### **TSH Receptor Mutations and Diseases**

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# TSH RECEPTOR MUTATIONS AND DISEASES

### **1. GAIN-OF-FUNCTION MUTATIONS.**

On a theoretical basis, for a hormone receptor, "gain of function" may have several meanings: (i) activation in the absence of ligand (constitutivity); (ii) increased sensitivity to its normal agonist; (iii) increased, or de novo sensitivity to an allosteric modulator; (iv) broadening of its specificity. When the receptor is part of a chemostat, as is the case for the TSH receptor, the first situation is expected to cause tissue autonomy, whereas the second would simply cause adjustment of TSH to a lower value. In the third and fourth cases, inappropriate stimulation of the target will occur because the illegitimate agonists or modulators are not expected to be subject to the normal negative feedback. If a gain-of-function mutation of the first category occurs in a single cell normally expressing the receptor (somatic mutation), it will become symptomatic only if the regulatory cascade controlled by the receptor is mitogenic in this particular cell type or, during development, if the mutation affects a progenitor contributing significantly to the final organ. Autonomous activity of the receptor will cause clonal expansion of the mutated cell. If the regulatory cascade also positively controls function, the resulting tumor may progressively take over the function of the normal tissue and ultimately result in autonomous hyperfunction. If the mutation is present in all cells of an organism (germline mutation) autonomy will be displayed by the whole organ.

From what we know of thyroid cell physiology it is easy to predict the phenotypes associated with gain-of-function of the cAMP-dependent regulatory cascade. Two observations provide pertinent models of this situation. Transgenic mice made to express ectopically the adenosine A2a receptor in their thyroid display severe hyperthyroidism associated with thyroid hyperplasia <sup>1</sup>. As the A2a adenosine receptor is coupled to Gs and displays constitutive activity due to its continuous stimulation by ambient adenosine <sup>2</sup>, this model mimics closely the situation expected for a gain-of-function germline mutation of the TSH receptor. Patients with the McCune-Albright syndrome are mosaïc for mutations in Gs (Gsp mutations) leading to the constitutive stimulation of adenylyl-cyclase <sup>3</sup>.

Hyperfunctioning thyroid adenomas develop in these patients from cells harboring the mutation, making them a model for gain-of-function somatic mutations of the TSH receptor. A transgenic model in which Gsp mutations are targeted for expression in the mouse thyroid has been constructed. Though with a less dramatic phenotype this represents also a pertinent model for a gain of function of the cAMP regulatory cascade <sup>4</sup>.

Since the TSH receptor is capable of activating both Gs and Gq (though with lower potency) the question arises whether mutations with a different effect on the two cascades would be associated with different phenotypes. Studies in mice <sup>5</sup> and rare patients <sup>6</sup> suggests that activation of Gq may be required to observe goitrogenesis in patients with non-autoimmune familial hyperthyroidism. However, when tested in transfected non-thyroid cells, all identified gain of function mutations of the TSHR stimulate constitutively Gs, with only a minority capable of stimulating both Gs and Gq <sup>7;8</sup>. Also, thyroid adenomas or multinodular goitre are frequent in McCune Albright syndrome, which is characterized by pure Gs stimulation <sup>9</sup>.

## **1.1. Familial non-autoimmune hyperthyroidism or hereditary toxic thyroid hyperplasia.**

The major cause of hyperthyroidism in adults is Graves' disease in which an autoimmune reaction is mounted against the thyroid gland and auto-antibodies are produced that recognize and stimulate the TSH receptor. This may explain why the initial description by the group of Leclère of a family showing segregation of thyrotoxicosis

