Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology & Therapeutics 106 (2005) 19–38

Pharmacology & Therapeutics

www.elsevier.com/locate/pharmthera

Associate editor: J. Wess

# Polymorphisms affecting gene regulation and mRNA processing: Broad implications for pharmacogenetics

Andrew D. Johnson[a,*], Danxin Wang[b], Wolfgang Sadee[c]

[a]*Integrated Biomedical Sciences Graduate Program, Program in Pharmacogenomics, Department of Pharmacology, College of Medicine and Public Health, The Ohio State University, 5072 Graves Hall, 333 W. 10th Avenue, Columbus, OH 43210-1239, United States*

[b]*Department of Pharmacology, College of Medicine and Public Health, The Ohio State University, Columbus, OH, United States*

[c]*Felts Mercer Professor of Medicine and Pharmacology, Chair, Department of Pharmacology, Director, Program in Pharmacogenomics, College of Medicine and Public Health, The Ohio State University, Columbus, OH, United States*

**Abstract**

Functional polymorphisms that alter gene expression and mRNA processing appear to play a critical role in shaping human phenotypic variability. Intensive studies on polymorphisms affecting drug response have revealed multiple modes of altered gene function, frequently involving *cis*-acting regulatory sequence variants. Experimental and in silico methods have advanced the search for such polymorphisms, but considerable challenges remain. Here, a survey of polymorphisms in drug-related genes indicates that: (a) a substantial proportion of genetic variability still remains unaccounted for; (b) a majority of these genes harbors known regulatory polymorphisms; and (c) a portion of polymorphisms affect splicing and mRNA turnover. Pharmacogenetic optimiziation of individual drug therapy may require a complete understanding of all functional sequence variants in key genes. This review surveys known noncoding polymorphisms in genes encoding cytochrome *P*450s and other drug-metabolizing enzymes, drug transporters, and drug targets and receptors. Current methods and challenges associated with the identification and characterization of functional polymorphisms are also discussed.


*Keywords:* *Cis*-Regulatory; Polymorphism; Pharmacogenetics; Pharmacogenomics; SNP; RNA; Allele-specific; Genotype-phenotype; Cytochrome; Review

*Abbreviations*: 5-FU, 5-fluorouracil; 5-HTT, serotonin transporter; 5-HTTLPR, serotonin gene-linked polymorphic region; 6-MP, 6-mercaptopurine; 6-TG, 6-thioguanine; ABCB, ATP-binding cassette (ABC), subfamily B; ACE, angiotensin I converting enzyme (peptidyl-dipeptidase A) 1; ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; ARE, adenylate-uridylate-rich element; ARED, adenylate-uridylate-rich element database; bp, base pair; BS, binding site; C/EBP, CCAAT/enhancer binding proteins; CAR, constitutive androstane receptor; CGAP, cancer genome anatomy project; CREATE, comprehensive research on expressed alleles in therapeutic evaluation; CRM, *cis*-regulatory module; CYP, cytochrome; DBP, albumin D-site binding protein; DPYD, dihydropyrimidine dehydrogenase; *Drd2*, dopamine D2 receptor; EM, extensive metabolizer; ESE, exonic splicing enhancer; Ets, E twenty six; FXR, farnesol X receptor; haploCHIP, haplotype-specific chromatin immunoprecipitation; hGRα, human glucocorticoid receptor alpha; hGRβ, human glucocorticoid receptor beta; HGVbase, human genome variation database; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HNF, hepatocyte nuclear factor; hnRNA, heterogeneous nuclear RNA; *hPepT2*, human solute carrier family 15 (H+/peptide transporter), member 2 gene; HTR2A, 5-hydroxytryptamine (serotonin) receptor 2a; IM, intermediate metabolizer; IVS, intronic splice site variation; kb, kilobases; LDL, low-density lipoprotein; LIPC, hepatic lipase; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight spectroscopy; MMP, matrix metalloproteinase; NAT, *N*-acetyltransferase; NF-Y, nuclear transcription factor Y; OATP, organic anion-transporting polypeptide; PABA, *para*-aminobenzoic acid; PGA, program for genomic applications; PharmGKB, the pharmacogenetics and pharmacogenomics knowledge base; PM, poor metabolizer; PPAR-α, peroxisome proliferator-activated receptor alpha; PTC, premature termination codon; PTP1N, protein tyrosine phosphatase, nonreceptor type 1; PXR, pregnane X receptor; SIFT, sorting tolerant from intolerant; SLC, solute carrier; SLCO, solute carrier organic anion transporter family; SM12502, (+)-*cis*-3,5-dimethyl-2(3-pyridyl) thiazolidin-4-one hydrochloride; SNP, single nucleotide polymorphism; SP-1, Sp1 transcription factor; SULT, sulfotranferase; TPMT, thiopurine (*S*)-methyltransferase; TSER, thymidylate synthase enhancer region; TYMS, thymidylate synthase; UGT1, UDP-glucuronosyltranferase-1; USF1, upstream transcription factor 1; UTR, unstranslated region; VDR, vitamin D receptor; VNTR, variable number tandem repeat.

* Corresponding author. Tel.: 614 292 6680; fax: 614 292 7232.
*E-mail address:* johnson.2060@osu.edu (A.D. Johnson).

0163-7258/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2004.11.001

**Contents**

1. Introduction . . . 20
2. Modes of *cis*-acting polymorphisms and methods for discovery . . . 21
   2.1. Experimental methods for discovering *cis*-acting polymorphisms . . . 21
   2.2. In silico methods for discovering *cis*-acting polymorphisms . . . 23
3. Drug-metabolizing enzymes . . . 24
   3.1. Cytochrome 1 family . . . 24
   3.2. Cytochrome 2 family . . . 25
   3.3. Cytochrome 3 family . . . 26
   3.4. Other cytochromes . . . 27
   3.5. Other classes of drug-metabolizing enzymes . . . 27
4. Drug transporters . . . 28
5. Drug targets and receptors . . . 29
6. *Cis*-acting polymorphisms in relevant *trans*-factors . . . 30
7. Conclusion . . . 30
Acknowledgments . . . 31
References . . . 31

## 1. Introduction

Regulation at the level of transcription initiation and RNA processing defines downstream biological effects. Such regulation occurs in *cis*, directly affecting the regulated gene, but it can also act in *trans* by altering activity of downstream genes (Fig. 1). Significant interindividual differences in gene expression patterns are common and may result from both environmental factors and *cis*- or *trans*-mediated genetic effects (Singer-Sam et al., 1992; Enard et al., 2002; Whitney et al., 2003; Pastinen & Hudson, 2004). There is growing evidence for abundant polymorphisms in *cis*-acting sequences that influence gene expression (Rockman & Wray, 2002; Yan et al., 2002; Bray et al., 2003; Lo et al., 2003) and indication that a significant portion of functional polymorphisms affect *cis*-acting regulatory elements (Stamatoyannopoulos, 2004; Wittkopp et al., 2004). Identifying the functional alleles that account for interindividual differences remains difficult (Ioannidis, 2003; Page et al., 2003; Sun et al., 2004). The genetic components of complex interindividual differences may require resolution of multiple modest variations in genotype which collectively yield a recognizeable phenotype such as disease susceptibility or drug response.

