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**An Interactive Web-Tool for Molecular Analyses links Naturally Occurring Mutation Data with Three-Dimensional Structures of the rhodopsin-like Glycoprotein Hormone Receptors**

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# An Interactive Web-Tool for Molecular Analyses Links Naturally Occurring Mutation Data with Three-Dimensional Structures of the Rhodopsin-like Glycoprotein Hormone Receptors



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**ABSTRACT:** The collection, description and molecular analysis of naturally occurring (pathogenic) mutations are important for understanding the functional mechanisms and malfunctions of biological units such as proteins. Numerous databases collate a huge amount of functional data or descriptions of mutations, but tools to analyse the molecular effects of genetic variations are as yet poorly provided. The goal of this work was therefore to develop a translational web-application that facilitates the interactive linkage of functional and structural data and which helps improve our understanding of the molecular basis of naturally occurring gain- or loss- of function mutations. Here we focus on the human glycoprotein hormone receptors (GPHRs), for which a huge number of mutations are known to cause diseases. We describe new options for interactive data analyses within three-dimensional structures, which enable the assignment of molecular relationships between structure and function. Strikingly, as the functional data are converted into relational percentage values, the system allows the comparison and classification of data from different GPHR subtypes and different experimental approaches. Our new application has been incorporated into a freely available database and website for the GPHRs (<http://www.ssfa-gphr.de>), but the principle development would also be applicable to other macromolecules. ©2010 Wiley-Liss, Inc.

**KEY WORDS:** Naturally occurring mutations, Database, Glycoprotein hormone receptors, TSHR, FSHR, LHCGR

## INTRODUCTION

Analyses of naturally occurring mutations are important for understanding the molecular mechanisms and malfunctions of biological macromolecules such as the G-protein-coupled receptors (GPCRs). These receptors are

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## 2 Kleinau et al.

the largest family of membrane proteins and mediate most cellular responses to hormones and neurotransmitters, as well as being responsible for vision, olfaction and taste (reviewed in (Schwartz et al., 2006)). Malfunction of GPCRs causes a variety of human diseases (reviewed in (Schoneberg et al., 2002; Tao, 2006)) and it has been estimated that GPCRs represent between 25-50% of current drug targets (Overington et al., 2006; Kristiansen, 2004; Flower, 1999). Investigation of mutations provides a powerful tool for deciphering causal relationships between such biological structures and patient phenotypes (Schoneberg et al., 2002; Schoneberg et al., 2004) and is also a prerequisite for the directed development of pharmacological treatments.

Several databases collate information and functional data on GPCRs: GPCR Database, <http://www.gpcr.org/7tm> (Horn et al., 1998); GRIS: Glycoprotein hormone Receptor Information System, <http://gris.ulb.as.be> (Van Durme et al., 2006); TSH Receptor mutation Database II, <http://innere.uniklinikum-leipzig.de/tsh> (Lachmund et al., 2004; Fuhrer et al., 2003); GPCR Natural Variants Database, <http://nava.liacs.nl> (Kazius et al., 2008). The unification and assignment of such information directly to the three-dimensional (3D) structure of GPCRs would facilitate the elucidation of causal relationships between sequence, structure, function and also malfunction. A direct and interactive link between mutation data and 3D structures would allow the molecular reasons for malfunction (which are ultimately responsible for patient phenotypes) to be studied more precisely.

Therefore we developed a web-application that facilitates interactive linkage between unified functional and 3D structural information. Our project focused on pathogenic mutations of the glycoprotein hormone receptors (GPHRs), which are available in a freely accessible database (<http://www.ssfa-gphr.de>), where our new tool is incorporated. A large number of GPHR mutations are known to cause various diseases such as hyperthyroidism (thyrotropin receptor, TSHR), hypogonadism (follitropin receptor, FSHR) or precocious puberty (lutropin/choriogonadotropin receptor, LHCGR). The system allows the comparison and semi-quantitative classification of mutant data from different GPHR subtypes and different experimental approaches. In our database and web-application the available structures (fragments) and homology models of GPHRs are used for projection of functional information on to 3D structures in order to facilitate molecular and interactive sequence-structure-function analyses of pathogenic mutations and receptor malfunction.