as an autosomal dominant trait in the absence of signs of autoimmunity was met with skepticism <sup>10</sup>. Re-investigation of this family together with another family from Reims identified two mutations of the TSH receptor gene, which segregated in perfect linkage with the disease <sup>11</sup>. A series of additional families have been studied since <sup>6;12-32</sup>. [See figure 1 and table 1. For a complete list of TSH receptor gene mutations with their functional characteristics, see "Glycoprotein-hormone receptor information system database", GRIS, at http://gris.ulb.ac.be/ 33 and 34 at http://www.ssfa-gphr.de/]. The functional characteristics of these mutant receptors confirm that they are constitutively stimulated (see below). This nosological entity, hereditary toxic thyroid hyperplasia (HTTH), sometimes called Leclère's disease, is characterized by the following clinical characteristics: autosomal dominant transmission; hyperthyroidism with a variable age of onset (from infancy to adulthood, even within a given family); hyperplastic goiter of variable size, but with a steady growth; absence of clinical or biological stigmata of auto-immunity. An observation common to most cases is the need for drastic ablative therapy (surgery or radioiodine) in order to control the disease, once the patient has become hyperthyroid <sup>11;35</sup>. The autonomous nature of the thyroid tissue from these patients has been elegantly demonstrated by grafting in nude mice <sup>36</sup>. Contrary to tissue from Graves' disease patients, HTTH cells continue to grow in the absence of stimulation by TSH or TSAb.

The prevalence of hereditary toxic thyroid hyperplasia is difficult to estimate. It is likely that many cases are still mistaken for Graves' disease. This may be explained by the high frequency of thyroid auto-antibodies (anti-thyroglobulin, anti-thyroperoxidase) in the general population. It is expected that wider knowledge of the

existence of the disease will lead to better diagnosis. This is not a purely academic problem, since presymptomatic diagnosis in children of affected families may prevent the developmental or

psychological complications associated with infantile or juvenile hyperthyroidism (for a review, see <sup>37</sup>). A country-wide screening for the condition has been performed in Denmark. It was found in one out of 121 patients with juvenile thyrotoxicosis (0.8%; 95% CI: 0.02-4.6%), which corresponds to one in 17 patients with presumed non-autoimmune juvenile thyrotoxicosis (6%; 95% CI:0.15-28.69) <sup>38</sup>.

### 1.2. Sporadic toxic thyroid hyperplasia.

Cases with toxic thyroid hyperplasia have been described in children born from unaffected parents <sup>32;39-48</sup>. Conspicuously, congenital hyperthyroidism was present in most of the cases and required aggressive treatment. Mutations of one TSH receptor allele were identified in the children, but were absent in the parents. As paternity was confirmed by mini- or microsatellite testing, these cases qualify as true neomutations. When comparing the

aminoacid substitutions implicated in hereditary and sporadic cases, for the majority, they do not overlap (see table 1 and <sup>37</sup> and GRIS, at http://gris.ulb.ac.be/<sup>33</sup> and <sup>34</sup> at http://www.ssfa-gphr.de/ ). Whereas most of the sporadic cases harbor mutations that are also found in toxic adenomas, most of the hereditary cases have "private" mutations. Although there may be exceptions, the analysis of the functional characteristics of the individual mutant receptors in COS cells, and the clinical course of individual patients, suggest an explanation for this observation: "sporadic" and somatic mutations seem to have a stronger activating effect than "hereditary" mutations <sup>49</sup>. From their severe phenotypes, it is likely that newborns with neomutations would not have survived, if not treated efficiently. On the contrary, from inspection of the available pedigrees, it seems that the milder phenotype of patients with 'hereditary" mutations has only limited effect on reproductive fitness. The fact that "hereditary" mutations are rarely observed in toxic adenomas is compatible with the suggestion that they would cause extremely slow tissue growth and, accordingly, would rarely cause thyrotoxicosis if somatic. There is, however, no a priori reason for neomutations to cause stronger gain of function than hereditary mutations. Accordingly, an activating mutation of the TSH receptor gene has been found in a six month child with subclinical hyperthyroidism presenting with weight loss as the initial symptom <sup>50</sup>.

