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Genetic Variations in Human G Protein-Coupled Receptors: Implications for Drug Therapy

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ABSTRACT Numerous genes encode G protein-coupled receptors (GPCRs)-a main molecular target for drug therapy. Estimates indicate that the human genome contains approximately 600 GPCR genes. This article addresses therapeutic implications of sequence variations in GPCR genes. A number of inactivating and activating receptor mutations have been shown to cause a variety of (mostly rare) genetic disorders. However, pharmacogenetic and pharmacogenomic studies on GPCRs are scarce, and therapeutic relevance of variant receptor alleles often remains unclear. Confounding factors in assessing the therapeutic relevance of variant GPCR alleles include 1) interaction of a single drug with multiple closely related receptors, 2) poorly defined binding pockets that can accommodate drug ligands in different orientations or at alternative receptor domains, 3) possibility of multiple receptor conformations with distinct functions, and 4) multiple signaling pathways engaged by a single receptor. For example, antischizophrenic drugs bind to numerous receptors, several of which might be relevant to therapeutic outcome. Without knowing accurately what role a given receptor subtype plays in clinical outcome and how a sequence variation affects drug-induced signal transduction, we cannot predict the therapeutic relevance of a receptor variant. Genome-wide association studies with single nucleotide polymorphisms could identify critical target receptors for disease susceptibility and drug efficacy or toxicity.

KEYWORDS: G Protein-Coupled, Receptors, Drug Therapy, Pharmacogenomics, Pharmacogenetics

INTRODUCTION

Sequence variations of the human genome.

This article provides an overview of the large superfamily of G protein-coupled receptors (GPCRs) and its variant alleles in the human population known to affect receptor function (**Table 1**) [1-132] . Sequencing of the human genome has introduced a flood of new information on the projected approximately 35 000 genes [133,134] ; however, the primary sequence is but a first step in understanding genomic organization, protein functions, communication networks, and cellular structure. Furthermore, the presence of sequence variations introduces a near-infinite variability in the genetic makeup of individuals. This is suspected to play a main role in disease susceptibility and variable response to drug therapy. The latter is the subject of pharmacogenetics-pharmacogenomics-with pharmacogenomics focusing on the entire genome or using genomic techniques to design and develop new drugs and guide therapy.

Polymorphisms refer to sequence variations with an allele frequency of greater than or equal to 1%; however, mutant alleles responsible for sporadic single-gene Mendelian diseases are often much less frequent. The exchange of a single nucleotide, commonly referred to as single nucleotide polymorphisms (SNPs), accounts for approximately 80% of all sequence variants. Current estimates of SNP frequency are 1:1200 [133], but this is clearly a function of coverage for genome sequencing (4- to 8-fold coverage). With increasing coverage (ie, more overlapping sequences analyzed from different individuals), SNP abundance will increase further.

Given a gene encoding a GPCR of an average length (1000-1500 base pair [bp] coding region), we would expect to find an average of 1 relatively common SNP and several more SNPs with a frequency of more than 1%. **Table 1** lists a selection of known sequence variants identified in human GPCR genes [1-132] . Because a majority of the listed SNPs have a relatively low allele frequency, an individual likely will not harbor a sequence variation at all in a GPCR gene. Even though this review focuses on sequence differences

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among individuals, we should recognize the extraordinary degree of conservation of a molecule as brittle as DNA across the human population. Sequence variants might accumulate in a population if they convey a selective advantage to the individual carrying the allele, but there is no such evidence for GPCR alleles. Chemokine receptors serving as coacceptors for the acquired immunodeficiency syndrome (AIDS) virus for penetration into cells could represent an exception (**Table 1**) because inactivating mutations appear to convey resistance to human immunodeficiency virus (HIV) infection [109,110] . However, AIDS has entered the human population only recently, precluding positive selection of inherited traits, which requires numerous generations.

Most polymorphisms in a GPCR gene are unlikely to affect receptor function, either because they occur in noncoding regions of the mature mRNA or in introns. Alternatively, SNPs occurring in coding regions can be silent (synonymous, no change in protein sequence) or occur in a region that can accommodate amino acid substitutions without functional consequences. Yet, we have only a partial understanding of all functional receptor domains that interact with ligands and numerous other proteins mediating receptor function. Polymorphisms in promoter regions or at splice junctions can have profound effects on the abundance of the encoded protein. A growing number of recognized polymorphisms in GPCR promoter regions suggests the importance of overall receptor expression of interindividual variability, but examples are still scarce. Here, we review general GPCR structure and function to facilitate a better understanding of how sequence variants might affect receptor signaling and drug interactions.

STRUCTURE AND FUNCTION OF GPCRS

GPCRs comprise a large class of membrane proteins that are encoded by approximately 600 human genes with broadly diverse functions [135] . Venter et al [133] predict the presence of 614 GPCRs, a number that requires further verification but is probably close to the true number of genes in this class. Ligands are extremely diverse and include hormones and neurotransmitters and neuromodulators such as biogenic amines, amino acids, peptides, glycoproteins, prostanoids, phospholipids, nucleosides and nucleotides, light-retinal, olfactants, and Ca^{2+} .

To understand the possible effects of sequence variations, it is necessary to analyze the molecular architecture of GPCRs. Moreover, we need to address the questions of whether and how GPCRs are related to each other in evolution. This might permit the prediction of functionally relevant domains where sequence variations are most likely to alter receptor function. Lastly, the extraordinary multiplicity of GPCRs represents a critical-and possibly a limiting-factor in our ability to predict the physiological effects of a mutation in a single receptor because of redundancy in signaling networks.

GPCR structure.

Biochemical and biophysical investigations show that GPCRs share a common overall structure characterized by 7 tandemly arranged transmembrane domains (TMDs) (**Figure 1**; for more snake-like views of GPCRs, see **Table 1**, link #3). Because of constraints imposed on their structures by their localization in the cellular membrane, TMDs can be identified by hydropathy analysis and are predicted to be α-helical structures, usually consisting of 20 to 24 amino acids each. These structures are linked through loops that intrude either into the extracellular space (e1-3) or the cytosol (i1-3) and are flanked by an extracellular N-terminal and an intracellular C-terminal tail. Whereas the transmembrane domains are highly conserved among closely related GPCRs, the loops are more variable in sequence and length, and the C- and N-terminal tails represent the most diverse elements.

A number of GPCR genes exist as a single exon, suggesting that gene duplications have involved a mechanism of retroposition. However, many GPCR genes are multiexonic; therefore, we must expect the existence of splice variants with distinct functions, as has been demonstrated for the prostaglandin EP3 receptor subtype. Alternative splicing of EP3 yields at least 4 isoforms that differ in their C-terminus and couple to different G proteins and second messengers[136] . Many more splice variants can be expected that have yet to be studied (for a review, see [137]).

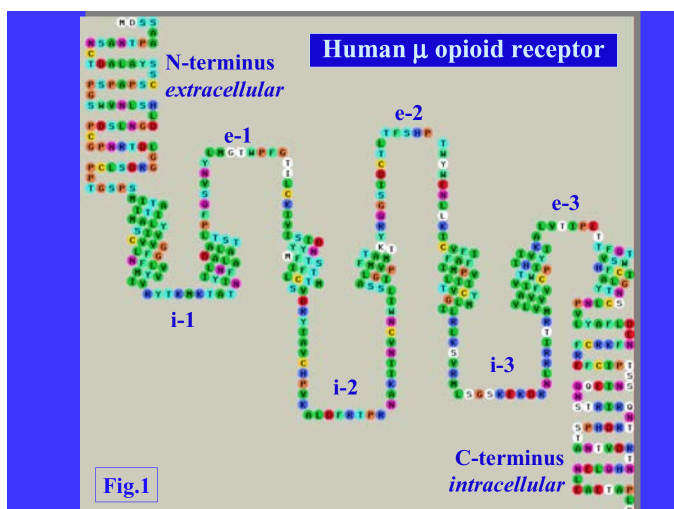

Figure 1.An example of the molecular G protein-coupled receptor architecture: proposed 7-transmembrane domain (TMD) topology of the human μ opioid receptor (MOR). The locations of the 7 TMDs are inferred from hydropathy analysis of the primary structure. The 3 extracellular and intracellular loops (e1-3 and i1-3) and the N- and C-terminal tails can vary considerably in length and sequence conservation among GPCRs.