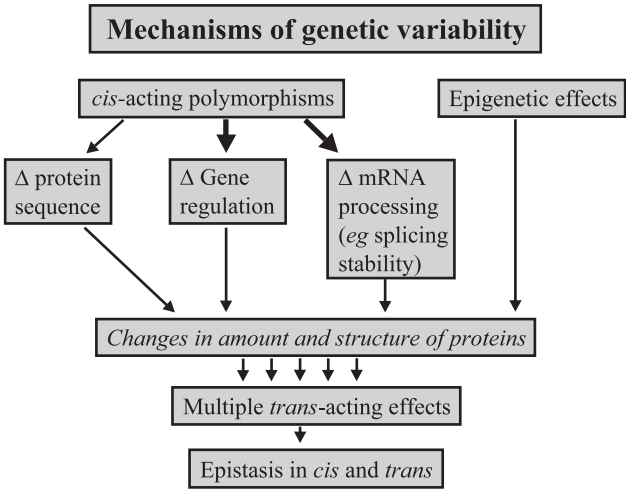


Fig. 1. Human genetic variability, involving *cis*- and *trans*-acting polymorphisms. *Cis*-acting regulatory polymorphisms appear to outnumber functional polymorphisms in coding regions affecting protein sequence. If *cis*-acting polymorphisms alter signaling or transcription factor activity, multiple *trans*-acting changes ensue. Epigenetic changes can mimic *cis*-acting polymorphisms. Lastly, epistatic effects (multiple interacting polymorphisms) are likely to play a role as well. Not shown are regulatory effects exerted by small RNAs, also subject to genetic variability.

Phenotypic differences can arise from genetic polymorphisms acting in *cis* by changing the protein coding sequence or at the level of RNA (Day & Tuite, 1998): affecting transcription (activation or inhibition through regulatory sites or structure of regulatory elements), mRNA processing, pre-mRNA splicing, exonic splicing enhancers (ESEs), exon skipping (Cartegni et al., 2003), mRNA stability (Sheets et al., 1990; Conne et al., 2000; Di Paola et al., 2002; Tebo et al., 2003), mRNA trafficking, or regulatory RNAs (Fig. 1). The most commonly studied polymorphisms, nonsynonymous changes that alter amino acid coding, appear in many cases insufficient to account for interindividual differences in disease aetiology and response to therapies. Further, it is estimated that functional polymorphisms that are *cis*-regulatory in the human genome outnumber those that alter protein sequence, and that the bulk of regulatory polymorphisms remain to be discovered (Ng & Henikoff, 2002; Rockman & Wray, 2002; Stamatoyannopoulos, 2004; Yan & Zhou, 2004). On the other hand, genomewide linkage analysis with mRNA expression as the quantitative trait demonstrates that interindividual differences in mRNA profiles appear to be largely caused by *trans*-acting factors (Morley et al., 2004). These statements are not incompatible since a single *cis*-acting polymorphism in a transcription factor or receptor could affect the expression of numerous other genes (Fig. 1). Therefore, to

understand consequences of genetic variations, we must first determine whether interindividual differences of a protein's activity are caused by polymorphisms in *cis* or *trans* (or both) or by environmental factors. If in *cis*, we must then find the functional polymorphism(s) in the candidate gene that can account for the observed variations and epistatic interactions among them if several are present. If in *trans*, we search for polymorphisms in *trans*-acting factors (e.g., transcription factors). Lastly, we must also consider epigenetic factors such as methylation, imprinting, and chromatin structure modulation that can be transmitted through the germline or observed in somatic cells without alterations in the primary DNA sequence (Grewal & Moazed, 2003; Yan & Zhou, 2004).

While many studies have addressed *cis*-regulatory variations, it is likely that a majority of functional variants are yet to be discovered. Novel techniques now enable broad investigation of this type of variation, which is likely to contribute substantially to knowledge of phenotypic variability of pharmacogenetic relevance. Previous extensive studies on *cis*-regulatory variations affecting disease susceptibilities (e.g., Loktionov, 2004) inform our thinking about functional variations with pharmacological-pharmacogenetic implications. Here, we review advances in the discovery of *cis*-regulatory variations within genes encoding drug-metabolizing enzymes, drug transporters, and drug targets and receptors (Table 1).

## 2. Modes of *cis*-acting polymorphisms and methods for discovery

### *2.1. Experimental methods for discovering cis-acting polymorphisms*

Measurement of sequence variants, primarily single nucleotide polymorphisms (SNPs), provides the fundamental units for linking genetic sequence to traits. Most genes harbor multiple sequence variations (e.g., SNPs, repeats, indels) showing a broad range of frequencies and linkage disequilibrium among them. In clinical genetic association studies with goals to pinpoint candidate genes, selection of polymorphisms yielding maximum information is difficult. Confounding factors, such as their relative frequencies in the targeted populations, population admixture, and the effects of age and sex (Pinsonneault & Sadee, 2003), account in part for the failure to replicate many association studies. Most polymorphisms are nonfunctional and thus serve as markers for functional alleles. Rather than using single polymorphisms, associations are now often made with the use of haplotypes, blocks of linked polymorphisms, that may demarcate trait-significant *cis*-regions of sequence. High-throughput SNP genotyping methods are now coming online, such as SNPlex, capable of screening thousands of SNP in many samples (Wenz, 2004). Such methods have been used to establish haplotype maps on a genomewide basis, including genes involved in drug metabolism, at significant marker density (Kamatani et al., 2004). Despite improvements in throughput for identification and association of sequence variants, the search for the specific identity of key regulatory variations is a difficult problem. Linking polymorphisms to transcriptional regulation has traditionally employed gene reporter constructs and in vitro DNA protein factor binding assays. For example, haplotype-specific chromatin immunoprecipitation (haploCHIP) takes advantage of the relation between the amount of phosphorylated RNA polymerase II and transcriptional activity (Knight et al., 2003). Via haploChIP, SNPs in regulatory regions can be investigated in conjunction with variation in transcription levels. However, these approaches provide incomplete pictures because they lack the physiological and structural context of a target tissue, or they currently lack high-throughput capability.

More broadly, mRNA expression measured by microarrays has been combined with genomewide linkage analysis, taking the expression level of each gene as the measured phenotype (Morley et al., 2004). Heritability of gene expression phenotypes can be explored through familial genotyping and transmission disequilibrium testing in nuclear families (Spielman & Ewens, 1996) or pedigree disequilibrium testing in larger pedigrees (Martin et al., 2000). Using target tissues (such as immortalized lymphocytes) from family members, this type of analysis is capable of distinguishing between *cis*- and *trans*-acting genetic factors and shows an abundance of functional genomic loci and a preponderance of *trans*-acting effects, as expected (see Fig. 1). However, the technology suffers from low sensitivity and therefore may limit the detection of functional variations in target genes.

An alternative approach involves the analysis of allele-specific expression in a relevant target tissue; each allele experiences its own regulation in the same cellular environment, with the other allele (for autosomal genes) serving as an internal control. As a result, the method controls for tissue conditions, *trans*-acting factors, and other environmental influences. Thus, SNPs in exonic and untranslated regions of message, can serve as markers for allele expression levels in individuals heterozygous for these markers. Taking the human solute carrier family 15 (H+/peptide transporter), member 2 gene (*hPepT2*) as one example, our laboratory has recently described a method for allele-specific measurement of mRNA expression through primer extension incorporation of fluorescent dideoxy-nucleotide terminating probes after RT-PCR amplification (Pinsonneault et al., 2004). Significant differences in the relative abundance of each allele in mRNA from kidney tissues demonstrated the presence of functional *cis*-acting factors. The primer extension reaction can be multiplexed (Bray et al., 2004) so that it will be possible to search for functional *cis*-acting polymorphisms in a large number of genes (Yan et al., 2002). Similar results can be achieved through methods employed on other platforms