### 1.3. Somatic mutations: autonomous toxic adenomas.

Soon after mutations of  $G_{s\alpha}$  had been found in adenomas of the pituitary somatotrophs <sup>51</sup>, similar mutations (also called Gsp mutations) were found in some toxic thyroid adenomas and follicular carcinomas <sup>52-55</sup>. The mutated residues (Arg201, Glu227) are homologous to those found mutated in the ras proto-oncogenes: i.e. the mutations decrease the endogenous GTPase activity of the G protein, resulting in a constitutively active molecule.

Toxic adenomas were found to be a fruitful source of somatic mutations activating the TSHr, probably because the phenotype is very conspicuous and easy to diagnose <sup>56</sup>. Whereas mutations are distributed all along the serpentine portion of the receptor and even in the extracellular aminoterminal domain <sup>7;56-65</sup>, there is clearly a hotspot at the cytoplasmic end of the sixth transmembrane segment (see figure 1). The clustering reflects the pivotal role of this portion of the molecule in the activation mechanisms mechanisms <sup>66-71</sup>. For a complete list of TSH receptor gene mutations with their functional characteristics, see "Glycoprotein-hormone receptor information system database",

GRIS, at <u>http://gris.ulb.ac.be/</u><sup>33</sup> and <sup>34</sup> at <u>http://www.ssfa-gphr.de/</u>].



Despite some dispute about the prevalence of TSH receptor mutations in toxic adenomas (which may be due to different origin of patients <sup>72;73</sup> or different sensitivity of the methodology) the current consensus is that activating mutations of the TSH receptor are the major cause of solitary toxic

adenomas and account for about 60 to 80% of cases <sup>7;61;74-76</sup>. Contrary to initial suggestions <sup>72</sup>, the same percentage of activating TSH receptor mutations is observed in Japan, an iodine-sufficient country with low prevalence of toxic adenomas<sup>77</sup>. In some patients with a multinodular goiter and two zones of autonomy at scintigraphy, a different mutation of the TSH receptor was identified in each nodule <sup>78-81</sup>. This indicates that the pathophysiological mechanism responsible for solitary toxic adenomas can be at work on a background of multinodular goiter. In agreement with this notion, activating mutations of the TSH receptor have been identified in hyperfunctioning areas of multinodular goiter <sup>14;24;74;80</sup>. The independent occurrence of two activating mutations in a patient may seem highly improbable at first. However, the multiplicity of the possible targets for activating mutations within the TSH receptor makes this less unlikely. It is also possible that a mutagenic environment is created in glands exposed to a chronic stimulation by TSH, resulting in H2O2 generation <sup>82;83</sup>. Finally the involvement of TSH receptor mutations in thyroid cancers has been implicated in a limited number of follicular thyroid carcinoma <sup>24;84-92</sup>.

## **1.4. Structure-function relationships of the TSH receptor, as deduced from activating mutations.**

An important observation has been that the wild-type receptor itself displays significant constitutive activity <sup>56;93</sup>.

This characteristic is not exceptional amongst GPCRs, but interestingly, it is not shared, at least to the same level, by its close relatives, the luteinizing hormone/chorionic gonadotropin (LH/CG) receptor and the follitropin (FSH) receptor. Compared to the TSH receptor, the LH/CG receptor displays minimal basal activity and the human FSH receptor is totally silent <sup>94</sup>. Together with the observation that mutations in residues distributed over most of the serpentine portion of the TSH receptor are equally effective in activating it (which does not seem to be a general characteristic in all GPCRs) this suggests that the unliganded TSH receptor might be less constrained than other GPCRs. As a consequence, being already "noisy," it would be more prone to further destabilization by a wide variety of mutations affecting multiple structural elements.