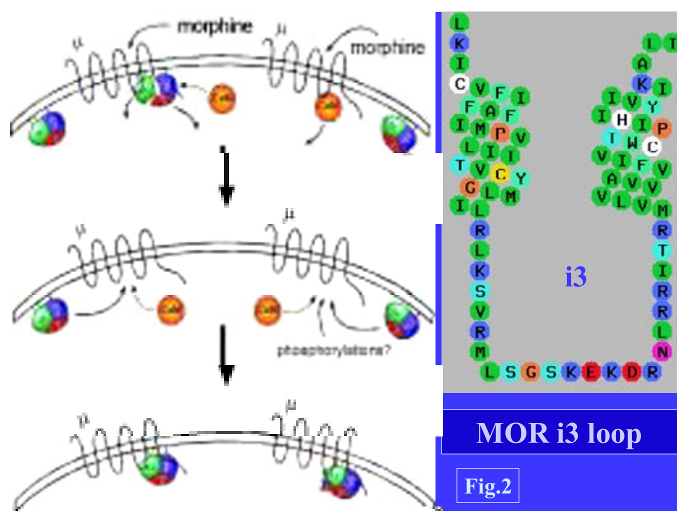

Figure 2.Schematics showing the proposed interactions of calmodulin and G proteins with the i3 loop of the μ opioid receptor (MOR). Calmodulin is thought to block basal G protein coupling, but it is released upon receptor activation by an agonist such as morphine. Conversely, activation of G protein is thought to dissociate the G protein from the receptor, allowing calmodulin to gain access to the receptor. (Calmodulin may also bind to the Gb subunit.). After chronic morphine pretreatment, calmodulin is depleted from the plasma membrane, which appears to permit enhanced access of G proteins to the receptor and, paradoxically, increase basal G protein coupling after morphine pretreatment. Receptor phosphorylation at S268 (a CaM-kinase II consensus site) might play a role in regulating access of G proteins and calmodulin. The i3 loop of MOR contains a calmodulin-binding motif in its C terminal portion, consisting of a predicted amphipathic a-helix with several positively charged residues. Adapted from J Biol Chem. 1999;274:22081-22088; J Neurochem. 2000;75:763-771; J Neurochem. 2000;74:1418-1425.

GPCR ancestry.

Despite compelling similarity in GPCRs' overall structure, the lack of statistically significant sequence similarity among several GPCR families raises the question of whether all GPCRs arose through common ancestry. Thus, vasoactive intestinal peptide, secretin, and metabotropic glutamate receptors show little sequence similarity to other peptide and biogenic amine receptors. In an attempt to understand evolutionary relationships, we have classified the sequences of approximately 1700 GPCRs and unrelated membrane proteins into clusters on the basis of sequence similarities [135] . Taking advantage of the dramatically increased number of cloned GPCRs from many species, this approach resulted in significant alignments between distant GPCR families, including receptors for the biogenic amine/peptide, vasoactive intestinal peptide/secretin, cyclic adenosine monophosphate (cAMP), STE3/MAP3 fungal pheromones, latrophilin, developmental receptors frizzled and smoothened, as well as the more distant metabotropic glutamate receptors. This study provides a refined view of GPCR ancestry, displays conserved sequence motifs for each receptor cluster, and serves as a reference database with hyperlinks to other sources [135] . Nevertheless, the numerous functionally diverse GPCR families often show marginal sequence similarities; therefore, care has to be taken when inferring structure-function relationships by comparing GPCRs from different families. Specifically, we cannot readily extrapolate the effect of sequence variations on structure and function of 1 receptor cluster to another.

GPCR coupling to G proteins and other signaling pathways.

As implied by the name, GPCRs are thought to couple to heterotrimeric G proteins composed of α, β and γ subunits. However, direct proof for G protein coupling remains elusive for the majority of the approximately 600 human GPCRs. G proteins also display considerable heterogeneity, with a predicted number of 27 different α, 5 β, and 13 γ subunits[133] . Upon receptor activation, GDP dissociates from the a subunit, and GTP binds to and activates the G protein. This leads to dissociation of Gα and Gβγ, each capable of triggering multiple downstream events. Main pathways include the regulation of adenylyl cyclases and cAMP phosphodiesterases, phospholipase C pathways, and regulation of ion channel activity. Taking advantage of the inherent GTPase activity of the Ga subunit, the activation process is reversed by production of Gα/GDP and its reassociation with Gβγ. Signal transduction is made more complex by the ability of a single receptor to engage multiple Gα proteins. Moreover, receptor recognition and signaling pathways are also determined by β andγ subunits[137] . Thus, overall effects of receptor activation can have opposite results in different tissues, as shown for the m4 muscarinic receptor-depending on which G proteins are expressed and which signaling molecules are present[138]. Main sites of contact between receptor and G proteins include the third intracellular loop (i3), but i1, i2, and the C-terminus have also been reported to contribute G protein coupling[139-141]. Therefore, the residues critical for coupling need to be determined individually for each receptor subgroup.

In addition to G proteins, GPCRs are known to interact with many other proteins, some of which may also serve signaling functions[142] . Receptor-associated proteins include arrestins, protein kinases and phosphatases, PDZ-domain binding proteins (if a C-terminal PDZ consensus sequence is present), and various modifying enzymes; for example, those introducing palmityl residues into the C-terminus. Each of these proteins modulates receptor functions at distinct domains that are possible targets for polymorphic effects in human GPCR signaling.

We have recently determined that the opioid receptor domain involved in G protein coupling (i3 loop) also interacts directly with calmodulin (**Figure 2**) [143,144] . Upon receptor activation, calmodulin is displaced from the receptor, thereby allowing G protein coupling to proceed while calmodulin itself appears to serve as a novel receptor messenger[145] . Hence, reported sequence variants of μ-opioid receptor (MOR) in its i3 loop could affect either G protein coupling, calmodulin binding, or both (see the following for polymorphic effects). It remains to be seen whether this is a general phenomenon for GPCRs.

GPCR binding pockets

The astounding diversity of receptor ligands begs the question of where the binding site resides and how it is structured. It is inconceivable that Ca^{2+} , acetylcholine, glutamate, bradykinin, prostaglandins, and the large polypeptide follicle-stimulating hormone all bind to the same site. Indeed, for each of these ligands, distinct binding sites appear to exist, either embedded within the pocket formed by the 7-TMD bundle within the membrane (biogenic amines), at pockets formed by the extracellular loops (peptides), or in the N-terminus (glutamate, Ca^{2+}, glycoprotein hormones)[146]. The latter may consist of an evolutionarily distinct protein module. For example, Ca^{2+}, glutamate, γ acid (GABA), and certain pheromones bind to a large N-terminal protein module related in evolution to the periplasmic binding proteins of gram-negative bacteria[147] (**Figure 3**). On the other hand, the thrombin receptor family represents a special case where the protease activity of the ligand thrombin cleaves a portion of the N-terminus. The newly generated N-terminus then serves as a tethered ligand for the receptor, rendering it constitutively active until degraded[148] .

These findings indicate that there is no parsimonious receptor-binding pocket as expected for the catalytic site of enzymes. Rather, GPCRs appear to be activated by ligand binding to many different sites of the protein. At the opioid receptors, peptide endorphins bind primarily to the extracellular loops, whereas opioid alkaloids dock deep into the 7-TMD core[149]. Thus, a single

receptor can be activated by various ligands binding to several distinct, often overlapping sites. Even within the same binding pocket, there is no invariant set of amino acid residues contributing to ligand binding. Studying a series of opioid ligands, Befort et al[150] have found that different residues appear to participate in the binding pocket of δ-opioidreceptor (DOR) even for closely related opioid compounds. This suggests that ligands can bind into the receptor pocket with different orientations, which may be affected by very small changes in chemical structure, including stereoisomers.

In summary, sequence variations in the receptor protein can affect ligand binding or the structural integrity of the receptor, indirectly changing ligand binding. Alternatively, mutations can alter G protein coupling or cellular trafficking such that the receptor is no longer expressed at the cell surface [149] .

Spontaneous GPCR signaling

GPCRs tend to show spontaneous, basal signaling activity in the absence of agonists (also referred to as constitutive activity)[151-153] . Constitutive activity of wild-type β2-adrenergic[154] , serotonin[155,156] , bradykinin[157] , d-opioid[158] , and muscarinic[159] receptors has been reported. Constitutive activity of MOR[160] , and in particular its up-regulation following chronic treatment with opiates, has been hypothesized to account for part of the regulatory mechanism underlying narcotic tolerance and dependence[144, 161] .

By altering the primary structure of GPCRs with site-directed mutagenesis, a number of investigators have found that exchange of single amino acid residues can lead to constitutive receptor activation[162] . Surprisingly, activating point mutations do not map to any specific area but are distributed throughout the receptor protein[146, 163] . This parallels the finding of different ligand binding activation domains. A possible conclusion derived from these studies is that GPCRs generally exist in a constrained inactive conformation that requires some trigger for activation by folding into a more relaxed structure. Indeed, a considerable number of human polymorphisms enhance signaling (gain of function) or even activate the

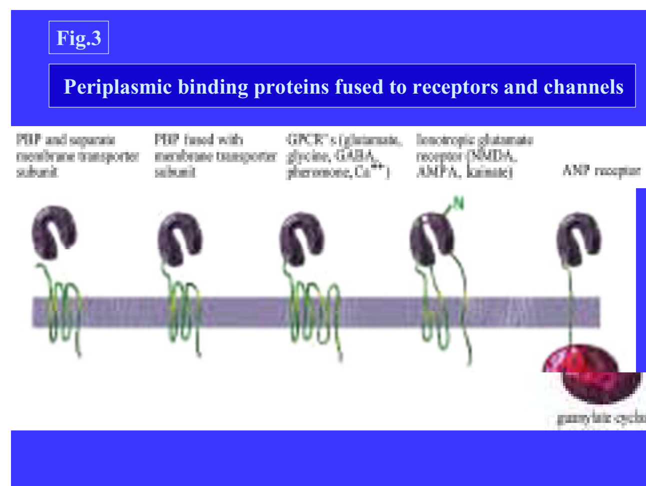

Figure 3.Schematics of the periplasmic binding protein module in various proteins (adapted from AAPS PharmSci. 1999; http://www.pharmsci.org/scientificjournals/pharmsci/journal/venus/index.html). The bacterial periplasmic binding proteins-serving as subunits of solute transporters and chemoreceptors-have fused with receptors and ion channels, providing the ligand-binding pocket. Several polymorphisms map to the PBP (periplasmic binding protein) module of G protein-coupled receptors (Table 1, calcium-sensing receptors).

receptor constitutively, causing serious genetic disorders (**Table 1**). Such mutant alleles are usually dominant and present opportunities for therapeutic intervention.

