



Universiteit
Leiden
The Netherlands

Computers and drug discovery : construction and data mining of chemical and biological databases

Kazius, J.

Citation

Kazius, J. (2008, June 11). *Computers and drug discovery : construction and data mining of chemical and biological databases*. Retrieved from <https://hdl.handle.net/1887/12954>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/12954>

Note: To cite this publication please use the final published version (if applicable).

Chapter 2

Natural Variants and their Impact on G Protein-Coupled Receptors

Natural variants in human DNA may contribute to inter-individual diversity. Such variants differ in allele frequency, their effect on protein functioning and thus their potential for disease association. Variants of G protein-coupled receptors (GPCRs) may be serious risk factors since these proteins regulate important biological functions and serve as prominent drug targets. In comparison to rare GPCR mutations, few GPCR polymorphisms have been convincingly linked to disease susceptibility. Copy number polymorphisms (CNPs) may be stronger than single nucleotide polymorphisms (SNPs) as predisposition factors for clinical manifestations. By cataloguing data of diverse small and large natural variants (NaVa) from various sources, the GPCR NaVa database facilitates studies into (pharmaco)genetics, genotype-phenotype and structure-function relationships of GPCRs.

Definitions used in Chapters 2, 3 and 4.

Variant	A genetic variation of low, high or unknown allele frequency, including rare mutations and common polymorphisms.
Mutation	A variant with an allele frequency close to 0%.
Polymorphism	A variant with an allele frequency of over 1%, which thus occurs in at least 2% of a human population.
Allele frequency	The fraction of human chromosomes that contain the reference or the mutated nucleotide(s), the major and minor allele, respectively. A minor allele frequency of 10% implies that, on average, 19% of the subjects have the minor allele in at least one chromosome (1% is homo- and 18% is heterozygous for the minor allele, while 81% is homozygous for the major allele).
Single nucleotide polymorphism (SNP)	A polymorphic single base-pair variant.
Copy number variant (CNV) or polymorphism (CNP)	A large segment of DNA, consisting of at least several kb, that can occur zero to several times at one site in a genome.
G protein-coupled receptor (GPCR)	Cell membrane-spanning receptor that selectively recognises a specific, extracellular ligand to produce an intracellular response.
Transmembrane domain (TMD)	A strand of amino acids that is shaped like an alpha helix and that spans the cell membrane.

INTRODUCTION

Inter-individual diversity exists in disease susceptibility, disease progression, drug response, and in characteristics of lesser clinical relevance. Genetic variation may be an important cause for this variability in phenotype. The post-genomic era has revealed millions of natural variants¹⁻³, which are defined as *variants* that occur naturally within human DNA. The more specific terms *mutations* or *polymorphisms* indicate variants with established low or high *allele frequency*. Allele frequencies quantify the fraction of human chromosomes that contain the reference or the mutated nucleotide(s), the major and minor allele, respectively. The term variants therefore includes genetic variations of low, high or unknown allele frequency, including rare mutations (allele frequency of 0-1%) and common polymorphisms (allele frequency of over 1%). Due to their abundance and frequency, human polymorphisms account for a significant part of the inter-individual variation in DNA sequences.

Focus on GPCRs

Relatively many natural variants occur in the superfamily of *G protein-coupled receptors (GPCRs)*^{4, 5}. As of August 2007, 774 full-length GPCRs are known in man⁶. Of these, 373 are olfactory GPCRs and 401 are non-olfactory GPCRs. As the latter regulate critical functions in diverse biological pathways, several non-olfactory GPCRs are key therapeutic targets. With respect to other protein families, the GPCR superfamily is most often targeted by today's medicines⁷. GPCR-targeted drugs show potencies and efficacies that differ between patients⁴. These inter-individual differences might, in part, be explained by the occurrence of natural variants in GPCRs⁷. Knowledge of GPCR polymorphisms that convincingly influence drug-induced clinical effects can aid the design of cohort stratification, diagnostics, clinical trials and therapies. Mutations in the vasopressin V₂ receptor, rhodopsin, and the melanocortin MC₄ receptor, respectively cause prominent adverse phenotypes, such as nephrogenic diabetes insipidus, retinitis pigmentosa and early-onset obesity^{8, 9}. Variants that affect GPCRs are therefore possible predisposition factors for clinical phenotypes. Though our discussion of variants is general, we will use specific examples from the GPCR superfamily. We will first briefly overview the subtypes of variants at the DNA level, then highlight example effects at the protein level that are caused by rare and common genetic variants, and finally discuss various associations of GPCR polymorphisms with clinical phenotypes. The impact on GPCR structure and function of selected subsets of natural variants have been discussed abundantly and are therefore outside the scope of this opinion article^{5, 7, 8, 10-16}.