Table 1
Polymorphisms affecting gene regulation and mRNA processing

| Gene*allele | Type | Functional reports | Reference |
|---|---|---|---|
| CYP1A2*1C | Enhancer (−3860G>A) | ↓ enzyme activity | Nakajima et al., 1999 |
| CYP1A2*1F | Intron 1 (−163C>A) | ↑ enzyme induction | Chida et al., 1999; Sachse et al., 1999; Shimoda et al., 2002; Nordmark et al., 2002 |
| CYP1A2*1K | Intron 1 | Disrupt Ets BS, ↓ RNA, metabolism | Aklillu et al., 2003 |
| CYP1A2*7 | Intron 6 splice donor SNP (3534G>A) | PM to clozapine (single individual) | Allorge et al., 2003 |
| CYP2A6*9 | TATA Box | ↓ RNA, protein, enzyme activity | Pitarque et al., 2001; Kiyotani et al., 2003; Yoshida et al., 2003 |
| CYP2A6*1D | Enhancer (−1013A>G) | ↓ transcription (reporter assay) | Pitarque et al., 2004 |
| CYP2A6*1H, J | Enhancer (−745A>G) | Disrupt NF-Y BS, ↓ transcription | von Richter et al., 2004 |
| CYP2A6*12 | Intron 1 2A6/7 crossover | ↓ enzyme activity in vitro and in vivo | Oscarson et al., 2002 |
| CYP2B6*9 | Splice variant | Skip exons 4–6 | Lamba et al., 2003 |
| CYP2B6*1G | Promoter (−750C>T) | ↓ RNA | Lamba et al., 2003 |
| CYP2B6*1B | Enhancer (−2320T>C) | ↓ protein in Caucasian females | Lamba et al., 2003 |
| CYP2B6*1C | ESE synonymous SNP | ↓ protein in Caucasian females | Lamba et al., 2003 |
| CYP2C9*6 | Frameshift | Null, severe toxicity | Kidd et al., 2001 |
| CYP2C19*2 | Exon 5 splice variant | PTC, PM phenotype | De Morais et al., 1994a |
| CYP2C19*3 | Exon 4 premature stop | PTC, PM phenotype | De Morais et al., 1994b |
| CYP2C19*4 | Initiation codon | Transcription ablation, PM | Ferguson et al., 1998 |
| CYP2C19*7 | Intron 5 splice donor SNP (IVS5+2 T>A) | PM phenotype | Ibeanu et al., 1999 |
| CYP2D6*4A-L | Intron 3 splice variant | PTC, PM phenotype | Kagimoto et al., 1990 |
| CYP2D6*11 | Intron 1 splice acceptor | PTC, PM phenotype | Marez et al., 1995 |
| CYP2D6*41 | Promoter | IM phenotype, ↑ expression | Lovlie et al., 2001; Zanger et al., 2001; Gaedigk et al., 2003 |
| CYP2D6 | Intron 6 (2988G>A) | IM phenotype prediction | Raimundo et al., 2004 |
| CYP2D6*44 | Intron 6 splice donor SNP | ↓ enzyme activity | Yamazaki et al., 2003 |
| CYP2D7 | 138delT | Pseudogene ORF, brain expression | Pai et al., 2004 |
| CYP2E1*1D | Enhancer VNTR | ↔ transcription, ↑ enzyme inducibility | McCarver et al., 1998; Hu et al., 1999 |
| CYP2J2*7 | SP-1 BS (−76G>T) | Disrupt SP-1 BS, ↓ transcription | King et al., 2002; Speicker et al., 2004 |
| CYP3A4*1B | Proximal promoter (−392A>G) | Disrupt nifedipine-specific repressor | Westlind et al., 1999; Spurdle et al., 2002; Amirimani et al., 2003; Floyd et al., 2003 |
| CYP3A4 | Far upstream enhancer (−11,129_−11,128 ins TGT) | Disrupt USF1BS, ↓ expression | Matsumura et al., 2004 |
| CYP3A5*3 | Exon 3B splice inclusion | PTC, ↓ protein, enzyme activity | Kuehl et al., 2001; Hustert et al., 2001a |
| CYP3A5*6 | Exon 7 (14690G>A) | Splice defect, Exon 7 deletion | Kuehl et al., 2001; Hustert et al., 2001a |
| CYP3A5*7 | Exon 11 (27131 T ins) | Predicted PTC and ↓ protein | Hustert et al., 2001a |
| CYP4F12*v1 | Intron 1 (146 bp del) | ↓ transcription | Cauffiez et al., 2004 |
| CYP4F12*v2 | 9-SNP promoter allele | ↓ transcription | Cauffiez et al., 2004 |
| CYP7A1 | Promoter (−204A>C) | ↓ response to atorvastatin | Kajinami et al., 2004 |
| CYP8A1*1D-F | Promoter VNTRs | ↑ number SP-1 BS, ↑ transcription | Chevalier et al., 2002 |
| UGT1A1*28 | $(TA_{5-8})$TAA repeat | ↓ transcription, protein, enz act, ↑ toxicity | Bosma et al., 1995; Iyer et al., 2002; Fang et al., 2004 |
| UGT1A9*22 | Promoter (T ins) | ↑ transcription | Yamanaka et al., 2004 |
| TPMT*V6a | Promoter VNTRs | ↓ transcription, ↓ in vivo activity, conflicting | Spire-Vayron de la Moureyre et al., 1999; Yan et al., 2000; Alves et al., 2001 |
| NAT1*16 | 3′ UTR AAA ins+C>A | ↓ protein, ↓ in vitro activity, disrupt predicted RNA structure | de Leon et al., 2000 |
| ADH4 | Promoter (−75A>C) | ↓ transcription | Edenberg et al., 1999; Iida et al., 2002 |
| ABCB1 | Synonymous (3435C>T) | ↓ RNA, protein, drug transport activity | Hoffmeyer et al., 2000; Sakaeda et al., 2001 |
| ABCB1 | Promoter haploypes | ↑ transcription | Takane et al., 2004 |
| SLC6A4 | 5′ HTTLPR (14 repeats) | ↓ RNA, protein, transport activity | Heils et al., 1996; Heinz et al., 2000; Hranilovic et al., 2004 |
| SLC6A4 | 3′ UTR PolyA | No quantitative assay | Battersby et al., 1999 |
| SLCO1B1*17 | Upstream promoter | ↑ pravastatin clearance | Niemi et al., 2004 |
| TSER*3 | Promoter VNTR | ↑ RNA, Poor 5-FU treatment outcome | Horie et al., 1995; Villafranca et al., 2001; Marsh et al., 2001 |
| TSER*3RG | SNP in 2nd VNTR | Disrupt USF-1 binding site | Mandola et al., 2003 |
| TYMS | 3′ UTR (1494 6bp del) | ↓ RNA, ↓ stability, ↓ intratumoral protein | Ulrich et al., 2000; Mandola et al., 2004 |

Table 1 (*continued*)

| Gene*allele | Type | Functional reports | Reference |
|---|---|---|---|
| HTR2A | Synonymous (102C>T) | (Conflicting reports on functionality) | Arranz et al., 1998; Bray et al., 2004 |
| HMGCR(SNP3) | Intron 5 | ↓ response to pravastatin | Chasman et al., 2004 |
| HMGCR(SNP29) | Intron 15 | ↓ response to pravastatin | Chasman et al., 2004 |
| MMP3 | Promoter (−1171 5A>6A) | ↓ expression, ↓ response to pravastatin | de Maat et al., 1999 |
| LIPC | Promoter (−480C>T) | Disrupt USF BS, ↓ transcription, ↓ enzyme activity, ↓ response to treatments | Botma et al., 2001; Zambon et al., 2001 |
| ACE | Intron 16 (287 bp ins) | ↓ response to fluvastatin | Marian et al., 2000 |
| PTP1B | 3′ UTR (1484 ins G) | ↑ RNA, ↑ mRNA stability | Di Paola et al., 2002 |
| hGRß | 3′ UTR AUUUA SNP | ↑ mRNA stability, ↑ protein | Schaaf et al., 2002 |

Polymorphisms with gene, allele denoted after '*' (if defined), the type of genetic alteration (if an allele defined by a single SNP exists, then the position and base change are given), reported functional observations, and related literature references.