Basal signaling activity is frequently observed in cell lines in which receptors are overexpressed[154] , but in some cases, basal activity is independent of receptor density[164,165] . This suggests that basal signaling represents an inherent physiological characteristic of the receptor. By definition, antagonists block agonist-mediated activation, but they can have distinct effects at basally active receptors. Those agonists suppressing basal signaling activity of the receptor are referred to as inverse agonists, or antagonists with negative intrinsic activity, whereas neutral antagonists or antagonists with no intrinsic activity do not affect basal signaling[153, 165] . Inverse agonists and neutral antagonists have been identified for a number of GPCRs. This becomes relevant for the treatment of inherited disorders caused by activating GPCR mutations, but inverse agonists have yet to be used clinically.

Lastly, activating mutations, or spontaneously active receptors, can serve as mitogens or oncogenes. This was first discovered by site-directed mutagenesis, rendering GPCRs spontaneously active. For example the α1B-adrenergic receptor becomes mitogenic upon introduction of activating mutations in the C-terminal portion of the third intracellular (i3) loop[166]. The physiological and pharmacological relevance of basal receptor signaling and the role of naturally occurring variant alleles will be discussed in more detail later (see also **Table 1**).

Multiple receptor conformations with distinct functions

Another possible explanation for unpredictable effects of receptor mutations on ligand binding is that GPCRs are flexible structures and may accommodate ligands in various ways. Indeed, GPCRs have been suspected to exist in multiple conformations. Moreover, recent evidence supports the view that discrete conformational states of GPCRs trigger distinct signaling pathways. For example, octopamine and tyramine each stimulate a separate signaling pathway at their common receptor in Drosophila [167]. An activating mutation of the α1B-adrenergic receptor selectively stimulates only 1 of 2 α1B signaling pathways examined[168]. Similarly, structurally distinct ligands differentially activate Gi and Go coupling of cannabinoid receptors[169]. Different MOR agonists vary dramatically in their ability to induce receptor internalization[170,171]. The opioid peptide DAMGO and etorphine, but not morphine, were shown to cause receptor internalization, even though all 3 strongly stimulate G protein coupling. This distinguishes receptor forms active in coupling and internalization. Using various ligands and site-directed mutagenesis, Thomas et al[172] have demonstrated the existence of multiple receptor conformations of the angiotensin II receptor, each supporting distinct functions: G protein coupling, internalization, and receptor phosphorylation. Lastly, numerous ligand-binding studies have revealed the existence of multiple receptor conformations[156, 173,174]. Clearly, this makes it difficult to predict which residues of a receptor will prove relevant for binding a given ligand, impeding rapid progress in receptor pharmacogenetics.

GPCR aggregation

Recent evidence suggests that essential molecules of GPCR signaling pathways are held in close proximity of each other in microdomains such as caveolae and are not freely floating or dependent on random collision to interact[175]. Therefore, access of ligands to receptor microdomains may differ between polar and lipophilic ligands. On the other hand, multiple receptor conformations and complexes might exist that are associated with different signaling pathways via the proteins contained within the complex. Target size analysis of GPCRs in the plasma membrane has revealed the existence of very large GPCR complexes exceeding 1 million d, which partially break up on agonist stimulation[176]. Receptor aggregation as a main organizing principle could lead to oligomeric receptors and functional complexes. Specifically, the DOR was shown to dimerize with itself and with the κ opioid receptor (KOR)[177], whereas the MOR forms oligomers with itself and DOR[178]. Homo- and hetero-oligomerization have been shown to affect the functional properties of these and other GPCRs[179,180]. The presence of low- and high-agonist affinity sites has been associated with formation of a receptor-G protein complex[173] but may also be related to receptor oligomerization as suggested for the m2 muscarinic receptor[174]. Lastly, GPCRs may be in physical contact with ion channels, as shown for a dopamine D5-GABA-A channel[181]. Some of the receptor domains responsible for aggregation have been described, often involving the intracellular C-terminus, but other domains are also likely to contribute. For example, the extracellular binding domains of metabotropic glutamate and GABA receptors are expected to dimerize in a fashion similar to that of periplasmic binding proteins[147]. Moreover, residues in the transmembrane segments may also support oligomerization. As a result, we expect numerous regions of GPCRs to interact with other proteins and promote aggregation in the membrane. Because most putative contact points are unknown, nonsynonymous sequence variants in any portion of the GPCR proteins must be analyzed for functional effect with all currently available assays to establish whether functional changes have occurred.

Receptor multiplicity and drug selectivity.

Through a variety of mechanisms, genes encoding GPCRs have duplicated and spread throughout eukaryotic genomes. Yeast, for example, contains several pheromone receptors and a glucose sensing GPCR, gpr1. The number of proposed GPCRs in the nematode, fruit fly, and human is 248, 146, and 616, respectively[133] . However, there are numerous additional chemo-attractant GPCR-like receptors in nematodes; classification of what constitutes a GPCR may not have been uniformly applied. Closely related genes (ie, those that have duplicated rather late in evolution) may locate next to each other on the same chromosome (tandem duplication, opsins, and olfactory receptors) or on separate chromosomes through translocations. Thus, at least 5 closely related human genes encode muscarinic cholinergic receptors, 5 encode dopamine receptors, and at least 15 encode serotonin receptors. Among these very closely related receptors, the TMDs are often most highly conserved. Because their binding pockets reside within the 7-TMD core, one can readily understand the difficulties in designing receptor subtype-specific drugs. Rather, most central nervous system-active drugs currently in clinical use bind to multiple drug receptors of the same subfamily and, furthermore, cross over to other receptor subfamilies. Chlorpromazine is one of the most promiscuous examples, binding to multiple dopamine, serotonin, and muscarinic acetylcholine receptors. Indeed, the spectrum of affinities to these receptors is thought to play a role in determining efficacy of antischizophrenic drugs, but it is exceedingly difficult to ascertain which receptor subtypes are critical.

Glutamate, GABA, serotonin, and acetylcholine are more commonly known as ligands for ion channels (ionotropic) rather than for GPCRs (metabotropic). Because of the considerably different structure of these ion channels, cross-reactivities at ionotropic receptors for drugs targeting GPCRs are less likely. However, for glutamate and GABA metabotropic and glutamate ionotropic receptors, binding sites share the same origin in evolution, namely the periplasmic binding proteins[147] . This module closes around the ligand-as in a firefly trap-thereby activating the ion channel or GPCR tethered to it (**Figure 3**). Because of this homology, the presence of cross-affinities between receptors and ion channels is conceivable.

Lack of receptor specificity presents a formidable challenge to pharmacogenetic-pharmacogenomic studies. Because schizophrenia is thought to have a multigenic origin, one might suspect that the spectrum of receptor subtype selectivities could determine clinical efficacy of a given drug in the individual patient. Genes involved in the etiology of schizophrenia are under intense investigation, providing the basis on which we may be able to rationally select the optimal drug regimen for individual patients. Likely, genes other than GPCRs will play key roles as well, such as the recently suspected RGS4 locus.

These examples illustrate the complexity of the GPCR signal transduction system. Each cell expresses countless GPCRs that trigger numerous interrelated signaling events. Loss of a functional receptor in a knockout experiment often has surprisingly little effect on overall physiology and behavior of the animal-as shown for the opioid receptors-either because the receptor does not display a basal tone in vivo or because other GPCRs compensate for the defect. However, drug effects can change profoundly. We must be most careful in interpreting sequence variants and their relevance to disease susceptibility and drug efficacy when taken together.

SEQUENCE VARIATIONS OF GPCRS AND ASSOCIATED DISEASES

In view of the large number of GPCRs in the human genome and their critical function in regulating cell behavior, a surprisingly small number of receptor variants have been linked to genetic diseases[146, 163, 182,183] (or search the OMIM; Online Mendelian Inheritance in man) database for GPCRs:http://www.ncbi.nlm.nih.gov:80/entrez/query.fcgi?db=OMIM . Use the pull-down menu and select OMIM, or use link 2 provided in **Table 1**). Similarly, only a few of the sequence variations are known to alter drug effects. We will first summarize sequence variants associated with disease or specific phenotypes, without attempting to be comprehensive (**Table 1**).