DISCUSSION

DNA variant subtypes

The simplest genetic variants are single base-pair substitutions such as single nucleotide polymorphisms (SNPs), which are polymorphic single base-pair variants. As millions of SNPs exist, they are also the most abundant type of variant: on average, single nucleotide variants occur about two to three times per kb². Slightly more complex variants are, for example, deletions, insertions and repeats as they cover one or more nucleotides. Though these variants appear infrequently in coding DNA, they occur roughly once per two kb in noncoding DNA².

More recently, researchers have identified hundreds of copy number variants (CNVs) and established their polymorphic character^{3, 17}. A CNV is a large segment of DNA, consisting of at least several kb, that can occur zero to several times at one site in the genome. Due to their size, CNVs can contain one or more complete genes. For example, one CNV in chromosome 1 is 600 kb long and contains 17 genes, of which 15 encode for olfactory receptors. Redon et al.³ estimated that all CNVs together cover more DNA than all SNPs and thus have a larger potential to explain inter-individual variability.

Table 2.1 shows the different databases that catalogue subsets of natural variants in all human genes/proteins. Of note, only the Database of Genomic Variants incorporates CNV data. The coverage of very frequent small polymorphisms by databases such as dbSNP may soon be exhaustive. However, the coverage of less common mutations, especially those that cause no monogenetic disease, might never become as extensive.

Table 2.1. Databases on natural variants.

Database name	Content	Website address
dbSNP	○ Small genetic variants (largest resource) ○ Allele frequency data	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=SNP
HapMap	○ Small genetic variants ○ Allele frequency data	http://www.hapmap.org
OMIM (Online Mendelian Inheritance in Man)	○ Rare, disease-related, small genetic variants ○ Phenotype description	http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
HGMD (Human Gene Mutation Database)	○ Rare, disease-related, small genetic variants ○ Phenotype description	http://www.hgmd.cf.ac.uk
Swiss-Prot Variant section of UniProt	○ Small missense variants ○ Phenotype description (brief)	http://www.uniprot.expasy.org
Database of Genomic Variants	○ Copy Number Variants	http://projects.tcag.ca/variation

Impact on protein

In general, effects at the protein level are least likely for small genetic variants that lie far from gene loci, at intergenic regions. Variants in introns, even those far from a splice-site, have the potential to affect the degradation and splicing of pre-mRNA. These processes can affect the expression levels of mRNA and thus of proteins and/or their splice variants. However, no conclusive associations with disease have been reported for polymorphisms in introns that lie far (over 15 bp) from a splice-site of a GPCR gene, perhaps because intronic variants have been granted little attention.

Though promoter variants cannot directly alter pre-mRNA mechanisms, they are more liable to influence phenotype as they can affect transcription efficiency and thereby the expression levels of premature and mature mRNA. Unfortunately, promoter sites are rarely defined and incorporated in genetic databases. Promoter polymorphisms of at least two GPCRs have been associated with an increased occurrence of aspirin-intolerant asthma and showed effects *in vitro*^{18, 19}. More specifically, SNPs of the prostaglandin E₂ receptor subtype 2 (EP₂) affected DNA-binding of an unidentified protein¹⁸ and SNPs of the cysteinyl leukotriene receptor subtype 1 (CysLT₁) altered gene expression¹⁹.

Noncoding variants in exons, either in 5' or 3' untranslated regions, and silent variants may interfere not only at the pre-mRNA stage, but also in the post-transcriptional phase, thus affecting the expression levels and/or stability of mature mRNA. Notably, effects on mRNA expression have been shown *in vitro* for several polymorphisms, like noncoding asthma-associated polymorphisms in the orphan receptor GPR44²⁰ and silent polymorphisms (including Pro319) in the dopamine D₂ receptor²¹.

