting through the TSH receptor, is the major stimulator of thyroid cell growth, differentiation and function. In Graves' disease, the TSH receptor is the target of stimulating antibodies that cause hyperthyroidism. Although still a topic of debate, the TSH receptor has been implicated in the pathogenesis of the endocrine ophthalmopathy associated with Graves' disease. Blocking antibodies against the TSH receptor are involved in the development of hypothyroidism in a subset of patients with autoimmune hypothyroidism. Transplacental passage of stimulating or blocking TSH receptor antibodies from a mother with autoimmune thyroid disease may result in transient hyper- or hypothyroidism in early infancy. During pregnancy, the placental hormone human choriogonadotropin (hCG) can cause gestational hyperthyroidism through cross-reaction with the TSH receptor. Gestational hyperthyroidism may also be involved in the pathogenesis of hyperemesis gravidarum. Trophoblast tumors secreting hCG are a rare cause of hyperthyroidism. Somatic activating mutations of the TSH receptor have been identified as a molecular cause of toxic adenomas, whereas activating mutations in the germline give rise to nonautoimmune familial hyperthyroidism or sporadic congenital hyperthyroidism. These gain-of-function mutations are dominant, and one mutated allele is sufficient to result in disease. Inactivating germline mutations of both TSH receptor alleles lead to variable degrees of resistance to TSH, encompassing a spectrum ranging from euthyroid hyperthyrotropinemia to overt hypothyroidism with thyroid hypoplasia.

Key words. Thyroid; thyrotropin; receptor; cAMP; hyperthyroidism; hypothyroidism; autoimmune thyroid disease; mutation.

Introduction

The thyroid is controlled by a classic hypothalamic-pituitary axis (fig. 1). The hypothalamic tripeptide TRH (thyrotropin-releasing hormone) stimulates the production and secretion of the pituitary glycoprotein hormone TSH (thyroid-stimulating hormone). TSH is composed of an α and a β subunit, the α subunit being common to the glycoproteins TSH, FSH (follicle-stimulating hormone) and LH/CG (luteinizing hormone/chorionic gonadotropin). TSH, acting through its specific receptor, stimulates growth and function of thyroid follicular cells and regulates the synthesis and secretion of the thyroid hormones, thyroxine (T4) and triiododothyronine (T3) (fig. 1). Thyroid hormones have multiple effects on differentiation, growth and metabolism. Their action is primarily mediated by nuclear receptors regulating gene transcription, although nongenomic actions are increasingly recognized [1, 2].

In disease, the TSH receptor is directly involved in the pathogenesis of Graves' disease, autoimmune hypothyroidism, toxic adenomas, familial and sporadic nonautoimmune hyperthyroidism, and certain forms of resistance to TSH (fig. 2, 3). Not surprisingly, the TSH receptor has been a primary focus of interest, generating an impressive increase in the amount and complexity of knowledge since its cloning in 1989 [3]. The ample information on the many facets of the TSH receptor in physiology and

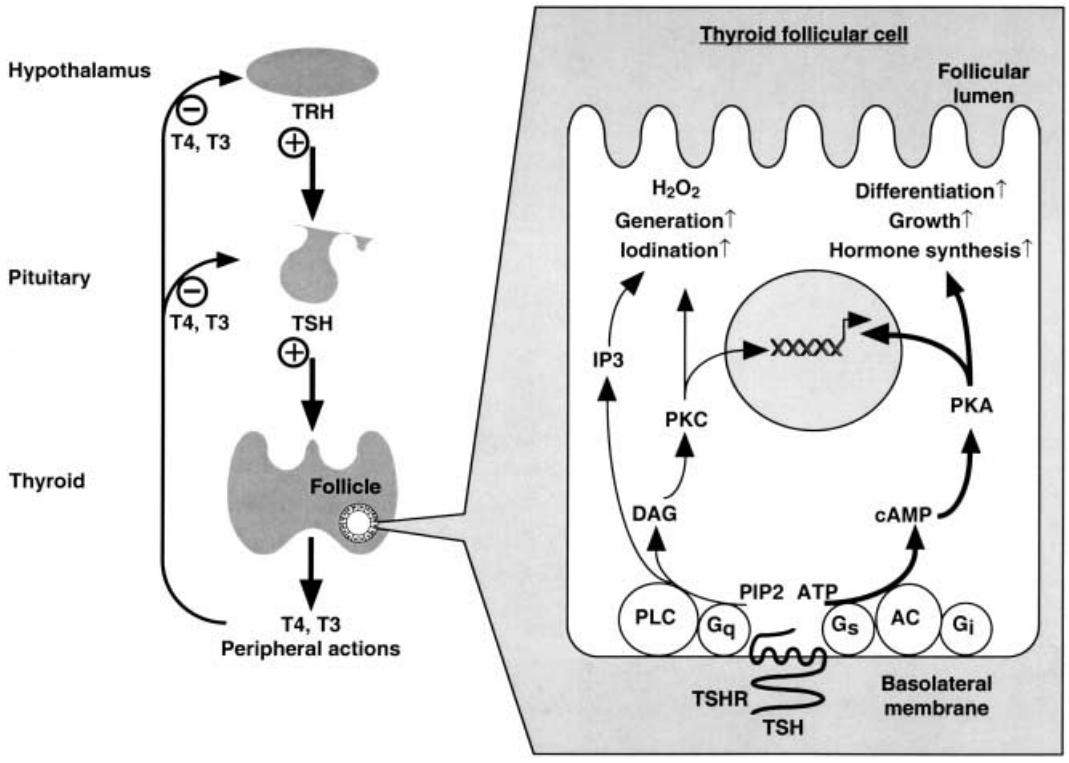

Figure 1. The hypothalamic-pituitary-thyroid axis and major signaling pathways in thyroid follicular cells. TRH, TSH releasing hormone; TSH, thyroid stimulating hormone; T4, tetraiodothyronine; T3, triiodothyronine; TSHR, TSH receptor; Gs/q/i, G proteins; AC, adenylyl cyclase; PKA, protein kinase A; PLC, phosophlipase C; DAG, diacylglycerol; PKC, protein kinase C; IP3, inositoltriphosphate.

disease is discussed in a number of reviews that contain further information and references not included here because of space constraints [4–18]. In addition to providing an overview of the implications of the TSH receptor in the pathogenesis of thyroid disorders, the primary emphasis of this review is directed towards the consequences of naturally occurring mutations in the TSH receptor.

Characteristics of the TSH receptor

Gene and protein structure

The TSH receptor is a member of the large superfamily of G-protein-coupled seven-transmembrane receptors (GPCRs) (fig. 1, 4). It belongs to subfamily A, which is related to rhodopsin and the β2-adrenergic receptor [19–21]. Together with the receptors for the other glycoprotein hormones FSH and LH/CG, the TSH receptor forms a distinct subgroup. The three glycoprotein hormone receptors share a high degree of homology (~70%) in the transmembrane domain [4, 6]. The main differences are found in the large amino-terminal extracellular domain involved in binding of the hormone. In the extracellular domain, the TSH receptor has 39% degree of homology with the FSH receptor, and 45% with the LH/CG receptor [4, 5, 8].

The canine TSH receptor complementary DNA (cDNA) was cloned using a reverse transcription polymerase chain raction (RT-PCR) approach in 1989 [3, 4]. Mammalian TSH receptor cDNAs have subsequently been characterized in humans, rat, mouse, cow, sheep and cat [22–30]. Species comparisons reveal a high degree of homology of more than 90%. The single-copy human gene is located on chromosome 14q13, spans more than 60 kb and contains 10 exons [31–33]. The first 9 exons encode the majority of the extracellular domain. Exon 10 codes for the carboxy-terminal part of the extracellular domain, the whole transmembrane domain and the intracellular carboxy-terminus. The open reading frame consists of 2295 nucleotides encoding a 764-amino acid protein (fig. 4). A 21-amino acid signal sequence precedes the long extracellular domain of 397 amino acids. Splice variants of unknown physiologic relevance have been reported [34]. The TSH receptor differs from the LH/CG receptor by the presence of two unique insertions of 8 (residues 38–45) and 50 amino acids (residues 317–366) in the extracellular domain [4, 8, 14]. Deletion of residues 38–45 disrupts TSH binding, whereas deletion of residues 317–366 does not affect TSH binding or TSH-mediated cyclic AMP (cAMP) accumulation in transfected cells [35]. Chimeras of the TSH receptor with the LH/CG receptor reveal primary roles for the extracellular domain

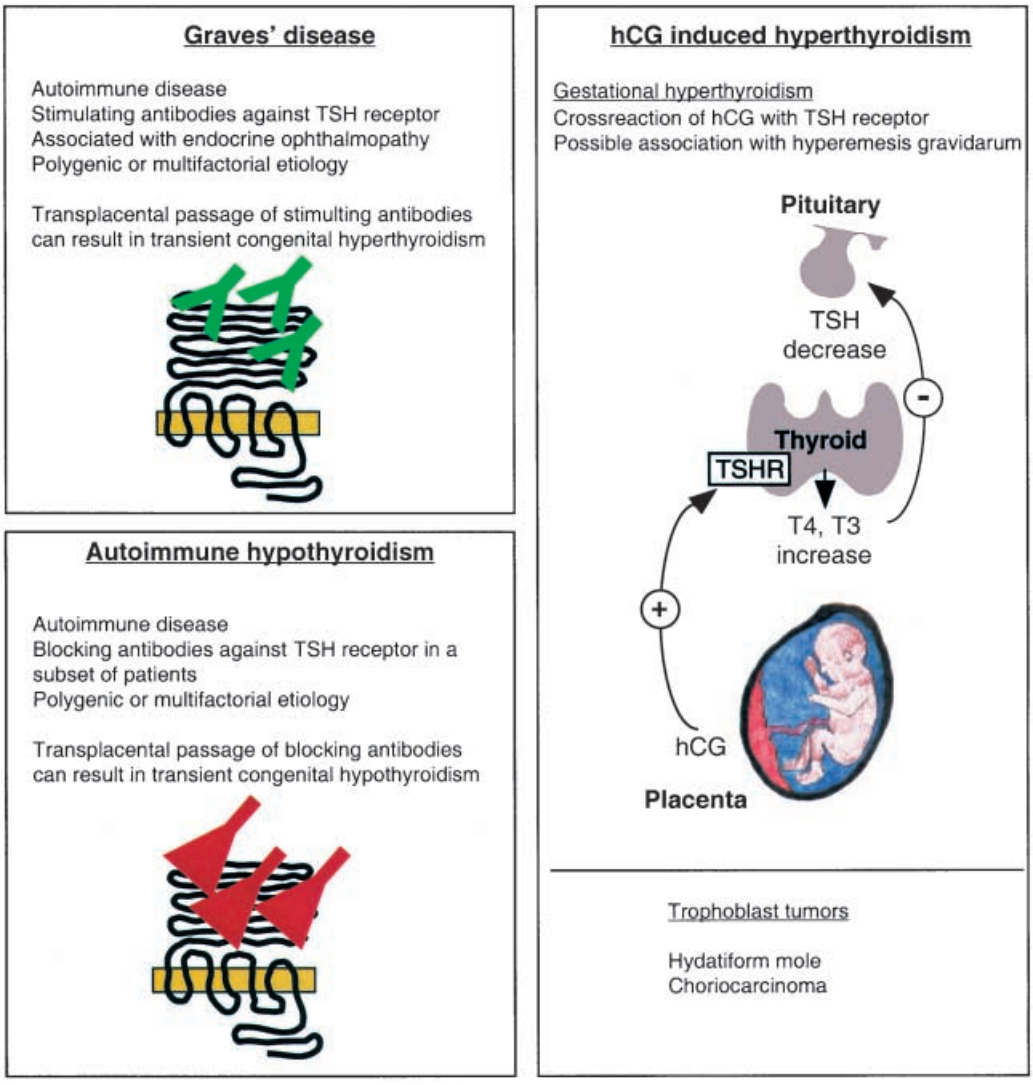

Figure 2. TSH receptor in disease without mutations.

in mediating the binding specificity, and for the transmembrane domain in signal transduction [36].

Currently, there is no information on the three-dimensional structure derived from crystallization of parts of the receptor, and expression of significant amounts of TSH-binding receptor remains a challenging task [14]. Based on the putative homology with the ribonuclease inhibitor, a three-dimensional model of the extracellular domain has been proposed [37]. Leucine-rich repeats are thought to form a horseshoe-shaped concave surface composed of parallel β sheets which interact with the ligand [7, 37, 38]. For the transmembrane domain, limited structural considerations have been proposed by comparing the TSH receptor to the helical wheel model proposed for GPCR and to a rhodopsin-derived model of the LH/CG receptor [39–42].

Expression of the TSH receptor

In addition to its expression on the basolateral membrane of thyroid follicular cells (fig. 1), there is evidence for expression of the TSH receptor in several nonthyroidal tissues. These include fibroblasts, white and brown adipocytes, lymphocytes, certain regions of the brain, the adrenal glands, cardiac myocytes, the kidney and the thymus [29, 43–52]. Some of these observations are based on the detection of messenger RNA (mRNA) transcripts by RT-PCR, and this may have led to the detection of illegitimate transcripts [53]. However, convincing evidence for TSH receptor protein expression and function does exist for some of the tissues studied [14]. For example, neonatal human adipocytes display sensitivity to TSH [54], and retroorbital preadipocyte fibroblasts may differentiate into TSH receptor-bearing adipocytes under appropriate culture conditions [44, 55]. The TSH receptor