The effect of activating mutations must accordingly be interpreted in terms of "increase in constitutive activity". Most constitutively active mutant receptors (also referred to as "CAMs") found in toxic adenomas and/or toxic thyroid hyperplasia share common characteristics: (1) they increase the constitutive activity of the receptor toward stimulation of adenylyl cyclase; (2) with a few notable exceptions (see Table 1 and below) <sup>95</sup>, they do not display constitutive activity toward the inositol phosphate/diacylglycerol pathway; (3) their expression at the cell surface is decreased (from slightly to severely); (4) most, but not all of them keep responding to TSH for stimulation of cAMP and inositol phosphate generation, with a tendency to do so at decreased median effective concentrations; and (5) they bind <sup>125</sup>I-labeled bovine TSH with an apparent affinity higher than that of the wild-type receptor. Of note, CAMs with mutations at Ser281 (to Ile) in the extracellular N-terminal part, at Ile486 (to Phe or Met) and Ile568 (to Thr) in the first and second extracellular loops, respectively, and at both Asp633 (to His) and Pro639 (to Ser) in transmembrane helix 6 are exceptional in that in addition to stimulating adenylyl cyclase, they cause constitutive activation of the inositol phosphate pathway. The constitutive activity of these mutants is interesting as it points to positions and structural fragments of the wild type receptor which may be of high relevance for its physiological coupling to both Gs and Gq (Figure

#### 1 and table 1).

No direct relationship is found between the level of cAMP achieved by different mutants in transfected COS cells and their level of expression at the cell membrane <sup>96</sup>, which means that individual mutants have widely different "specific constitutive activity" (measured as the stimulation of cAMP accumulation/receptor number at the cell surface). Although this specific activity may tell us something about the mechanisms of receptor activation, it is not a measure of the actual phenotypic effect of the mutation in vivo. Indeed, one of the relatively mild mutations, observed up to now only in a family with HTTH (Cys672Tyr), is among the strongest according to this criterion. It would be logical to expect the best correlation to be found between the phenotype and the actual level of cAMP achieved, irrespective of the level of receptor expression.

Differences between the effects of the mutants in transfected COS or HEK293 cells and thyrocytes in vivo render these correlations a difficult exercise. Indeed, most of the activating mutations of the TSH receptor have been studied by transient expression in COS or HEK293T cells and there is no guarantee that the mutants will function in an identical way in these artificial systems as they do in thyrocytes <sup>97</sup>. In thyrocytes, a better relation as been observed between adenylylcyclase stimulation and differentiation than with growth <sup>97</sup>. However, the built-in amplification associated with transfection of constructs in COS or HEK 293T cells has the advantage of allowing detection of even slight increases in constitutive activity of certain TSH receptor mutants.

According to a current model of G protein–coupled receptor (GPCR) activation, the receptor would exist under at least two interconverting conformations: R (silent conformations) and R\* (the active forms) <sup>66</sup> (Figure 2). The unliganded wild type receptor would shuttle between both forms, the equilibrium being in favor of R. Binding of the agonistic ligand is believed to stabilize the R\* conformation.



The resulting R-to-R\* transition is supposed to involve a conformational change that modifies the relative position of transmembrane helices, which in turn would translate into conformational changes in the cytoplasmic domains interacting with hetero-trimeric G proteins. This model is strongly supported by recently solved crystal structures of active GPCR conformations (reviewed in <sup>98</sup>). They

revealed that activation involves movements of transmembrane helices 5, 6 and 7 leading to modification of their distances relative to each other. Helix 6 is a major player in this process, its cytoplasmic end moving away from that of helix 3 by turning around a pivotal helix-kink. The result is an "opening" of the cytoplasmic crevice of the receptor allowing interaction with the G protein. A more detailed description of the activation mechanics, adapted to a model of the TSH receptor, is given in the legend to figure 3B. This essential function of helix 6 for determination of an active state <sup>99</sup> might explain, why most activating TSHR mutations were found in this particular helix (Fig. 1). This conclusion is in accordance with the early concept that the silent form of GPCRs would be submitted to structural constraints requiring the wild-type primary structure of the helix 6 and the connected third intracellular loop, and explains why these constraints could be released by a wide spectrum of amino acid substitutions in this segment <sup>66;100</sup>.