Missense and more severe coding variants cannot only affect mRNA, they can also interfere with quality control systems in the endoplasmic reticulum (ER) and with the subsequent transport of proteins to, for instance, the cell membrane. The majority of disease-causing mutations in GPCRs alter both the gene and the protein sequence and they act at this step to cause a loss of function⁸⁻¹¹. Moreover, most gain of function-phenotypes are also caused by missense variants¹⁴. Examples of rare mutations that distort post-translational mechanisms are the Thr4Lys and Asn15Ser mutations in rhodopsin, which affect N-glycosylation and have been linked to retinitis pigmentosa⁵. A polymorphism that has been associated with delayed AIDS progression is the Δ 32 polymorphism in the chemokine receptor 5 (CCR5). This Δ 32 polymorphism deletes 32 base pairs and thus causes a frameshift and an early truncation of the CCR5, which is retained in the ER²². The polymorphism thereby abolished the expression of functional CCR5 in transfected cells and in homozygous natural lymphocytes²².

Small polymorphisms and their disease association

The challenge for years to come remains to discover which of the human variants are causally involved in clinical phenotypes. Most natural variants probably have no effect as millions of polymorphisms lack an obvious phenotype², while ‘only’ a few thousand rare mutations cause monogenetic diseases⁹.

The least disputed case of a GPCR polymorphism that has been causally linked to human phenotype, and to effects *in vitro*, is the CCR5 $\Delta 32$ polymorphism described in the previous paragraph. The polymorphism is exceptional in that about 80% of the studies reported a positive association when testing for its delay of the progression of AIDS in man²². Such strong agreement on clinical effects may relate to the severe effect the CCR5 $\Delta 32$ polymorphism has *in vitro*²².

On the other hand, missense polymorphisms in GPCRs can lack functionality *in vitro* as well as in man. For one, the Arg347Cys polymorphism in the α_{1A} adrenoceptor did not cause a change in ligand binding, signal transduction or receptor desensitization properties²³. In agreement with such findings, the Arg347Cys polymorphism has not been linked to a predisposition for an adverse human phenotype, though it has been tested for association with clozapine-induced side effects, benign prostatic hyperplasia/hypertrophy, hypertension and numerous mental disorders²⁴. Similarly, silent DNA variants (Ile178, Gly183, Lys294) in the α_{1B} adrenoceptor lack an effect *in vitro* and in the clinic²⁴.

Even when multiple studies suggest an effect on a phenotype, links with data *in vitro* are not always intuitive. The Val103Ile polymorphism in the melanocortin MC₄ receptor, for instance, was recently associated with a decreased chance for obesity in a meta-analysis²⁵ and with higher energy expenditure in healthy volunteers²⁶, but also with weight gain in elderly subjects²⁶. Yet, this Val103Ile polymorphism is indistinguishable from the wild-type receptor in tests *in vitro*²⁵. Associations²⁷, but also the lack thereof²⁸, have been reported for the Ala986Ser SNP in the calcium-sensing receptor and its effects on serum calcium levels, bone mineral density, and other indices of calcium homeostasis, while this polymorphism appears not to differ from wild-type *in vitro*^{27, 28}.

Unfortunately, most polymorphisms lack a consensus on their effects *in vitro* and often also on their effects in man. In fact, for those cases where polymorphisms have been retested for functionality, whether *in vitro* or in man, results often proved inconsistent. In that respect, fewer disagreements exist about the effects of rare disease-causing variants⁹. As an example of a polymorphism with conflicting data, the Asn40Asp polymorphism in the μ opioid receptor was first reported to increase agonist affinity *in vitro*, though this effect could not be confirmed in more recent studies²⁹. Reported associations of this Asn40Asp polymorphism with subtypes of human addiction are not considered conclusive²⁹. Contradicting effects on agonist affinity and constitutive activity *in vitro* have also been reported for the Cys23Ser polymorphism of the serotonin 2_C receptor^{30, 31}. The clinical effects

of this Cys23Ser SNP on clozapine response and noradrenaline metabolite concentrations appear inconsistent^{32, 33}. The Arg990Gly polymorphism in the calcium-sensing receptor showed conflicting effects *in vitro* on the potency of extracellular calcium^{27, 28} and reported associations with various indices of calcium homeostasis are also under debate.