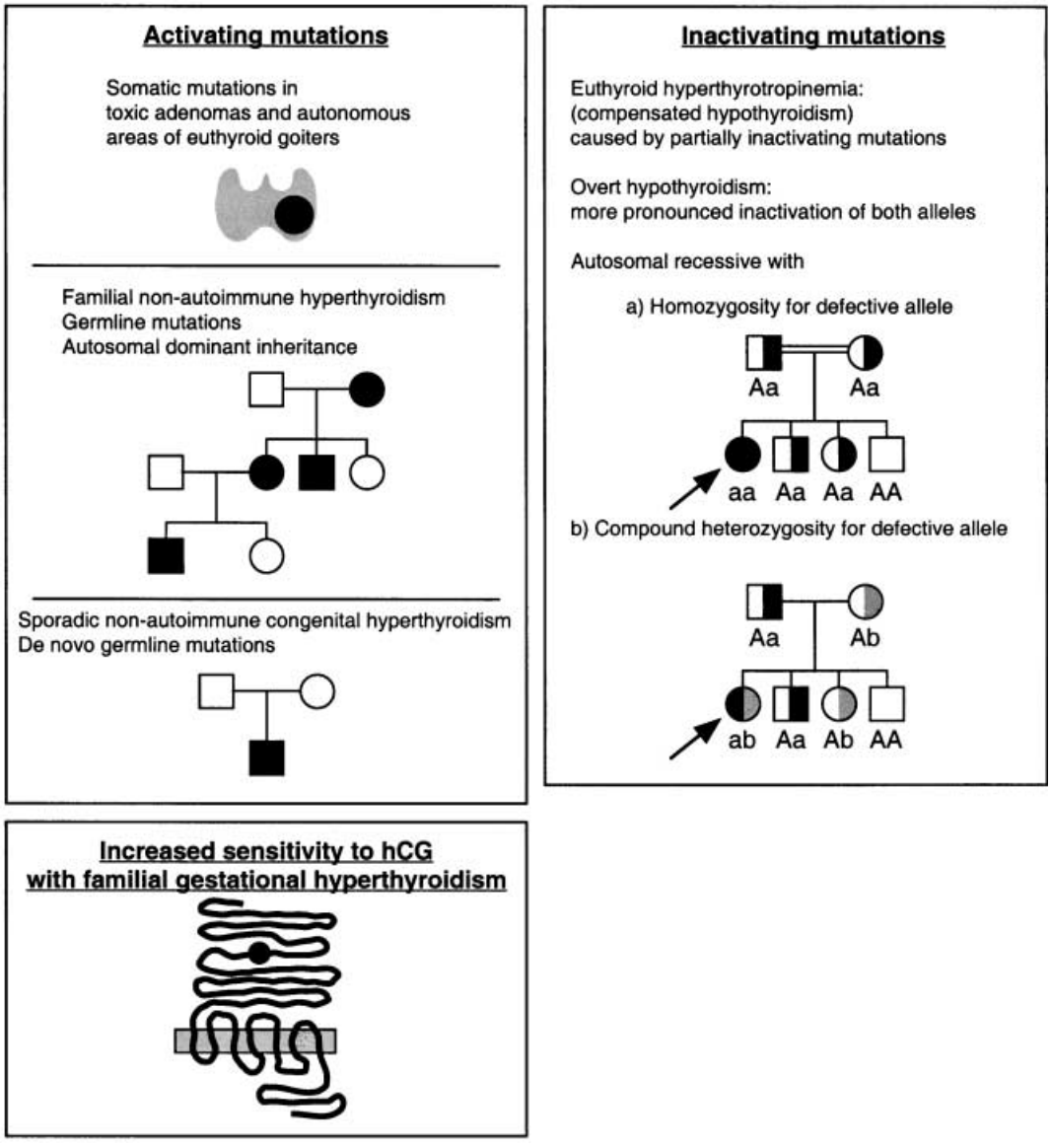

Figure 3. TSH receptor in disease with mutations.

cDNA has been cloned from ovine hypothalamus and appears to be expressed throughout the ovine brain [29]. In the anterior human pituitary, the TSH receptor was found to be expressed in folliculo-stellate cells, suggesting that it could be involved in an intrapituitary ultrashort loop regulating TSH secretion [48]. In the gastrointestinal tract, the TSH receptor has been detected in intestinal T cells but not in epithelial cells [46]. Interestingly, the *hyt/hyt* mouse, which is homozygous for an inactivating mutation in the TSH receptor, has a selectively impaired intestinal T cell repertoire, and it has been proposed that TSH may be a key immunoregulatory mediator in the intestine [46]. Dendritic cells were reported to have high levels of TSH receptors, and they display an enhanced phagocytic response as well as interleukin 1 and 12 secretion after stimulation with TSH [47]. The functional relevance of TSH and its receptor in extrathyroidal tissues needs to be further delineated.

Glycosylation and palmitoylation

The human extracellular domain contains six potential asparagine-linked glycosylation sites (fig. 4). Based on studies in transfected CHO cells, the primary translation product of 85 kDa is glycosylated, resulting in a high-mannose form of ~100 kDa, with subsequent maturation to a 120-kDa form with complex carbohydrates [12, 14, 56]. TSH binding is increased on the mature, glycosylated form of the receptor, and mutation of glycosylation sites reduces TSH binding [12, 57]. The mature TSH re-

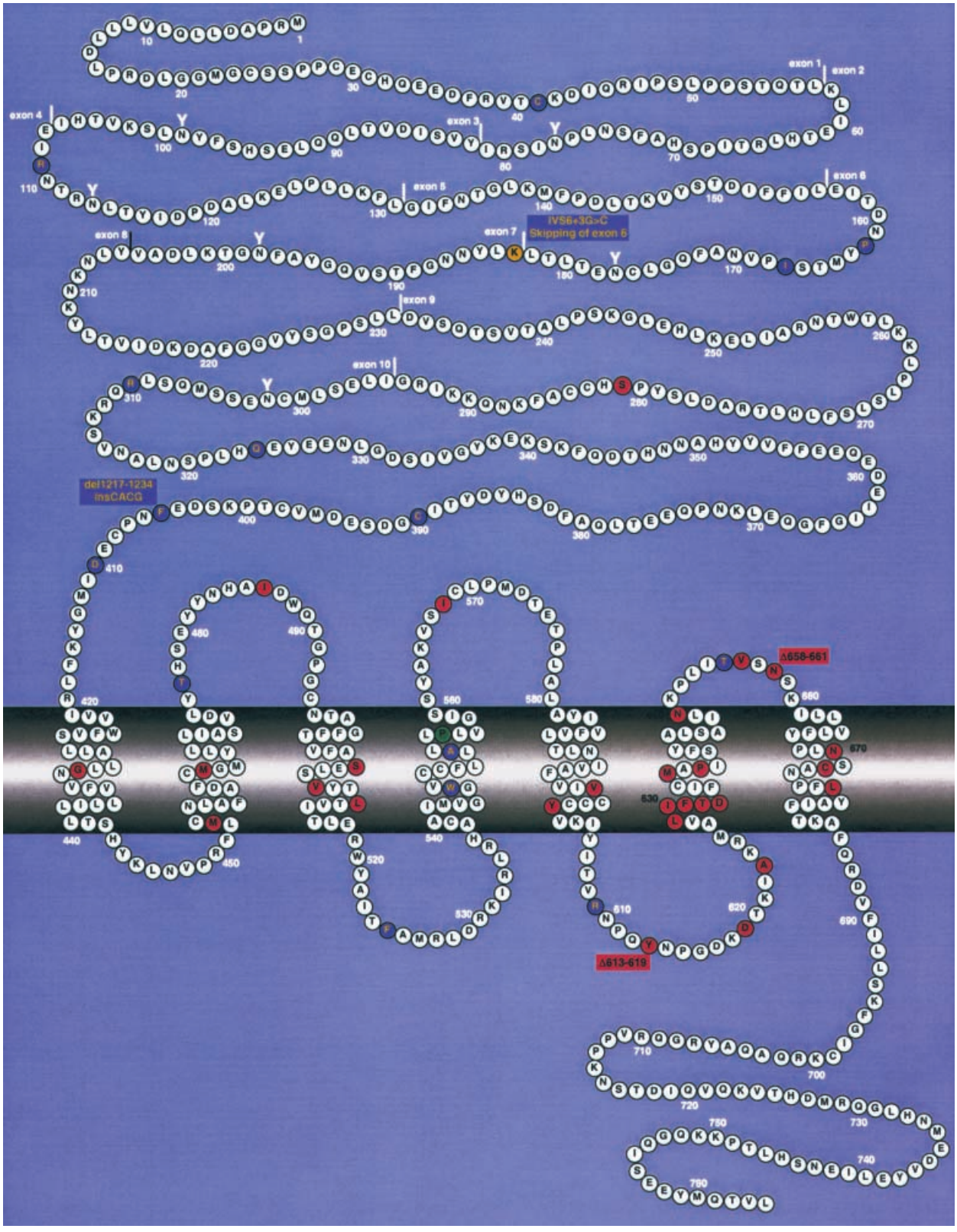

Figure 4. Naturally occurring mutations in the TSH receptor. ● Activating mutations, ● inactivating mutations, ● mutation conferring increased sensitivity to hCG, ● mutation in the hypothyroid *hyt/hyt* mouse. **Y** asparagine-linked glycosylation sites. The figure, and tables 1 and 2 include mutations published up to December 2000. The mutations are designated according to the *Recommendations for a nomenclature system for human gene mutations* [223].

ceptor is palmitoylated at cysteine 699 in the cytoplasmic tail of the receptor [58]. Mutation of cysteine 699 to alanine (C699A) delays surface expression, but the mutant remains fully active in terms of TSH binding and signaling.

Two subunit model of the TSH receptor

Considerable interest has been directed towards the two-subunit model of the TSH receptor [14]. In vitro studies with thyroid cells in culture and mammalian cells transfected with the recombinant TSH receptor suggest that the receptor is present not only as a single chain, but also in a two-subunit form [59–61]. The glycosylated extracellular domain, the α subunit, is cleaved from the unglycosylated membrane-spanning β subunit [14, 62]. The α and β subunits (also referred to as A and B subunits) are linked by disulfide bonds [14]. The transmembranous β subunit is more prevalent in membrane preparations of thyroid follicular cells, suggesting that the α subunit may undergo shedding from the transmembranous part of the receptor [61]. The cleavage sites that give rise to the two subunits flank the unique insertion found in the extracellular domain of the TSH receptor [17]. The amino-terminal cleavage site is located between residues 302 and 317 in proximity to a basic domain formed by residues 310–313 (RQR) [56, 63–65]. Deletion mutants of this region shift the cleavage site towards the amino terminus, suggesting that cleavage is not dependent on a specific amino acid sequence but rather occurs at a fixed distance from a protease attachment site [66]. The carboxy-terminal cleavage site seems to be located between residues 366 and 378 at or in proximity to a GQE motif at positions 367–369 [63–65, 67, 68]. The impossibility of recovering a connecting peptide suggests that the initial cleavage at the amino-terminal site is followed by progressive cleavage or degradation to the amino-terminal site or, alternatively, rapid disintegration of the putative connecting peptide [63–65, 67–69]. Initially, it was proposed that cleavage is performed by a metalloproteinase. More recent data, however suggest, that cleavage may be exerted by an enzyme that is related to TACE (tumor necrosis factor α-converting enzyme) [64]. The enzyme is not thyroid specific since cleavage also occurs in transfected nonthyroidal cells expressing the TSH receptor.

The physiologic significance of the cleavage and shedding phenomenon remains unresolved. The existence of various isoforms generated from the initial holoreceptor raises the possibility that their biologic behavior is not uniform [56, 67]. However, affinity for TSH and signaling do not differ between the single-chain and the two-subunit forms of the receptor. Moreover, artificial TSH receptor mutations that prevent intramolecular cleavage do not abolish its constitutive activity [67]. Whether shedding of the extracellular α subunit or the release of fragments of the connecting peptide are involved in initiation of an immune response leading to the development of autoantibodies in Graves' disease remains unresolved [62].

Desensitization

Analogous to many other GPCRs, the TSH receptor undergoes homologous desensitization after exposure to TSH [5]. Desensitization involves G protein receptor kinases (GRK 2 and GRK 5) which phosphorylate the receptor [70–72]. Subsequently, β-arrestin binds to the phosphorylated receptor, resulting in its uncoupling from $G_{s\alpha}$ [71]. Transcriptional downregulation of the TSH receptor gene has been shown to involve a splice variant of CREM (cAMP response element modulator), the transcriptional repressor ICER (inducible cAMP early repressor) [73].

Intracellular signaling in thyroid follicular cells

Binding of TSH to its receptor leads primarily to coupling to $G_{s\alpha}$ and subsequent activation of adenylyl cyclase [74, 75] (fig. 1). At higher doses of TSH, the receptor couples to $G_{q/11}$, resulting in activation of phospholipase C. Additional interactions have been demonstrated with other G protein families [76]. More recently, TSH-mediated phosphorylation of the intracellular Janus kinases (JAK) 1 and 2 and the JAK family substrate STAT3 (signal transducer and activators of transcription) has been demonstrated in rat thyroid cells and transfected Chinese hamster ovary (CHO) cells [77].

The TSH-dependent cAMP cascade is the major regulator of growth, differentiation and hormone secretion of thyroid follicular cells [78]. The increase in cAMP leads to phosphorylation of protein kinase A and to activation of targets in the cytosol and the nucleus, such as the transcription factor CREB. Stimulation of $G_{q/11}$ and the phospholipase C-dependent inositol phosphate/diacylglycerol pathway at higher doses of TSH activates hydrogen peroxide generation and iodination [74, 75, 79, 80]. In contrast to the receptors for LH/CG and FSH, the TSH receptor displays constitutive basal activity, i.e. readily measurable spontaneous activity in the absence of ligand. The predominant role of the cAMP pathway in thyroid cell growth and function has been corroborated by several transgenic models with chronic overstimulation or disruption of this pathway [81–84]. Its importance is furthermore underscored by the consequences of antibodies that stimulate or block the TSH receptor, as well as naturally occurring activating and inactivating mutations of the TSH receptor [13].

The TSH receptor in disease

Disorders affecting thyroid function or growth are common and may occur independently or in combination. Functional disturbances result in hyper- or hypothyroidism. In iodine-sufficient regions, the autoimmune thyroid disorders, Hashimoto thyroiditis (hypothyroidism) and Graves' disease (hyperthyroidism), are by far the most common clinical entities affecting the thyroid gland. Their prevalence is significantly higher in women [85, 86]. Benign growth alterations are equally common and include diffuse and multinodular goiter, cysts, nodules and adenomas. Thyroid carcinomas are relatively infrequent neoplasms that account for ~0.6–1.6 % of all malignancies [87]. Figures 2 and 3 summarize the disorders in which the TSH receptor plays a pivotal role in the pathogenesis.

Graves' disease

Graves' disease is a complex autoimmune disease. Its exact pathogenesis, which involves T and B cells, remains unresolved, and a discussion of these aspects is beyond the scope of this review. In addition to the signs and symptoms of thyrotoxicosis, patients with Graves' disease often develop Graves' ophthalmopathy, and they may present with skin infiltrations, most commonly pretibial myxedema.

In Graves' disease, stimulatory antibodies (TSAbs) directed against the TSH receptor, the major antigen in this form of autoimmune thyroid disease, mimic TSH action and result in hyperthyroidism and goiter [88, 89] (fig. 2). Antibodies against the TSH receptor are heterogeneous. In addition to TSAb, blocking antibodies (TBAb) may be present concomitantly, and fluctuations in their relative concentrations may result in periods of hyper- or hypothyroidism [90, 91]. Routine assays do not differentiate between TSAb and TBAb, but merely measure the binding of immunoglobulins to the receptor by a radioreceptor assay using porcine or human TSH receptor (TBI, TSH-binding inhibition or TBIIs, TSH-binding inhibitory immunoglobulins). More sensitive second-generation TBI assays use a monoclonal-antibody-capturing recombinant TSH receptor in a coated tube format [92]. Determination of TBIIs can be useful in the differential diagnosis of hyperthyroidism, and they can be helpful in the prediction of fetal and neonatal thyroid dysfunction in neonates born to a mother with a history of autoimmune thyroid disease. There is also some suggestion that TBII levels may be useful as predictors in the course of Graves' disease [91]. Functional characterization of TRAb is possible using thyroid cells or cell lines stably transfected with the human TSH receptor [91]. Most commonly these assays rely on the determination of intracellular cAMP accumulation. More recent developments include measurement of cAMP-dependent luciferase reporter gene activity [93, 94].

The TSH receptor epitopes for TSAb and TBAb are not well defined [9, 14, 17]. Studies with linear epitopes suggest that TSAbs bind predominantly to the amino-terminal part of the extracellular domain, whereas TBAbs bind primarily to an ectodomain region in proximity to the transmembrane domain [8, 95]. However, it is more likely that the majority of these autoantibodies recognize conformational epitopes that involve segments of both the amino- and carboxy-terminal segments of the ectodomain [14, 96]. Chimeras of the TSH and LH/CG receptor not only confirm that autoantibodies interact with the TSH receptor ectodomain but provide evidence that TSH and autoantibody-binding sites are overlapping, but not identical [96].