In addition to the release of structural locks stabilizing the inactive conformation of GPCRs, activation of the glycoprotein hormone receptors has been shown to involve a triggering mechanism exerted by an "activated module" constituted by segments of the exoloops and the C-terminal portion of their ectodomain. According to this model, it is this module, activated by the binding of TSH, thyroid stimulating auto-antibodies, or mutations (see below) which would be the immediate agonist of the serpentine portion of the receptor (Figure 3A) <sup>71;101</sup>. In all cases, however, mutations are expected to affect the local three dimensional structure of the receptor with a resulting global effect on its activation state. Amongst these are modification of "knob and hole" interactions (e.g. by repulsion) in tightly packed local microdomains and breakage, or creation of intramolecular interactions by changing the biophysical characteristics of side chains. As examples of these, mutations at Asp633 or Asp619 are expected to break interhelical locks between transmembrane helices 6 and 7 or 3, respectively. Interestingly, even mutations affecting an important residue of the trigger in the ectodomain (Ser281) seem also to be responsible for a "loss of local structure". Indeed, substitution of the wild type residue (serine) by almost any amino acid results in constitutive activation<sup>102</sup>. This implicates that predictions of phenotype-genotype relationships must always be considered with much caution if they are not backed by detailed structural and functional knowledge.





### 1.5. Familial gestational hyperthyroidism.

Some degree of stimulation of the thyroid gland by human chorionic gonadotrophin (hCG) is

commonly observed during early pregnancy. It is usually responsible for decrease in serum thyrotropin with an increase in free thyroxine concentrations that remains within the normal range (for references see <sup>103</sup>). When the concentrations of hCG are abnormally high, like in molar pregnancy, true hyperthyroidism may ensue. The pathophysiological mechanism is believed to be the promiscuous stimulation of the TSH receptor by excess hCG, as suggested by the rough direct or inverse relation between serum hCG and free T4 or TSH concentrations, respectively <sup>104</sup>. A convincing rationale is provided by the close structural relationships of the glycoprotein hormones and their receptors, respectively <sup>105</sup>.

A new syndrome has been described in 1998 in a family whith dominant transmission of hyperthyroidism limited to pregnancy (see figure 4)<sup>106</sup>. The proposita and her mother had severe thyrotoxicosis together with hyperemesis gravidarum during the course of each of their pregnancies. When non pregnant they were clinically and biologically euthyroid. Both patients were heterozygous for a K183R mutation in the extracellular aminoterminal domain of the TSH receptor gene. When tested by transient transfection in COS cells, the mutant receptor displayed normal characteristics towards TSH. However, providing a convincing explanation to the phenotype, it showed higher sensitivity to stimulation by hCG, when compared with wild type TSH receptor <sup>106</sup>.

The aminoacid substitution responsible for the promiscuous stimulation of the TSHr by hCG is surprisingly conservative. Also surprising is the observation that residue 183 is a lysine in both the TSH and LH/CG receptors. When placed on the available three dimensional model of the hormone-binding domain of the TSH receptor <sup>107</sup>, residue 183 belongs to one of the beta sheets which constitute the putative surface of interaction with the hormones. Detailed analysis of the effect of the Lys183Arg mutation by site-directed mutagenesis indicated that any amino acid substitution at this position confers a slight increase in stability to the illegitimate hCG/TSH receptor complex <sup>108</sup>. This increase in stability would be enough to cause signal transduction by the hCG concentrations achieved in pregnancy, but not by the LH concentrations observed after menopause. Indeed, the mother of the proposita remained euthyroid after menopause. This finding is compatible with a relatively modest gain of function of the Lys183Arg mutant upon stimulation by hCG.