Copy number variants and their potential for disease association

Copy number variants (CNVs) can contain several complete genes and this notion contrasts with smaller variants, whose effects depend on their position relative to the gene. Due to their probable impact on protein expression, copy number polymorphisms (CNP) that contain vital genes are strong candidates for disease association studies³. The therapeutic importance of gene copy numbers was first shown for cytochrome P450 CYP2D6, where the number of active genes determined the rate of debrisoquine metabolism, likely by affecting expression levels³⁴. More recently, high gene copy numbers of the epidermal growth factor receptor, which is a receptor tyrosine kinase, have been associated with increased protein expression and with improved clinical response to tyrosine kinase inhibitors in lung cancer patients³⁵.

So far, 69 non-olfactory GPCRs have been catalogued to overlap with CNVs in the Database of Genomic Variants¹⁷, and these include the α_{2C} adrenoceptor³, the dopamine D₄ receptor³ and the histamine H₃ receptor. Notably, mice with one or zero copies of the α_{2C} adrenoceptor gene have been shown to possess copy number-dependent effects on: increased urinary excretion of adrenaline *in vivo*, decreased expression levels of mRNA and decreased inhibition of noradrenalin release in isolated adrenal glands *in vitro*³⁶. Such findings support the hypothesis that CNV-dependent copy numbers of GPCRs affect protein expression levels and clinical phenotypes. Particularly strong risk factors for GPCR-related phenotypes are CNPs that contain non-olfactory GPCRs or peptides/proteins that interact with them³⁷. We anticipate that future association studies of GPCR-related CNPs will spur new diagnostic tools and therapies.

The GPCR NaVa database

Data on natural variants (NaVas) are distributed over numerous sources, see also Table 2.1, such as databases on disease-related mutations⁹, candidate polymorphisms² and large genetic variants¹⁷. In particular, this is a drawback for researchers that focus on one protein or on a single protein family, such as GPCRs. Therefore, we have recently developed the GPCR NaVa database, which catalogues over 75,000 small and large natural variants in human GPCR genes (<http://nava.liacs.nl>). Chapter 3 describes its construction and how the variant data can be accessed. For a GPCR of choice, variant subtypes can be selected (intronic, silent, etc.) and an overview orders the resulting DNA variants by their genetic

position. Alternatively, details on missense and silent variants can be accessed via a protein-specific page, an example of which is shown in Figure 2.1.