Although several studies suggested that certain naturally occurring sequence variations of the TSH receptor may have immunogenic properties or result in enhanced signaling activity, it seems unlikely that any of these polymorphisms is of relevance in the pathogenesis of Graves' disease (for review see [9]). The possibility that autoantibodies against the TSH receptor arise in response to a foreign antigen with structural similarity, for example infection with *Yersinia enterocolitica*, has been raised, but remains unresolved [14, 97].

A genetic component in the pathogenesis of Graves' disease is supported by family and twin studies [98, 99]. Given the key role of TSAbs in the pathogenesis of Graves' disease, the TSH receptor gene itself is an obvious candidate gene. Most population-based case-control studies argue, however, against a significant role of the TSH receptor locus [100–102], although a positive association has been reported in one study on women with Graves' disease [103]. Linkage studies and transmission equilibrium analyses have equally been negative [102, 104]. Susceptibility loci that may be involved in the pathogenesis of Graves' disease currently include the *HLA* locus (6p21), the *CTLA-4* gene (2q33), and loci referred to as *GD-1* (14q31), *GD-2* (20q11.2) and *GD-3* (Xq21.33–22) [99].

Ophthalmopathy and skin manifestations in Graves' disease

Graves' disease is often associated with ophthalmologic symptoms, endocrine ophthalmopathy or Graves' ophthalmopathy, and skin manifestations [105–107]. It is important to differentiate signs of increased sympathicomimetic activity associated with all forms of thyrotoxicosis from the tissue involvement defining endocrine ophthalmopathy. The latter includes edema, chemosis, congestion of conjunctival vessels, proptosis, extraocular muscle involvement (diplopia), corneal lesions due to lagophthalmos and compression of the optic nerve [106]. Minor ocular involvement is present in the majority of

patients more pronounced ophthalmopathy occurs in ~25–50% [106]. Skin manifestations are most commonly seen in the pretibial area, but Graves' dermopathy may occur in any area of the body in response to trauma or pressure [107].

Because of the fact that the TSH receptor is the major antigen in Graves' disease, and given the well-established role of TSAbs in the pathogenesis of hyperthyroidism and goiter, these molecules form obvious candidates in the pathogenesis of eye and skin disease. Reports on the identification of TSH receptor mRNA transcripts in orbital tissues from patients with endocrine ophthalmopathy by RT-PCR were met with some skepticism due to concern that these messages may be illegitimate transcripts without physiologic relevance [53, 108, 109]. However, TSH receptor mRNA has also been detected using a ribonuclease protection assay in orbital tissue from patients with Graves' disease, but not from normal controls, suggesting that the receptor is indeed expressed in this tissue [44]. Moreover, retroorbital preadipocyte fibroblasts appear to differentiate into TSH receptor-bearing adipocytes under appropriate culture conditions [55]. These studies suggest that the TSH receptor may indeed be expressed in orbital tissue in Graves' disease. However, it remains unclear whether the TSH receptor is the orbital antigen responsible for the development of endocrine ophthalmopathy. There are currently no studies that document reactivity of orbital T cells against the TSH receptor, an, as of yet, the autoantigen(s) and specific pathogenic autoantibodies have not been identified.

The documented expression of the TSH receptor in adipocytes and fibroblasts may also be of relevance for the development of skin manifestations [14, 110, 111]. Interaction of autoantibodies with the TSH receptor or a cross-reacting antigen would, however, not explain the predilection of extrathyroidal manifestations in retroorbital tissue and pretibial skin, nor the occurrences at other locations in response to trauma. Chronic inflammation, in combination with local physical factors, may therefore be required for the development of the extrathyroidal manifestations of Graves' disease [111].

Animal models for Graves' disease

Graves' disease probably only occurs in humans. Cats are the only other mammalian species in which spontaneous thyrotoxicosis occurs frequently [112]. The pathogenic mechanisms underlying feline hyperthyroidism remain controversial, as both an autonomous mechanism of growth and function, and an autoimmune etiology have been proposed [112, 113]. However, a recent study, using cells transfected with the feline TSH receptor, provides further evidence against the presence of TSH receptor antibodies in hyperthyroid cats [30].

Initial attempts to create murine models of Graves' disease by immunization with soluble TSH receptor and adjuvant were not successful in eliciting appropriate T cell and B cell responses, and histological analyses only revealed lymphocytic infiltrates in a subset of these experiments [14]. However, several promising approaches in generating mouse models of Graves' disease have been generated during the last few years [16, 114–120]. For example, the immunization of mice with fibroblasts stably transfected with cDNA encoding the human TSH receptor and major histocompatibility complex (MHC) class II antigen resulted in thyrotoxicosis and goiter in ~25% of the animals [115]. The sera contained detectable stimulating antibodies against the TSH receptor, as determined in CHO cells expressing the TSH receptor. However, there was no lymphocytic infiltration in thyroid tissue and no ocular involvement [115]. Inbred mice immunized with a plasmid encoding the human TSH receptor developed TBII and thyroiditis in the majority of the animals [117]. This approach was also successful in inducing lymphocytic and mast cell infiltration of the orbits [118]. Moreover, there was accumulation of adipocytes and edema in the orbital tissue, periodic acid Schiff-positive material, probably corresponding to glycosaminoglycans, dissociation of muscle fibers and TSH receptor immunoreactivity. This interesting model may be a first example of endocrine ophthalmopathy caused by the transfer of T cells sensitized to the TSH receptor, and it supports the concept that this disease has indeed an autoimmune basis [118]. Particularly promising is a recent model with outbred mice immunized with human TSH receptor cDNA in a eukaryotic expression vector [120]. All mice developed antibodies capable of recognizing the recombinant TSH receptor in CHO cells. Consistent with the higher prevalence of Graves' disease in women, 17 of 29 females, but only 1 of 30 males, developed overt hyperthyroidism, goiter with lymphocytic infiltration and ocular signs reminiscent of Graves' ophthalmopathy [120].

Graves' disease during pregnancy

Hyperthyroidism is second to diabetes mellitus as the most common endocrinopathy in pregnancy. Its prevalence is ~0.05–0.2% [121, 122]. Its clinical assessment may be difficult, because many of the symptoms suggestive for hyperthyroidism are also associated with normal pregnancy. Hyperthyroidism during pregnancy is most commonly caused by Graves' disease or hCG-induced gestational hyperthyroidism (discussed below). The natural history of Graves' disease in pregnancy is typically characterized by aggravation during the first trimester, amelioration in the second and third trimesters, and recurrence a few months after pregnancy [123]. In mothers with autoimmune thyroid disease, the fetus may rarely present with congenital hyperthyroidism caused by maternal-to-fetal transfer of thyroid-stimulating antibodies. This form of hyperthyroidism is transient because the

stimulating antibodies are cleared from the fetal circulation. Very rarely, congenital hyperthyroidism is caused by activating mutations in the TSH receptor, and in these infants it is persistent (see below) [124].

Human chorionic gonadotropin-induced gestational hyperthyroidism and hyperemesis gravidarum

During early pregnancy, secretion of the placental hormone human chorionic gonadotropin (hCG) may result in thyroid dysfunction. At high doses, hCG cross-reacts with the TSH receptor, and this stimulation can lead to an increase in secretion of T4 and T3, with subsequent suppression of TSH secretion [123, 125] (fig. 2). The increased secretion of hCG may result exclusively in suppression of TSH, or in overt hyperthyroidism. Estimates for hCG-induced hyperthyroidism predict that it occurs in ~30–40 patients in 1000 pregnancies [123]. Elevations of hCG are particularly pronounced in twin pregnancies [126]. Because of the decrease in the levels and bioactivity of hCG later in pregnancy, this form of hyperthyroidism is typically transient and limited to the first 3–4 months of gestation. Gestational hyperthyroidism is frequently associated with hyperemesis gravidarum [123, 127]. The hyperemesis might be caused by a marked hCG-induced increase in estradiol levels [128]. However, the relation between hyperemesis and gestational hyperthyroidism varies among patients, and additional, unidentified mechanisms may be involved. Hyperthyroidism is only rarely caused by trophoblastic tumors, hydatiform moles and choriocarcinomas that secrete high amounts of hCG [129]. In men, choriocarcinomas can arise in the testis and cause hyperthyroidism by secreting hCG [130].

Hypersensitivity to hCG due to mutation of the TSH receptor

A remarkable form of familial gestational hyperthyroidism caused by a mutant thyrotropin receptor displaying hypersensitivity to normal levels of hCG has been identified by Rodien et al. (table 1; fig. 3, 4) [131]. The proband had a history of two miscarriages that were accompanied by hyperemesis. Subsequently, she had two pregnancies that were complicated by hyperthyroidism, severe nausea and vomiting. Of note, she was negative for antibodies against the TSH receptor or thyroid peroxidase (TPO). Her hCG levels, determined during the second pregnancy, were in the normal range for the first trimester. The patient's mother had a history of one miscarriage and two pregnancies that were complicated by hyperemesis gravidarum. Direct analysis of the TSH receptor gene in the proband and her mother revealed the presence of a heterozygous point mutation in exon 7, resulting in the substitution of K183R (fig. 4). Functional studies in COS-7 cells transfected with the mutated receptor documented no differences in membrane expression, and similar levels of basal and TSH stimulated cAMP accumulation. Whereas the wild-type TSH receptor reacts only minimally to high doses of hCG, the K183R mutant is hypersensitive to hCG, although it is still 1000 times less responsive to hCG than the LH/CG receptor. Aside from explaining the recurrent hyperthyroidism in these two patients, the K183R TSH receptor mutation is unique because sensitivity is increased for hCG but remains unaltered for the cognate ligand TSH [131]. This observation also supports the possibility of a hCG-independent connection between hyperthyroidism and hyperemesis gravidarum.

Autoimmune hypothyroidism

In adults living in iodine-replete regions, autoimmune thyroiditis is the most common cause of hypothyroidism [85, 86]. Analogous to Graves' disease, it is a complex autoimmune disorder with an abnormal humoral and cellular immune response to the thyroid. Several forms of autoimmune thyroiditis are distinguished on clinical grounds [132]. In Hashimoto's thyroiditis, the thyroid shows the signs of widespread chronic lymphocytic infiltration, and at least in early stages of the disease, it is mildly enlarged [133]. In addition to an increase in TSH and a decrease in thyroid hormone levels, most patients have elevated titers of autoantibodies against thyroperoxidase (Anti-TPOAb) and thyroglobulin (Anti-TGAb) [133]. The progressive destruction of thyroid tissue by chronic inflammation may ultimately lead to a decrease in thyroid hormone synthesis and hypothyroidism. In about 15% of patients with autoimmune hypothyroidism, the thyroid dysfunction is thought to be the consequence of blocking antibodies directed against the TSH receptor (fig. 2) [134–136]. Of note, transplacental passage of TBAb may be a cause of transient congenital hypothyroidism [137]. Linkage and association studies indicate a role for the HLA and CTLA-4 genes in the development of autoimmune hypothyroidism, but the TSH receptor locus itself has not been implied as a susceptibility locus [138].

Activating mutations in the TSH receptor

The discovery that site-directed mutagenesis of a critical residue in the third intracellular loop of the α_{1b}-adrenergic receptor can lead to constitutive activation in the absence of ligand subsequently led to the search and detection of naturally occurring activating mutations in numerous GPCRs [139, 140]. Gain of function mutations are by definition dominant, and alteration of one allele is thus sufficient for generating the pathologic phenotype. Activating TSH receptor mutations can occur somatically in solitary toxic adenomas or toxic adenomas within multinodular goiters. Germline TSH receptor mutations give rise to autosomal dominant nonautoimmune hyperthy-

Table 1. Activating TSH receptor mutations.

Residue	Toxic adenoma[a]	Thyroid cancer[b]	Familial nonautoimmune hyperthyroidism[b]	Sporadic congenital hyperthyroidism[b]
Activating somatic and germline mutations				
S281	S281I			
	S281T			
	S281N			S281N [194, 186]
G431			G431S [186]	
M453	M453T	M453T [181]		M453T [179, 180]
M463			M463V [187]	
I486	I486F	I486F [175]		
	I486M			
S505				S505N [193, 196]
			S505R [39]	
V509			V509A [155]	
L512	L512R			
I568	I568T			
V597				V597L [195]
Y601	Y601N			
613-619	Del613-619			
	YNPGDKDTK			
D619	D619G			
A623	A623I			
	A623S			
	A623V	A623V [171]	A623V [183]	
L629	L629F		L629F [184]	
I630	I630L			
F631	F631L			F631L [124]
	F631C			
T632	T632I	T632I [172]		T632I [192]
	T632A	T632A [172]		
D633	D633A			
	T633Q			
	T633Y			
	D633H	D633H [173]		
M637	M637R			
P639	P639S		P639S [185]	
N650			N650Y [39]	
V656	V656F			
658-661	Del658-661			
	NSK			
N670			N670S [39]	
C672			C672Y [155]	
L677		L677V [174]		
Increased sensitivity to hCG				
Residue	Familial gestational hyperthyroidism[b]			
K183	K183R [131]			

[a] The reports on TSH receptor mutations in toxic adenomas are too numerous to cite individually. The interested reader is referred to the following articles and reviews [7, 10, 13, 146].
[b] References are indicated in brackets.

roidism or, in the case of de novo mutations, to sporadic nonautoimmune congenital hyperthyroidism (table 1; fig. 3, 4).

TSH receptor mutations in toxic adenomas

Chronic stimulation of the cAMP cascade results in enhanced proliferation and function of thyroid follicular cells [78]. Consequently, any molecular alteration leading to constitutive activation of the cAMP pathway in a thyroid cell is predicted to result in clonal autonomous growth and function, and ultimately in a toxic adenoma [78]. Such somatic mutations were discovered in the stimulatory $G_{s\alpha}$ subunit (*gsp* mutations) in toxic adenomas, and have also been found in a subset of nonfunctioning adenomas and differentiated thyroid carcinomas [141–143]. $G_{s\alpha}$ mutations, most commonly found in amino acids arginine 201 and glutamine 227, impair the hydrolysis of GTP to GDP, resulting in persistent activation of adenylyl cyclase. The same $G_{s\alpha}$ mutations are found in 35–40% of somatotroph tumors in acromegalic

patients [144]. Mosaicism for $G_{s\alpha}$ mutations that occur early in development cause the McCune Albright syndrome and may affect multiple tissues, including the thyroid [145].

Somatic mutations in the TSH receptor were first discovered in toxic adenomas [146]. The first identified mutations were clustered in the third intracellular loop and the sixth transmembrane domain of the receptor, but a wide variety of activating somatic mutations have been found in subsequent studies (table 1; fig. 4) [7, 10, 13, 147]. Mutations conferring constitutive activity occur in the entire transmembrane domain, including the extracellular loops, as well as the carboxy-terminal region of the extracellular domain. All these mutations increase basal cAMP levels, but only a few amino acid substitutions activate the phospholipase C cascade in a constitutive manner [13]. Inositoltriphosphate (IP3) accumulation in response to TSH is, however, usually retained. A naturally occurring mutation in the fifth transmembrane domain, Y601N, is distinct in this respect in that it results in constitutive activation of the cAMP pathway, while losing coupling to $G_{q/11}$ [148]. A polymorphic variant at this position, Y601H, loses basal cAMP accumulation while retaining responsiveness to TSH, and it also fails to activate the PLC pathway [41, 148].