Contrary to other mammals, human and primates rely on chorionic gonadotropin for maintenance of corpus luteum in early pregnancy <sup>109</sup>. The frequent partial suppression of TSH observed at peak hCG levels during normal pregnancy indicates that evolution has selected physiological mechanisms operating very close to the border of thyrotoxicosis. This may provide a rationale to the observation that, in comparison to other species, the glycoprotein hormones of primates display a lower biological activity due to positive selection by evolution of specific aminoacid substitutions in their alpha subunits <sup>110</sup>. Up to now no spontaneous mutation has been identified which would increase the bioactivity of hCG. An interesting parallel may be drawn between familial gestational hyperthyroidism and cases of spontaneous ovarian hyperstimulation syndrome (sOHSS) <sup>94;111</sup>. In sOHSS, mutations of the FSH receptor gene render the receptor abnormally sensitive to hCG. The result is recurrent hyperstimulation of the ovary, on the occasion of each pregnancy



### 2. LOSS OF FUNCTION MUTATIONS.

Loss-of-function mutations in the TSH receptor gene are expected to cause a syndrome of "resistance to TSH." The expected phenotype is likely to resemble that of patients with mutations in TSH itself. These mutations have been described early because of the prior availability of information on TSH alpha and beta genes <sup>110</sup>. Mouse models of resistance to TSH are available as natural (hyt/hyt mouse) <sup>112</sup> or experimental TSH receptor mutant lines <sup>113;114</sup>. Interestingly, and contrary to the situation in human (see below), the thyroid of newborn TSH receptor knockout mice is of normal size. As expected, the homozygote animals displayed profound hypothyroidism. Their thyroids do not express the sodium-iodide symporter, but showed significant (non-iodinated) thyroglobulin production. From this information one would expect patients with two TSH receptor mutated alleles to exhibit a degree of hypothyroidism in accordance with the extent of the loss of function, going from mild, compensated, hypothyroidism, to profound neonatal hypothyroidism with absent iodide trapping. Heterozygous carriers are expected to be normal or display minimal increase in plasma TSH.

### Clinical cases with the mutations identified (figure 5).

A few patients with convincing resistance to TSH had been described before molecular genetics permitted identification of the mutations <sup>115;116</sup>. The first cases described in molecular terms were euthyroid siblings with elevated TSH <sup>117</sup>. Sequencing of the TSH receptor gene identified a different mutation in each allele of the affected individuals, which made them compound heterozygotes. The substitutions were in the extracellular amino-terminal portion of the receptor (maternal allele, Pro162Ala; paternal allele, Ile167Asn). The functional characteristics of the mutant receptors showed that the paternal allele was virtually completely nonfunctional, whereas the maternal allele displayed an increase in the median effective TSH concentration for stimulation of cAMP production. Current knowledge of the tridimensional structure of part of the ectodomain of the receptor <sup>107;118</sup> allows to establish structure-function relationships for mutations affecting this portion of the receptor <sup>119</sup>.



A large number of familial cases with loss-of-function mutations of the TSH receptor have been identified in the course of screening programs for congenital hypothyroidism <sup>120-133</sup> ([For a complete list of TSH receptor gene mutations with their functional characteristics, see <sup>33</sup> at <u>http://gris.ulb.ac.be/</u> and <sup>34</sup> at <u>http://www.ssfa-gphr.de/</u>]. Some of the patients displayed the usual criteria for congenital hypothyroidism, including high TSH, low free T4, and undetectable trapping of 99Tc . In some cases, plasma thyroglobulin levels were normal or high. The patients can be compound heterozygotes for complete loss of function mutations <sup>121</sup>, or homozygotes, born to consanguineous <sup>120</sup> or apparently unrelated parents <sup>127</sup>. In agreement with the phenotype of knock-out mice with homozygous invalidation of the TSH receptor, patients with complete loss-of-function of the receptor display an inplace, thyroid with completely absent iodide or 99Tc trapping. However, in contrast with the situation in mice, the gland is hypoplastic. Activation of the cAMP pathway, while dispensable for the anatomical development of the gland and thyroglobulin production, is thus absolutely required for expression of the NIS gene and, at least in human, for normal growth of the tissue during fetal life. This explains that in the absence of thyroglobulin measurements or expert echography, loss-of-function mutations of the TSH receptor may easily be misdiagnosed as thyroid agenesis.

