MINI REVIEW

The human thyrotropin receptor is highly mutable: a review of gain-of-function mutations

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Abstract

Objectives: To find whether germline and somatic gain-of-function mutations of the thyrotropin receptor (TSHR) differ in location and/or mutational mechanisms, as well as to explore the degree to which these mutations are specific to TSHR compared with pituitary glycoprotein hormone receptors.

Methods: We examined the data on the TSHR website (www.unvi-leipzig~innerre/TSH) supplemented with recent literature. Comparisons were also made with gain-of-function mutations of lutropin/choriogonadotropin (LH/CGR) and follicle-stimulating hormone receptors (FSHR).

Results: Some mutations (at residues 183, 505, 509 and 597) are exclusively germline, whereas mutations at 630 and 633 are characteristic of somatic mutations. Several residues located mainly in a mutation cluster region (619–639) are shared by both. Germline mutations are more likely to be transitions than transversions compared with somatic mutations. The lack of mutations involving deamination of CpG dinucleotides, a common mechanism for C→T transitions, reflects the low CG prevalence in the mutable regions of TSHR. Comparison of the mutation sites with the equivalent positions in LH/CGR showed a significant difference ($P < 0.0001$), whereas those in the mutation cluster region comprising the sixth transmembrane helix (TM6) and the adjoining third intracellular loop were concordant ($P > 0.90$). We suggest that there is specific clustering of mutations in the juxtacytoplasmic end of TM6 in LH/CGR, a hydrophobic patch that is tightly packed with a face on TM5 whose sequences diverge from those of TSHR.

Conclusions: TSHR exhibited frequent mutations outside the mutation cluster region. A role for a mutagenic environment created by the thyroid for other TSHR-specific codons cannot be discounted, nor can genetic factors, when accounting for the variation in the prevalence of TSHR-activating mutations worldwide.

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Introduction

The thyrotropin receptor (TSHR) is a member of the leucine-rich repeat-containing G protein-coupled receptor (LRG) family. In addition to the pituitary glycoprotein hormone receptors, two new members, of unknown ligands, each having an extended leucine-rich region, were recently identified. The TSHR stands out by virtue of a 50-residue insertion distal to the C-flanking cysteine-rich region characteristic of LRGs (1). The homologies within the transmembrane (TM) helices, particularly TM3, -6 and -7, reflect the structural constraints related to location and signal transduction. The recent modeling of the TM helical bundles of the lutropin/choriogonadotropin receptor (LH/CGR) has proved useful in clarifying the structural and functional consequences of receptor mutations (2).

The description (3) of a constitutively activating mutation of the LH/CGR in familial male precocious puberty coincided with that of mutations of a similar nature in autonomous thyroid nodules (4). These studies triggered the search for other mutations within the glycoprotein hormone receptor genes. Nowhere has this search been as successful as in the case of TSHR (5). Germline mutations were found to result in loss of function and sporadic or familial non-autoimmune hyperthyroidism. A variant mutation enhances the TSHR response to chorionic gonadotropin (CG) and results in familial gestational hyperthyroidism (6). Much more commonly encountered were the somatic or acquired mutations of TSHR seen in single autonomous nodules and those found in multinodular goiters as well as those among an unusual subgroup of thyroid carcinomas (see reference 5 for details, 6).

Here we review the details of all published TSHR-activating mutations and address the following questions: 1) do the locations of germline mutations differ from those of the somatic variety?; 2) what form do these mutations take?; and 3) is the apparently high susceptibility of TSHR in general and certain sites in particular to mutations related or unrelated to the structure of TSHR and its encoding gene? Our review is based on the TSHR mutations database (5), supplemented by two somatic and two germline mutations more recently described and gleaned from the literature (6–9).

Differences and overlap in mutation sites

Fifteen germline mutations at 14 codons (Table 1) and 94 somatic activating mutations at 15 codons were identified in TSHR. Two short deletions associated with constitutive activation were also recognized (Table 2).

In general, activating mutations tend to be concentrated in the TM helices and intervening loops. Two interesting exceptions worthy of note are the Lys 183 Arg mutation (6) and mutations at Ser 281. The Lys 183 Arg mutation is associated with familial gestational hyperthyroidism, apparently related to increased sensitivity of TSHR to CG. The lysine in that position is conserved between the two receptors, but not in the follicle-stimulating hormone receptor (FSHR), and falls within the sixth leucine-rich repeat in a transition region in which domain-exchange experiments between the two receptors suggested a switch in specificity. The Lys 183 Arg also stands separate from the remaining activating mutations in that it is 'conditional', i.e. its expression is dependent on high serum concentrations of CG. Ser 281 is part of a motif (YPSHCCAF) in the C-flanking cysteine-rich region completely conserved

between the three glycoprotein receptors. Ser 281 probably maintains the receptor at this important hinge region in a constrained state, which is released by missense mutations.