The reported prevalence of TSH receptor mutations in toxic adenomas varies widely, but may be as high as 80% [147, 149]. Differences in sampling technique and methodological approach, as well as variations in iodine intake, may contribute to the reported differences in prevalence rates [150, 151].

The vast majority of activating mutations is located in the β subunit of the receptor. However, naturally occurring substitutions at serine 281 (S281I/N/T), a residue located in the extracellular α subunit, also confer constitutive activation to the receptor (fig. 4) [152, 153]. The exact mechanisms resulting in gain of function are still poorly defined. Some of the mutations may alter the positions of the transmembrane helices, thus mimicking the conformational changes induced by binding of ligand. Alternatively, some mutations may alter the structure of domains that inhibit receptor coupling to G proteins in the absence of TSH [154, 155]. Activating mutations in the extracellular domain are thought to result in the relief of a negative constraint present in the unliganded carboxy-terminal part of the extracellular domain [147, 152, 153, 156, 157], and a subset of mutations may alter desensitization of the receptor.

TSH receptor mutations in multinodular goiters

Multinodular goiters are common, particularly under conditions of scarce iodine supply, and there is an age-dependent increase in their prevalence [85]. The mechanisms underlying nodular alteration are complex [158], and the following discussion is limited to hyperfunctioning adenomas within multinodular goiters or autonomous areas within euthyroid goiters. Somatic TSH receptor mutations have been identified in toxic adenomas isolated from multinodular goiters [159–161]. Consistent with studies demonstrating distinct clonal origins of different thyroid adenomas within the same multinodular goiter [162], the mutations may differ among these adenomas. For example, in two adenomas from the same goiter, one neoplasm harbored a mutation M453T, the second adenoma a T632I substitution [159]. In another study, L632I and F631L were found in two distinct lesions within the same goiter, whereas another patient had two distinct toxic nodules with the same mutation (I630L) [160]. These studies demonstrate that the pathogenesis of hyperfunctioning adenomas does not differ between multinodular goiters and solitary toxic adenomas. In contrast, TSH receptor mutations are rare in nonfunctioning adenomas, even if of monoclonal origin [161, 163]. Therefore, distinct mechanisms must be implicated in the abnormal growth leading to nonfunctioning nodules [158].

A germline polymorphism in the cytoplasmic tail of the receptor, D727E, has been associated with the development of multinodular goiters [164]. This polymorphism was found in 8 of 24 patients, and in vitro studies by these authors suggested an increased cAMP accumulation of the D727E variant in response to TSH. These findings contrast with a study characterizing basal and TSH-stimulated cAMP-dependent trancriptional activity using a luciferase reporter gene that was unable to document a difference between the wild type and the D727E variant [165]. In a more recent study, the association between the codon 727 polymorphism and toxic thyroid adenomas or toxic multinodular goiters could not be confirmed [166].

In a study analyzing hyperfunctioning and nonfunctioning areas from patients with toxic multinodular goiters, activating TSH receptor mutations were detected in 14 of 20 hyperfunctioning areas, whereas no mutation was identified in nonfunctioning nodules [167]. On microscopic analysis, only two of the hyperfunctioning areas corresponded to classic adenomas surrounded by a capsule, whereas the remainder had the characteristic features of hyperplastic lesions. The clonal composition of the analyzed tissues was not determined, and it has to be emphasized that histologic analysis does not allow to discriminate monoclonal from polyclonal lesions [168].

Constitutively activating TSH receptor mutations have also been detected in autoradiographically hyperfunctioning areas of goiters from euthyroid patients [169]. These findings contribute to the understanding of the mechanisms underlying thyroid autonomy and functional heterogeneity in multinodular goiters [167, 169].

TSH receptor mutations in thyroid carcinomas

In well-differentiated thyroid cancers, mutations in the $G_{s\alpha}$ subunit and the TSH receptor genes occur only rarely [143, 170–175]. Although constitutive activation of the cAMP pathway results in enhanced growth, it is not thought to be sufficient for malignant transformation of otherwise normal thyrocytes.

Interesting aspects have been gained from the study of a patient presenting with hyperthyroidism and increased uptake in two nodules, but suppressed uptake in the remainder of the gland [173]. After surgical removal of the right thyroid lobe, histological examination revealed the presence of a papillary carcinoma of the insular subtype with lymph node metastases. On follow-up examination with a whole-body scan, this patient had diffuse uptake in both lungs, but no uptake in residual tissue in the neck. Therefore, the diagnosis of autonomously functioning thyroid cancer was made. Mutational analysis of the TSH receptor gene documented a somatic mutation, D633H, located in the sixth transmembrane domain, in DNA isolated from the primary tumor and metastatic tissue. This aspartate residue is conserved among the glycoprotein hormone receptors. In the LH receptor, the corresponding mutation D578H has been found in Leydig cell tumors [176]. The D578H LH receptor displays constitutive activation of both the cAMP and phospholipase C (PLC) signaling pathways in transfected cells [176]. Recently, the substitution of this aspartate to histidine has been analyzed in more detail in the LH, TSH and FSH (D581H) receptors [177]. Similar to the D578H LH receptor, the D633H TSH receptor produces strong constitutive activation of both the cAMP and PLC pathways. In the FSH receptor, D581H does not spontaneously stimulate inositol phosphate production, but it confers the ability to mediate FSH-stimulated PLC activity, a property absent in the wild-type FSH receptor. PLC stimulation is pertussis toxin insensitive, indicating that a member of the Gq family mediates the response. No other amino acid that has been substituted for this conserved aspartate in helix 6 of glycoprotein hormone receptors has the same functional impact as histidine. This suggests that this histidine stabilizes a receptor conformation with increased ability to couple to Gq. The ability of the D578H LHR and D633H TSH receptor mutants to costimulate the cAMP and PLC pathways constitutively may thus have direct relevance to the pathogenesis of endocrine neoplasia [177]. Another mutation that activates both pathways, I486F, was found in a hyperfunctioning well-differentiated follicular carcinoma in a patient presenting with hyperthyroidism and increased radioiodine uptake within the thyroid mass (table 1). This supports the notion that concomitant activation of these two signaling cascades may promote transformation [175]. In a patient with a Hürthle cell carcinoma, Russo et al. identified a L677V TSH receptor mutation [174]. Basal cAMP levels were increased in transfected CHO cells, but IP3 accumulation has not been determined with this mutant.

Overexpression of the naturally occurring M453T mutation in the FRTL-5 rat cell line was sufficient to induce neoplastic transformation as assessed by growth in semisolid medium and athymic mice [178]. This mutation only activates the cAMP pathway in transfected COS (green monkey fibroblast-like) cells, and the neoplastic transformation in FRTL-5 cells may be linked to inherent properties of this cell line, which is known to be tumorigenic under conditions of prolonged TSH stimulation. In humans, this mutation has been found in the germline of two patients with congenital hyperthyroidism, but there was no suggestion that it is oncogenic on its own (table 1) [179, 180]. However, a somatic M453T substitution has been identified in a 11-year-old girl with a hyperfunctioning nodule and a papillary carcinoma [181]. This raises the possibility that this mutation could itself be associated with neoplasia, or that one or several mutations in other growth-controlling genes have occurred in cells harboring the M453T mutation.

Germline mutations in familial nonautoimmune hyperthyroidism

Autosomal dominant familial hyperthyroidism without evidence of an autoimmune etiology was first recognized by Thomas et al. [182]. The typical signs associated with autoimmune hyperthyroidism, i.e. exophthalmos, myxedema, TSAb and lymphocytic infiltration of the thyroid gland, are absent in this form of hyperthyroidism. Because all thyroid follicular cells display an increased growth rate, these patients have a diffuse goiter. The molecular basis of inherited nonautoimmune hyperthyroidism was elucidated by detecting activating germline mutations in the TSH receptor in the family reported by Thomas et al. [155], and subsequently confirmed in several other families (table 1; fig. 3, 4) [39, 183–187]. The onset of hyperthyroidism may vary in carriers of the same mutation in a given kindred. Thus, other factors, for example genetic background and/or iodine intake, appear to modulate the phenotypic expression [182, 188, 189]. In a Chinese family with a P639S germline mutation and hyperthyroidism in the father and three children, two of the children and the father also had mitral valve prolapse (MVP) associated with mitral regurgitation [185]. The close temporal relationship between the onset of thyrotoxicosis and the diagnosis of mitral valvular disease was taken as an indication that an increased clinical expression of MVP may exist in genetically predisposed individuals.

Sporadic germline mutations in congenital hyperthyroidism

Congenital hyperthyroidism is usually caused by transplacental passage of stimulating TSH receptor autoantibodies [190]. Autoimmune neonatal hyperthyroidism is

rare and occurs in less than 2% of infants born to a mother with a history of Graves' disease [191], a condition with an estimated incidence of about 2 of every 1000 pregnancies [182]. Antibody-induced neonatal hyperthyroidism typically resolves within the first 3–7 months as the maternal antibodies are cleared from the circulation. A fluctuating course may be seen because of the concomitant presence of TBAbs [90].

Constitutively activating de novo mutations of the TSH receptor have been found in a few patients with sporadic congenital nonautoimmune hyperthyroidism (table 1; fig. 3, 4) [124, 179, 180, 186, 192–196]. Congenital hyperthyroidism due to a toxic adenoma harboring a somatic TSH receptor mutation was reported as another unusual variant of congenital hyperthyroidism [153]. These rare cases with nonautoimmune congenital hyperthyroidism due to TSH receptor mutations must be differentiated from the much more frequent and transient autoimmune form of hyperthyroidism, because most of these patients have pronounced hyperthyroidism requiring a more aggressive therapeutic approach that may necessitate surgery and ablative radiotherapy. It is noteworthy that several of these children with severe neonatal hyperthyroidism seem to have mild mental retardation [124, 192, 193], suggesting that high levels of thyroid hormone may have a negative impact on brain development [197]. A subset of these children had proptosis [179, 180]. Computertomography of the retroorbital tissue in one of these children failed, however, to demonstrate infiltration of the eye muscles [179].

Inactivating mutations in the TSH receptor

Resistance to TSH may be caused by various molecular mechanisms. In pseudohypoparathyroidism type 1a (PHP 1a), the inability of TSH to stimulate the cAMP pathway is the consequence of inactivating mutations in $G_{s\alpha}$. PHP1a is typically associated with resistance to other hormones such as gonadotropins. Moreover, these patients present with characteristic somatic features that include short stature, brachydactyly and round face (Albrights's hereditary osteodystrophy) [198]. Resistance to TSH can also occur as an isolated phenomenon without the features of PHP 1a (for review see [11, 15]). In a subset of these patients, the molecular cause consists of inactivating mutations in the TSH receptor that are partially or completely inactivating (table 2; fig. 3) [199–206]. The mode of inheritance is recessive and affected individuals are homozygous or compound heterozygous for inactivating mutations. Among these patients the phenotype encompasses a wide spectrum ranging from isolated TSH elevation to severe hypothyroidism, and there is a clear correlation between genotype and phenotype. In other patients with sporadic or familial resistance to TSH, the TSH receptor gene was found to be normal, indicating locus heterogeneity due to defects in other genes [207–209]. Obvious candidate genes include genes encoding elements of the TSH-dependent signaling cascades or regulators of thyroid development and gene expression.

The *hyt*/*hyt* mouse

A loss-of-function mutation in the TSH receptor gene as a cause of TSH resistance was first discovered in the hypothyroid *hyt/hyt* mouse [26, 27]. The phenotype of this inbred mouse strain is defined by congenital hypothyroidism, retarded growth, mild anemia, hearing loss and infertility [210–214]. The mode of inheritance is autosomal recessive. The hypoplastic thyroids of these mutant mice are located in the proper position. Histologically, the thyroid follicular cells are developed, but incompletely differentiated, and the epithelial cells are not organized into structures recognizable as follicles [211]. The thyroid tissue of homozygous mice is unresponsive to TSH in vivo and *i*n vitro, but activators of $G_{s\alpha}$ induce a normal cAMP response [214]. Analysis of the mouse TSH receptor cDNA ultimately led to the identification of a missense mutation resulting in the substitution of a highly conserved proline (P556L) in the fourth transmembrane domain by leucine (fig. 4) [26, 27]. The mutation eliminates TSH binding, and thus normal receptor function, although the membrane localization of the receptor appears to be preserved [27].

Euthyroid hyperthyrotropinemia caused by TSH receptor mutations

The first human case with TSH resistance due to a defect in the TSH receptor was documented in three sisters, offspring of unrelated parents, who were found to have normal peripheral thyroid hormone but high TSH levels, a constellation referred to as euthyroid hyperthyrotropinemia (table 2) [199]. Both parents only showed discrete TSH elevations. None of the family members had clinical signs of hypothyroidism. The three affected siblings were found to be compound heterozygous for mutations in the extracellular TSH binding domain of the receptor (P162A and I167 N) (table 2; fig. 4) [11, 199]. In vitro studies documented that the I167N mutation had almost no biologic activity, whereas P162A displayed reduced activity [199]. Cell surface expression of the P162A mutant was reduced about twofold, and the EC50 for TSH stimulation was increased twofold [38]. In contrast, the I167N mutant did not reach the cell surface. Both amino acids are part of a putative α helix formed by the fifth leucine rich repeat motif in the extracellular domain and are thought to influence interaction of the receptor with the ligand [7, 11, 37]. Based on the current model of the three-dimensional structure of the TSH receptor, the P162A substitution maps to the surface of the molecule, whereas the I167N mutation affects a residue whose side chain contributes to

Table 2. Inactivating TSH receptor mutations.