(Wojnowski & Brockmoller, 2004) including the use of matrix-assisted laser desorption/ionization time-of-flight spectroscopy (MALDI-TOF; Ding et al., 2004) and allele-specific RT-PCR methodologies (Zhang et al., 2004). These techniques may be extended to unprocessed heterogeneous nuclear RNA (hnRNA) if exonic and untranslated markers are unavailable and hnRNA is abundant enough in the target samples (Hirota et al., 2004).

Taken together, these approaches allow rapid determination of the extent of *cis*- or *trans*-genetic variation in a locus and the heritibility of that component. Further determination of the functional alleles is challenging because regulatory regions span across large genomic DNA segments. Therefore, in silico methods have proven helpful.

### 2.2. In silico methods for discovering cis-acting polymorphisms

Bioinformatics complements experimental investigations of regulatory polymorphisms, allowing investigators to interpret whether polymorphisms exist in a sequence region with predicted functional importance (Wasserman & Sandelin, 2004). Table 2 provides weblinks for tools and databases. Most tools employ phylogenetic footprinting to compare regions of sequence conservation that may highlight regulatory regions and then match these sequences against models predicting transcription factor binding sites (BSs). Pharmacogenetics-centered examples can be found in conjunction with the comprehensive research on expressed alleles in therapeutic evaluation (CREATE) website (Table 2: PromoLign, ReguLign). This general approach provides improvements over earlier methods but still fails to identify many regulatory sites (Wasserman & Sandelin, 2004). Acknowledging the combinatorial nature of factors binding regulatory regions, some tools use combinations of *cis*-regulatory modules (CRM) for specific tissues or gene types to make successful predictions (Liu et al., 2003).

A recent tool, PupaSNP, integrates available information on the potential for individual SNPs to alter expression or function (Conde et al., 2004). PupaSNP takes into account predicted transcription factor binding sites, intron/exon boundaries, predicted ESEs (Cartegni et al., 2003), amino acid changes in predicted motifs, and additional annotation information. ESE prediction is of interest since defects in splicing represent *cis*-regulatory variants, constitute a small but significant portion of known disease-causing mutations (Cooper & Mattox, 1997), and provide potential therapeutic targets (Sierakowska et al., 1996).

Posttranscriptional mRNA turnover represents another potentially important cause of genetic variability arising from *cis*-polymorphisms. mRNA stability can fluctuate through modulation of a number of pathways and changes in RNA structure and protein-RNA binding sites. For example, destabilizing adenylate-uridylate-rich sequence elements (ARE) are found in the 3′ untranslated regions (3′ UTR; Tebo et al., 2003) of 5–8% of human genes (Bakheet et al., 2003). Mutations in these elements are linked to disease pathology in human insulin resistance and have been suggested as stratifiers for administration of

Table 2
Polymorphism databases and related tools

| Resource name | URL reference |
|---|---|
| ARED (AU-rich element database) | rc.kfshrc.edu.sa/ared |
| CeleraSNPs | (proprietary) |
| CGAP | gai.nci.nih.gov |
| CREATE | pharmacogenetics.wustl.edu |
| dbSNP | www.ncbi.nlm.nih.gov/SNP |
| Environmental Genome DB | www.niehs.nih.gov/envgenom |
| GeneSNPs | www.genome.utah.edu/genesnps |
| Human Gene Mutation DB (Cardiff) | www.hgmd.org |
| HGVbase | hgvbase.cgb.ki.se |
| Human Genome Variation Society | www.hgvs.org |
| Hapmap Project | www.hapmap.org |
| Innate Immunity PGA | innateimmunity.net |
| JSNP Database | snp.ims.u-tokyo.ac.jp |
| Perlegen | (proprietary) |
| PharmGKB | www.pharmgkb.org |
| PromoLign | polly.wustl.edu/promolign/main.html |
| PupaSNP | pupasnp.bioinfo.cnio.es |
| ReguLign | polly.wustl.edu/regulign/default.html |
| Seattle SNPs (UW-FHCRC) | pga.mbt.washington.edu |
| SIFT | blocks.fhcrc.org/sift/SIFT.html |
| The SNP Consortium | snp.cshl.org |

Web references for tools and databases useful in finding and characterizing functional *cis*-regulatory polymorphisms.

protein tyrosine phosphatase, nonreceptor type 1 (PTP1N) inhibitors (Di Paola et al., 2002). Human glucocorticoid receptor beta (hGRβ) acts as a dominant negative factor for the steroid-responsive human glucocorticoid receptor alpha (hGRα, Bamberger et al., 1995), and increased hGRβ expression has been associated with steroid resistance in asthmatics (Hamid et al., 1999; Sousa et al., 2000). A SNP in the 3′ UTR of hGRβ disrupts an ATTTA motif and leads to increased mRNA stability suggesting the functional cause of steroid resistance (Derijk et al., 2001; Schaaf & Cidlowski, 2002). AREs collected in a database (Bakheet et al., 2003) demonstrate how the identification of consensus motifs and novel regulatory elements in combination with integrated genomic polymorphism databases may allow parallel characterization of functional polymorphisms. Further effects of 'silent' polymorphisms in transcribed regions will likely be uncovered as methods improve for the difficult problem of predicting RNA tertiary structure (Chen et al., 1999; Zuker, 2003). Silent mutations predicted to change mRNA folding in drug-related genes have previously been supported experimentally: dopamine D2 receptor (*Drd2*; Duan et al., 2003) and *NAT1* (de Leon et al., 2000). Newer algorithms that include evolutionary weighting schemes will likely lead to further experimentally validated examples (e.g., comRNA; Ji et al., 2004).

## 3. Drug-metabolizing enzymes

Numerous genes encoding proteins involved in drug metabolism and transport have been categorized, and nomenclature of polymorphic variations is being standardized (see Table 3 for references). Accounting for the largest proportion of genetic variation affecting drug therapy are the cytochrome *P*450s, a large, highly polymorphic family of heme-containing mono-oxygenases (http://www.imm.ki.se/CYPalleles/; Rendic, 2002; Guengerich, 2004). The human genome encodes at least 57 cytochrome *P*450s and contains 58 cytochrome *P*450 pseudogenes (Nelson et al., 2004), which are organized into 18 families (enzymes sharing >40% identity) and 43 subfamilies (enzymes sharing >55% identity). The most important cytochrome *P*450s involved in drug metabolism are the members of the CYP1, CYP2, and CYP3 families (Danielson, 2002). Comprehensive cytochrome genotyping assays are becoming feasible and are now applied in pharmaceutical trials, but a key question is whether we have sufficiently determined the bulk of the functional alleles in human populations. Many alleles have been reported, but there is an inherent bias toward sequencing and genotyping of coding regions. As a result, a portion of phenotypic variation remains unaccounted for by genetic factors. Large-scale sequencing projects continue to reveal noncoding alterations that could affect expression and function of drug-relevant genes (Iida et al., 2001b; Adjei et al., 2003; Aklillu et al., 2003; Allorge et al., 2003; Saito et al., 2003; Blaisdell et al., 2004; Cauffiez et al., 2004; Fukushima-Uesaka et al., 2004; Murayama et al., 2004). Table 1 lists known functional noncoding polymorphisms, which include crucial drug-relevant functional alleles.