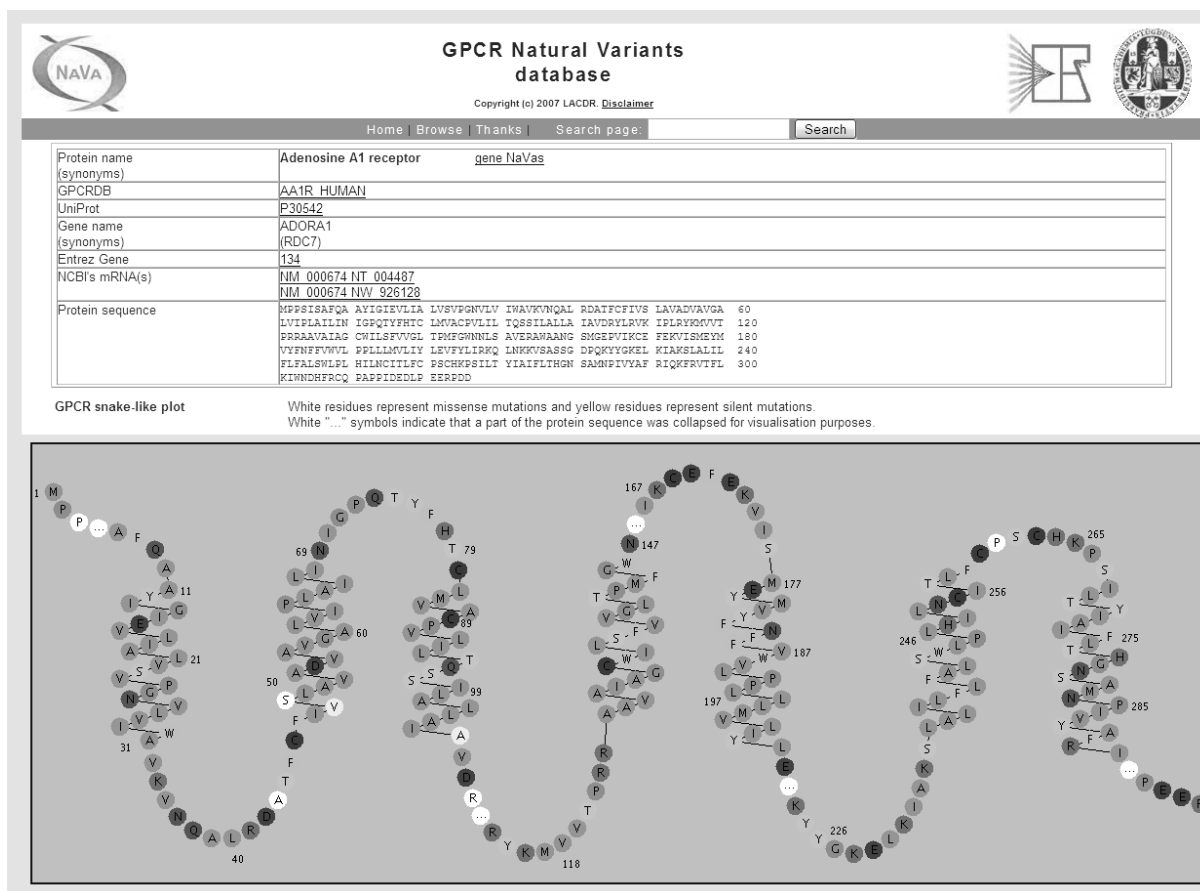


Figure 2.1: A typical page of the GPCR NaVa database. For the depicted receptor, the adenosine A₁ receptor, five missense variants are shown in white and two silent variants are shown in light grey (at positions 49 and 102, online the colour is yellow). These clickable variants direct users to more specific information. The figure shows the seven α helix-like *transmembrane domains*, which are characteristic for GPCR families. For visualisation purposes, parts of extracellular (top) and intracellular (bottom) regions that lack variants are collapsed in white symbols as (...). Noncoding variants are accessible from other pages of the GPCR NaVa database.

Detailed analyses of natural GPCR variants may yet reveal insights into the GPCR transmembrane structure. For one, differences between disease mutations and variants that lack an obvious phenotype may indicate regions in this structure where more or less variability is tolerated. A three-dimensional analysis, see Figure 2.2, shows that relatively many ‘buried’ variants (those in the GPCR core) are disease mutations. This supports the hypothesis that more changes are tolerated at the GPCR surface, while changes in the GPCR core likely cause receptor malfunction.

