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Genetic Vulnerability of GPCRs: A call to action

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Abstract

G protein-coupled receptors (GPCRs) are the targets for many drugs, but the response shows interindividual variability. The “one-drug-fits-all” approach has been challenged by evidence showing multiple human genetic variants of GPCRs. Identification and characterization of GPCR variants must be undertaken for rational, personalized, and economically sound prescribing practices.

Keywords

Polymorphism; mutation; G protein; coupling; asthma; heart failure

G protein-coupled receptors (GPCRs) are the largest superfamily of proteins in the body. They carry out a diverse array of signaling, controlling every cell and organ. This includes receptors for neurotransmission, hormonal/endocrine, autocrine and paracrine actions, metabolic products, immune modulators, and even taste, smell, and sight. This control over critical systems such as hypothalamic/pituitary, cardiovascular, gastrointestinal, lung, renal, reproduction, and brain has resulted in a large number of drugs targeted to GPCRs. In fact, these are the most prescribed drugs in medicine, and include agonists (activators) and antagonists (blockers) to these receptors. GPCRs have a similar topography (Figure 1A), with an extracellular amino-terminus, seven transmembrane spanning domains (TMDs), three extracellular and three intracellular loops, and an intracellular carboxy-terminus. Signaling occurs when agonist binding stabilizes the receptor in a conformation that binds to a heterotrimeric G protein, whose components act at other proteins termed effectors, which serve to generate a “second messenger” initiating the intracellular function. A classic example is the β_2 -adrenergic receptor (β_2 AR), which couples to Gs, thus activating the effector adenylyl cyclase, catalyzing the production of the second messenger cAMP.

It is widely recognized that the responses to these drugs (and virtually all therapeutic agents) display interindividual variability, typically in the form of a bell-shaped curve (Figure 1B). Unfortunately, most publications (or governmental approval agencies) assess efficacy and safety based on the *average* response. However, when responses are binned as shown in Figure 1B, it becomes clear that there are “non-responders”, “responders” and “super-responders” within the study population for most drugs. How could we identify these

patients? For drugs acting at GPCRs, this question may be dependent on whether these receptors display genetic variation in the population. Such variation has been considered to have several potential consequences: 1) alter the risk of developing a disease, 2) define physiological or clinical phenotypes of healthy individuals or those with a disease, or 3) alter the response to therapy (termed pharmacogenetics or pharmacogenomics).

In a recent paper, Hauser et al [1] explored the genetic variability of GPCPs to new depths to reveal considerable functional variation. Historically, we have known about such variability, typically for certain classes of GPCRs, for more than two decades [2, 3]. The first DNA variation leading to a change in the amino acid sequence (termed nonsynonymous or missense) was published in 1993 by our group [3]. We found missense variations (MV) in the coding region of β_2 AR by sequencing genomic DNA from normal subjects and those with asthma. β_2 ARs are expressed on airway smooth muscle, and when activated relax the muscle and open the airways; they are the targets for β -agonists used for treating asthma, and considerable interindividual variation in the response to β -agonists is well recognized. This initial study was followed by defining the signaling phenotypes of these MVs in transfected model cells, isolated human airway smooth muscle cells, and targeted transgenic mice [2]. Ultimately clinical studies in asthma and heart failure (β_2 ARs are also expressed in the heart) were performed [4, 5]. And indeed, patients displayed clinically significant phenotypic differences or response to therapy when grouped by genotype. Additional sequencing into the 5'UTR and promoter region of this intronless gene showed even more variability, which altered transcription factor binding and receptor expression [6]. Soon it became clear that certain combinations of variants(haplotypes) were the best predictors of certain asthmatic responses to β -agonists [6]. All of this, and it was still just one GPCR: was such variability present in other GPCRs? The same discovery, *in vitro*, mouse, and human subject studies (Figure 1C) were performed with the β_1 AR, α_{2A} , α_{2B} , and α_{2C} ARs [2, 7, 8]. This was enough to convince us that the largest druggable family of proteins displayed substantial variability, and would be the segue into modern pharmacogenomics for common diseases, leading to personalized medicine. When the human genome projects were published in 2001, I stated that personalized medicine would be in common practice within 10 years [9]. But this did not happen, and I contend that we are now realizing the consequences at the clinical and economic levels of this inaction. There are virtually no routine tests or guidelines for tailoring GPCR-targeted drugs based on genetic variation, and pharmaceutical companies continue to attempt to market with a "one drug fits all" approach.