Impaired or enhanced agonist signaling efficacy.

Several inactivating sequence variants of peptide receptors have been associated with congenital disorders. For example, a point mutation causing truncation of the thyrotropin-stimulating hormone (TSH) receptor leads to Leydig's cell hypoplasia, and inactivating mutations of the adrenocorticotropic hormone (ACTH) receptor (MC2 receptor) are associated with familial glucocorticoid deficiency [72] . Some receptor variants display enhanced sensitivity to agonists, as reported for the angiotensin II type 1 receptor and the D72E variant of the TSH receptor. The latter mutation occurs in the large N-terminus, the binding site for glycoprotein hormone receptors, leading to toxic multinodular goiter [86] .

V2 vasopressin receptor

A number of mutations in the gene encoding the V2 vasopressin receptor lead to functionally inactive receptor protein and are causative for nephrogenic diabetes insipidus (**Figure 4**)[88,89] . V2 receptors recruit aquaporin-2 channels in the renal collecting ducts responsible for water retention. Thus, inactivating mutations of aquaporin-2 also result in nephrogenic diabetes insipidus[184] . This is a clear indication that receptor activity depends on intact signaling pathways with multiple components, each of which is subject to genetic variability.

The truncation mutation of the V2 vasopressin receptor provides a specific example, which suggests a possible therapeutic intervention. One of the more prevalent missense mutations inserts a termination codon leading to a receptor truncated within the i3 loop[88,89] . The N-terminal fragment consisting of TMDs 1-5 is nonfunctional as a GPCR. If one coexpresses the C-terminal fragment consisting only of the i3 loop, TMDs 6 and 7, and the C-tail, the 2 receptor fragments combine, traffic to the plasma membrane, and display at least partial receptor signaling activity[89] . This could provide an attractive strategy for gene therapy because the C-fragment per se would be inactive and functional receptor would be reconstituted only where the N-terminal fragment is expressed under the normal promoters of the V2 receptor gene.

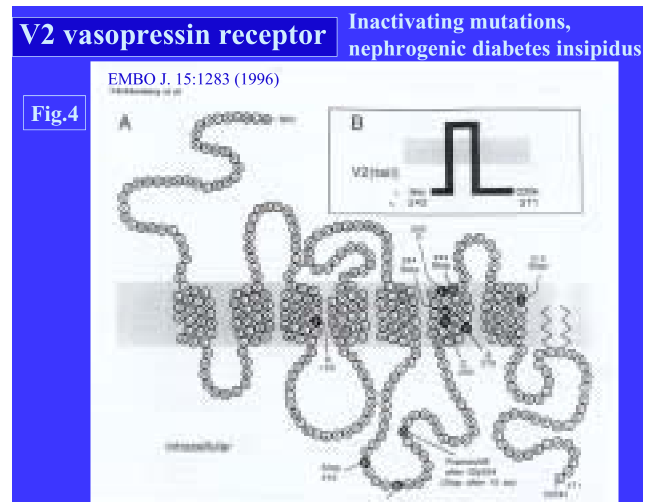

Figure 4.Inactivating mutations of the V2 vasopressin receptor. Note the introduction of a termination codon at position 242 of the polypeptide, leading to a truncated receptor. Cotransfection of the missing C-terminus can restore receptor activity. Reproduced with permission from J Clin Endocrinol Metab. 1999;84:1483-1486.

Thromboxane A2 (TBXA2) receptor

The TBXA2 receptor performs an essential role in hemostasis by inducing platelet aggregation. An R60L amino acid substitution in the first cytoplasmic loop of the TBXA2 receptor causes a dominantly inherited bleeding disorder characterized by defective platelet response to TBXA2[120,121] . The mutant receptor showed decreased agonist-induced second messenger formation despite normal ligand binding affinities. Dominant inheritance of the disorder suggests that the mutation produces a dominant-negative effect by an unknown mechanism. Two isoforms of the human TXA2 receptor with different C-terminals have been cloned, TXR-α and TXR-β, both expressed in human platelets[120,121] . The 2 isoforms show similar ligand-binding characteristics and phospholipase C activation but regulate adenylyl cyclase activity in opposite directions: TXR-α activates adenylyl cyclase, while TXR-β inhibits it. The R60L mutation of TXR-α impairs phospholipase C and adenylyl cyclase stimulation, whereas TXR-β with the same mutation retained its ability to inhibit adenylyl cyclase (**Table 1**; select the OMIM link). Hence, the interaction between splice variants and polymorphisms determines the biological activity of the receptor.

P2Y 12 ADP receptor

Another example of a rare bleeding disorder involving ADP receptors led to the cloning of the elusive Gi-linked P2Y12 receptor and the discovery of a 2-nucleotide deletion in a region mapping to the end of TMD6, associated with the disorder in an affected family[122] . This ADP receptor subtype was then shown to be the target for antithrombotic drugs such as ticlopidine and clopidogrel. In this fashion, the cloning of a gene causing an inherited disorder can serve in the discovery of new therapeutic agents targeted toward this receptor.

Chemokine receptors

Of considerable current interest are sequence variants of chemokine receptors[185] . At least 2 of these (LESTR/fusin and CKR5) have been identified as coreceptors for cellular entry of HIV[186,187] . Similarly, certain chemokines were found to block HIV entry into cells[188,189], presumably by competing with the virus for binding to the chemokine receptor. Hence, natural resistance to HIV infection could occur either by high endogenous levels of chemokines or by mutations of the receptors. Indeed, Samson et al[110] discovered that a 32 bp deletion in CCR5 with high allele frequency in a Caucasian population (0.092), leading to a frame shift and a nonfunctional protein, appeared to protect homozygous carriers against HIV infection and blocked HIV entry into macrophages lacking functional CCR5. Furthermore, Val64 substitution with Ile was shown to result in heterodimerization of CCR2 with CCR5 or CXCR4, thereby promoting resistance to AIDS[101,104,105]. On the other hand, certain CCR5 and CX3CR1 alleles may be correlated with AIDS progression[109,112]. However, in a subsequent communication extending the studies on CX3CR1 and AIDS progression, a group of investigators failed to confirm an association with receptor polymorphisms and concluded that the results "do not support a clear and consistent role for CX3CR1 in HIV pathogenesis"[133].

Virally induced or encoded receptors

The virus-GPCR nexus turns out to be pervasive. Epstein-Barr virus induces the expression of

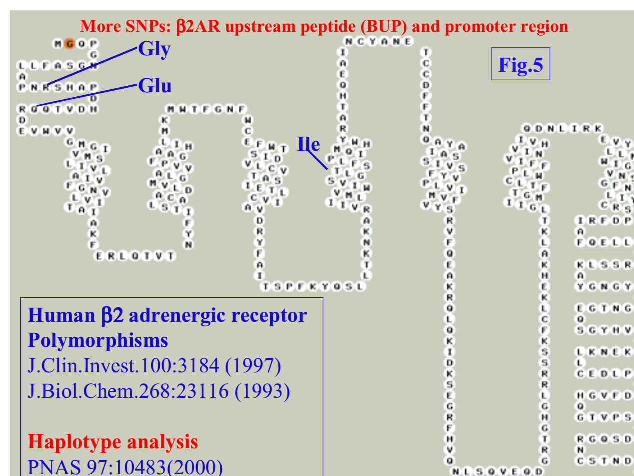

Figure 5. Sequence variants of the β2 adrenergic receptor. Functional consequences are summarized in Table 1 . Q27E introduces resistance to agonist-induced down-regulation if present alone; however, it largely occurs in the same haplotype with R16G. The latter causes more rapid down-regulation and negates the sparing effect of Q27E (see Table 1).

human GPCRs (EBI 1 and EBI 2) in B-lymphocytes as possible mediators of EBV effects[113] . On the other hand, virally encoded GPCRs-apparently hijacked from mammalian genomes-appear to function as essential promoters of infection. For example, the CMV (cytomegalovirus)-encoded GPCR, US28, is a functional β-chemokine receptor[114] and serves also as a coreceptor for HIV-1 entry[115]. In yet another twist of the viral plot, Kaposi's sarcoma-associated herpesvirus harbors 4 genes that mimic the cytokine signaling pathway at various junctions, including genes homologous to the chemokines MIP and IL-6[190]. These examples reveal a complex interplay between the genome of the virus and the host. A viral GPCR gene harbored by Kaposi's sarcoma-associated herpesvirus displays spontaneous activity and serves as a viral oncogene and angiogenesis activator[116]. This provides yet another example of a GPCR as mediator of viral effects, in this case those of Kaposi's sarcoma. Thus, interindividual variability in receptor activity can result from external factors such as viral infections.