### 3.1. Cytochrome 1 family

CYP1A2 has roles in metabolism of clozapine, paracetamol, phenacetin, theophylline, imipramine, and tacrine and is generally probed with caffeine since it specifically demethylates this substrate. There is evidence for a number of functional, noncoding alleles in the sequence of *CYP1A2*. A moderately frequent single nucleotide change at a demonstrated protein binding site in the enhancer region (CYP1A2*1C allele) correlates with a decrease in enzyme activity (Nakajima et al., 1999). A single base change (−163C>A) in intron 1 (CYP1A2*1F), occurring frequently in a Japanese population (Chida et al., 1999), is correlated with high enzyme induction in Caucasians (Sachse et al., 1999). However, a study on the plasma concentrations of haloperidol in Japanese schizophrenics carrying CYP1A2*1F (Shimoda et al., 2002) and another on Swedish pregnant women with the same allele (Nordmark et al., 2002) dispute this connection. Another polymorphism in intron 1 (−729C>T) was found in an Ethiopian population 10 base pair (bp) upstream from one previously reported

Table 3
Nomenclature guides for selected human gene families and alleles

| Gene class | URL reference | Reference |
|---|---|---|
| Alcohol dehydrogenases | www.gene.ucl.ac.uk/nomenclature/genefamily/ADH.shtml | Duester et al., 1999 |
| Aldehyde dehydrogenases | www.aldh.org/ | Vasilou et al., 1999 |
| Arylamine *N*-acetyltransferases | www.louisville.edu/medschool/pharmacology/NAT.html | Hein et al., 2000 |
| Cytochrome P450s | www.imm.ki.se/CYPalleles/ | Nelson et al., 2004 |
| Dihydropyrmidine dehydrogenase | (none available) | McLeod et al., 1998 |
| Organic anion transporting polypeptides | www.bioparadigms.org/slc/ (see Glatt & Meinl, 2004 for amino acid changing alleles) | Hagenbuch & Meier, 2004 |
| Sulfotranferases | | Blanchard et al., 2004; Glatt & Meinl, 2004 |
| UDP glucuronosyltransferases | som.flinders.edu.au/FUSA/ClinPharm/UGT/ | Mackenzie et al., 1997 |

Literature and web references for gene and allele nomenclature of families with pharmacogenetic importance. Definitive allele lists are not available for all families.

(−739T>G; Aklillu et al., 2003). This novel allele (CYP1A2*1K) associates with decreased in vivo metabolism, decreased expression in reporter constructs, and disruption of binding within intron 1 by a member of the E twenty six (Ets) family of proteins (Aklillu et al., 2003). The results suggest that the discrepancy between previous intron 1 studies may be due to failure to completely determine haplotype structures since CYP1A2*1K (−163C>A, −729C>T, −739T>G) was shown to be functionally significant, but CYP1A2*1F (−163C>A) and CYP1A2*1J (−163C>A, −739T>G) were not. Another SNP (3534G>A) in the donor splice site of intron 6 (CYP1A2*7) was suggested to account for extremely high clozapine concentrations at normal doses in a single individual, but this has not been replicated (Allorge et al., 2003). These results illustrate difficulties in assigning functional properties to polymorphisms in regulatory regions and combinations of polymorphisms in haplotypes and, moreover, in relating them to pharmacokinetic differences in vivo where more than one factor contributes to the phenotype.

### 3.2. Cytochrome 2 family

CYP2A6 accounts for approximately 10% of human liver microsome CYPs and is the major player in oxidation of nicotine, cotinine, and a few pharmaceuticals (e.g., fadrozole, halothan, losigamone, letrozole, methoxyflurane, SM12502; Pelkonen et al., 2000). Interindividual expression levels vary more than 10-fold and are attributed to environmental and genetic factors, with Asian populations having a high proportion of poor metabolizers (PMs; Pelkonen et al., 2000). An uncommon allele (CYP2A6*12) results in a crossing over event between *CYP2A6* and *CYP2A7* in intron 2, addition of 10 amino acids and subsequently lower 7-hydroxylation activity of the enzyme (Oscarson et al., 2002). A TATA box allele (CYP2A6*9) with ~23% frequency in Asian populations and ~5% in Caucasians correlates with lower expression level (mRNA and protein) and enzyme activity (Pitarque et al., 2001; Kiyotani et al., 2003). Recently an additional functional promoter allele (CYP2A6*1D) with high prevalence in Caucasians has been described which appears to disrupt an enhancer element in reporter assays (Pitarque et al., 2004). A novel regulatory polymorphism (CYP2A6*1H) that disrupts binding of nuclear transcription factor Y (NF-Y) to the *CYP2A6* enhancer region affects expression and was assayed alone and in combination with CYP2A6*1D (CYP2A6*1J; von Richter et al., 2004).

The proteins encoded by *CYP2C* genes account for approximately 20% of the total liver cytochrome *P*450 content in humans (Imaoka et al., 1996) and are responsible for metabolizing approximately 20% of clinically administered drugs. CYP2C19 is the cytochrome *P*450 isoform primarily responsible for metabolism of the anticonvulsant agent (*S*)-mephenytoin. Individuals can be characterized as either extensive metabolizers (EMs) or PMs. The PM phenotype occurs in 2–5% of Caucasian populations and 18–23% of Asian populations (Kupfer & Preisig, 1984; Nakamura et al., 1985). The major genetic defect responsible for the CYP2C19 PM phenotype is a single base pair (681G>A) mutation in exon 5 of *CYP2C19* (CYP2C19*2), which creates an aberrant splice site. This alters the reading frame of the mRNA starting with amino acid 215 and produces a premature termination codon (PTC) 20 amino acids downstream, resulting in a truncated nonfunctional protein (De Morais et al., 1994a, 1994b). Another SNP (636G>A; *CYP2C19*3*) in exon 4, also creates a PTC and is responsible for the PM phenotype in Japanese populations but not in Caucasian populations (De Morais et al., 1994a, 1994b). The PM phenotype in Caucasian populations is also partially explained by other SNPs: disruption of the ATG initiation codon (change to GTG; CYP2C19*4; Ferguson et al., 1998), changes in amino acids (CYP2C19*5A, CYP2C19*5B, CYP2C19*6), and a single nucleotide transversion (intronic splice site variation [IVS]5+2T>A) in the GT 5′ donor splice site of intron 5 (CYP2C19*7; Ibeanu et al., 1999). Overall, variations in splicing contribute to a significant extent to the PM phenotype.

*CYP2C9* is the most highly expressed member of the *CYP2C* subfamily in hepatic tissue, and metabolizes 16% of drugs in current clinical use, including some drugs with narrow therapeutic indices such as the hypoglycemic tolbutamide, the anticonvulsant phenytoin, and the anticoagulant (*S*)-warfarin (Schwarz, 2003). Several SNPs that change amino acids and result in reduced enzyme activity have been identified and associated with adverse drug reactions or toxicity to drugs metabolized by CYP2C9 (Aithal et al., 1999; Lee et al., 2002; Ho et al., 2003; Schwarz, 2003). The deletion of an adenine at base pair 818 of the mRNA causes frame shift and yields a nonfunctional protein (CYP2C9*6; Kidd et al., 2001). While the allele frequency of this variant is <1%, it has been associated with toxicity after treatment with normal doses of phenytoin (Kidd et al., 2001).

*CYP2D6* is the most polymorphic cytochrome gene, constitutes 2% of total hepatic cytochrome *P*450 content (Shimada et al., 1994; Imaoka et al., 1996), and supports oxidative metabolism of more than 70 pharmaceuticals. Genetic polymorphisms in the coding region of the *CYP2D6* gene have been extensively investigated (see review Zanger et al., 2004). More than 70 SNPs have been identified so far, and the focus has been on the coding region and mRNA splice sites that are responsible for the PM phenotype (7–10% in Caucasian populations and ~1% in Asian populations; Zanger et al., 2004). One of the main functional defects, a splicing defect mutation (1846G>A; CYP2D6*4, with an allele frequency of 20–25%) in the intron 3/exon 4 boundary causes a shift of the consensus acceptor splice site of the third intron by one base pair, yielding a spliced mRNA with one additional base, an altered reading frame and a PTC (Kagimoto et al., 1990).