We subsequently extended these findings to 64 GPCRs by targeted sequencing of a group of 82 ethnically diverse individuals [10]. And there it was again: over 400 variants with mean allele frequencies (MAF) of 1%, and no GPCR was found without a variation. Hauser et al [1] have moved the field forward by thinking about GPCR variants in a somewhat different way. Yes, of course we want to know about the common variants, and many were found. But rare variants that affect the receptor in the same manner (such as altered ligand affinity or functional coupling to G protein) can be grouped. The group, then, can represent a phenotype (and thus drug response) that is more common than when each variant is considered alone. This knowledge would then have potential consequences for drug development, clinical trial design, personalized prescribing, and predictions of the economic burden of genetic variation in regards to altered efficacy or adverse effects. They examined

GPCR sequences *in silico* from 2,504 individuals that had undergone sequencing in the 1000 Genomes Project. On average, they found that an individual has 68 MVs within the coding region of one-third of the 23 GPCRs examined (for 78 drugs). In addition, using the exome aggregation consortium, sequence for 108 GPCR genes from 60,000 individuals were investigated. On average 25% of positions contained a MV, a rather remarkable level of variability. On average each receptor had 3.7 MVs with MAF = 0.1% and 128 MVs with MAF <0.1%. MVs were more frequent in the TMDs and loops (Figure 1A) areas known to define ligand binding and functional coupling to G proteins. Although many studies have examined the effects of MVs using recombinant expression, the authors tested variants found in the μ -opioid and cholecystokinin receptor. In both cases, altered agonist-promoted signaling was observed with a MV receptor compared to wild-type. However, these functional changes *were not* readily predicted from a previous structure/function or crystal structure information. This represents an interesting conundrum. Do we perform *in vitro* studies for all variants detected? I have always contended that all MVs should be studied, but had no real idea as to the extent of GPCR variability. Nevertheless, using some newer high-throughput techniques it can be done efficiently, and in a practical manner, if MAF cut-offs and a priority of GPCRs are established. As described by Hauser et al [1], the economic benefit of tailoring therapy could be immense. In the UK, the cost of prescribing ineffective drugs targeting GPCRs was estimated to range from 14–501 GBP. This sort of estimate will require refinement as we gain additional information. For example, a loss of function variant due to a low affinity of the receptor for agonist might be overcome by increasing the dose or using an agonist with different structural features.

Nevertheless, the body of work, now extending over 20 years, and significantly bolstered by the elegant work of Hauser et al [1], lays to rest the question of whether these important drug targets display relevant genetic variability, and represent a call for action. This will serve to match patients to the right drug, and, may salvage drugs where efficacy is found in only a subpopulation (Figure 1B). An international consortium to phenotype these variants (Figure 1C), and to then establish prescribing guidelines based on this genetic and phenotype information, is clearly in order, both to improve patient care and decrease economic burden.

References

1. Hauser AS, et al. Pharmacogenomics of GPCR Drug Targets. *Cell*. 2018; 172(1–2):41–54.e19. [PubMed: 29249361]
2. Small KM, et al. Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu Rev Pharmacol Toxicol*. 2003; 43:381–411. [PubMed: 12540746]
3. Reihnsaus E, et al. Mutations in the gene encoding for the beta 2-adrenergic receptor in normal and asthmatic subjects. *Am J Respir Cell Mol Biol*. 1993; 8(3):334–9. [PubMed: 8383511]
4. Israel E, et al. Use of regularly scheduled albuterol treatment in asthma: genotype-stratified, randomised, placebo-controlled cross-over trial. *Lancet*. 2004; 364:1505–1512. [PubMed: 15500895]
5. Wagoner LE, et al. Polymorphisms of the β_2 -adrenergic receptor determine exercise capacity in patients with heart failure. *Circ Res*. 2000; 86:834–840. [PubMed: 10785504]
6. Drysdale CM, et al. Complex promoter and coding region β_2 -adrenergic receptor haplotypes alter receptor expression and predict *in vivo* responsiveness. *Proc Natl Acad Sci U S A*. 2000; 97:10483–10488. [PubMed: 10984540]

7. Miallet-Perez J, et al. β_1 -adrenergic receptor polymorphisms confer differential function and predisposition to heart failure. *Nat Med.* 2003; 9(10):1300–1305. [PubMed: 14502278]
8. Liggett SB, et al. A polymorphism within a conserved β_1 -adrenergic receptor motif alters cardiac function and b-blocker response in human heart failure. *Proc Natl Acad Sci U S A, U.S.A.* 2006; 103(30):11288–11293.
9. Liggett SB. Pharmacogenetic applications of the Human Genome Project. *Nat Med.* 2001; 7:281–283. [PubMed: 11231618]
10. Small KM, et al. Gene and protein domain-specific patterns of genetic variability within the G-protein coupled receptor superfamily. *Am J Pharmacogenomics.* 2003; 3(1):65–71. [PubMed: 12562217]

Figure 1. GPCR structure, clinical variability, and a variant phenotyping scheme

A) The typography of a GPCR, depicting the TMD segments, the extracellular loops (ECL), intracellular loops (ICL), and amino-terminal (extracellular) and carboxy-terminal (intracellular) regions. Each circle represents an amino-acid. Shown is the β_2 AR. B) The distribution of clinical responses grouped by the magnitude of the response. The data represent a hypothetical example. C) Variant discovery from a multiethnic DNA repository is utilized to ascertain potential functional implications. Cloning or mutagenesis is used to make the variant cDNA for transfection of model cells to ascertain basic pharmacology. Additional studies of pharmacology and physiology can also be obtained using genetically altered mice, human tissue, or human physiological studies. Taken together, they can lead to a hypothesis-driven clinical trial (prospective or retrospective) which may show associations between variant and drug response. Data can also provide leads for additional variant screening of other genes.