Biogenic amine receptors

Numerous polymorphisms/variants have been described for biogenic amine receptors. The R16G substitution in the β2 adrenoceptor has been

associated with nocturnal asthma (**Figure 5**), whereas W64R in the β3 receptor-expressed in adipocytes and involved in energy metabolism-is linked with obesity[16] . Because of the pervasive role of adrenergic receptors, specifically the β2 adrenoceptor, in cardiovascular and pulmonary functions, there has been an intense search for receptor gene variants predisposing to disease, including heart failure, hypertension, and asthma **Table 1**. However, these earlier studies have relied on the analysis of single nucleotide polymorphisms, whereas the physiological relevance of chromosomally phased multiple SNPs (haplotypes) remained unknown. The study of Drysdale et al[15] has demonstrated clearly the need to consider haplotypes, because multiple SNPs on the same strand of DNA can result in different effects from what would have been expected from independent contributions of each SNP alone. This is particularly relevant to the β2 adrenoceptor because the SNPs identified within this gene have distinct effects on receptor function. We will discuss the relevance of β2 adrenoceptor haplotype in more detail later in the context of pharmacological implications.

Intensive studies have also focused on dopaminergic and serotonergic receptors because of their presumed relevance to mental disorders (**Table 1**). However, linking sequence variations of multiple dopamine and serotonin receptors to mental disease has proven difficult at best. Several possible associations between single nucleotide variants and specific disorders are listed in **Table 1** . We have already discussed some of the reasons for this lack of clear association linking receptor variants to disease-this topic is not the main focus of the present review. Lack of strong penetrance and multigenic disease origin are the main complicating factors, along with limited ability to classify the phenotype accurately.

Activating mutations

For several receptors, single nucleotide variants have been reported to lead to activation rather than to inactivation. For example, whereas several inactivating point mutations of the calcium sensing receptor cause familial hypercalcemias, different activating mutations result in hypocalcemias[123-128] . Most of these mutations reside in the large N-terminus, the Ca^{2+} -binding module related to periplasmic binding proteins[147] (**Figure 3**). Because this soluble protein module has a well-defined structure and shares homology with numerous other GPCRs fused to it, we might expect similar, yet-to-be-discovered mutations to occur throughout this receptor subgroup.

Activating mutations are likely to be dominant; thus, a single allele expressing a constitutively active receptor is sufficient and can have profound pathophysiological effects[146,163] . Basally active TSH receptor variants cause thyroid adenomas; the D619G and A623I variants are somatic mutations[87] . Further receptor mutants that signal in the absence of agonists include the parathyroid hormone receptor[77,78] and rhodopsin[129-132] . As pointed out previously, these mutations can occur in various regions of the receptor protein. Of particular interest is the Lys 296 mutation of rhodopsin, which abrogates an ionic bridge to the counterion in TMD3, thereby allowing the receptor to assume an active conformation[131,132] . The result is retinitis and, eventually, blindness. This finding supports the notion that GPCRs are normally constrained in an inactive conformation but can relax into the active conformation after the constraint is released.

Further, spontaneously active GPCRs include a variant of smoothened, which is part of the hedgehog/patched signaling pathway having a key role in basal cell carcinoma. The sonic hedgehog signaling pathway (Shh) proceeds from the soluble Shh to the tumor suppressor patched (PTCH) and the proto-oncogene smoothened (SMO)[117] . Whereas SMO is a member of the 7-TMD GPCR class, PTCH is an integral membrane protein with approximately 9 TMDs unrelated in sequence to the GPCRs[135] . Oncogenic mutations in both PTCH and SMO result in enhanced signaling via the Shh pathway, leading to basal cell carcinoma, medulloblastoma, and other human tumors. Specifically, somatic mutation in SMO has been associated with basal cell carcinoma[119] , the most prevalent cancer worldwide, which is caused by ultraviolet irradiation. This led to a search for drugs capable of suppressing the basal activity of SMO, so-called inverse agonists, or antagonists

with negative intrinsic activity. Cyclopamine, a plant steroidal alkaloid shown to affect the Shh in embryonic development, suppressed basal and stimulated SMO activity and abnormal cell growth associated with SMO and PTCH oncogenic mutations[119] . This provides an intriguing example of a drug discovery taking advantage of activating mutations as a genetic cause of disease. A similar approach may prove valuable for drug discovery targeting activated GPCR variants in general.

The melanocortin receptors MC1-5 have diverse functions throughout the body. With primary location in the skin, the MC1 receptor affects skin pigmentation; receptor variants are associated with skin color[66] but may also play a role in melanoma[67] . Multiple variants have also been reported for the MC4 receptor, a recent focus of interest because of its role in appetite suppression, caloric utilization, and body weight[70] . By integrating signals from melanocortin and Agouti-related protein, an endogenous melanocortin antagonist, MC4 regulates food intake stimuli in the hypothalamus. The presence of endogenous GPCR antagonists is a rare observation; yet, we have found that the opioid peptide dynorphin also serves as an endogenous antagonist, which may regulate melanocortin function under physiological conditions[191] . A rare mutation in the MC4 receptor has recently been shown to account for approximately 4% of cases of extreme obesity[70] . Interestingly, obesity-associated mutations range from inactivating to activating MC4 variants. In contrast, no polymorphisms were associated with morbid obesity in the genes encoding a-MSH or AGRP. Targeting activating variants of MC4 with inverse agonists may lead to therapy of affected individuals.

Spontaneously active wild-type receptors

Spontaneously active variant receptors are to be distinguished from wild-type receptors already endowed with basal signaling activity, including serotonin 5-HT2C, dopamine 1B (D5), B2 bradykinin, MOR, and DOR. The physiological role of basal signaling activity is under debate for these receptor types, but for the histamine H3 receptor, basal signaling contributed to the regulation of histaminergic neurons in vivo[54] . We will discuss MOR polymorphisms separately as an example of the range of polymorphic effects on receptor function, specifically basal signaling.

In all cases of monogenic Mendelian disorders, sequence variations are rare, and in most cases, treatment options are scarce. Yet, it may be possible to design effective therapies for some of these disorders attributed to variant receptor alleles, particularly by designing inverse agonists (antagonists with intrinsic negative activity) for receptors carrying activating mutations.

SEQUENCE VARIATIONS OF GPCRS AND DRUG EFFECTS

Biogenic amine receptors.

Ligand-receptor binding is readily quantified so that a number of variant receptors have been shown to display well-documented altered affinities for their ligands (**Table 1**). However, a single substitution in the binding pocket may affect only 1 type of ligand and not others. This is indeed the case for the T164I variant of the β2 receptor. Thr164 provides a hydrogen bond to the catechol moiety of adrenaline that is absent in β2 antagonists; hence, this mutation strongly reduces catechol binding without having any effect on antagonist binding[11,13] (**Figure 5**).

Similarly, several single-residue variants of the dopamine D1B receptor selectively affect agonist binding[17] . Variant D2 and D3 receptors may also lead to altered drug response and toxicity-for example, increased tardive dyskinesia caused by antipsychotics. However, none of these variant alleles have been conclusively linked to altered drug response, possibly because the frequency of homozygous carriers is low or because the drug effect is mediated by multiple receptors and penetrance of the variant allele is low.

Recently, a sequence variation (N251K) has been mapped to the i3 loop of the α2A adrenergic receptor[1] . Unlike previously described variants of G protein-coupled receptors, where the minor species causes a loss of function, the phenotype of Lys-251 α2A AR represents a gain of agonist-promoted function. Similarly, a G389R polymorphism in the intracellular cytoplasmic tail near the seventh transmembrane-spanning segment

of the human β1 AR leads to a gain of function, enhancing both basal and agonist-stimulated G protein coupling[2] . Occurring at amino acid position 389, Gly or Arg can be found with allele frequencies of 0.26 and 0.74, respectively; the minor allele was previously considered to be the human wild-type β1 AR.