Other genetic mechanisms for null alleles include frame shifts resulting from single or multiple base pair insertion or deletion (CYP2D6*3, CYP2D6*6, CYP2D6*15, CYP2D6*19, CYP2D6*20, CYP2D6*38, CYP2D6*42; Kagimoto et al., 1990; Saxena et al., 1994; Sachse et al., 1996; Marez-Allorge et al., 1999), a SNP that creates a PTC (CYP2D6*8; Broly et al., 1995), and other splicing defect mutations (CYP2D6*11, CYP2D6*44; Marez et al., 1995; Yamazaki et al., 2003), in addition to entire gene deletion (CYP2D6*5) or duplications (Gaedigk et al., 1991) and amino acid changes (CYP2D6*7,12; Evert et al., 1994; Marez et al., 1996). However, only a few SNPs in the promoter region have been identified, and phenotypic consequences have not been demonstrated. Since 'normotypic' *CYP2D6* carriers still display large variations in metabolic capacity, including the intermediate metabolizer (IM) phenotype, we need to discern whether this is due to yet unrecognized polymorphisms acting in *cis* or *trans*, or to enzyme induction effects. A SNP in the promoter region (−1548C>G) within the CYP2D6*41 allele has been associated with CYP2D6 expression, with the G allele correlating with higher levels of expression (Lovlie et al., 2001; Zanger et al., 2001). However, because this SNP is in linkage with other SNPs known to affect expression (Raimundo et al., 2000), it is not clear which is functional. The mutation has been used as a marker to rule out CYP2D6 PM status (Gaedigk et al., 2003). An intron 6 SNP (2988G>A) with a frequency of 8.4% in Caucasians was found to be an improved predictive marker for the IM phenotype over CYP2D6*41 (Raimundo et al., 2004).

Although most frameshift mutations in cytochrome *P*450 genes cause nonfunctional proteins, a frameshift mutation in the *CYP2D7* pseudogene generates a functional enzyme (Pai et al., 2004). This common single base pair deletion (138delT) generates an open reading frame in the *CYP2D7* pseudogene and a spliced variant containing partial inclusion of intron 6. This transcript produces a functional protein, that is expressed in the brain, but not liver or kidney. The variant CYP2D7 metabolizes codeine to morphine more efficiently than CYP2D6 in Neuro2a cells and also colocalizes with μ-opoid receptors in brain tissue, suggesting a possible role in metabolism at the drug site of action.

Less than 1% of hepatic cytochrome *P*450 content is due to CYP2B6, yet it is involved in the metabolism of ~70 clinically employed drugs, including alfentanil, ketamine, bupropion, verapamil, tamoxifen, efavirenz, and drugs of abuse such as methylenedioxymeth-amphethamine and nicotine (Lang et al., 2001). CYP2B6 activity in liver microsomes varies more than 100-fold among different individuals (Yamano et al., 1989; Ekins et al., 1998), with female subjects having higher levels of mRNA, protein and enzyme activity than males (Lamba et al., 2003). Large interracial differences are also observed for CYP2B6 with Hispanic females having higher CYP2B6 activity than Caucasian or African-American females (Lamba et al., 2003).

Many variants have been identified for *CYP2B6* including mRNA splice variants (Lamba et al., 2003). These variants paint a complex picture because multiple potentially functional polymorphisms are found in linkage and thus produce epistatic interactions. The most common splice variant skips exons 4–6 (CYP2B6*9), while others lack the first 29 bp of exon 4 or contain an intron 3 insertion. Because of the presence of PTCs, all of these variants encode truncated proteins. A nonsynonymous SNP in exon 4 (15631G>T) that disrupts an ESE and a SNP (15582C>T) in an intron 3 branch site are correlated with these splicing variants. Several SNPs in the promoter region have also been reported. The −750T>C SNP (CYP2B6*1G) correlates with lower levels of expression. The −2320T>C SNP (CYP2B6*1B) in the hepatocyte nuclear factor 4 (HNF4) binding site in the promoter and the SNP in the intron 3 branch point (15582C>T) show a high degree of linkage disequilibrium and associate with low quantities of CYP2B6 protein in Caucasian females. Recently, several missense SNPs forming null alleles were identified, but their combined frequency is only 2.6% in a Caucasian population (Lang et al., 2004). In combination these genetic variants do not fully explain the phenotypic variability in CYP2B6 activity. Because *CYP2B6* expression is correlated with constitutive androstane receptor (CAR) expression (Lamba et al., 2003), it is possible that polymorphisms in the CAR gene might affect *CYP2B6* expression in *trans*. Recently, several novel tissue-specific variant isoforms of CAR have been identified (Lamba et al., 2004), but their association with *CYP2B6* expression remains to be established.

### 3.3. Cytochrome 3 family

The CYP3A subfamily of proteins is highly homologous but exhibits wide tissue expression differences, implying a diversity of regulatory control. CYP3A4 is a critical enzyme because it is involved in the metabolism of over 30% of clinically used drugs including cyclosporin, erythromycin, and nifedipine. Interindividual expression differences of *CYP3A4* in liver are substantial and have not been sufficiently ascribed to known functional polymorphisms (Hirota et al., 2004). Principal among these has been a promoter mutation (CYP3A4*1B) that disrupts a nifedipine-specific repressor element (Westlind et al., 1999; Amirimani et al., 2003) which has been suggested to associate with treatment-related leukemia (Felix et al., 1998). However, different reporter constructs and confounding results suggest the need for consideration of a more complex regulatory picture (Spurdle et al., 2002; Floyd et al., 2003). Clarification of polymorphic *trans*-factors also provided insufficient explanation (Zhang et al., 2001). A recent attempt to elucidate genetic components of variation scanned up to 13 kilobase (kb) upstream of *CYP3A4* and found a novel far upstream enhancer element with a polymorphism (−11,129_−11,128 ins TGT) that disrupts a upstream transcription factor 1 (USF1) binding site and reduces

expression, but is relatively uncommon (3.1%) in a French population (Matsumura et al., 2004). The complexity of the yet unfolding regulatory story of *CYP3A4* underlies the importance of careful analysis of regulatory regions and the need for application of new technologies to resolve the nature and extent of genetic factors contributing to variabilities in expression and activity.

Polymorphic *CYP3A5* expression may also contribute to interindividual and interracial differences in CYP3A-dependent drug clearance (Kuehl et al., 2001). CYP3A5 was formerly considered to be an extraheptic enzyme (Wrighton et al., 1989; Schuetz et al., 1992; Murray et al., 1995; Haehner et al., 1996; Kivisto et al., 1996), only sporadically found in the liver tissue of some individuals (Wrighton et al., 1989). Kuehl et al. (2001) discovered a SNP (6986A>G) in intron 3 (CYP3A5*3) creates a cryptic splice site and an extra exon (exon 3b) and is responsible for polymorphic expression of *CYP3A5* in the liver. This CYP3A5*3 allele encodes an aberrantly spliced mRNA with a PTC resulting in an unstable mRNA and no detectable CYP3A5 mRNA and protein in the liver. The allele frequency of CYP3A5*3 is reported to be 75.9% in a Japanese population (Saeki et al., 2003) and ~73% in an African-American population (Hustert et al., 2001a). CYP3A4 and CYP3A5 have overlapping substrate specificity (Bargetzi et al., 1989; Gorski et al., 1994; Guitton et al., 1997), and CYP3A5 can represent over 50% of the total hepatic CYP3A content in some individuals (Kuehl et al., 2001). Thus, genetic polymorphism of *CYP3A5* may play a role in the variability in CYP3A targeted drug response, in some human populations.