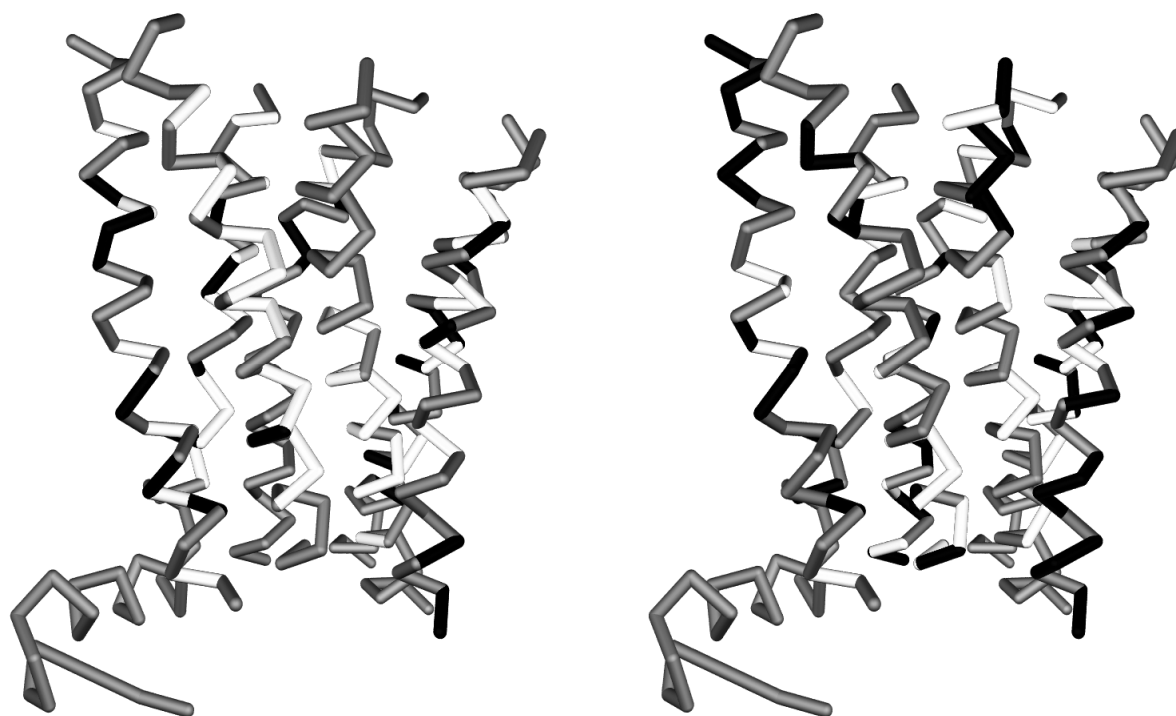


Figure 2.2: Disease mutations (left panel) and variants without an obvious phenotype (right panel) that occur in transmembrane domains. GPCR variants from Lee, et al.³⁸ were overlaid on the crystal structure of bovine rhodopsin³⁹. From left to right, transmembrane domains 1, 2, 3 and 4 are visible at the front. Transmembrane positions without variants are shown in grey. Buried variants with low accessibility (<10%⁴⁰) are shown in white. Variants on the GPCR surface have high accessibility (>10%) and are shown in black. The fraction of disease mutations (left panel) that occur at buried positions, 68% (63 of 93 disease mutations), is higher than the corresponding fraction of non-disease variants (right panel) that occur at buried positions, 41% (48 of 118 variants) ($p_{\chi} < 0.01$, $\chi^2 = 15.3$). This suggests that variants are more prone to cause disease if they occur at buried positions rather than at surface positions.

CONCLUSION

Natural variants exist in a variety of forms and their data are distributed over numerous sources, including databases, patents, and thousands of peer-reviewed papers. Almost all natural variants, both coding and noncoding, may affect clinical phenotypes by interfering with pre- and post-transcriptional processes. However, in comparison to mutations in GPCRs that have clear effects on phenotype, relatively few convincing links with clinical manifestations have been reported for GPCR polymorphisms. For those cases where copy number polymorphisms and small polymorphisms affect clinically relevant GPCRs, we expect CNPs to be stronger candidates than SNPs for causal links with disease. A catalogue of class-specific natural variants such as the GPCR NaVa database can be a valuable starting point for easy access to, and further analysis of, genetic diversity.