### THE DATABASE AND NEW ANALYSIS TOOLS

#### Background

Published data and information on the thyrotropin receptor (TSHR), follitropin (FSHR) and lutropin (LHCGR) receptors are collected in a web accessible database ([www.ssfa-gphr.de](http://www.ssfa-gphr.de)). We updated and redesigned our database (SSFA-GPHR) and web-interface, which combines protein sequences, multiple sequence alignments, protein structures, homology models and data from mutagenesis experiments of GPHRs (Kleinau et al., 2007a). As the functional data are converted into relational percentage values, the system allows the comparison and semi-quantitative classification of mutagenesis data from different GPHR subtypes and different experimental approaches. We implemented new data, types of data (e.g. double mutations) and bioinformatic tools like 3D search tools.

#### Interactive linkage of functional information with three-dimensional structures

One section of the database presents germline and somatic single side-chain substitutions of the GPHRs (website-link: "Natural mutations"). In a sub-section ("3D structural-search") we provide a novel 3D search tool whereby positions of known naturally occurring mutations are automatically highlighted on homology models of the leucine-rich repeat domain (LRRD), the serpentine domain (SD) (modeling procedures have been previously described in detail (Kleinau et al., 2007a; Kleinau et al., 2007b; Kleinau et al., 2008) or known crystal structures of receptor parts (Fan and Hendrickson, 2005; Sanders et al., 2007). These positions are colored according to the occurrence of inactivating or activating mutations (Figure 1A). Such residues are linked with specific information such as the type of mutation, converted functional data into relational percentage values, or the original source of publication. This information can be retrieved by clicking on the interactive links at the colored C-alpha atoms of mutated positions (Figure 1B, yellow sphere), which leads to a results-overview in a separate browser-window (Figure 1C). In this website section we also added a tabulated overview and a sequence alignment where positions of pathogenic mutations are colored according to their mutation phenotype (*gain- or loss- of function*).

### Technical data

The Sequence-Structure-Function-Analysis of Glycoprotein hormone receptors application was built by combining an Apache web server (<http://www.apache.org>), PHP Hypertext Pre-processor scripts (PHP 5) and a relational database management system (MySQL 5). The web interface is based on HTML, JavaScript and CSS (Cascading Style Sheets). 3D structures are displayed using the Jmol structure viewer (Jmol: an open-source Java viewer for chemical structures in 3D. <http://www.jmol.org/>). To display the GPHRs in 3D, the viewer is embedded in the application as a Java applet. The Jmol scripting language, in combination with JavaScript, is used to change the rendering of the molecules in order to illustrate important structural features and to allow the linkage of the crystal structures or homology models to the functional information stored in the database.

### LINKAGE BETWEEN STRUCTURAL AND FUNCTIONAL INFORMATION OF GLYCOPROTEIN HORMONE RECEPTORS

The three glycoprotein hormone subtypes are of high physiological importance. Whereas the thyroid-stimulating hormone (TSH) regulates growth and function of thyroid follicular cells, the gonadotrophic hormones LH/CG and follicle-stimulating hormone (FSH) play an important role in human reproduction (Ascoli et al., 2002; Rapoport et al., 1998; Simoni et al., 1997; Szkudlinski et al., 2002). Therefore their receptors, TSHR, FSHR, and LHCGR (review (Kleinau and Krause, 2009)), are of central importance to medical, pharmaceutical and biological research (reviewed in (Corvilain et al., 2001; Rapoport and McLachlan, 2007; Schoneberg et al., 2004; Simoni et al., 1997; Smit et al., 2007; Tao, 2006; Tao, 2008; Davies et al., 2005)).