### 3.4. Other cytochromes

Other human cytochrome genes have reported functional regulatory polymorphisms, and their diversity indicates that more will likely be uncovered. A relatively new family of cytochromes, CYP4F, contains members important for inflammation physiology. CYP4F12 is expressed in liver and intestine and ω-hydroxylates eicosanoids, fatty acids, and antihistamines (e.g., ebastine; Bylund et al., 2001; Hashizume et al., 2001). Cauffiez et al. (2004) recently published the first study of the *CYP4F12* promoter region and revealed 2 alleles (CYP4F12*v1, CYP4F12*v2) common in the French population that associate with significantly reduced expression levels in HepG2 cells (Cauffiez et al., 2004). CYP4F12*v1 is a 192 bp deletion in intron 1 (21% frequency) that diminishes expression. CYP4F12*v2 is a nine SNP-phased haplotype (8.5% frequency) which also associates with lower expression. In silico analysis suggests functional sites within these alleles, but further characterization is needed. The extrahepatically expressed CYP2J2 metabolizes diclofenac, bufuralol, and astemizole and contains a common mutation in a putative Sp1 transcription factor (SP-1) site (King et al., 2002). Contrary to previous reports the functional effects of this SNP were only speculated upon after discovery, while effects for less common amino acid changes were actually investigated. However, a recent article verifies that CYP2J2*7 disrupts an SP-1 binding site and reduces transcription significantly (Speicker, 2004). Other proposed functional *CYP* polymorphisms include variable number tandem repeats (VNTRs) in the 5′ regulatory regions of *CYP2E1* (McCarver et al., 1998; Hu et al., 1999) and *CYP8A1* (Chevalier et al., 2002), and point mutation in the CYP7A1 promoter recently linked with reduced response to atorvastatin (Wang et al., 1998; Kajinami et al., 2004). Taken together the mutational record of the *CYP* superfamily argues for extensive consideration of regulatory mutations in all families critical to pharmacology and disease.

### 3.5. Other classes of drug-metabolizing enzymes

Drug-metabolizing enzymes other than the cytochromes also display significant interindividual allelic differences. Non-*CYP* genes and their variations may play increasingly important roles as pharmaceutical companies design drugs that evade the challenges of cytochrome variability (Ingelman-Sundberg, 2001). The cluster of *UDP-glucuronosyltranferase-1* (*UGT1*) genes (*UGT1* superfamily) at *2q37* exhibits exon sharing and harbors identified promoter and missense mutations that have been associated with 2- to 6-fold lower conjugation activity (Kohle et al., 2003). Pathways involving members of the *UGT1* superfamily act on approximately 35% of all drugs metabolized by phase II drug-metabolizing enzymes (Evans & Relling, 1999). Over and above many rare polymorphisms in the *UGT1A1* gene, a common promoter region dinucleotide repeat (5–8 repeats; UGT1A1*28) accounts for reductions in expression level via alteration of transcription initiation (Bosma et al., 1995; Guillemette, 2003). The polymorphism correlates with lower protein levels and reduced conjugation activity (Fang & Lazarus, 2004), and is implicated in toxicity to treatment with irinotecan via altered glucuronidation of its active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin; Iyer et al., 1999) and adverse reactions including neutropenia and diarrhea (Iyer et al., 2002). UGT1A1*28 is relatively frequent in many populations: Caucasian (~32%), Asian non-Japanese (~15%), African (~41%), Italian (~36%; Guillemette, 2003). A promoter region polymorphism in *UGT1A9* (UGT1A9*22) common in Japanese, Caucasians and African-Americans has recently been associated with higher expression via HepG2 reporter constructs, but related enzyme induction or metabolism phenotypes are unreported (Yamanaka et al., 2004).

Thiopurine (*S*)-methyltransferase (TPMT) is a prominent example of how pharmacogenetics can impact individual treatment. The enzyme is involved in the metabolism of thiopurines: the cytotoxic drugs 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), and the immunosupressant azathioprine, which is rapidly converted to 6-MP. Amino acid changing polymorphisms account for most of the

variability in the RBC activity of TPMT, although their frequency varies widely in different populations (Vuchetich et al., 1995; McLeod & Siva, 2002). VNTR in the promoter region of TPMT have been identified (Spire-Vayron de la Moureyre et al., 1998, 1999) and suggested as modulators of expression and thus enzyme activity (Fessing et al., 1998; Spire-Vayron de la Moureyre et al., 1998; Yan et al., 2000; Alves et al., 2001). These repeats appear to be in linkage disequilibrium with the nonsynonymous alleles making definitive declaration of their functional role difficult (Marinaki et al., 2003).

*N*-acetyltransferases (NAT) catalyze acetyltransfer from acetylcoenzyme A to an array of carcinogens and arylamine and hydrazine drugs (e.g., *para*-aminobenzoic acid [PABA], isoniazid, sulfamethazine, procainamide, nitrazepam, dapsone). The 2 human *NATs*, *NAT1* and *NAT2*, are intronless and so far have mainly nonsynonymous polymorphisms that associate with enzyme activity (Hein et al., 2000; Butcher et al., 2002). Slow acetylation genotypes correlate with adverse effects after combined isoniazid and rifampicin therapy (Ohno et al., 2000) and co-trimoxazole treatment (Zielinska et al., 1998). The extent of *N*-acetylation also accounts for variability in toxicity to amonafide treatment in cancer patients, with rapid acetylators experiencing significantly greater toxicity (Ratain et al., 1995). A relatively uncommon allele (NAT1*16) is an AAA insertion and C to A transversion in the 3′ UTR region and correlates with predicted structural variation, 2-fold lower expression and a similar reduction in *N*-acetylation of substrates (de Leon et al., 2000). Another 3′ UTR variant NAT1*10 is suggested to correlate with higher enzyme activity (Bell et al., 1995; Payton & Sim, 1998), but this has not been supported in a number of studies perhaps due to linkage with an amino acid change not detected in some of the former assays (Bruhn et al., 1999; de Leon et al., 2000; Hein et al., 2000).

The sulfotransferase (*SULT*) family forms sulfate conjugates with a variety of xenobiotics and endogenous small molecules and now numbers 17 distinct genes (Freimuth et al., 2004). Multiple studies report interindividual differences in SULT expression, drug-related response, and realization of a heritable component (Reiter & Weinshilboum, 1982; Bonham Carter et al., 1983; Weinshilboum, 1990; Glatt & Meinl, 2004). Noticeably, no functional studies on noncoding variants have been reported despite a substantial number of such variants identified in sequencing studies (Freimuth et al., 2001; Iida et al., 2001b; Glatt & Meinl, 2004).

A few aldehyde (ALDH) and alcohol (ADH) dehydrogenases are responsible for 90% of the metabolism of ethanol, a compound with potential for pharmacological interactions. Well-known coding variants in ADH2 and ALDH2 have been described which associate with resistance to alcoholism. ADH4 is expressed in specific portions of the GI tract (Vaglenova et al., 2003) and is essential for vitamin A metabolism (Duester et al., 2003), but may also have a role in first-pass metabolism of ethanol (Yin et al., 2003). A reporter assay indicated that a single functional promoter allele affects ADH4 expression (Edenberg et al., 1999), and no coding variations were identified in a subsequent sequencing study (Iida et al., 2002), but in vivo characterizations of allelic protein or tissue level effects are yet unreported. These examples show that further understanding of polymorphic expression in many types of genes beyond the cytochrome *P*450 superfamily is important to understanding the genetic basis of interindividual differences in drug responses.