β2 Adrenoceptor

Altered drug response of variant β2 adrenoceptor ranks among the most cited examples of therapeutic consequences resulting from receptor polymorphisms[3-15] . Several SNPs were shown have profound effects on β2 adrenoceptor function when expressed as single mutations of the wild-type receptor in heterologous cells. A R16G β2 adrenoceptor variant was shown to down-regulate more rapidly upon agonist activation (**Table 1**). In contrast, a Q27E substitution protects the receptor against down-regulation (**Figure 5**)[11]. Thus, children with asthma carrying the rather common R16G variant have been suggested to be less responsive clinically to β2 agonists, presumably because the receptor is down-regulated by therapy in vivo[3,4] . However, not all studies have supported this finding, which is based on predictions from in vitro results obtained with β2 adenoceptors containing only a single SNP[15] . Clearly, it is important to consider the haplotype in order to understand the in vivo significance of variant receptor genes. For example, enhanced sensitivity to agonists in individuals with the Q27E β2 receptors (protected from receptor downregulation) may have been expected; however, in the vast majority of cases, R16G and Q27E are located on the same allele-forming a haplotype with 2 sequence variants on the same strand of DNA. It turns out that the R16G substitution overrides the effect of Q27E, causing rapid down-regulation regardless of the presence of the Q27E substitution **Figure 5**) [11] . Recognizing the importance of haplotype, Drysdale et al[15] have determined the β2 adrenoceptor haplotypes of 13 polymorphic sites. Sequence variants included the promoter region of the gene, a T/C allele in the β2 adrenoceptor 5'-leader cistron (β2 adrenoceptor upstream peptide [BUP]), and nonsynonymous SNPs leading to the R16G, Q27D, and T164I substitutions (**Figure 5**). Each of these amino acid substitutions has

Table 2 Haplotype analysis of β2AR gene

Nucleotide Alleles	-47 T/C	46 G/A	79 C/G	491 C/T	Ca	A-A	As	H-L
Haplotype								
1	T	A	C	C	0.7	25	12	10
2	C	G	G	C	48	6	10	28
4	T	A	C	C	33	30	45	40
6	T	G	C	C	13	31	30	13
7	T	G	C	T	1	1.6	0	3.3
	BUP	R16G	Q27E	T164I				

Response (FEV1) to albuterol: haplotype 2 > haplotype 4

Ca: Caucasian
A-A: African American
As: Asian
H-L: Hispanic-Latino

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significant and often opposing effects on receptor function when analyzed in isolation. Of the 8192 possible haplotypes, only 12 were actually found in the study population, and only 4 accounted for the vast majority of all haplotypes (**Table 2**) [15] . Comparing homozygous carriers of either 1 of the 2 most common haplotypes (2/2 and 4/4) revealed a significantly increased response (FEV1) to albuterol in patients with asthma having the 2/2 genotype[15] . Allele frequencies differed substantially between various ethnic populations (**Table 2**). These results demonstrate that at least in the case of the β2 adrenoceptor, single allelic sites fail to predict therapeutic outcome-contradicting earlier results-but rather that the combination of SNPs in a haplotype determines the functionality of the receptor[15] . On the basis of these observations, reassessment of the association between variant β2 adrenoceptors and disease outcome, not only in asthma but also in cardiovascular disorders where adrenergic receptors play major roles as well, is needed.

Although β2 adrenoceptor genotyping appears to offer the opportunity of individualized medication, the clinical value remains to be established. Common clinical protocol stipulates that therapy should proceed to alternative drugs such as steroids if β2 agonists are ineffective. Therefore, potential benefits of genotyping remain to be documented for guiding asthma therapy; however, a better understanding of the functional roles of β2 adrenoceptor haplotypes in disease and therapy might prove valuable for determining factors predisposing to disease and optimizing early treatment.

Schizophrenia and clozapine therapy

A number of GPCR variations have been tested for association with schizophrenia, yielding mixed results (33,39,52,53,192). Some association was reported for dopamine D3 and D4 receptor variants, among others, but the penetrance of these variants was marginal. Nevertheless, it is possible that GPCR variants play a significant role in the etiology of schizophrenia. Because most antischizophrenic agents target GPCRs-predominantly dopamine and serotonin receptors[193,194] -it is likely that GPCR variants may also affect the therapeutic response. Altered ligand binding and drug response have been reported for variants of the serotonin 5-HT2A and C receptors (**Table 2**)[40-46]. In the case of the 5-HT2A and C receptors, these variants maybe associated with altered response to clozapine in the treatment of schizophrenia. This has been tested in some detail for clozapine by Arranz et al [41,44] (**Figure 6**). This atypical antipsychotic agent interacts not only with dopamine receptors, the originally intended target, but also with serotonergic, histaminergic, and muscarinic receptors[193-195] , and moreover with ionotropic GABA-A receptors, a unique property of clozapine[196] . To make matters even more complex, clozapine interacts variably with the 5 muscarinic receptor types either as antagonist or partial agonist[195] . Because of this promiscuity, the therapeutically relevant receptor interactions of clozapine remain elusive, although the 5-HT2A and -2C receptors were proposed as a main target[41] . Only 30% to 60% of treated patients respond favorably to clozapine.

To test whether sequence variants can be identified that determine therapeutic outcome with clozapine, Arranz et al[44] screened a series of polymorphisms in the α2, 5-HT2a, 5-HT2C, and H2 receptors and in the serotonin transporter gene. A combination of 6 polymorphisms resulted in 76% to 77% success in predicting clozapine response[44] (**Figure 6**)-a remarkable result that could presage future clinical applications of pharmacogenetics. These variant alleles involve the genes encoding 5-HT2A and -2C receptors, H2 receptor, and serotonin transporter. However, this analysis not only leaves out several receptors thought to play a role in schizophrenia, it also leaves unclear how the H2 receptor would affect the response and what its role in schizophrenia might be. Therefore, the association of each variant with therapeutic outcome needs to be validated separately before these results can serve in the prediction of therapeutic outcome. Much work remains before genotyping can become useful for optimizing clozapine therapy. Moreover, the results are not readily transferable to other antipsychotic drugs. Antipsychotics and antidepressants are prime candidates for prospective genotyping to select the optimally effective drug because therapeutic response may take weeks to become apparent.

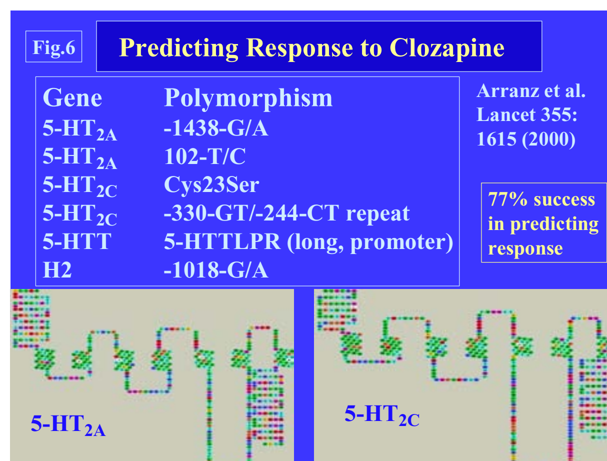

Figure 6.Predicting clinical response to clozapine therapy of schizophrenic patients. Shown are the 6 polymorphisms used by Arranz et al[44] to predict response of individual patients with a 76% to 77% success rate. The predicted secondary structures of 5HT-2A and -2C are also shown as the presumed main targets of clozapine.

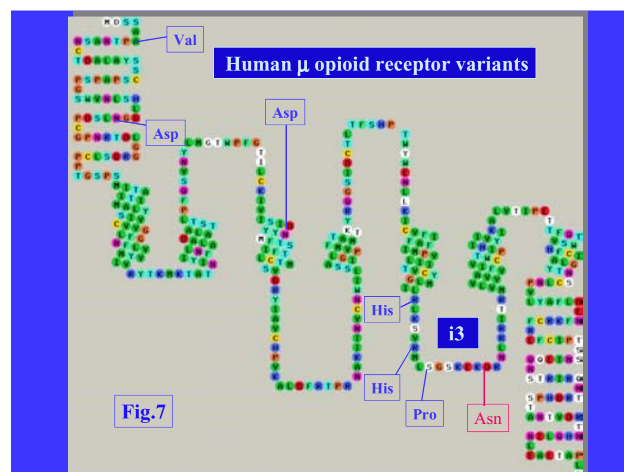

Figure 7.Selected polymorphisms of the μ-opioid receptor (MOR).

Administration of an ineffective drug, therefore, places an undue burden on the patient, both in terms of failure to alleviate symptoms and economics.

Peptide receptors

Protease activated receptor (PAR).

The PAR family includes several receptor subtypes and involves thrombin as 1 of the substrates. The inherent protease activity of the ligand thrombin cleaves the N-terminus, yielding a new N-terminus that serves as a tethered agonist[146] . Recently, a F240S variant of the PAR2 receptor-affecting the second extracellular loop-has been shown to display altered ligand-binding sensitivity[99] The authors speculate that the F240S allele with a frequency of 0.084 may contribute to, or be predictive of, inflammatory disease.

The μ opioid receptor: multiple sequence variations and multiple effects.

Drug addiction involves a strong genetic component. Whereas addiction generally increases with increasing use, the susceptibility to addiction and its severity appears to be largely determined by genetic factors. MOR is the immediate target and mediator of narcotic addiction; moreover, opioid pathways have been implicated in contributing to drug addiction in general-for example, to alcohol and cocaine-by impinging on dopaminergic pathways to the nucleus accumbens, a central reward locus. This has led to compelling incentives for the study of MOR gene variants as possible contributors to genetic predisposition to addiction. Spread over a fairly large genomic region, the multiexonic MOR harbors numerous SNPs in the coding region as well as in noncoding flanking regions[96] . None of these variants has been positively linked to narcotic addiction thus far, suggesting that (multiple) other factors play a role in genetic predisposition to drug abuse. However, human MOR variants altering its primary structure (**Figure 7**) have been studied as to their effect on ligand binding. One of the variant MOR receptors, carrying a relatively frequent N40D substitution, displays 2-fold enhanced binding of β-endorphin[92] . The authors suggest that this change might be relevant to narcotic effects, including addiction; however, it is not clear what, if any, role β-endorphin plays in the process of addiction. An N152D-MOR variant was expressed in reduced quantities upon in vitro transfection, implying some defect in protein folding and trafficking[95] .