Certain mutations in the ectodomain and the proximal TM domain appear to be specific for germline mutations as opposed to somatic mutations. Thus, mutations at codons 183, 505, 509 and 597 are exclusively germline. In contrast, those at codons 630 and 633 are limited to somatic mutations.

On the other hand, several codons between 619 and 633 (the mutation cluster region) represent mutational 'hot-spots' for both.

More of the germline mutations (67%), compared with the somatic mutation (53%), are transitions ($\chi^2 = 1.323$, NS). It remains to be seen to what extent this difference in codon preference and mechanism of mutation is frequency-dependent or merely reflects the small sample size for germline mutations.

The remainder of the TSHR mutations are in the TM helices and intervening loops. The distal TM region is indeed a mutation cluster region. Met 453 is located in TM2, Ser 505 and Val 509 are in TM3 and Val 597 is in TM5. These residues are highly conserved in glycoprotein hormone receptors and, in the case of 505, also in all LRGs identified (1).

The Met 543 TSHR mutation is identical to that at the corresponding residue (398) in LH/CGR. Mutations resulting in conservative residue changes are also noted in the corresponding position of TM5: Val 597 Leu in TSHR and Ile 542 Leu in LH/CGR. In contrast, mutations at residues 505 and 509 are specific to TSHR (Tables 1 and 2).

Two of the codons in the mutable distal TM region stand out: 632 because of its high frequency of C→T transition, and 633 because of the variety of mutations

Table 1 Activating TSHR germline mutations.

	Codon	Wild type	Mutation	Position ^a	Nature ^b
1	183*	AAA	A → G	2	Ts
2	281	AGC	G → A	2	Ts
3	453	ATG	T → C	2	Ts
4	505	ACC	C → A	2	Tv
5	505	AGC	C → A	3	Tv
6	509	GTG	T → C	2	Ts
7	597	GTC	G → C	1	Tv
8	623	GCC	C → T	2	Ts
9	629	TTG	G → T	3	Tv
10	631	CTC	C → T	1	Ts
11	639	CCA	C → T	1	Ts
12	632	ACC	C → T	2	Ts
13	650	AAC	A → T	1	Tv
14	670	AAC	A → G	2	Ts
15	672	TGT	G → A	2	Ts

* The activating mutation at residue 183 is a special case. It is only expressed in female family members during pregnancy when serum levels of CG are elevated.

^a 'Position' refers to the relevant codon position at which the mutation takes place.

^b Ts and Tv refer to transitions and transversions respectively.

Table 2 Activating TSHR somatic mutations.

	Codon	Wild type	Mutation ^a	Position ^b	Nature ^c
1	281	AGC	G → T	2	Tv
2			G → A (2)	2	Ts
3			G → C (2)	2	Tv
4	453	ATG	T → C (2)	2	Ts
5	486	ATC	C → G (2)	3	Tv
6			A → T (2)	1	Tv
7	586	ATC	T → C (5)	2	Ts
8	619	GAT	A → G (7)	2	Ts
9	del. 613–621				del.
10	623	GCC	G → T (7)	1	Tv
11			GC → AT	1 & 2	Ts
12			C → T (7)	2	Ts
13	629	TTG	G → C	3	Tv
14			G → T (2)	3	Tv
15	630	ATC	A → C (5)	1	Tv
16	631	TTC	T → A	1	Tv
17			T → C	1	Ts
18			T → A (7)	3	Tv
19	632	ACC	C → T (21)	2	Ts
20			A → G	1	Ts
21	633	GAC	A → C	2	Tv
22			G → C (3)	1	Tv
23			G → T (3)	1	Tv
24			C → G (2)	3	Tv
25			C → A (4)	3	Tv
26	639	CCA	C → T	1	Ts
27	656	GTT	G → T	1	Tv
28	666	TTC	T → C	2	Ts
29	677	CTG	C → G	1	Ts
30	del. 658–661				del.

^a The figures in brackets signify frequency of the particular mutation.^b 'Position' refers to the relevant codon position at which the mutation takes place.^c Ts, Tv and del. refer respectively to transitions, transversions and deletions.

in all three codon positions. Codon 623 features both transition (G→T) at the first codon position and a 2 bp transition: GC→AT (Table 1).

Are the mutations related to LRG structure ?

We wondered whether these TSHR mutational hot-spots are related to LRG receptor structure, particularly to shared codon usage among members of the family, or to the cellular location of the TSHR domain. It is likely that both factors apply.