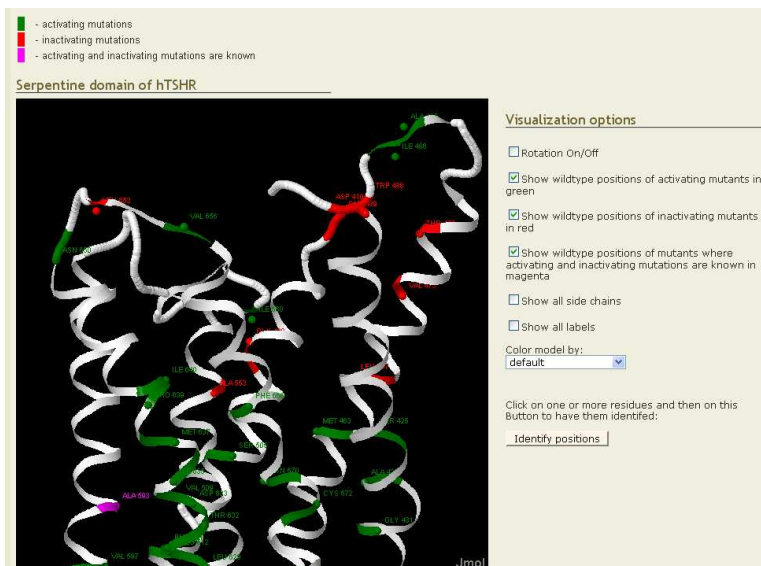
We designed an information resource to collect data for the GPHRs and made them available for investigation of sequence-structure-function relationships (Kleinau et al., 2007a). It provides a condensed overview of functional data from mutagenesis studies across the GPHRs using converted relational percentage values and allows searching of the database via different specific tools. This information resource and publicly accessible web interface (<http://www.ssfa-gphr.de>) was updated and redesigned to contribute to improve the analyses and understanding of malfunctions caused by mutations. We designed and provide web-access to known 3D structures or structural homology models of the receptors with the positions of pathogenic mutations highlighted in different colors according their different function (e.g. gain or loss of function) and also provide links to information from published reports. Furthermore, we have introduced a novel interactive search tool. User-driven picking of particular amino acid positions directly on a 3D structure or homology model provides the user with information about side-chain variations and their particular functional effects. Different effects can be classified and highlighted in different colors. This allows interactions of residues located close in space to be investigated and the comparison of functional data of mutations. Thus, conclusions concerning potential interactions can be drawn according to comparable mutant receptor phenotypes or attractive/repulsive side-chain effects.

Examples of different scenarios for exploring *gain-of-function* mutations are given in Figure 2A. The first example comprises experimentally validated interacting wild type amino acids (Karges et al., 2005) at which positions mutations are known that lead to a *gain-of-function* phenotype (wild type residues Val509 in TMH3 and Ala593 in TMH5). The two wild-type amino acids can be seen to interact in the 3D TSHR model. The second example focuses on three tightly-packed triad-like interacting hydrophobic residues, which constitute an hydrophobic patch for stabilization of the basal TSHR conformation. Several naturally occurring single CAMs are known at these positions (Figure 2B) and might be related to hyperfunctioning thyroid nodules (Alberti et al., 2001; Esapa et al., 1999; Gozu et al., 2006; Holzapfel et al., 1997; Kosugi et al., 2000; Trulzsch et al., 2001).

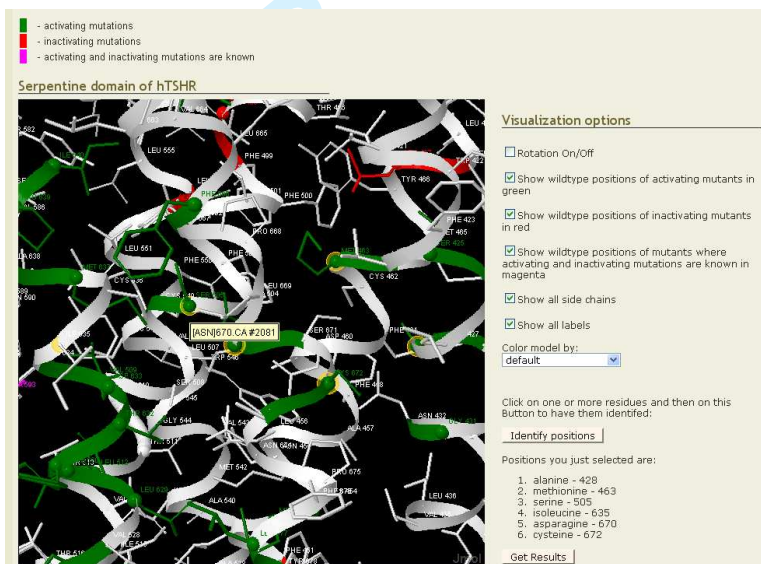
Each of these CAMs causes disruption of the interacting triad, leading to constitutive TSHR activation. This important core of amino acid interaction and molecular interrelationship has not yet been experimentally investigated in detail but according to our model is suggested to be an essential component of the observed mutant phenotypes. Further information regarding the particular amino acids and mutation of interest can be obtained by following the interactive links implemented in our tool.