Protein	Gene[a]	Membrane expression	Basal and TSH-induced cAMP	Thyroid size	Thyroglobulin	Comments	Ref.
Euthyroid hyperthyrotropinemia							
P162A	484C > G	reduced	shifted to the right	normal	not reported	three affected sisters	38, 199
I167N	500T > A	retained intracellularly	almost none				
C41S	122G > C	studied in [224]	studied in [224]	normal ^{123}iodine scan	normal		201
F525L	155C > A	expressed on membrane, TSH binding similar to wt	reduced basal and TSH stimulated activity				
Q324X	970C > T	not analyzed	not analyzed	slightly enlarged on ^{123}iodine scan	normal		201
D410N	1228G > A	expressed on membrane, TSH binding similar to wt	reduced basal and TSH stimulated activity				
C390W	1170T > G	expressed on membrane, TSH binding reduced	basal activity similar to wt reduced response to TSG	slightly enlarged on ^{123}iodine scan	normal		201
W546X	1637G > A	not analyzed	not analyzed				
P162A	484C > G	studied in [38, 199]	Studied in [38, 199]	Normal on ^{123}iodine scan	normal	consanguinity	201
R310C	928C > T	TSH binding reduced	constitutive activity no response to TSH	normal ultrasound and scan	not reported	consanguinity discrepancy in vitro and in vivo (see text)	215
Hypothyroidism							
R109Q	326G > A	TSH-binding 60% of wt	right-shifted response to TSH	normal ultrasound and scan	not reported	mild hypothyroidism	200
W546X	1637G > A	no specific binding	inactive				
Frameshift at F406 > 420X	Del1217-1234/ins CACG	retained intracellularly inserted into membrane	absent	hypoplastic on ultrasound	normal off levothyroxine	mild hypothyroidism	202
C390W	1170T > G	total binding similar to wt	reduced basal levels, right-shifted response to TSH				
A553T	1657G > A	Normal translation, very low levels of membrane expression K_d reduced	reduced basal and TSH-induced cAMP accumulation no coupling to Gq11	hypoplastic on ultrasound Tc-scan negative	high normal range	consanguinity profound hypothyroidism	203
Altered splicing with skipping of exon 6 T656X	IVS6 + 3 G > C	not reported	not reported	hypoplastic on ultrasound TC-scan negative	normal range	profound hypothyroidism maternal great aunt with congenital hypothyroidism of distinct etiology	204
R609X	1825C > T	not reported	not reported	hypoplastic on ultrasound normal location Tc-scan negative	below or within the normal range	profound hypothroidism hypocortisolism consanguinity ACTH resistance, reason unknown	205
T4771	1430C > T	very low cell surface expression receptor retained in intracellular compartments K_d reduced	no basal activity no significant increase in response to TSH	hypoplastic on ultrasound ^{131}iodine scintigraphy: reduced volume, low uniform uptake	undetectable	profound hypothyroidism common ancestors	206

[a] The mutations are designated according to the *Recommendations for a nomenclature system for human gene mutations* [223].

the hydrophobic core characteristic of proteins harboring leucine repeat motifs [37, 38]. I167N seems to cause a gross destabilization of receptor structure that is incompatible with its normal routing through the intracellular membrane system of the cell [38].

Euthyroid hyperthyrotropinemia due to mutations in the TSH receptor gene has subsequently been reported in several other families (table 2; fig. 4) [201, 215]. The size of the thyroid gland is either normal or slightly increased in these patients**.** Partial resistance to TSH associated with a homozygous R310C replacement was found in two brothers with compensated hypothyroidism [215]. In transfected cells, this mutation has reduced TSH binding and absent responsiveness to TSH, but it appears to have constitutive activity in terms of cAMP accumulation in comparison to the wild-type receptor. If constitutive activity of the R310C mutations is indeed responsible for the compensated hypothyroid state, it is not clear why TSH levels were elevated in the two patients homozygous for the mutation.

Congenital hypothyroidism due to TSH receptor mutations

More pronounced or complete inactivation of both TSH receptor alleles leads to mild or severe congenital hypothyroidism (table 2; fig. 3, 4) [202–206]. Because of absent uptake of the radioisotope, scintigraphic studies typically do not reveal any thyroid tissue. However, ultrasound of the neck will reveal the presence of a normally located hypoplastic gland. Intriguingly, many of these patients have normal or elevated thyroglobulin levels. For example, in an inbred kindred studied by Abramowicz et al., the hypothyroid index patient had no detectable thyroid tissue on scintigraphy prompting the diagnosis of thyroid agenesis [203]. His thyroglobulin levels were, however, in the high normal range, and ultrasonography documented a normally located hypoplastic thyroid gland. The patient and his sister, who had been diagnosed earlier with hypothyroidism, were found to be homozygous for a A553T mutation in the fourth transmembrane domain (table 2; fig. 4). Functional analysis of the mutated receptor in transfected cells demonstrated an extremely low level of expression of the mutated receptor at the cell surface despite normal intracellular synthesis. The mutated receptor was able to bind TSH and stimulate cAMP accumulation in transfected cells, but appeared to have lost the ability to activate the PLC pathway. The finding of high thyroglobulin levels in this situation is not entirely clear. It suggests that thyroglobulin measurements, combined with ultrasound of the neck, may be helpful in distinguishing congenital athyreosis from thyroid hypoplasia, an entity which may eventually escape detection by scintigraphy [203, 204].

In other patients with inactivation of both TSH receptor alleles, thyroglobulin levels were undetectable or low (table 2). For example, in a female patient with severe hypothyroidism, thyroid hypoplasia and a homozygous mutation in the first extracellular loop (T477I) with loss of function, thyroglobulin levels were undetectable [206]. In a large inbred Bedouin kindred in which profound congenital hypothyroidism and hypocortisolism occurred alone or together in eight family members, serum thyroglobulin levels varied among the affected individuals and were below or within the normal range [205]. The patients were homozygous for an inactivating mutation truncating the TSH receptor in the third intracellular loop (R609X). The molecular cause of the adrenal insufficiency remained unclear, but was not linked to the ACTH receptor. The presence of hypothyroidism did not affect the timing, severity and manner of clinical manifestation of hypocortisolism [205].

Significance of inactivating TSH receptor mutations for thyroid development

The *hyt/hyt* mouse and patients with TSH-resistant congenital hypothyroidism with correctly located hypoplastic glands confirm that development and migration of the thyroid is independent of TSH stimulation [202, 203, 211]. This is consistent with the observation that the genes for thyroperoxidase, thyroglobulin and the TSH receptor are only expressed once the gland has reached its pretracheal location [216, 217]. Although early events of thyroid development are not dependent on TSH and its signaling pathway, this cascade is essential for complete differentiation, growth and function of thyroid follicular cells [26, 84, 211].

Whereas congenital hypothyroidism is a relatively frequent disorder affecting ~1:3000 newborns, thyroid hypoplasia is only found in about 5% of all patients with congenital hypothyroidism and inactivating mutations in the TSH receptor only accounts for a subset of these patients. A study of familial or consanguinous cases of congenital hypothyroidism was negative for linkage to the TSH receptor locus [218]. Moreover, mutational analysis of the coding sequence of the TSH receptor did not reveal sequence alterations that would explain the phenotype in several studies of patients presenting with thyroid hypoplasia, hypothyroidism and resistance to TSH [201, 207–209]. It has also become apparent that hypoplasia can be associated with several genetic defects, including mutations in the TSHβ subunit, the TSH receptor and the $G_{s\alpha}$ subunit. More recently, mutations in the paired domain transcription factor PAX8 have been identified as a further cause of thyroid hypoplasia [219]. This is supported by the murine knockout model; mice homozygous for a disrupted *Pax8* gene have a phenotype with hypothyroidism, severely hypoplastic thyroid glands and absence of follicular structures [220]. Homozygosity for mutations in the forkhead transcription factor TTF2 (thyroid transcription factor 2; FKHL15) has been demonstrated

in two siblings with thyroid agenesis, cleft palate, choanal atresia and spiky hair [221]. Similarly, targeted disruption of *Ttf2* in the mouse leads to cleft palate and either a sublingual or completely absent thyroid gland [222].

Perspective

Although an overwhelming wealth of new insights into the function of the TSH receptor in physiology and disease has been obtained, numerous challenging aspects remain to be solved or refined [4, 6, 14]. They include, among others, further clarification of the exact role of the TSH receptor in the pathogenesis of Graves' disease and endocrine ophthalmopathy, further refinement of the current animal models for Graves' disease, development of new assay systems for the determination of autoantibodies directed against this receptor and last but not least, attempts to elucidate parts of its structure.

Acknowledgements. This work was in part supported by a New Investigator Award from the Howard Hughes Medical Institute, and an Intramural Grant from Northwestern University.

1 Jameson J. L. (2001) Mechanisms of thyroid hormone action. In: Endocrinology, 4th ed., pp. 1327–1344, DeGroot L. J., Jameson J. L. (eds), W. B. Saunders, Philadelphia 1
2 Davis P. J. and Davis F. B. (1996) Nongenomic actions of thyroid hormone. Thyroid **6:** 497–504
3 Parmentier M., Libert F., Maenhaut C., Lefort A., Gerard C., Perret J. et al. (1989) Molecular cloning of the thyrotropin receptor. Science. **246:** 1620–1622
4 Vassart G. and Dumont J. E. (1992) The thyrotropin receptor and the regulation of thyrocyte function and growth. Endocr. Rev. **13:** 596–611
5 Nagayama Y. and Rapoport B. (1992) The thyrotropin receptor 25 years after its discovery: new insights after its molecular cloning. Mol. Endocrinol. **92:** 145–156
6 Vassart G., Parma J., van Sande J. and Dumont J. E. (1994) The thyrotropin receptor and the regulation of thyrocyte function and growth: update 1994. In: Endocrine Society Press, Behesda M. D., Braverman L. E. and Refetoff S. (eds), Endocrine Review Monographs, pp. 77–80
7 Van Sande J., Parma J., Tonacchera M., Swillens S., Dumont J. and Vassart G. (1995) Somatic and germline mutations of the TSH receptor gene in thyroid disease. J. Clin. Endocrinol. Metab. **9:** 2577–2585
8 Kohn L. D., Shimura H., Shimura Y., Hidaka A., Giuliani C., Napolitano G. et al. (1995) The thyrotropin receptor. Vit. Horm. **50:** 287–384
9 Tonacchera M., van Sande J., Parma J., Duprez L., Cetani F., Costagliola S. et al. (1996) TSH receptor and disease. Clin. Endocrinol. **44:** 621–633
10 Paschke R. and Ludgate M. (1997) The thyrotropin receptor in thyroid diseases. New Engl. J. Med. **337:** 1675–1681
11 Refetoff S., Sunthornthepvarakul T., Gottschalk M. and Hayashi Y. (1996) Resistance to thyrotropin and other abnormabilities of the thyrotropin receptor. Rec. Prog. Hormone Res. **51:** 97–122
12 Sanders J., Oda Y., Roberts S. A., Maruyama M., Furmaniak J. and Smith B. R. (1997) Understanding the TSH receptor function-structure relationship. Ballière's Clin. Endocrinol. Metab. **11:** 451–480
13 Vassart G. (1998) Hypo- and hyperthyroidism caused by mutations of the TSH receptor. In: G Proteins, Receptor, and Disease, pp. 119–138, Spiegel A. M. (ed.), Totowa N. J.: Humana Press
14 Rapoport B., Chazenbalk G. D., Jaume J. C. and McLachlan S. M. (1998) The thyrotropin (TSH) hormone receptor: interaction with TSH and autoantibodies. Endocr. Rev. **19:** 673–716
15 Kopp P. (1999) Resistance to TSH. In: Hormone resistance syndromes pp. 111–144, Jameson J. (ed), Totowa, Humana Press, Totowa NJ
16 Ludgate M. (2000) Animal models of Graves' disease. Eur. J. Endocrinol. **142:** 1–8
17 Graves P. N. and Davies T. F. (2000) New insights into the thyroid-stimulating hormone receptor: the major antigen of Graves' disease. Endocrinol. Metab. Clin. North Amer. **29:** 267–286
18 Bahn R. S. (2000) Understanding the immunology of Graves' ophthalmopathy: is it an autoimmune disease. Endocrinol. Metab. Clin. North Amer. **29:** 287–296
19 Probst W. C., Snyder L. A., Schuster D. I., Brosius J. and Sealfon S. C. (1992) Sequence alignement of the G-protein coupled receptor superfamily. DNA Cell. Biol. **11:** 1–20
20 Kolakowski L. F. J. (1994) GCRDb: a G-protein-coupled receptor database. Receptors Channels. **2:** 1–7
21 Gether U. (2000) Uncovering molecular mechanisms involved in activation of G protein coupled receptors. Endocr. Rev. **21:** 90–113
22 Nagayama Y., Kaufman K. D., Seto P. and Rapoport B. (1989) Molecular cloning, sequence and functional expression of the cDNA for the human thyrotropin receptor. Biochem. Biophys. Res. Commun. **165:** 1184–1190
23 Libert F., Lefort A., Gerard C., Parmentier M., Perret J., Ludgate M. et al. (1989) Cloning, sequencing and expression of the human thyrotropin (TSH) receptor: evidence for binding of autoantibodies. Biochem. Biophys. Res. Commun. **165:** 1250–1255
24 Misrahi M., Loosfelt H., Atger M., Sar S., Guiochon-Mantel A. and Milgrom E. (1990) Cloning, sequencing and expression of human TSH receptor. Biochem. Biophys. Res. Commun. **166:** 394–403
25 Akamizu T., Ikuyama S., Saji M., Kosugi S., Kozak C., McBride O. W., et al. (1990) Cloning, chromosomal assignment and regulation of the rat thyrotropin receptor: expression of the gene is regulated by thyrotropin, agents that increase cAMP levels and thyroid autoantibodies. Proc. Natl. Acad. Sci. USA. **87:** 5677–5681
26 Stein S. A., Oates E. L., Hall C. R., Grumbles R. M., Fernandez L. M., Taylor N. A. et al. (1994) Identification of a point mutation in the thyrotropin receptor of the hyt/hyt hypothyroid mouse. Mol. Endo. **8:** 129–138
27 Gu W. X., Du G. G., Kopp P., Rentoumis A., Albanese C., Kohn L. D. et al. (1995) The thyrotropin (TSH) receptor transmembrane domain mutation (Pro556-Leu) in the hypothyroid hyt/hyt mouse results in plasma membrane targeting but defective TSH binding. Endocrinology **136:** 3146–3153
28 Silversides D. W., Houde A., Ethier J. F. and Lussier J. G. (1997) Bovine thyrotropin receptor cDNA is characterized by full-length and truncated transcripts. J. Mol. Endocrinol. **18:** 101–112
29 Bockmann J., Winter C., Wittkowski W., Kreutz M. R. and Bockers T. M. (1997) Cloning and expression of a brain-derived TSH receptor. Biochem. Biophys. Res. Commun. **238:** 173–178
30 Nguyen L. Q., Stein B. S., Jameson J. L. and Kopp P. (1998) Molecular cloning of the cat thyrotropin (TSH) receptor: evidence against an autoimmune etiology of feline hyper-