Functional studies on the effect of single SNPs determined in isolation neglects the combined effect of multiple SNPs on the same haplotype, as discussed for the adrenergic receptors. Hoehe et al[96] analyzed MOR variations in all known functionally relevant regions of the gene, including 6.7 kilobase regulatory, exonic, and partial intronic sequences. They identified 43 sequence variants in 250 cases (individuals with drug dependence) and controls. By applying a statistical approach to deduce the haplotype (ie, the combination of variants on the same strand of DNA), the authors were able to cluster the haplotypes into 2 functionally related categories. One of these was significantly more frequent in substance-dependent individuals of African American descent, but not in other ethnic groups studied. This reveals ethnic admixture as an important factor in such association studies involving complex traits because ethnic populations are likely to carry distinct sets of polymorphisms and haplotypes. As a result, ethnic admixture is a confounding factor in pharmacogenetic studies unless rigorously controlled for. The results also provide another example of how haplotype analysis can serve to identify complex genotype/phenotype relationships. Although potentially of broad significance, these results need to be validated. In a more limited analysis considering the haplotype associated with only 2 SNPs in the coding region of exon I of MOR (leading to A6V and D40N), Gelernter et al[197] were unable to establish either single polymorphisms or the haplotypes as risk factors in alcohol- and drug-dependent subjects. More work is needed to clarify MOR haplotype contributions to drug addiction.

We are investigating functional changes resulting from variations leading to altered primary structure in the i3 loop of MOR (H260R, H265R, S268P), which represent the primary domain for receptor-G protein coupling (**Figure 7**). Hoellt et al[94] had already demonstrated that the S268P substitution results in a diminution of receptor desensitization, apparently because of the disruption of this CaMK-

II phosphorylation consensus site. Whereas this work was performed with a rat-MOR gene, we have obtained a similar result with the human MOR having an identical i3 loop (Wang et al, submitted). On the other hand, Befort et al [95] have shown that the S268P variant has a reduced maximal capacity for coupling to G proteins, whereas the H265R variant receptor did not show any obvious effects. We have identified yet another sporadic SNP affecting the i3 loop structure of MOR (D274N), but the functional consequences remain unknown[93a].

We have recently established that the i3 loop of MOR interacts with both Gi/Go coupling proteins and with calmodulin[143] because of overlapping sequence motifs required for interaction with either of these 2 major second messengers and cellular regulators. Calmodulin appears to interact with MOR at the i3 loop in such a way that it competes with G proteins binding to MOR (**Figure 2**). One important consequence of MOR-calmodulin binding is to reduce basal (spontaneous) signaling of MOR, which we have proposed plays a key role in narcotic addiction[160,161] . Our results clearly demonstrate that calmodulin serves an important function in regulating basal MOR signaling during morphine exposure[144] . Moreover, calmodulin also may serve as a second messenger of MOR; upon receptor stimulation, it is released from the plasma membrane and calmodulin translocates to the nucleus where it regulates CREB (cAMP response element binding protein) phosphorylation[145] -an event thought to contribute to narcotic dependence. Therefore, we were interested in determining the effects of each polymorphic substitution in the i3 loop on both G protein coupling (in particular, basal coupling) and calmodulin binding. The results show that H260R and H265R have low spontaneous basal G protein-coupling activity, whereas H265R- and S268P-MOR are deficient in calmodulin binding (**Figure 8**)[93a] .

Because these sequence variants are relatively rare (a single allele found in a population sample of 250 individuals), they cannot account for a substantial portion of the genetic predisposition for drug abuse. However, even if rare, these MOR variants might provide new insights into the mechanism underlying narcotic addiction. Conceivably, both

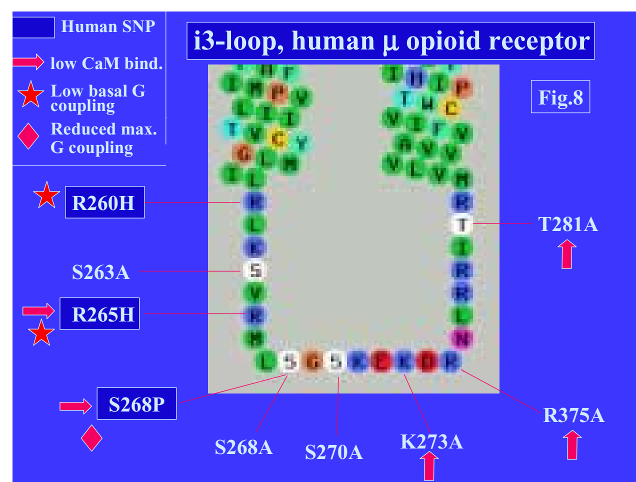

Figure 8.Schematics of the i3 loop of the μ-opioid receptor (MOR), showing the effects of mutations introduced by sit-directed mutagenesis or human polymorphisms (indicated by dark blue boxes) on G protein coupling and interaction with calmodulin. Basal signaling activity is reduced for R260H- and R265H-MOR, whereas calmodulin binding is reduced for R265H- and S268P-MOR (143-145,93a). Note that most variants with low calmodulin binding are located toward the C-terminal portion; however, the N-terminal portion of i3 also appears to play a role.

spontaneous signaling and calmodulin-MOR interactions might play a significant role in the addictive process. This would permit us to search for candidate genes in diverse signaling pathways or to design novel approaches to therapy of addiction. For example, we have recently identified neutral antagonists that do not suppress the up-regulated basal MOR activity in dependent tissue, observed with naloxone and naltrexone. As predicted from the hypothesis that high basal activity plays a role in narcotic dependence, these neutral antagonists caused significantly reduced withdrawal symptoms in morphine-dependent mice[165] .

FUTURE DIRECTIONS

Our knowledge about receptor polymorphisms reveals growing insights into the nature and significance of sequence variations in GPCRs. However, because of structural heterogeneity, receptor multiplicity, and redundancy in complex receptor signaling pathways, identifying the relevance of a single receptor variant is difficult. We suspect that receptor signaling may frequently be impaired by variants of downstream signaling

molecules, rather than the receptor itself. Moreover, genetic disorders, in particular mental illness and neurodegenerative disorders, are multigenic. We have relied on the study of candidate genes or linkage analysis involving finite chromosomal locations. However, progress has been slow, and a new approach is needed to resolve the main questions-which genes predispose to disease and which are linked to drug response, either desired effect or toxicity. A promising approach to resolving these questions comes from genome-wide linkage studies using SNPs. Clinical drug trials with genome-wide scanning were first started by Genset Co., using DNA-array technology with 60 000 SNPs. The SNP projects of Celera, a consortium of leading drug companies (SNP consortium), and the public genome sequencing effort now have amassed in excess of 3 million SNPs, promising to enhance the power of genome-wide association studies. This approach might eventually enable researchers to pinpoint genes that contribute to complex disease and therapeutic outcomes. This could lead to more efficacious therapy tailored toward small patient populations. Before this scenario can be played out, however, we need to develop novel methodologies for extensive SNP analysis and statistical treatment of the resulting complex data sets. This process could likely take decades before it becomes the mainstream approach of the pharmaceutical industry. However, the beacons guiding these developments have been planted, and a compelling future direction for novel drug therapies is beginning to emerge.

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Table 1 Sequence Variants of Human G Protein Coupled Receptors*†

Receptors	**Variant/Allele**	**Disease/Phenotype** **Cellular Mechanism/Event**	**Reference**
Bioaminergic receptors			
Adrenergic receptors			
a2A adrenergic receptor 1 2 3	N251K (i3 loop)	Enhanced agonist-dependent G protein coupling	1
ß1 adrenergic receptor 1 2 3	G389R (C-terminus)	Enhanced basal and agonist-dependent G protein coupling	2
ß2 adrenergic receptor 1 2 3	R16G Q27E T164I C341G Haplotype analysis	Nocturnal asthma Enhanced agonist-promoted downregulation of receptor Decreased response to albuterol Increased severity of asthma Susceptibility to hypertension Depressed exercise performance in heart failure Reduced lung function at maturity Susceptibility to myasthenia gravis? IgE variability? Resistance to down-regulation (unless coupled with R16G) Susceptibility to hypertension Obesity? *Decreased bOH-agonist affinity* Altered coupling to Gs/adenylate cyclase system *Depressed exercise performance in heart failure* Uncoupling from Gs/adenylate cyclase system *Genotype associated with response to albuterol (haplotype* *2/2 greater response than 4/4)*	3 3 4 5 6 7 8 9 10 11 6 12 11 13 7 14 15
ß3 adrenergic receptor 1 2 3	W64R	Obesity	16
Dopaminergic receptors			
D1B (D5) 1 2 3	L88F N351D	Increased affinity of agonist Decreased affinity of agonist	17 17
D2 S/L (414/443)	A1 (TaqI rflp)	Reward deficiency syndrome	18,19