In the heterozygous state, complete loss of function mutations of the TSH receptor is a cause of moderate hyperthyrotropinemia (subclinical hypothyroidism), segregating as an autosomal dominant trait <sup>134</sup>.

### Resistance to thyrotropin not linked to the TSH receptor gene

Finally, it must be stressed that an autosomal dominant form of partial resistance to TSH has been demonstrated in families in which linkage to the TSH receptor gene has been excluded <sup>135</sup>. A locus has been identified on chromosme 15q25.3-26.1 but the gene responsible for the phenotype has not been identified yet <sup>136</sup>.

### **3-Polymorphism**

A series of single nucleotide polymorphisms affecting the coding sequence have been identified in the TSH receptor gene. After the initial suggestion that some of these (Asp36His, Pro52Thr, Asp727Glu) would be associated with susceptibility to autoimmune thyroid diseases <sup>137-139</sup> the current consensus is that they represent neutral alleles with no pathophysiological significance <sup>140-143</sup>. However, a genome-wide study involving a large cohort of patients has recently demonstrated association between non-coding SNPs at the TSH receptor gene locus and Graves'disease <sup>144;145</sup>. The genetic substratum responsible for this association is still under study <sup>144</sup>. However, a large meta analysis of genome wide association studies failed to identify the TSH receptor as a locus affecting plasma TSH values <sup>146</sup>.

One polymorphic residue deserves special mention: position 601 was found to be a tyrosine or a histidine in the two initial reports of TSH receptor cloning <sup>147;148</sup>. Characterization of the two alleles by transfection in COS cells

indicated interesting functional differences: the Tyr601 allele displayed readily detectable constitutive activity, whereas the His601 was completely silent; the Tyr601 allele responded to stimulation by TSH by activating both the adenylylcyclase and phospholipase C dependent regulatory cascades, when the His601 allele was only active on the cAMP pathway <sup>149</sup>. The Tyr601 allele is by far the most frequent in all populations tested. A Tyr601Asn mutation was found in a toxic adenoma. Characterization of the mutant demonstrated increase in constitutive activation of the cAMP regulatory cascade <sup>149</sup>, making the 601 residue an interesting target for structure function studies.

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#### Legend to figures.

#### Legend to Figure 1.

Structural model of the TSH receptor with indication of gain-of-function mutations. Known activating mutations are indicated by red spheres displaying the position of C-alpha atoms of wild type amino acids. The single letter amino acid abbreviations are used. The model shows different parts of the receptor for which homologous structural information is available. The leucine-rich repeat domain (LRRD) and the hinge region are both harboring determinants for hormone and antibody binding. The hinge region (colored pink) structurally links the LRRD with the serpentine domain made of transmembrane helices (H) 1-7 connected by intracellular (IL) and extracellular (EL) loops. Two cysteine bridges (yellow sticks) between the C-terminal LRRD and the C-terminus of the hinge region are indicated that are required for correct receptor arrangement and function. The majority of activating mutations are distributed over the entire serpentine portion of the receptor structure with clustering in the central core and specifically in helix 6. In contrast to other glycoprotein-hormone

receptors, naturally occurring activating mutations were also identified in the extracellular loops and in the hinge region (Ser281). They indicate amino acids of high importance for regulation of signaling activity. Mutation of lysine 183 in the LRRD leads to promiscuous hormone binding.

#### Legend to figure 2

#### Left.

Schematic representation of the equilibria between inactive (R) and active (R\*) conformations of TSH receptor. The triangles indicate the equilibrium point of the wild type receptor (pink) and hypothetical mutants with increasing constitutive activity (brown, red). The situation of a receptor which would be devoid of basal activity is also indicated (blue triangle). Note that the wild type receptor (pink) has basal activity.

#### Right.

The concentration action curves corresponding to the hypothetical mutants and wild type receptors are indicated with the same color code.

#### Legend to figure 3

A-Model of the intramolecular interactions involved in the activation of the TSH receptor.