REFERENCES

1. Venter, J. C.; Adams, M. D.; Myers, E. W.; Li, P. W.; Mural, R. J.; Sutton, G. G., et al. The sequence of the human genome. *Science* **2001**, 291, 1304-51.
2. Sherry, S. T.; Ward, M. H.; Kholodov, M.; Baker, J.; Phan, L.; Smigielski, E. M., et al. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res* **2001**, 29, 308-11.
3. Redon, R.; Ishikawa, S.; Fitch, K. R.; Feuk, L.; Perry, G. H.; Andrews, T. D., et al. Global variation in copy number in the human genome. *Nature* **2006**, 444, 444-54.
4. Small, K. M.; Tanguay, D. A.; Nandabalan, K.; Zhan, P.; Stephens, J. C.; Liggett, S. B. Gene and protein domain-specific patterns of genetic variability within the G-protein coupled receptor superfamily. *Am J Pharmacogenomics* **2003**, 3, 65-71.
5. Rana, B. K.; Shiina, T.; Insel, P. A. Genetic variations and polymorphisms of G protein-coupled receptors: functional and therapeutic implications. *Annu Rev Pharmacol Toxicol* **2001**, 41, 593-624.
6. the UniProt Consortium. The Universal Protein Resource (UniProt). *Nucleic Acids Res* **2007**, 35, D193-7.
7. Insel, P. A.; Tang, C. M.; Hahntow, I.; Michel, M. C. Impact of GPCRs in clinical medicine: monogenic diseases, genetic variants and drug targets. *Biochim Biophys Acta* **2007**, 1768, 994-1005.
8. Schöneberg, T.; Schulz, A.; Biebermann, H.; Hermsdorf, T.; Rompler, H.; Sangkuhl, K. Mutant G-protein-coupled receptors as a cause of human diseases. *Pharmacol Ther* **2004**, 104, 173-206.
9. Pearson, P.; Francomano, C.; Foster, P.; Bocchini, C.; Li, P.; McKusick, V. The status of online Mendelian inheritance in man (OMIM) medio 1994. *Nucleic Acids Res* **1994**, 22, 3470-3.
10. Tang, C. M.; Insel, P. A. Genetic variation in G-protein-coupled receptors--consequences for G-protein-coupled receptors as drug targets. *Expert Opin Ther Targets* **2005**, 9, 1247-65.
11. Tao, Y. X. Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. *Pharmacol Ther* **2006**, 111, 949-73.
12. Small, K. M.; McGraw, D. W.; Liggett, S. B. Pharmacology and physiology of human adrenergic receptor polymorphisms. *Annu Rev Pharmacol Toxicol* **2003**, 43, 381-411.
13. Sadee, W.; Hoeg, E.; Lucas, J.; Wang, D. Genetic variations in human G protein-coupled receptors: implications for drug therapy. *AAPS PharmSci* **2001**, 3, E22.
14. Spiegel, A. M.; Weinstein, L. S. Inherited diseases involving G proteins and G protein-coupled receptors. *Annu Rev Med* **2004**, 55, 27-39.
15. Thompson, M. D.; Burnham, W. M.; Cole, D. E. The G protein-coupled receptors: pharmacogenetics and disease. *Crit Rev Clin Lab Sci* **2005**, 42, 311-92.
16. Smit, M. J.; Vischer, H. F.; Bakker, R. A.; Jongejan, A.; Timmerman, H.; Pardo, L., et al. Pharmacogenomic and structural analysis of constitutive G protein-coupled receptor activity. *Annu Rev Pharmacol Toxicol* **2007**, 47, 53-87.
17. Zhang, J.; Feuk, L.; Duggan, G. E.; Khaja, R.; Scherer, S. W. Development of bioinformatics resources for display and analysis of copy number and other structural variants in the human genome. *Cytogenet Genome Res* **2006**, 115, 205-14.
18. Jinnai, N.; Sakagami, T.; Sekigawa, T.; Kakihara, M.; Nakajima, T.; Yoshida, K., et al. Polymorphisms in the prostaglandin E2 receptor subtype 2 gene confer susceptibility to aspirin-intolerant asthma: a candidate gene approach. *Hum Mol Genet* **2004**, 13, 3203-17.
19. Kim, S. H.; Oh, J. M.; Kim, Y. S.; Palmer, L. J.; Suh, C. H.; Nahm, D. H., et al. Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. *Clin Exp Allergy* **2006**, 36, 433-9.