thyroidism. 80th Meetings of the Endocrine Society, New Orleans, Abstract P3–110
31 Gross B., Misrahi M., Sar S. and Milgrom E. (1991) Composite structure of the human thyrotropin receptor gene. Biochem. Biophys. Res. Commun. **177:** 679–687
32 Rousseau-Merck M. F., Misrahi M., Loosfelt H., Atger M., Milgrom E. and Berger R. (1990) Assignment of the human thyroid stimulating hormone receptor (TSHR) gene to chromosome 14q31. Genomics **8:** 233–236
33 Libert F., Passage E., Lefort A., Vassart G., Mattei M. G. (1990) Localization of human thyrotropin receptor gene to chromosome region 14q31 by in situ hybridization. Cytogene.t Cell. Genet. **54:** 82–83
34 Graves P. N., Tomer Y. and Davies T. F. (1992) Cloning and sequencing of a 1.3 kb variant of human thyrotropin receptor mRNA lacking the transmembrane domain. Biochem. Biophys. Res. Commun. **187:** 1135–1143
35 Wadsworth H. L., Chazenbalk G. D., Nagayama Y., Russo D. and Rapoport B. (1990) An insertion in the human thyrotropin receptor critical for high affinity hormone binding. Science **249:** 1423–1425
36 Nagayama Y., Wadsworth H. L., Chazenbalk G. D., Russo D., Seto P. and Rapoport B. (1991) Thyrotropin-luteinizing hormone/chorionic gonadotropin receptor extracellular domain chimeras as probes for thyrotropin receptor function. Proc. Natl. Acad. Sci. USA **88:** 902–905
37 Kajava A. V., Vassart G. and Wodak S. J. (1995) Modeling of the three-dimensional structure of proteins with the typical leucine-rich repeats. Structure **3:** 867–877
38 Costagliola S., Sunthorntepvarakul T., Migeotte I., Van Sande J., Kajava A., Refetoff S. et al. (1999) Structure-function relationships of two loss-of-function mutations of the thyrotropin receptor gene. Thyroid **9:** 995–1000
39 Tonacchera M., van Sande J., Cetani F., Swillens S., Schvartz C., Winizewski P. et al. (1996) Functional characteristics of three new germline mutations of the thyrotropin receptor gene causing autosomal dominant toxic thyroid hyperplasia. J. Clin. Endocrinol. Metab. **81:** 547–554
40 Baldwin J. M. (1993) The probable arrangement of the helices in G protein-coupled receptors. EMBO J. **12:** 1693–1703
41 Biebermann H., Schoneberg T., Schulz A., Krause G., Gruters A, Schultz G. et al. (1998) A conserved tyrosine residue (Y601) in transmembrane domain 5 of the human thyrotropin receptor serves as a molecular switch to determine G-protein coupling. FASEB J. **12:** 1461–1471
42 Lin Z., Shenker A. and Pearlstein R. (1997) A model of the lutropin/choriogonadotropin receptor: insights into the structural and functional effects of constitutively activating mutations. Protein Eng. **10:** 501–510
43 Davies T. F., Teng C. S., McLachlan S. M., Smith B. R. and Hall R. (1978) Thyrotropin receptors in adipose tissue, retroorbital tissue and lymphocytes. Mol. Cell. Endocrinol. **9:** 303–310
44 Bahn R. S., Dutton C. M., Natt N., Joba W., Spitzweg C. and Heufelder A. E. (1998) Thyrotropin receptor expression in Graves' orbital adipose/connective tissues: potential autoantigen in Graves' ophthalmopathy. J. Clin. Endocrinol. Metab. **83:** 998–1002
45 Spitzweg C., Joba W., Hunt N. and Heufelder A. E. (1997) Analysis of human thyrotropin receptor gene expression and immunoreactivity. Eur. J. Endocrinol. **136:** 998–1002
46 Wang J., Whetsell M. and Klein J. R. (1997) Local hormone networks and intestinal T cell homeostasis. Science **275:** 1937–1939
47 Bagriacik E. U. and Klein J. R. (2000) The thyrotropin (thyroid-stimulating hormone) receptor is expressed on murine dendritic cells and on a subset of CD45RBhigh lymph node T cells: functional role for thyroid-stimulating hormone during immune activation. J. Immunol. **164:** 6158–6165
48 Prummel M. F., Brokken L. J., Meduri G., Misrahi M., Bakker O. and Wiersinga W. M. (2000) Expression of the thyroid-stimulating hormone receptor in the folliculo-stellate cells of the human anterior pituitary. J. Clin. Endocrinol. Metab. **85:** 4347–4353
49 Dutton C. M, Joba W., Spitzweg C., Heufelder A. E. and Bahn R. S. (1997) Thyrotropin receptor expression in adrenal, kidney, and thymus. Thyroid **7:** 879–884
50 Sellitti D. F., Hill R., Doi S. Q., Akamizu T., Czaja J., Tao S., et al. (1997) Differential expression of thyrotropin receptor mRNA in the porcine heart. Thyroid **7:** 641–646
51 Sellitti D. F., Akamizu T., Doi S. Q., Kim G. H., Kariyil J. T., Kopchik J. J. et al. (2000) Renal expression of two 'thyroid-specific' genes: thyrotropin receptor and thyroglobulin. Exp. Nephrol. **8:** 235–243
52 Spitzweg C., Joba W. and Heufelder A. E. (1999) Expression of thyroid-related genes in human thymus. Thyroid. **9:** 133–141
53 Paschke R., Vassart G., Ludgate M. (1995) Current evidence for and against the TSH receptor being the common antigen in Graves' disease and thyroid associated ophthalmopathy. Clin. Endocrinol. **42:** 565–569
54 Marcus C., Ehren H., Bolme P. and Arner P. (1988) Regulation of lipolysis during the neonatal period. Importance of thyrotropin. J. Clin. Invest. **82:** 1793–1797
55 Valyasevi R., Erickson D., Harteneck D., Dutton C., Heufelder A., Jyonouchi S. et al. (1999) Differentiation of human orbital preadipocyte fibroblasts induces expression of functional thyrotropin receptor. J. Clin. Endocrinol. Metab. **84:** 2557–2562
56 Graves P., Vlase H., Bobovnikova Y. and Davies T. (1996) Multimeric complex formation by the thyrotropin receptor in solubilized thyroid membranes. Endocrinology **137:** 3915–3920
57 Russo D., Chazenbalk G., Nagayama Y., Wadsworth H. L. and Rapoport B. (1991) Site-directed mutagenesis of the human thyrotropin receptor: role of asparagine-linked oligosaccharides in the expression of a functional receptor. Mol. Endocrinol. **5:** 29–33
58 Tanaka K., Nagayama Y., Nishihara E., Namba H., Yamashita S. and Niwa M. (1998) Palmitoylation of human thyrotropin receptor: slower intracellular trafficking of the palmitoylation-defective mutant. Endocrinology. **139:** 803–806
59 Furmaniak J., Hashim F. A., Buckland P. R., Petersen V. B., Beever K., Howells R. D. et al. (1987) Photoaffinity labeling of the TSH receptor on FRTL5 cells. FEBS Lett. **215:** 316–322
60 Russo D., Chazenbalk G. D., Nagayama Y., Wadsworth H. L., Seto P. and Rapoport B. (1991) A new structural model for the thyrotropin (TSH) receptor as determined by covalent cross-linking of TSH to the recombinant receptor in intact cells: evidence for a single polypeptide chain. Mol. Endocrinol. **121:** 2119–2130
61 Loosfelt H., Pichon C., Jolivet A., Misrahi M., Caillou B., Jamous M. et al. (1992) Two-subunit structure of the human thyrotropin receptor. Proc. Natl. Acad. Sci. **89:** 3765–3769
62 Misrahi M. and Milgrom E. (1997) Cleavage and shedding of the TSH receptor. Eur. J. Endocrinol. **137:** 599–602
63 Tanaka K., Chazenbalk G. D., McLachlan S. M. and Rapoport B. (1998) Thyrotropin receptor cleavage at site 1 does not involve a specific amino acid motif but instead depends on the presence of the unique, 50 amino acid insertion. J. Biol. Chem. **273:** 1959–1963
64 De Bernard S., Misrahi M., Huet J. C., Beau I., Desroches A., Loosfelt H. et al. (1999) Sequential cleavage and excision of a segment of the thyrotropin receptor ectodomain. J. Biol. Chem. **274:** 101–107
65 Graves P., Pritsker A. and Davies T. F. (1999) Post-translational processing of the natural human TSH receptor: demonstration of more than two cleavage sites. Endocrinology **84:** 2177–2181

66 Tanaka K., Chazenbalk G. D., McLachlan S. M. and Rapoport B. (2000) Evidence that cleavage of the thyrotropin receptor involves a 'molecular ruler' mechanism: deletion of amino acid residues 305–320 causes a spatial shift in cleavage site 1 independent of amino acid motif. Endocrinology **141:** 3573–3577
67 Chazenbalk G., Tanaka K., McLachlan S. and Rapoport B. (1999) On the functional importance of thyrotropin receptor intramolecular cleavage. Endocrinology **140:** 4516–4520
68 Tanaka K., Chazenbalk G., McLachlan S. and Rapoport B. (1999) Subunit structure of thyrotropin receptors expressed on the cell surface. J. Biol. Chem. **274:** 33979–33984
69 Chazenbalk G. D., Tanaka K., Nagayama Y., Kakinuma A, Jaume J. C., McLachlan S. M. et al. (1997) Evidence that the thyrotropin receptor ectodomain contains not one, but two, cleavage sites. J. Clin. Endocrinol. Metab. **138:** 2893–2899
70 Nagayama Y., Tanaka K., Haras T., Namba H., Yamashita S., Taniyama K. et al. (1996) Involvement of G protein-coupled receptor kinase 5 in homologous desensitization of the thyrotropin receptor. J. Biol. Chem. **271:** 10143–10148
71 Iacovelli L., Franchetti R., Masini M. and De Blasi A. (1996) GRK2 and beta-arrestin1 as negative regulators of thyrotropin receptor-stimulated response. Mol. Endocrinol. **10:** 1138– 1146
72 Iacovelli L., Franchetti R., Grisolia D. and De Blasi A. (1999) Selective regulation of G protein-coupled receptor-mediated signaling by G protein-coupled receptor kinase 2 in FRTL-5 cells: analysis of thyrotropin, alpha(1B)-adrenergic, and A(1) adenosine receptor-mediated responses. Mol. Pharmacol. **56:** 316–324
73 Lalli E. and Sassone-Corsi P. (1995) Thyroid-stimulating hormone (TSH)-directed induction of the CREM gene in the thyroid gland participates in the long-term desensitization of the TSH receptor. Proc. Natl. Acad. Sci USA **92:** 9633–9637
74 Laurent E., Mockel J., van Sande J., Graff I. and Dumont J. E. (1987) Dual activation by thyrotropin of the phospholipase C and cAMP cascades in human thyroid. Mol. Cell. Endocrinol. **52:** 273–278
75 Allgeier A., Offermans S., van Sande J., Spicher K., Schultz G. and Dumont J. E. (1994) The human thyrotropin receptor activates G-protein Gs and Gq11. J. Biol. Chem. **269:** 13733–13735
76 Laugwitz K. L., Allgeier A., Offermanns S., Spicher K., van Sande J., Dumont J. E. et al. (1996) The human thyrotropin receptor: a heptahelical receptor capable of stimulating members of all four G protein families. Proc. Natl. Acad. Sci. USA **93:** 116–120
77 Park E. S., Kim H., Suh J. M., Park S. J., You S. H., Chung H. K. et al. (2000) Involvement of JAK/STAT (Janus kinase/signal transducer and activator of transcription) in the thyrotropin signaling pathway. Mol. Endocrinol. **14:** 662–670
78 Dumont J. E., Lamy F., Roger P. and Maenhaut C. (1992) Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol. Rev. **72:** 667–697
79 Van Sande J., Raspe E., Perret J., Lejeune C., Maenhaut C., Vassart G. et al. (1990) Thyrotropin activates both the cyclic AMP and the PIP2 cascades in CHO cells expressing the human cDNA of TSH receptor. Mol. Cell. Endocrinol. **74:** R1–R6
80 Raspé E., Laurent E., Andry G. and Dumont J. E. (1991) ATP, bradykinin, TRH and TSH activate the Ca2+-phosphatidylinositol cascade of human thyrocytes in primary culture. Mol. Cell. Endocrinol. **81:** 175–183
81 Ledent C., Dumont J. E., Vassart G. and Parmentier M. (1992) Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J. **11:** 537–542
82 Michiels F. M., Caillou B., Talbot M., Dessarps-Freichey F., Maunoury M. T., Schlumberger M. et al. (1994) Oncogenic potential of guanine nucleotide stimulatory factor alpha subunit in thyroid glands of transgenic mice. Proc. Natl. Acad. Sci. USA **91:** 10488–10492
83 Zeiger M. A., Saji M., Gusev Y., Westra W. H., Takiyama Y., Dooley W. C. et al. (1997) Thyroid-specific expression of cholera toxin A1 subunit causes thyroid hyperplasia and hyperthyroidism in transgenic mice. Endocrinology **138:** 3133–3140
84 Nguyen L. Q., Kopp P., Martinson F., Stanfield K., Roth S. I. and Jameson J. L. (2000) A dominant negative CREB (cAMP Response Element-Binding Protein) isoform inhibits thyrocyte growth, thyroid-specific gene expression, differentiation, and function. Mol. Endocrinol. **14:** 1448–1461
85 Vanderpump M. P. J., Tunbridge W. M. G., French J. M., Appleton D., Bates D., Clarks F., et al. (1995) The incidence of thyroid disorders in the community: a twenty-year follow-up of the Wickham survey. Clin. Endocrinol. **43:** 55–68
86 Helfand M. and Redfern C. C. (1998) Screening for thyroid disease: an update. Ann. Intern. Med. **129:** 144–158
87 Ries L. A. G, Eisner M. P., Kosary C. L., Hankey B. F., Miller B. A., Clegg L. et al. (2000) Surveillance, Epidemiology and End Results (SEER) Cancer Statistics Review 1973–1997, National Cancer Institute, Bethesda, MD
88 Rees Smith B., McLachlan S. M. and Furmaniak J. (1988) Autoantibodies to the thyrotropin receptor. Endocr. Rev. **9:** 106–121
89 Weetman A. P. and McGregor A. M. (1994) Endocr. Rev. **15:** 788–830
90 Zakarija M., McKenzie J. M. and Hoffmann W. H. (1986) Prediction and therapy of intrauterine and late-onset neonatal hyperthyroidism. J. Clin. Endocrinol. Metab. **62:** 368–371
91 Orgiazzi J. (2000) Anti-TSH receptor antibodies in clinical practice. Endocrinol. Metab. Clin. North Amer. **29:** 339–355
92 Costagliola S., Morgenthaler N. G., Hoermann R., Badenhoop K., Struck J., Freitag D. et al. (1999) Second generation assay for thyrotropin receptor antibodies has superior diagnostic sensitivity for Graves' disease. J. Clin. Endocrinol. Metab. **84:** 90–97
93 Watson P. F., Ajjan R. A., Phipps J., Metcalfe R. and Weetman A. P. (1998) A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease. Clin. Endocrinol. **49:** 577–581
94 Evans C., Morgenthaler N. G., Lee S., Llewellyn D. H., Clifton-Bligh R., John R. L. et al. (1999) Development of a luminescent bioassay for thyroid stimulating antibodies. J. Clin. Endocrinol. Metab. **84:** 374–377
95 Kosugi S., Ban T., Akamizu T. and Kohn L. (1992) Identification of separate determinants on the thyrotropin receptor reactive with Graves' thyroid-stimulating antibodies and with thyroid-stimulating blocking antibodies in idiopathic myxedema: these determinants have no homologous sequence on gonadotropin receptors. Mol. Endocrinol. **6:** 168–180
96 Nagayama Y., Wadsworth H. L., Russo D., Chazenbalk G. D. and Rapoport B. (1991) Binding domains of stimulatory and inhibitory thyrotropin (TSH) receptor autoantibodies determined with chimeric TSH-lutotropin/chorionic gonadotropin receptors. J. Clin. Invest. **88:** 336–340
97 Tomer Y. and Davies T. F. (1993) Infection, thyroid disease, and autoimmunity. Endocr. Rev. **14:** 107–120
98 Brix T. H., Kyvik K. O. and Hegedüs L. (1998) What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. Thyroid **8:** 727–734
99 Gough S. (1999) The immunogenetics of Graves' disease. Curr. Opin. Endocrinol. Diabetes **4:** 270–276
100 Watson P., French A., Pickerill A., McIntosh R. and Weetman A. (1995) Lack of association between a polymorphism in the coding region of the thyrotropin receptor gene and Graves' disease. J. Clin. Endocrinol. Metab. **80:** 1032–1035