The basal state of the receptor is characterized by an inhibitory interaction between the ectodomain and the serpentine domain. The ectodomain would function as a tethered inverse agonist. Note that the unliganded receptor is not silent. Removal of the ectodomain releases the serpentine domain from the inhibitory interaction, resulting in partial activation. Mutation of Ser281 into Leu switches the ectodomain from an inverse agonist into a full agonist of the serpentine domain. Binding of TSH (or thyroid stimulating autoantibodies) to the ectodomain are proposed to have a similar effect, converting it into a full agonist of the serpentine portion. (adapted from <sup>101</sup>)

B-Model of TSH receptor structure with illustration of putative activation mechanisms. Left: the receptor is displayed as a backbone cartoon in complex with space filling representations of the dimeric hormone and trimeric G protein. For the serpentine portion of the receptor, the model is based on the solved structure of the  $\beta$ 2-adrenergic/Gs crystal 78. The ectomain (in complex with TSH) and the hinge region are modeled from the published FSHR-FSH structure 127. Right: a blow-up of the hinge and serpentine portions of the model. A selection of residues affected by known activating mutations are shown as red spheres and identified by their position in the primary structure of the protein. Their positions tentatively illustrate the "path" followed by the activation signal, from outside the membrane (in the ectodomain) to the cytoplamsic surface of the receptor, via transmembrane helices. Briefly, the hormone binds to both the Leucine rich repeat domain (LRRD) and the hinge region (brown ribbon). This "initial signal" is transduced into a conformational change (large gray arrows) of a module constituted by the "hinge" region of the ectodomain and the exoloops (EL) of the serpentine portion of the receptor. In favor of this model, several residues belonging to this module (Ser281 in the ectodomain; D403 and D406 at the ectodomain-serpentine domain border; Ile486, Ile568, Val656 in the exoloops) can activate the receptor constitutively when mutated. Together with the observation that a truncated receptor devoid of ectodomain displays significant increase in constitutive activity, this suggests that activation of the TSHR involves switching of specific extracellular portions from a tethered inverse agonist (maintenance of the basal state) to an intramolecular agonist <sup>101</sup>. The resulting structural changes affecting the exoloops are expected to be directly conveyed to the transmembrane helices with the resulting breakage of silencing locks

(horizontal arrows). From comparison of the inactive and active beta2 adrenoreceptor structures <sup>99</sup>, the largest spatial movement affects H6, involving a combination of horizontal and rotational (wound arrow) movements around a pivotal helix-kink at Pro639. These global changes result in the partial "opening" of the intrahelical crevice on the cytoplasmic side of the receptor (horizontal double-head arrows), allowing an activating interaction with Galphas.

#### Legend to figure 4.

Pathophysiology of familial gestational hyperthyroidism secondary to mutation of the TSH receptor gene. Upper-left panel illustrates binding of TSH, or hCG to the ectodomain of the TSH receptor, according to crystallographic data from <sup>150</sup>. The yellow dot indicates the position of the K183R mutation. Lower-left panel documents the thyrotropic activity of hCG during normal pregnancy (adapted from <sup>104</sup>). Upper right panel displays the pedigree, with the two ladies affected, together with a "snake plot "of the TSH receptor, with the mutation indicated. Lower right panel illustrates the increased sensitivity of the K183R mutant TSH receptor vis-à-vis hCG.

#### Legend to figure 5.

Structural model of the TSH receptor with indication of loss-of-function mutations. The location and substitutions responsible for known loss-of-function mutations (blue spheres of the wild type C-alpha atoms) are indicated on a three-dimensional receptor model. Single letter abbreviations of amino-acids are used. In contrast to activating mutations, the majority of inactivating mutations is located in the loops and in the LRRD. In most cases they diminish hormone or G-protein binding, or lead to a decrease of receptor cell surface expression. For a complete list of inactivating mutations with their functional characteristics, see <a href="http://gris.ulb.ac.be/">http://gris.ulb.ac.be/</a> and <a href="http://www.ssfa-gphr.de">http://www.ssfa-gphr.de</a>).

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