4 Kleinau et al.

A)



B)



C)

**Results for 3D - Structural Search**

Search for mutation:  
 • by receptor: hTSHR  
 • at position: 428, 463, 505, 635, 670, 672

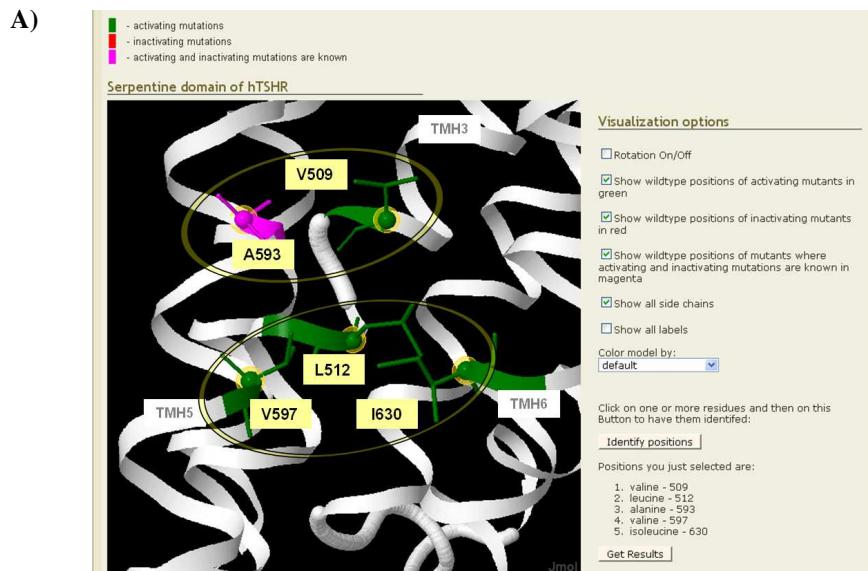
**Results**

hTSHR Swissprot 3d model

Substructure	No	Ba-We	wildtype	mutant	pubmed
TMH1	428	1.46	Ala	Val	no data available <a href="#">Borgel K, Pohlentz J,...</a>
TMH2	463	2.53	Met	Val	<a href="#">details</a> <a href="#">Fuhrer D, Warner J,...</a>
TMH3	505	3.36	Ser	Asn	<a href="#">details</a> <a href="#">Holzapfel HP, Woneru...</a>
TMH3	505	3.36	Ser	Arg	no data available <a href="#">Van Sande J, Parma J,...</a>
TMH6	635	6.46	Ile	Val	<a href="#">details</a> <a href="#">Castro I, Lima L, Se...</a>
TMH7	670	7.45	Asn	Ser	<a href="#">details</a> <a href="#">Tonacchera M, Van Sa...</a>
TMH7	672	7.47	Cys	Thr	no data available <a href="#">Duprez L, Parma J, V...</a>

**Figure 1.** Sections of the SSFA-GPDR database for investigation, visualization and data collection of naturally occurring single side-chain variations of GPHRs. Three types of search tools are provided: 1. From an alignment of human TSHR, FSHR and LHCGR; 2. from a tabulated and referenced overview and 3. by selecting a structural domain (the leucine-rich repeat domain or serpentine domain). **A)** Highlighted and color-differentiated mutations of hTSHR are displayed on a 3D homology model. Wild type positions of reported naturally occurring mutations (“gain-“ (green) and “loss- of function” (red) mutations) are shown. Several additional visualization options allow the user to customize how the molecule is displayed, for example color coding of different types of mutations and display/un-display switches for side-chains are provided. **B)** User-driven picking of selected side chains (yellow sphere) on the 3D structure will retrieve mutation information of each residue. **C)** Such a request will lead to a result overview opening in a separate browser window and presenting all available information.