101 Kotsa K. D., Watson P. F. and Weetman A. P. (1997) No association between a thyrotropin receptor gene polymorphism and Graves' disease in the female population. Thyroid **7:** 31–33

102 Allahabadia A., Heward J. M., Mijovic C., Carr-Smith J., Daykin J., Cockram C. et al. (1998) Lack of association between polymorphism of the thyrotropin receptor gene and Graves' disease in United Kingdom and Hong Kong Chinese patients: case control and family-based studies. Thyroid **8:** 777–780

103 Cuddihy R. M., Dutton C. M. and Bahn R. S. (1995) A polymorphism in the extracellular domain of the thyrotropin receptor is highly associated with autoimmune thyroid disease in females. Thyroid **5:** 89–95

104 De Roux N., Shields D. C., Misrahi M., Ratanachaiyavong S., McGregor A. M. and Milgrom E. (1996) Analysis of the thyrotropin receptor as a candidate gene in familial Graves' disease. J. Clin. Endocrinol. Metab. **81:** 3483–3486

105 Burch H. B. and Wartofsky L. (1993) Graves' ophthalmopathy: current concepts regarding pathogenesis and management. Endocr. Rev. **14:** 747–793

106 Bahn R. S. and Heufelder A. E. (1993) Mechanisms of disease: pathogenesis of Graves' ophthalmopathy. N. Engl. J. Med. **329:** 1468–1475

107 Smith T. J., Bahn R. S. and Gorman C. A. (1989) Connective tissue, glyosaminoglycans and diseases of the thyroid. Endocr. Rev. **10:** 366–391

108 Feliciello A., Porcellini A., Ciullo I., Bonavolonta G., Avvedimento E. V. and Fenzi G. (1993) Expression of thyrotropin-receptor mRNA in healthy and Graves' disease retro-orbital tissue. Lancet. **342:** 337–338

109 Heufelder A. E., Dutton C. M., Sarkar G., Donovan K. A. and Bahn R. S. (1993) Detection of TSH receptor RNA in cultured fibroblasts from patients with Graves' ophthalmopathy and pretibial dermopathy. Thyroid **3**: 297–300

110 Endo T., Ohta K., Haraguchi K. and Onaya T. (1995) Cloning and functional expression of a thyrotropin receptor cDNA from rat fat cells. J Biol Chem. **270:** 10833–10837

111 Rapoport B., Alsabeh R., Aftergood D. and McLachlan S. M. (2000) Elephantiasic pretibial myxedema: insight into and a hypothesis regarding the pathogenesis of the extrathyroidal manifestations of Graves' disease. Thyroid **10:** 685–692

112 Peter H., Gerber H., Studer H., Peterson M., Becker D. and Groscurth P. (1991) Autonomous growth and function of cultured thyroid follicles from cats with spontaneous hyperthyroidism. Thyroid **1:** 331–338

113 Peter P., Gerber H., Studer H., Becker D. and Peterson M. (1987) Autonomy of growth and of iodine metabolism in hyperthyroid feline goiters transplanted onto nude mice. J. Clin. Invest. **80:** 491–498

114 Soliman M., Kaplan E., Straus F., Fisfalen M. E., Hidaka Y., Guimaraes V. et al. (1995) Graves' disease in severe combined immunodeficient mice. J Clin Endocrinol Metab. **80:** 2448–2455

115 Shimojo N., Kohno Y., Yamaguchi K., Kikuoka S., Hoshioka A., Niimi H. et al. (1996) Induction of Graves-like disease in mice by immunization with fibroblasts transfected with the thyrotropin receptor and a class II molecule. Proc. Natl. Acad. Sci. USA. **93:** 11074–11079

116 Costagliola S., Many M. C., Stalmans-Falys M., Vassart G. and Ludgate M. (1996) Transfer of thyroiditis, with syngeneic spleen cells sensitized with the human thyrotropin receptor, to naive BALB/c and NOD mice. Endocrinology **137:** 4637–4643

117 Costagliola S., Rodien P., Many M. C., Ludgate M. and Vassart G. (1998) Genetic immunization against the human thyrotropin receptor causes thyroiditis and allows production of monoclonal antibodies recognizing the native receptor. J. Immunol. **160:** 1458–1465

118 Many M. C., Costagliola S., Detrait M., Denef F., Vassart G. and Ludgate M. C. (1999) Development of an animal model of autoimmune thyroid eye disease. J Immunol. **162:** 4966–4974

119 Kaithamana S., Fan J., Osuga Y., Liang S. and Prabhakar B. (1999) Induction of experimental autoimmune Graves' disease in BALB/c mice. J. Immunol. **163:** 5157–5164

120 Costagliola S., Many M. C., Denef J. F., Pohlenz J., Refetoff S. and Vassart G. (2000) Genetic immunization of outbred mice with thyrotropin receptor cDNA provides a model of Graves' disease. J. Clin. Invest. **105:** 803–811

121 Burrow G. N. (1985) The management of thyrotoxicosis in pregnancy. N. Engl. J. Med. **313:** 562–565

121 Davis L. E., Lucas M. J. and Hankins G. D. V. (1989) Thyrotoxicosis complicating pregnancy. Am. J. Obstet. Gynecol. **160:** 63–70

123 Glinoer D. (1998) Thyroid hyperfunction during pregnancy. Thyroid **8:** 859–864

124 Kopp P., van Sande J., Parma J., Duprez L., Gerber H., Joss E. et al. (1995) Congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. N. Engl. J. Med. **332:** 150–154

125 Burrow G. (1993) Thyroid function and hyperfunction during gestation. Endocr. Rev. **14:** 194–202

126 Grun J. P., Meuris S., De Nayer P., Glinoer D. (1997) The thyrotrophic role of human chorionic gonadotrophin (hCG) in the early stages of twin (versus single) pregnancies. Clin. Endocrinol. **46:** 719–725

127 Glinoer D. (1997) The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev. **18:** 404–433

128 Yoshimura M. and Hershman J. M. (1995) Thyrotropic action of human chorionic gonadotropin. Thyroid **5:** 425–434

129 Hershman J. M. (1995) Trophoblastic tumors. In: The Thyroid, 7th ed., pp. 573–576, Braverman L. E, Utiger R. D. (eds), Lippincott-Raven, Philadelphia

130 Gleason P. E., Elliott D. S., Zimmerman D., Smithson W. A. and Kramer S. A. (1994) Metastatic testicular choriocarcinoma and secondary hyperthyroidism: case report and review of the literature. J. Urol. **151:** 1063–1064

131 Rodien P., Brémont C., Raffin Sanson M. L., Parma J., van Sande J., Costagliola S. et al. (1998) Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. N. Engl. J. Med. **339:** 1823–1826

132 Utiger R. D. (1994) Hypothyroidism. In: Endocrinology 3nd, pp. 752–768, DeGroot L. J. (ed), W. B. Saunders, Philadelphia

133 Hayashi Y., Tamai H., Fukata S., Hirota Y., Katayama S., Kuma K. et al. (1985) A long-term clinical, immunological, and histological follow-up study of patients with goitrous chronic lymphocytic thyroiditis. J. Clin. Endocrinol. Metab. **61:** 1172–1178

134 Arikawa K., Ichikawa Y., Yoshida T., Shinozawa T., Homma M., Momotani N. et al. (1985) Blocking type antithyrotropin receptor antibody in patients with nongoitrous hypothyroidism: its incidence and characteristics of action. J. Clin. Endocrinol. Metab. **60:** 953–959

135 Kraiem Z., Lahat N., Glaser B., Baron E., Sadeh O. and Sheinfeld M. (1987) Thyrotropin receptor blocking antibodies: incidence, characterization and in vitro synthesis. Clin. Endocrinol. **27:** 409–421

136 Rieu M., Portos C., Lissak B., Laplanche S., Sambor B., Berrod J. et al. (1996) Relationship of antibodies to thyrotropin receptors and to thyroid ultrasonographic volume in euthyroid and hypothyroid patients with autoimmune thyroiditis. J. Clin. Endocrinol. Metab. **80:** 641–645

137 Brown R. S., Bellisario R. L., Botero D., Fournier L., Abrams C. A., Cowger M. L. et al. (1996) Incidence of transient congenital hypothyroidism due to maternal thyrotropin receptor-blocking antibodies in over one million babies. J. Clin. Endocrinol. Metab. **81:** 1147–1151

138 Barbesino G. and Chiovato L. (2000) The genetics of Hashimoto's disease. Endocrinol. Metab. Clin. North Amer. **29:** 357–374
139 Kjelsberg M. A., Cotecchia S., Ostrowski J., Caron M. G. and Lefkowitz R. J. (1992) Constitutive activation of the alpha 1B-adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. J. Biol. Chem. **267:** 1430
140 Spiegel A. M. (1996) Mutations in G proteins and G protein-coupled receptors in endocrine disease. J. Clin. Endocrinol. Metab. **81:** 2434–2442
141 Lyons J., Landis C. A., Harsh G., Vallar L., Grünewald K., Feichtinger H. et al. (1990) Two G protein oncogenes in human endocrine tumors. Science **249:** 655–659
142 O'Sullivan C., Barton C. M., Staddon S. L., Brown C. L. and Lemoine N. R. (1991) Activating point mutations of the gsp oncogene in human thyroid adenomas. Molec Carcinogen. **4:** 345–349
143 Suarez H. G., du Villard J. A., Caillou B., Schlumberger M., Parmentier C. and Monier R. (1991) Gsp mutations in human thyroid tumors. Oncogene **6:** 677–679
144 Spada A., Vallar L., Faglia G. (1992) G protein oncogenes in pituitary tumors. Trends Endocrinol Metab. **3:** 355–360
145 Weinstein L. S., Shenker A., Gejman P. V., Merino M. J., Friedman E. and Spiegel A. M. (1991) Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. N. Engl. J. Med. **325:** 1688–1695
146 Parma J., Duprez L., van Sande J, Cochaux P., Gervy C., Mockel J. et al. (1993) Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature **356:** 649–651
147 Parma J., van Sande J., Swillens S., Tonacchera M., Dumont J. and Vassart G. (1995) Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3′,5′-monophosphate and inositol phosphate-Ca2+ cascades. Mol. Endocrinol. **9:** 725–733
148 Karamanoglu Arseven O., Wilkes W. P., Jameson J. L. and Kopp P. (2000) Substitutions of tyrosine 601 in the human thyrotropin receptor result in increase or loss of basal activation of the cyclic adenosine monophosphate pathway and disrupt coupling to Gq/11. Thyroid **10:** 3–10
149 Takeshita A., Nagayama Y., Yokoyama N., Ishikawa N., Ito K., Yamashita S. et al. (1995) Rarity of oncogenic mutations in the thyrotropin receptor of autonomously functioning thyroid adenomas. J. Clin. Endocrinol. Metab. **80:** 2607–2611
150 Derwahl M., Hamacher C., Russo D., Broecker M., Manole D., Schatz H. et al. (1996) Constitutive activation of the Gs alpha protein-adenylate cyclase pathway may not be sufficient to generate toxic thyroid adenomas. J. Clin. Endocrinol. Metab. **81:** 1898–1904
151 Tonacchera M., Cetani F., Parma J., van Sande J., Vassart G. and Dumont J. (1996) Oncogenic mutations in thyroid adenoma: methodological criteria. Eur. J. Endocrinol. **135:** 444–446
152 Duprez L., Parma J., Costagliola S., Hermans J., van Sande J., Dumont J. E. et al. (1997) Constitutive activation of the TSH receptor by spontaneous mutations affecting the N-terminal extracellular domain. FEBS Lett. **409:** 469–474
153 Kopp P., Muirhead S., Jourdain N., Gu W. X., Jameson J. L. and Rodd C. (1997) Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281→isoleucine) in the extracellular domain of the thyrotropin receptor. J. Clin. Invest. **100:** 1634–1639
154 Paschke R., Tonacchera M., van Sande J., Parma J. and Vassart G. (1994) Identification and functional characterization of two new somatic mutations causing constitutive activation of the thyrotropin receptor in hyperfunctioning autonomous adenomas of the thyroid. J. Clin. Endocrinol. Metab. **79:** 1785–1789
155 Duprez L., Parma J., van Sande J., Allgeier A., Leclère J., Schvartz C. et al. (1994) Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. Nat. Genet. **7:** 396–401
156 Zhang M. L., Sugawa H,. Kosugi S. and Mori T. (1995) Constitutive activation of the thyrotropin receptor by deletion of a portion of the extracellular domain. Biochem. Biophys. Res. Commun. **211:** 205–209
157 Van Sande J., Massart C., Costagliola S., Allgeier A., Cetani F., Vassart G. et al. (1996) Specific activation of the thyrotropin receptor by trypsin. Mol Cell Endocrinol. **119:** 161–168
158 Studer H. and Derwahl M. (1995) Mechanisms of non-neoplastic endocrine hyperplasia and hyperfunction: a changing concept. Endocr. Rev. **16:** 411–426
159 Duprez L., Hermans J., Van Sande J., Dumont J. E., Vassart G. and Parma J. (1997) Two autonomous nodules of a patient with multinodular goiter harbor different activating mutations of the thyrotropin receptor gene. J. Clin. Endocrinol. Metab. **82:** 306–308
160 Holzapfel H. P., Führer D., Wonerow P., Weinland G., Scherbaum W. A. and Paschke R. (1997) Identification of constitutively activating somatic thyrotropin receptor mutations in a subset of toxic multinodular goiters. J. Clin. Endocrinol. Metab. **82:** 4229–4233
161 Tonacchera M., Chiovato L., Pinchera A., Agretti P., Fiore E., Cetani F. et al. (1998) Hyperfunctioning thyroid nodules in toxic multinodular goiter share activating thyrotropin receptor mutations with solitary toxic adenoma. J. Clin. Endocrinol. Metab. **83:** 492–498
162 Kopp P., Kimura E. T., Aeschimann S., Oestreicher M., Tobler A., Fey M. F. et al. (1994) Polyclonal and monoclonal thyroid nodules coexist within human multinodular goiters. J. Clin. Endocrinol. Metab. **79:** 134–139
163 Kopp P., Wilkes B., Gu W. X. and Jameson J. L. (1996) Absence of mutations in the TSHR receptor and the Gsα subunit in nodules and adenomas of multinodular goiters and identification of a new mutation (tyrosine601 → asparagine) in the fifth transmembrane domain in a toxic adenoma. Sixty-ninth meeting of the American Thyroid Association, San Diego. Thyroid **6:** S–9, Abstract P17
164 Gabriel E. M., Bergert E. R., Grant C. S., van Heerden J. A. and Thompson G. B., Morris J. C. (1999) Germline polymorphism of codon 727 of human thyroid-stimulating hormone receptor is associated with toxic multinodular goiter. J. Clin. Endocrinol. Metab. **84:** 3328–3335
165 Nogueira C. R., Kopp P., Arseven O. K., Santos C. L., Jameson J. L. and Medeiros-Neto G. (1999) Thyrotropin receptor mutations in hyperfunctioning thyroid adenomas from Brazil. Thyroid **9:** 1063–1068
166 Mühlberg T., Herrmann K., Joba W., Kirchberger M., Heberling H. and Heufelder A. (2000) Lack of association of non-autoimmune hyperfunctioning thyroid disorders and a germline polymorphism of codon 727 of the human thyrotropin receptor in a European Caucasian population. J. Clin. Endocrinol. Metab. **85:** 2640–2643
167 Tonacchera M., Agretti P., Chiovato L., Rosellini V., Ceccarini G., Perri A. et al. (2000) Activating thyrotropin receptor mutations are present in nonadenomatous hyperfunctioning nodules of toxic or autonomous multinodular goiter. J. Clin. Endocrinol. Metab. **85:** 2270–2274
168 Aeschimann S., Kopp P., Kimura E. T., Zbaeren J., Tobler A., Fey M. F. et al. (1993) Morphological and functional polymorphism within clonal thyroid nodules. J. Clin. Endocrinol. Metab. **77:** 846–851
169 Krohn K., Wohlgemuth S., Gerber H. and Paschke R. (2000) Hot microscopic areas of iodine deficient euthyroid goiters