1 2 3	 A1 A2 S311C, P310S, V96A V154I	Obesity Alcoholism? Reduced receptor expression Pathological gambling Tardive dyskinesia? Altered drug affinity Myoclonus dystonia	19 20-22 23 24 25 26 27
D3 1 2 3	BalI S9G (MscI rflp) S9G + 3 other SNPs	No association with schizophrenia Unipolar depression No association with schizophrenia Tardive dyskinesia in schizophrenic patients Haplotype associated with schizophrenia	28 29 30 31,32 33
D4 1 2 3	48 bp repeat in i3 loop coding region V194G C-521C (promoter)	Effect on clozapine binding Effect on G protein coupling Pathological gambling (females) Decreased sensitivity to dopamine and clozapine *Elevated D4-like sites in schizophrenic brains*	34,35 36 37 38 39
Serotonergic Receptors			
5HT2A 12 3	102 T/C (silent) H452Y 1438 G/A	Psychotic symptoms in AD *Decreased response to clozapine* Blunting of calcium mobilization *Decreased response to clozapine* *Effects on clozapine response?* Susceptibility to eating disorders?	40 41-43 44 45 43,44 46
5HT2C 1 2 3	C23S Promoter SNPs	Psychotic symptoms in AD *Increased clozapine response?* Associated with obesity and type II diabetes	40 47 48
5HT1B 1 2 3	F124C	Increased affinity for ligand	49
5HT6 13	C267T (267C-allele)	Increased risk for AD	50
Histaminergic receptors			
H1 12 3	Multiple SNPs	No association with asthma	51
H2 1 2 3	R649G Promoter SNPs (1018 G/A)	Increased incidence in schizophrenia No association with schizophrenia	52 53
H3 1 2	High basal activity of native H3 R	Unknown association with disease	54
Peptide receptors			
Angiotensin II	1166 A/C	Coronary atheroma?	55

type 1 1 2 3	Multiple SNPs	Increased risk of ischemic events Influences aortic stiffness Increased angiotensin response	56 57 58,59
Endothelin A 1 2 3 **Endothelin B** 1 2 3	Multiple SNPs Multiple SNPs W276C	No association with myocardial infarction Affect pulse pressure? No association with myocardial infarction Hirschsprung's disease	60 60 60 61
Luteinizing hormone 12 3	Truncated TM5 D578G T398M	Leydig's cell hyperplasia Precocious puberty in male children Constitutively activated LH receptor *Activating mutation;* variable association with familial precocious male puberty	62 63 63 64
FSH 1 23	A189V	Ovarian dysgenesis Altered protein folding, inactivation of receptor	65
Melanocortin (MC1R) 1 2 3 MC4R 1 2 3	D294H D84E V92M Multiple SNPs	Red hair/fair skin Development of melanoma Red hair/fair skin Development of melanoma? Low affinity of receptor for ligand Activating or inactivating SNPs Morbid obesity	66 67 68 68,69 68 70 70,71
ACTH (MC2R) 1 2 3	S120R, R201Stop, S74I, V254C Promoter polymorphism	Fam. glucocorticoid def., altered/loss of receptor function Reduced expression; loss of heterozygosity in adrenocortical tumors	72-75 76
Parathyroid hormone (PTH) 1 2 3	H223R, T410P, I458R P132L Delet. bp 1122 (frame shift),1176 G/A	Jansen's metaphyseal chondrodysplasia, constitutes active receptor Blomstrand's chondrodysplasia, no accumulation of cAMP Blomstrand's chondrodysplasia	77-79 80 81,82
Thyrotropin (TSH) 1 23	P52T D727E D619G, A623I (somatic)	Possible influence on autoimmune thyroid disease (Graves disease); *altered receptor function/ conformation* Toxic multinodular goiter Increased cAMP response *Hyperfunctioning thyroid adenomas* Constitutively activation of adenylate cyclase	83,84,85 86 86 87
Vasopressin V2-receptor	Multiple SNPs	Nephrogenic diabetes insipidus Decreased ligand binding	88,89

1 2 3	R113W R137H	Reduced expression of receptor Decreased ligand affinity, decreased coupling to the Gs/adenylate cyclase system *Decreased stimulation of the Gs/adenylate cyclase system*	90 90 91
Opioid Receptors **μ-opioid receptor (MOR)** 1 2 3 **d-opioid receptor (DOR)** 1 2 3	N40D H260R H265R S268P N273D N152D Haplotype analysis 921 T/C	Increased affinity/ potency of b-endorphin Idiopathic absence epilepsy? Reduced basal G-protein coupling Reduced CaM binding, reduced basal G-protein coupling *Reduced CaM binding, reduced basal G-protein coupling, reduced desensitization, and maximal agonist activation* Unknown functional change Reduced receptor expression in vitro Association with substance dependence Susceptibility to substance abuse still under review	92 93 93a 93a 93a 94, 95, 93a 95 96 97,98
Protease activated receptor PAR2 **1 2**	F240S	Altered PAR2 ligand binding/activation	99
FMLP (N-formyl peptide) receptor **1 2 3**	F110S, C126W (i2 loop)	Possible association with decreased chemotactic activity in patients with localized juvenile periodontitis	100
Chemokine receptors **CCR2** 12 3 **CCR3** 1 2 3 **CCR5** 1 2 3 **CX3CR1** 1 2 3	V64I R275Q, L351P, 51 T/C (silent), 240 L/T (silent) CCR5P1 alleles Δccr5 (32 bp deletion) 59029 A/G -homozygotes -heterozygotes V249I, T280M	Delayed progression of AIDS Delayed progression of sarcoidosis Heterodimer with CCR5 or CXCR4 Susceptibility to insulin dependent diabetes mellitus? Unknown functional change or influence on disease Increased progression of AIDS Altered binding affinity Resistance to HIV infection Delayed progression of AIDS? Decreased risk of non-Hodgkin's lymphoma Increased progression of AIDS	101,102 103 104 105 106 107,108 107 109,110 102 109 111 112
Virally induced or encoded receptors **EBI 1 (CCR7, homologous to**	EBV-induced	Mediator of EBV effects on B lymphocytes	113

IL8 R) 1 23 **EBI 2 (homologous to thrombin R)** 1 2 3 **US28 (viral homolog of RANTES R)** 1 **KSHV GPCR (viral homolog of IL8R)** 1	EBV-induced Human CMV encoded Kaposi's sarcoma-associated herpesvirus encoded	Mediator of EBV effects on B lymphocytes b-chemokine like receptor with role in CMV infection; serves as coreceptor for HIV-1 entry (cell line adapted for CXCR4) Candidate viral oncogene in Kaposi's sarcoma, high constitutive signaling activity	113 114 115 116
Other GPCRs			
Smoothened 1 23	W535L Multiple oncogenic mutations	Activating somatic mutation Basal cell carcinoma (hedgehog-patched path) reversal by cyclopamine	117,118 119
Thromboxane A2 (TRXa and TRXß) 123	R60L	Bleeding disorder*. Impaired coupling to phospholipase C and adenylyl cyclase (but R60L-TRXß inhibits AC normally).*	120,121
ADP receptor P2Y$_{12}$ 12	Del of 2 nt (TTCATT) in coding region (end of TMD6)	*Nonfunctional Gi-linked ADP receptor.* Bleeding disorder.	122
Ca-Sensing 123	L174R,P40A, R63M, R67C, G144E, T139M, R228Q, R198E, E298K, R796W, E128A N118K, F128L, T151M, E191K, F612S (N-term)	Familial hypocalciuric hypercalcemia/neonatal severe hyperparathyroidism Familial hypocalcemia, increased IP3-response Hypocalcemia	123-126 127 128
Rhodopsin 12 3	G90D, A292E, K296E/ Glu 113 E134Q E134D	Retinitis pigmentosa, congenital night blindness Constitutively activated receptor Increased activity of opsin *Decreased activity of opsin*	129-131 132 132

*Each receptor is linked to ENTREZ GenPept Report, Online Mendelian Inheritance in Man (OMIM) database, and GPCRDB Viseur's snake-like view of the protein structure. Changes in receptor function are listed in italics. Click on: 1 = Entrez (and further links); 2 = OMIM database; 3 = Snake-like view (Viseur, available at G protein-coupled receptor (GPCR) database :http://www.gpcr.org/7tm/). This site also contains alignments, phylogenetic trees, 3-dimensional models, chromosomal locations, ligand binding constants, and mutation data. For modeling the 3-dimensional structure of any GPCR, go to: http://www.expasy.ch/swissmod/SWISS-MODEL.html.

†A indicates alanine; C, cysteine; D, aspartate; E, glutamate; F, phenylalanine; G, glycine; H, histamine; K, lycine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine; AD, Alzheimer's disease; AIDS, acquired immunodeficiency syndrome; cAMP, cyclic adenosine monophosphate; CMV, cytomegalovirus; EBV, Epstein-Barr virus; FSH, follicle-stimulating hormone; HIV, human immunodeficiency virus; LH, luteinizing hormone; SNP, single nucleotide polymorphisms.