20. Huang, J. L.; Gao, P. S.; Mathias, R. A.; Yao, T. C.; Chen, L. C.; Kuo, M. L., et al. Sequence variants of the gene encoding chemoattractant receptor expressed on Th2 cells (CRTH2) are associated with asthma and differentially influence mRNA stability. *Hum Mol Genet* **2004**, 13, 2691-7.
21. Duan, J.; Wainwright, M. S.; Comeron, J. M.; Saitou, N.; Sanders, A. R.; Gelernter, J., et al. Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet* **2003**, 12, 205-16.
22. Arenzana-Seisdedos, F.; Parmentier, M. Genetics of resistance to HIV infection: Role of co-receptors and co-receptor ligands. *Semin Immunol* **2006**, 18, 387-403.
23. Shibata, K.; Hirasawa, A.; Moriyama, N.; Kawabe, K.; Ogawa, S.; Tsujimoto, G. Alpha 1a-adrenoceptor polymorphism: pharmacological characterization and association with benign prostatic hypertrophy. *Br J Pharmacol* **1996**, 118, 1403-8.
24. Schaak, S.; Miale-Perez, J.; Flordellis, C.; Paris, H. Genetic variation of human adrenergic receptors: from molecular and functional properties to clinical and pharmacogenetic implications. *Curr Top Med Chem* **2007**, 7, 217-31.
25. Geller, F.; Reichwald, K.; Dempfle, A.; Illig, T.; Vollmert, C.; Herpertz, S., et al. Melanocortin-4 receptor gene variant I103 is negatively associated with obesity. *Am J Hum Genet* **2004**, 74, 572-81.
26. Rutanen, J.; Pihlajamäki, J.; Karhapää, P.; Vauhkonen, I.; Kuusisto, J.; Moilanen Mykkanen, L., et al. The Val103Ile polymorphism of melanocortin-4 receptor regulates energy expenditure and weight gain. *Obes Res* **2004**, 12, 1060-6.
27. Vezzoli, G.; Terranegra, A.; Arcidiacono, T.; Biasion, R.; Coviello, D.; Syren, M. L., et al. R990G polymorphism of calcium-sensing receptor does produce a gain-of-function and predispose to primary hypercalciuria. *Kidney Int* **2007**, 71, 1155-62.
28. Harding, B.; Curley, A. J.; Hannan, F. M.; Christie, P. T.; Bowl, M. R.; Turner, J. J., et al. Functional characterization of calcium sensing receptor polymorphisms and absence of association with indices of calcium homeostasis and bone mineral density. *Clin Endocrinol (Oxf)* **2006**, 65, 598-605.
29. Mayer, P.; Holtt, V. Pharmacogenetics of opioid receptors and addiction. *Pharmacogenet Genomics* **2006**, 16, 1-7.
30. Okada, M.; Northup, J. K.; Ozaki, N.; Russell, J. T.; Linnoila, M.; Goldman, D. Modification of human 5-HT(2C) receptor function by Cys23Ser, an abundant, naturally occurring amino-acid substitution. *Mol Psychiatry* **2004**, 9, 55-64.
31. Fentress, H. M.; Grinde, E.; Mazurkiewicz, J. E.; Backstrom, J. R.; Herrick-Davis, K.; Sanders-Bush, E. Pharmacological properties of the Cys23Ser single nucleotide polymorphism in human 5-HT2C receptor isoforms. *Pharmacogenomics J* **2005**, 5, 244-54.
32. Reynolds, G. P.; Templeman, L. A.; Zhang, Z. J. The role of 5-HT2C receptor polymorphisms in the pharmacogenetics of antipsychotic drug treatment. *Prog Neuropsychopharmacol Biol Psychiatry* **2005**, 29, 1021-8.
33. Jönsson, E. G.; Bah, J.; Melke, J.; Abou Jamra, R.; Schumacher, J.; Westberg, L., et al. Monoamine related functional gene variants and relationships to monoamine metabolite concentrations in CSF of healthy volunteers. *BMC Psychiatry* **2004**, 4, 4.
34. Johansson, I.; Lundqvist, E.; Bertilsson, L.; Dahl, M. L.; Sjoqvist, F.; Ingelman-Sundberg, M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci U S A* **1993**, 90, 11825-9.
35. Weydert, J. A.; Cohen, M. B. Small peripheral pulmonary adenocarcinoma: morphologic and molecular update. *Adv Anat Pathol* **2007**, 14, 120-8.

36. Gilsbach, R.; Brede, M.; Beetz, N.; Moura, E.; Muthig, V.; Gerstner, C., et al. Heterozygous alpha(2C)-adrenoceptor-deficient mice develop heart failure after transverse aortic constriction. *Cardiovasc Res* **2007**, 24, 24.
37. Gonzalez, E.; Kulkarni, H.; Bolivar, H.; Mangano, A.; Sanchez, R.; Catano, G., et al. The influence of CCL3L1 gene-containing segmental duplications on HIV-1/AIDS susceptibility. *Science* **2005**, 307, 1434-40.
38. Lee, A.; Rana, B. K.; Schiffer, H. H.; Schork, N. J.; Brann, M. R.; Insel, P. A., et al. Distribution analysis of nonsynonymous polymorphisms within the G-protein-coupled receptor gene family. *Genomics* **2003**, 81, 245-8.
39. Palczewski, K.; Kumasaka, T.; Hori, T.; Behnke, C. A.; Motoshima, H.; Fox, B. A., et al. Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **2000**, 289, 739-45.
40. Rost, B.; Sander, C. Bridging the protein sequence-structure gap by structure predictions. *Annu Rev Biophys Biomol Struct* **1996**, 25, 113-36.