The FSHR was found to harbor only one activating mutation (Asp 567 Gly) corresponding to position 619 in TSHR and position 564 in LH/CGR (Fig. 1) (10). Several of the mutational hot-spots are encoded by identical nucleotides in all three receptors and yet they only mutate in TSHR and/or LH/CGR. Position 578 in the latter and the equivalent 633 in the former are encoded by GAC in TSHR and GAT in LH/CGR and are highly mutable. As noted above, a variety of transversions are involved in the three codon positions in TSHR, whereas the vast majority of mutations described in LH/CGR are limited to transitions in the second adenine.

TSHR 610	AAT CCG	CAG TAC	AAC CCA	GGG GAC	AAA GAT	620	ACC AAA	ATT GCC	AAG AGG	ATG GCT	GTG TTG	630	ATC TTC	ACC GAC	TTC ATA	TGC ATG	GCC CCA	ATC 640
LH/CGR 555	AAC CCA	GAA TTA	ATG GCT	ACC ATT	AAA GAT	ACA AAG	ATT GCT	AAG AAA	ATG GCA	ATC CTC	ATC TTC	ACC GAT	TTC ACC	TGC ATG	GCA CCT	ATC 585		
FSHR 558	AAC CCC	AAC ATC	GTG TCC	TCC TCT	AGT GAC	ACC AGG	ATC GCC	AAG CGC	ATG GCC	ATG CTC	ATC TTC	ACT GAC	TTC CTC	TGC ATG	GCA CCC	ATT 588		

Figure 1 DNA sequences spanning residues 610–640 of the TSHR and their counterpart regions of LH/CGR and FSHR. The activating mutations cluster regions of the former two receptors are included within this sequence. Asterisks are placed underneath codons at which mutations have been reported. Note that all three receptors are mutable at GAT/GAC at position 619 in TSHR and equivalents in the other two receptors.

Table 3 LH/CGR mutations and their equivalents in TSHR in TM6 and the distal third intracellular loop. The table shows LH/CGR mutations, and shows only the equivalent wild-type codon position in TSHR. It emphasizes the less-than-complete overlap of mutations in the corresponding receptor regions (2, 11, 12).

LH/CGR		TSHR	
Codon	Mutation	Position	Wild type
564	gat → ggt	619	gat
568	gct → gat	623	gcc
571	atg → ata		
572	gca → gta		
575	atc → ctc	630	atc
577	acc → act	632	acc
578	gat → ggt	633	gac
	gat → gaa		
	gat → tat		
581	tgc → cgc		

Single examples of transversions at the first and third codon positions of LH/CGR 578 have been described (11, 12; Table 3).

Codons 632 of TSHR and 577 of LH/CGR are both encoded by ACC and both undergo transition mutation to ATC.

Codon 631 (TTC) is the only one conserved between LH/CGR and/or FSHR and TSHR and in which mutations have only been reported in TSHR; the other conserved codons, 623 GCC and 630 ATC, are subject to mutations in both TSHR and their equivalents in LH/CGR and 619 GAT in all three receptors (Fig. 1). There is a suggestion that LH/CGR-activating mutations are clustered at the juxtacytoplasmic region of TM6 and are clustered in TSHR (and LH/CGR) in the 629–633 interval of that helical domain. The apparent relative specificity of that region of the LH/CGR almost certainly reflects the interface of that domain with distal TM5, which shows notable sequence divergence between the two receptors (Figs 1 and 2 and see below).

It is interesting that none of the TSHR germline or somatic mutations involves CpG dinucleotides and, by implication, spontaneous deamination. This may be an attribute of the TSHR codon usage. Thus we found only one potential (codon 611 CCG) site between codons 610 and 640, which encompasses the mutation cluster region (Fig. 1).

Modelling TSHR gain-of-function mutations

In order to understand how these mutations result in the constitutive activation of TSHR, we look to the model of LH/CGR proposed recently (2). The inactive state of the receptor is maintained by specific inter-helical hydrogen bonds between TM6 and TM7. Maintenance of native orientation of TM6 is crucial to maintaining the inactive state of the receptor. Loss of these bonds may destabilize contact surfaces and reduce packing specificity. A hydrophobic patch on TM6 is formed by Ala 623, Met 626, Ala 627 and Val 630 (Ile in LH/CGR) and is involved in tight packing with TM5. In contrast, a polar patch is formed by residues Thr 632, Asp 633 and Cys 636 and faces TM7. A Pro 638 kink in TM6 increases the side-chain packing of the distal halves of TM5 and TM6. The contact between these helices results from the interaction of the side-chains of Tyr 605, Ile 604, His 601 (Tyr in LH/CGR), Val 597 (Ile in LH/CGR) and Phe 594 on TM5 and the side-chains of Ile 622, Ala 623, Met 626, Ala 627 and Val 630 and Phe 634 on TM6. Strong hydrogen bonding between the mid-regions of TM6 and TM7 are formed by the interactions between Thr 633 and Asn 674, between Asp 634 and Asn 674 and between Asp 634 and Asn 670. It is apparent that mutations on the contact faces of the three adjacent helical domains can disrupt their packing and result in the constitutive activation of the receptor (2).