contain constitutively activating TSH receptor mutations. J. Pathol. **192:** 37–42
170 Matsuo K., Friedman E., Gejman P. V. and Fagin J.A. (1993) The thyrotropin receptor (TSH-R) is not an oncogene for thyroid tumors: structural studies of the TSH-R and the alpha-subunit of Gs in human thyroid neoplasms. J. Clin. Endocrinol. Metab. **76:** 1446–1451
171 Russo D., Arturi F., Schlumberger M., Caillou B., Monier R., Filetti S. et al. (1995) Activating mutations of the TSH receptor in differentiated thyroid carcinomas. Oncogene. **11:** 1907–1911
172 Spambalg D., Sharifi N., Elisei F., Gross J. L., Medeiros-Neto G. and Fagin J. A. (1996) Structural studies on the thyrotropin receptor and Gsα in human thyroid cancers: low prevalence of mutations predicts infrequent involvement in malignant transformation. J. Clin. Endocrinol. Metab. **81:** 3898–3901
173 Russo D., Tumino S., Arturi F., Vigneri P., Grasso G., Pontecorvi A. et al. (1997) Detection of an activating mutation of the thyrotropin receptor in a case of an autonomously hyperfunctioning thyroid insular carcinoma. J. Clin. Endocrinol. Metab. **82:** 735–738
174 Russo D., Wong M. G., Costante G., Chiefari E., Treseler P. A., Arturi F. et al. (1999) A Val 677 activating mutation of the thyrotropin receptor in a Hurthle cell thyroid carcinoma associated with thyrotoxicosis. Thyroid **9:** 13–17
175 Camacho P., Gordon D., Chiefari E., Yong S., DeJong S., Pitale S. et al. (2000) A Phe 486 thyrotropin receptor mutation in an autonomously functioning follicular carcinoma that was causing hyperthyroidism. Thyroid **10:** 1009–1012
176 Liu G., Duranteau L., Carel J. C., Monroe J., Doyle D. A. and Shenker A. (1999) Leydig-cell tumors caused by an activating mutation of the gene encoding the luteinizing hormone receptor. N. Engl. J. Med. **341:** 1731–1736
177 Liu G., Kopp P., Monroe J. and Shenker A. (2000) Comparative function of glycoprotein hormone receptors containing an activating aspartate to histidine substitution in transmembrane helix 6. Eighty-second Annual Meeting of the Endocrine Society, Toronto, Abstract 1065
178 Fournes B., Monier R., Michiels F., Milgrom E., Misrahi M. and Feunteun J. (1998) Oncogenic potential of a mutant human thyrotropin receptor expressed in FRTL-5 cells. Oncogene **16:** 985–990
179 De Roux N., Polak M., Couet J., Legher J., Czernichow P., Milgrom E. et al. (1996) A neomutation of the thyroid-stimulating hormone receptor in a severe neonatal hyperthyroidism. J. Clin. Endocrinol. Metab. **81:** 2023–2026
180 Lavard L., Sehested A., Brock Jacobsen B., Muller J., Perrild H., Feldt-Rasmussen U. et al. (1999) Long-term follow-up of an infant with thyrotoxicosis due to germline mutation of the TSH receptor gene (Met453Thr). Horm. Res. **51:** 43–46
181 Mircescu H., Parma J., Huot C., Deal C., Oligny L. L., Vassart G. et al. (2000) Hyperfunctioning malignant thyroid nodule in an 11-year-old girl: pathologic and molecular studies. J. Pediatr. **137:** 585–587
182 Thomas J. L., Leclère J., Hartemann P., Duheille J., Orgiazzi J., Petersen M. et al. (1982) Familial hyperthyroidism without evidence of autoimmunity. Acta Endocrinol. **100:** 512–518
183 Schwab K. O., Söhlemann P., Gerlich M., Broecker M., Petrykowski W., Holzapfel H. P. et al. (1996) Mutations of the TSH receptor as cause of congenital hyperthyroidism. Exp. Clin. Endocrinol. Diabetes **104** (Suppl 4)**:**124–128
184 Führer D., Wonerow P., Willgerodt H. and Paschke R. (1997) Identification of a new thyrotropin receptor germline mutation (Leu629Phe) in a family with neonatal onset of autosomal dominant nonautoimmune hyperthyroidism. J. Clin. Endocrinol. Metab. **82:** 4234–4238
185 Khoo D. H., Parma J., Rajasoorya C., Ho S. C. and Vassart G. (1999) A germline mutation of the thyrotropin receptor gene associated with thyrotoxicosis and mitral valve prolapse in a Chinese family. J. Clin. Endocrinol. Metab. **84:** 1459–1462
186 Biebermann H., Schoneberg T., Krude H., Gudermann T., Gruters A. (2000) Constitutively activating TSH-receptor mutations as a molecular cause of non-autoimmune hyperthyroidism in childhood. Langenbecks Arch. Surg. **385:** 390–392
187 Fuhrer D., Warner J., Sequeira M., Paschke R., Gregory J., Ludgate M. (2000) Novel TSHR germline mutation (Met463Val) masquerading as Graves' disease in a large Welsh kindred with hyperthyroidism. Thyroid **10:** 1035–1041
188 Horton G. L. (1987) Hyperthyroidie héréditaire par hyperactivité diffuse non-autoimmune de la thyroide avec autonomie de fonction et de croissance. Thesis, University of Lausanne, Switzerland.
189 Leclère J., Béné M. C., Aubert V., Klein M., Pascal-Vigneron V., Weryha G. et al. (1997) Clinical consequences of activating germline mutations of TSH receptor, the concept of toxic hyperplasia. Horm. Res. **47:** 158–162
190 Zakarija M. and McKenzie J. M. (1983) Pregnancy-associated changes in the thyroid-stimulating antibody of Graves' disease and the relationsship to neonatal hyperthyroidism. J. Clin. Endocrinol. Metab. **57:** 1036–1040
191 Ramsay I., Kaur S. and Krassas G. (1983) Thyrotoxicosis in pregnancy: results of treatment by antithyroid drugs combined with T4. Clin. Endocrinol. **18:** 73–85
192 Kopp P., Roe T. and Jameson J. L. (1997) Congenital non-autoimmune hyperthyroidism in a non-identical twin caused by a sporadic germline mutation in the thyrotropin receptor gene. Thyroid **7:** 765–770
193 Holzapfel H. P, Wonerow P., von Petrykowski W., Henschen M., Scherbaum W. A. and Paschke R. (1997) Sporadic congenital hyperthyroidism due to a spontaneous germline mutation in the thyrotropin receptor gene. J. Clin. Endocrinol. Metab. **82:** 3879–3884
194 Gruters A., Schoneberg T., Biebermann H., Krude H., Krohn H., Dralle H. et al. (1998) Severe congenital hyperthyroidism caused by a germ-line neo mutation in the extracellular portion of the thyrotropin receptor. J. Clin. Endocrinol. Metab. **83:** 1431–1436
195 Esapa C., Duprez L., Ludgate M., Mustafa M., Kendall-Taylor P., Vassart G. et al. (1999) A novel thyrotropin receptor mutation in an infant with severe thyrotoxicosis. Thyroid **9:** 1005–1010
196 Führer D., Mix M., Wonerow P., Richter I., Willgerodt H. and Paschke R. (1999) Variable phenotype associated with Ser505Asn-activating thyrotropin-receptor germline mutation. Thyroid **9:** 757–761
197 Hollingsworth D. R. (1985) Neonatal hyperthyroidism. In: Pediatric Thyroidology, pp. 210–222, Delange F., Fisher D. A. and Malvaux P. (eds), Karger, Basel
198 Weinstein L. (1998) Albright hereditary osteodystrophy, pseudohypoparathyroidism, and Gs deficiency. In: G proteins, receptors, and disease, pp. 23–56, Spiegel AM (ed), Humana Press, Totowa, NH
199 Sunthornthepvarakul T., Gottschalk M. E., Hayashi Y. and Refetoff S. (1995) Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. New Engl. J. Med. **332:** 155–160
200 Clifton-Bligh R. .J, Gregory J. W., Ludgate M., John R., Persani L, Asteria C. et al. (1997) Two novel mutations in the thyrotropin (TSH) receptor gene in a child with resistance to TSH. J. Clin. Endocrinol. Metab. **82:** 1094–1100
201 De Roux N., Misrahi M., Brauner R., Houang M., Carel J. C., Granier M. et al. (1996) Four families with loss of function mutations of the thyrotropin receptor. J. Clin. Endocrinol. Metab. **81:** 4229–4235
202 Biebermann H., Schöneberg T., Krude H., Schultz G., Gudermann T. and Grüters A. (1997) Mutations of the human thyrotropin receptor gene causing thyroid hypoplasia and persis-

tent congenital hypothyroidism. J. Clin. Endocrinol. Metab. **82:** 3471–3480
203 Abramowicz M. J., Duprez L., Parma J., Vassart G., Heinrichs C. (1997) Familial congenital hypotyroidism due to inactivating mutation of the thyrotropin receptor causing profound hypoplasia of the thyroid gland. J. Clin. Invest. **99:** 3018–3024
204 Gagné N., Parma J., Deal C., Vassart G. and van Vliet G. (1998) Apparent congenital athyreosis contrasting with normal plasma thyroglobulin levels and associated with inactivating mutations in the thyrotropin receptor gene: are athyreosis and ectopic thyroid distinct entities ? J. Clin. Endocrinol. Metab. **83:** 1771–1775
205 Tiosano D., Pannain S., Vassart G., Parma J., Gershoni-Baruch R., Mandel H. et al. (1999) The hypothyroidism in an inbred kindred with congenital thyroid hormone and glucocorticoid deficiency is due to a mutation producing a truncated thyrotropin receptor. Thyroid **9:** 887–894
206 Tonacchera M., Agretti .P, Pinchera A., Rosellini V., Perri A., Collecchi P. et al. (2000) Congenital hypothyroidism with impaired thyroid response to thyrotropin (TSH) and absent circulating thyroglobulin: evidence for a new inactivating mutation of the TSH receptor gene. J. Clin. Endocrinol. Metab. **85:** 1001–1008
207 Takeshita A., Nagayama Y., Yamashita S., Takamatsu J., Ohsawa N., Maesaka H. et al. (1994) Sequence analysis of the thyrotropin (TSH) receptor gene in congenital primary hypothyroidism associated with TSH unresponsiveness. Thyroid **4:** 255–259
208 Xie J., Pannain S., Pohlenz J., Weiss R. E., Moltz K., Morlot M. et al. (1997) Resistance to thyrotropin (TSH) in three families is not associated with mutations in the TSH receptor or TSH. J. Clin. Endocrinol. Metab. **82:** 3933–3940
209 Nogueira C., Nguyen L., Coelho-Neto J., Arseven Karamanoglu O., Jameson J. L., Kopp P. et al. (1999) Structural analysis of the thyrotropin receptor in four patients with congenital hypothyroidism due to thyroid hypoplasia. Thyroid. **9:** 523–529
210 Beamer W. G., Eicher E. M., Maltais L. J. and Southard J. L. (1981) Inherited primary hypothyroidism in mice. Science **212:** 61–62
211 Beamer W. D. and Creswell L. A. (1982) Defective thyroid ontogenesis in fetal hypothyroid *(hyt/hyt)* mice. Anat. Rec. **202:** 387–393
212 Adams P. M., Stein S. A., Palnitkar M., Anthony A. and Gerrity L. (1989) Evaluation and characterization of the hyt/hyt hypothyroid mouse. I. Somatic and behavioral studies. Neuroendocrinology **49:** 138–143
213 Stein S. A., Shanklin D. R., Kruilich L., Roth M. G., Chubb C. M. and Adams P. M. (1989) Evaluation and characterization of the hyt/hyt hypothyroid mouse. II. Abnormabilities of TSH and the thyroid gland. Neuroendocrinology **49:** 509–519
214 Stein S. A., Zakarija M., McKenzie J. M., Shanklin D. R., Palnitkar M. B. and Adams P. M. (1991) The site of the molecular defect in the thyroid gland of the hyt/hyt mouse: abnormabilities in the TSH receptor-G protein-adenylyl cyclase complex. Thyroid **1:** 257–265
215 Russo D., Betterle C., Arturi F., Chiefari E., Girelli M. E. and Filetti S. (2000) A novel mutation in the thyrotropin (TSH) receptor gene causing loss of TSH binding but constitutive receptor activation in a family with restistance to TSH. J. Clin. Endocrinol. Metabol. **85:** 4238–4242
216 Kimura S., Hara Y., Pineau T., Fernandez-Salguero P., Fox C. H., Ward J. M. et al. (1996) The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev. **10:** 60–69
217 Damante G. and DiLauro R. (1994) Thyroid-specific gene expression. Biochim. Biophys. Acta. **1218:** 255–266
218 Ahlbom B. E., Yaqoob M., Larsson A., Illicki A., Annerén G., Wadelius C. (1997) Genetic and linkage analysis of familial congenital hypothyroidism: exclusion of linkage to the TSH receptor. Hum. Genet. **99:** 186–190
219 Macchia P. E., Lapi P., Krude H., Pirro M. T., Missero C., Chiovato L. et al. (1998) PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nat. Genet. **19:** 83–86
220 Mansouri A., Chowdhury K. and Gruss P. (1998) Follicular cells of the thyroid gland require Pax8 gene function. Nat. Genet. **19:** 87–90
221 Clifton-Bligh R. J., Wentworth J. M., Heinz P., Crisp M. S., John R., Lazarus J. H. et al. (1998) Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. Nat. Genet. **19:** 399–401
222 De Felice M., Ovitt C., Biffali E., Rodriguez-Mallon A., Arra C., Anastassiadis K. et al. (1998) A mouse model for hereditary thyroid dysgenesis and cleft palate. Nat. Genet. **19:** 395–398
223 Antonarakis S. E. and the Nomenclature Working Group. (1998) Recommendations for a nomenclature system for human gene mutations. Hum. Mut. **11:** 1–3
224 Wadsworth H. L., Russo D., Nagayama Y., Chazenbalk G. D. and Rapoport B. (1992) Studies on the role of amino acids 38–45 in the expression of a functional thyrotropin receptor. Mol. Endocrinol **6:** 394–398