B)

■ Results for 3D - Structural Search

Search for mutation:  
 • by receptor: hTSHR  
 • at position: 509, 512, 593, 597, 630

Results

hTSHR Swissprot 3d model

Substructure	No	Ba-We	wildtype	mutant	pubmed
TMH3	509	3.40	Val	Ala	<a href="#">Duprez L, Parma J, V...</a>
TMH3	509	3.40	Val	Ala	<a href="#">Karges B, Krause G...</a>
TMH3	512	3.43	Leu	Gln	<a href="#">Trulzsch B, Krohn K...</a>
TMH3	512	3.43	Leu	Arg	<a href="#">Kosugi S, Hai N, Oka...</a>
TMH5	593	5.50	Ala	Asn	<a href="#">Sykiotis GP, Neumann...</a>
TMH5	593	5.50	Ala	Val	no data available <a href="#">Fricke-Otto S, Pfarr...</a>
TMH5	597	5.54	Val	Phe	<a href="#">Alberti L, Proverbio...</a>
TMH5	597	5.54	Val	Leu	<a href="#">Esapa CT, Duprez L...</a>
TMH6	630	6.41	Ile	Leu	<a href="#">Holzapfel HP, Fuhrer...</a>
TMH6	630	6.41	Ile	Met	<a href="#">Gozu H, Avsar M, Bir...</a>

C)

■ Results for Point Mutation

Search for mutation:  
 • by receptor: hTSHR  
 • at position: 630

Highlight result values

Functional parameter	in red below [%]	in green above [%]	
binding			<input type="button" value="Update"/>
Gs_basal activation	179	180	<input type="button" value="Update"/>
Gs_max stimulation	79	80	<input type="button" value="Update"/>
expression			<input type="button" value="Update"/>
Gq_basal activation	179	180	<input type="button" value="Update"/>
Gq_max stimulation	79	80	<input type="button" value="Update"/>

Results

hTSHR Swissprot 3d model 3d morph

Substructure	No	Ba-We	wildtype	mutant	binding	Gs_basal activation	Gs_max stimulation	expression	Gq_basal activation	Gq_max stimulation
TMH6	630	6.41	Ile	Leu	62	250	-	-	-	-
TMH6	630	6.41	Ile	Leu	42	476	106	-	-	-
TMH6	630	6.41	Ile	Leu	-	640	80	36	92	24
TMH6	630	6.41	Ile	Met	46	500	87	29	61	32

**Figure 2.** Interacting wild type amino acids of known naturally occurring constitutively activating mutations mapped on to a 3D TSHR model. Five wild type amino acids with known naturally occurring activating mutations are highlighted after performing a 3D search (interactive mouse-click, yellow sphere) A) Double mutation experiments have shown that two of the residues interact with one another (Karges et al., 2005): Val509 in TMH3 and Ala593 in TMH5 (magenta). In spatial neighbourhood a triad of interacting hydrophobic wild type residues Leu512 (TMH3), Ile630 (TMH6) and Val597 (TMH5) can be observed on this 3D projection, but has not yet been investigated experimentally in detail. It can be speculated that this patch stabilizes the basal conformation of TSHR and that interruption of this interaction by mutation (thereby resulting in a release of flexibility between the helices) leads to TSHR activation. B) By pressing the “Identify positions” and “Get result” buttons detailed information regarding the single substitutions e.g. known mutations identified in vivo, are provided. The source of information from the original publication can be reached via Pubmed links. C) Additionally, mutagenesis data obtained by in vitro studies for the specific amino acid of interest can be retrieved via the `details` link, if available. Here, a direct comparison of the mutation data (converted into relational percentage values, wt = 100%) is possible using the highlighting feature. The user defined red/green classes indicate that the pathogenic mutations cause strong “gain of function” by constitutively activating effects on the cAMP pathway (> 180%: increased basal Gs; >80% Gs max. stimulation, green) but in fact also a “loss of function” on the IP pathway (<180%: (not) increased basal Gq; <80% Gq max. stimulation, red).

## 6 Kleinau et al.

Our new tool is therefore suitable for exploring and recognizing spatial interrelationships between side-chains and causally-induced malfunctions of proteins. The 3D mapping and visualization can be used to: *a.* evaluate mutation as already known or as new, *b.* get entrance to published information, *c.* identify direct interaction partners, *d.* evaluate potential mechanisms of molecular malfunction caused by specific mutations. Strikingly, under respect of converted data into comparable percentage values the discrimination of *loss- and gain- of function* mutations mapped onto the 3D structures helps to both identify hot-spots of signaling mechanisms and integrate and classify new results. Notably, this feature is of universal principle and is not restricted to the GPHRs or other GPCRs, it is also applicable to molecules like DNA or RNA. This tool may help link different scientific fields like biology, biochemistry, medicine, chemistry, genetics and bioinformatics.

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