This model, however, may be limited. The use of peptide mimics has suggested (13) that it is not only the juxtacytoplasmic domain of TM6 but also those of TM4, TM5 and TM7 that may form a pocket which interacts with Gs. Constitutive mutations of LH/CGR (and, by extension, TSHR) may involve the opening of that pocket to expose the Gs-binding sites on the helices. Interestingly, Asp 578 of LH/CGR (633 in TSHR) placed centrally in TM6 has a dampening effect in the peptide mimicking studies, probably by virtue of its role in maintaining an inactive conformation of the receptor (14). *In vitro* mutations at residue 576 in LH/CGR, whose equivalent mutates spontaneously in TSHR appear to be pleomorphic in that mutations which result in a local extended conformation of TM6 constitutively activate the receptor, whereas those that increase its helicity cripple the receptor (15).

567	I A K K M A I L I F T D F T C M P I S F	578	LH/CGR
			TM6
	I A K R M A V L I F T D F I C M P T F		
622		642	TSHR
	A F F I I C A C Y I K I Y	550	LH/CGR
			TM5
	A F V I V C C C H V K I Y		TSHR
		605	

Figure 2 Relevant TM5 and TM6 residues of the TSHR and the LH/CGR.

Met 453, which exhibits both germline and somatic activating TSHR mutations, is located near the cytoplasmic end of the second TM helix, facing the interior of the bundle and surrounded by TM3 and TM7. The side-chains of Met 453 point towards Glu 518 and Arg 519, which represent part of a motif that is highly conserved throughout the G protein-coupled receptor. The consensus sequence D/ERY is apparently important for signal transduction (2).

The third intracellular loop is thought to be directly involved in mediating the interaction of the receptor with the G protein. Receptor occupancy results in conformational changes in the third intracellular loop which extend to adjacent TM domains. The nine-residue deletion (Table 2) in that loop results in constitutive TSHR activation (16), probably because of tethering of the juxtacytoplasmic TM6 domain and the adjoining third intracellular loop into a conformation that is permanently receptive to Gs.

The role of mutagens and background genes

The notion that the high frequency of mutations of TSHR is dictated by the intrinsic characteristics of its structure is on firm ground (Table 4). This overall picture of differences in the location of mutations between TSHR and LH/CGR, however, contrasts with the overlap between the corresponding residues in the two receptors over the mutation cluster region in the juxtacytoplasmic part of TM6 and the third intracytoplasmic loop (Tables 3 and 4). The TSHR shows much higher rates of activating mutations elsewhere in its structure.

Kitahori *et al.* (18) explored the possibility that activating mutations may be inducible by mutagens. Nitrosamine-inducible rat thyroid tumors showed a low

rate of Gs and K-ras mutations and only one synonymous mutation at codon 636 of TSHR. The mutation rate of TSHR is negligible and does not involve the G→A transition noted for Gs and K-ras induced mutations. Either other mutagens, e.g. oxygen radicals or oxidized iodide products, are relevant or mutagens are not involved at all in TSHR mutations.

The uneven distribution of germline and somatic mutations worldwide is unlikely to be accounted for entirely by the differences in interest and vigor with which TSHR mutations are being pursued. It is possible that these differences may be related to heritable and/or environmental co-factors (7) which vary between different geographic regions or ethnic groupings. The finding that the repertoire of activating LH/CGR is different in European compared with US families lends credence to this notion (19).

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Table 4 Differences in, and concordance of, mutable residues in equivalent positions of TSHR and LH/CGR.

TSHR	+	--
LH/GCR +	8	4
--	15	722

The lack of concordance between mutations is significant at the $P < 10^{-4}$ level using Fisher's exact test (data on the LH/CG receptor are based on references 2, 11 and 17). When only the 21 residues spanning the mutation cluster region (619–639 in TSHR) are considered significant, concordance is seen ($P > 0.90$ using Fisher's exact test). The data are arrayed as a 2×2 table such that cell 1 (left upper corner) contains residues concordant for both receptors and cell 4 (right lower corner) contains residue positions found in both receptors and not involved in mutations; cells 2 and 3 contain residues in which mutations were discordant for one or the other receptor. By definition, TSHR-specific insertions are excluded from the analysis.

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