## 4. Drug transporters

Modulation in drug transporter expression potentially affects the uptake and efficacy of many compounds. Numerous genes encode solute carriers (*SLC* families, >300 genes) and the class of primary active ATP-binding cassette (ABC) transporters (48 genes). Because of the size of these gene families, we only address a few examples here. The multiple drug resistance polypeptide ATP-binding cassette, subfamily B 1 (ABCB1: MDR1, Pgp) is an energy-dependent protein efflux pump that acts upon a wide range of natural and pharmacological substrates (see review Sun et al., 2004). A synonymous SNP (3435C>T) has been associated with low expression and altered pharmacokinetics in a number of studies (Hoffmeyer et al., 2000), but others have reported conflicting results (Sakaeda et al., 2001) or dismissed this as an unlikely functional SNP since it is not a coding alteration. In these cases, it is suggested that a functional allele (perhaps 2677G>A,T) must be in linkage, with 3435C>T being an indicator SNP. However, the synonymous polymorphism could affect mRNA structure, stability, or translational efficiency. Recently, Takane et al. (2004) conducted a functional analysis of *ABCB1* variants. They found 10 promoter variants (7 were novel), an association of 3435C>T with lower expression, association of promoter haplotypes with transcription level differences independent of 3435C>T, a novel transcription factor binding site which is disrupted in a haplotype correlating with lower expression, and ruled out differences in methylation status as a principal cause (Takane et al., 2004). Three promoter variants found in rare haplotypes were associated with higher transcriptional expression, including one (−129T>C) that was previously reported to be associated with high transport activity in hematopoietic stem cells (Calado et al., 2002). While there are numerous studies associating *ABCB1* polymorphisms with altered drug disposition and effect, these results are often not reproducible in different populations. This points to a lack of sufficient understanding of the interaction among multiple genetic factors determining *ABCB1* expression and function.

Two noncoding polymorphisms of the serotonin transporter (5-HTT) gene *SLC6A4* have been studied extensively based on allelic differences in expression in brain and other tissues (Lesch et al., 1994; Heils et al., 1996; Hranilovic et al., 2004). The contribution of serotonin neurophysiology to

psychiatric disorders is great, and thus the impact of these variants may be far-reaching. In particular, the short form of the promoter region polymorphism (serotonin gene-linked polymorphic region [5-HTTLPR]) correlates with lower transcriptional and translational activity (Heinz et al., 2000; Hranilovic et al., 2004), blunted fenfluramine-induced prolactin release (Reist et al., 2001), greater amygdala neuronal activity in response to fearful stimuli (Hariri et al., 2002), poorer efficacy of antidepressants (e.g., citalopram, Eichhammer et al., 2003; fluoxetine, Rausch et al., 2002; paroxetine, Pollock et al., 2000), and antidepressant induced mania in bipolar disorder (Mundo et al., 2001). The presence of a genetic component to variability in response to antidepressants is also suggested by family studies (e.g., O'Reilly et al., 1994). Contributions of this polymorphism to variation in colonic uptake of alosetron (Scherl & Frissora, 2003) and heroin dependence (Gerra et al., 2004) have also been suggested. However, there are dissenting results, and the debate over the extent of the functional importance of these alleles is yet to be settled. There are alternative alleles, splice variants, and polyadenylation signals that might play roles (Delbruck et al., 1997; Battersby et al., 1999; Michaelovsky et al., 1999; Frisch et al., 2000; Nakamura et al., 2000; Cigler et al., 2001; Sun et al., 2002) and significant population differences in genotype to consider (Gelernter et al., 1999; Lotrich et al., 2003).

The members of the organic anion-transporting polypeptide (OATP) superfamily, encoded by the solute carrier organic anion transporter family (*SLCO*) genes, are broadly expressed and mediate transport of a wide range of endogenous and exogenous compounds including anions, cations, neutral compounds, and peptidomimetic agents (Tirona & Kim, 2002; Hagenbuch & Meier, 2004). Regulatory region polymorphisms have been reported in a number of *SLCO* genes (Iida et al., 2001a; Tirona & Kim, 2002). OATP-A (*SLCO1A2*) is expressed in brain capillary endothelial cells suggesting a role in blood-brain barrier permeability of solutes and this transmembrane protein transports analgesic opoid peptides (Gao et al., 2000). There is a *SLCO1A2* SNP localized to a HNF1α binding site (Kullak-Ublick et al., 1997), but there are no functional reports on it or any of the identified nonsynonymous polymorphisms in the gene (Source: dbSNP). A newer far upstream polymorphism (−11187G>A) in OATP-C (*SLCO1B1*) was associated with 98% higher clearance (AUC) of pravastatin in Caucasian males and combined in a haplotype (SLCO1B1*17) with coding polymorphisms which exhibited a similar association (Niemi et al., 2004). Drug transporters will likely provide further examples of clinically relevant *cis*-regulatory polymorphisms.

## 5. Drug targets and receptors

Modulation of expression of drug targets is another avenue for studying interindividual differences in therapeutic response. In the field of cancer pharmacogenetics, understanding the expression patterns in patients' tumors or their untransformed genomes in somatic cells can guide selection or administration of treatment. The principal downstream target of the common chemotherapeutic 5-fluorouracil (5-FU) is thymidylate synthase (TYMS). Three copies of a 28 bp tandem repeat (thymidylate synthase enhancer region [TSER]*3) in the promoter of *TYMS* have been associated with higher TYMS levels (Horie et al., 1995) and poorer outcomes with 5-FU treatment (Horie et al., 1995; Villafranca et al., 2001). Here, again, we see a complex regulatory picture that needs dissection as further polymorphisms show similar association: a SNP within the TSER*3 (TSER*3RG) that abolishes a USF1 binding site (Mandola et al., 2003) and a 3′ UTR SNP now associated with message stability (Ulrich et al., 2000; Mandola et al., 2004). Inactivation of 5-FU is principally mediated via dihydropyrimidine dehydrogenase (DPYD), and variations in its activity can have fatal consequences (Van Kuilenburg et al., 2002). A splice site transition (DPYD*2A) accounts for some observed toxicity but has low frequency (<1%; Wei et al., 1996). Despite description of many other polymorphisms (Wei et al., 1996; McLeod et al., 1998; Collie-Duguid et al., 2000), 5-FU toxicity remains only partially understood. Other examples in cancer pharmacogenetics include applications of genotyping to treatment with well-known tyrosine kinase inhibitors (e.g., herceptin; Arteaga & Baselga, 2004). Functional explanations for these stratifying mutations, often involving genomic instability and high expression or loss of heterozygosity, are becoming understood (Sordella et al., 2004).

Drug targets in the brain and the degree to which their genetic variation explains differences in psychoses and their treatment have been the focus of much research. A synonymous 102C>T polymorphism of the 5-hydroxytryptamine (serotonin) receptor 2a (HTR2A) was previously proposed to associate with responsiveness to clozapine (Arranz et al., 1998), implying that it is in linkage disequilibrium with *cis*-acting regulatory polymorphisms. However, using this SNP as a marker for measuring relative allelic mRNA abundance, no difference in expression was detected in adult brain tissues (Bray et al., 2004). This argues against the presence of *cis*-acting regulatory polymorphisms. These findings do not address possible effects on translation but do argue for a shift of focus in future research on variants in this gene region.

An intriguing study in a large cohort of individuals treated with pravastatin recently revealed a significant association between reduction in low-density lipoprotein (LDL) levels after treatment and 2 intronic SNPs in the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) gene (Chasman et al., 2004). The SNPs are in linkage disequilibrium but are not near intron-exon borders or CpG dinucleotides. The functional explanation remains an open question, but there is disequilibrium with a 3′ UTR variant present in mRNA, raising the possibility of altered

pretranslational regulation. If a functional allele is identified and generalized to other statins, it may help explain why a significant proportion of treated individuals does not respond readily to this blockbuster class of drugs (Schmitz & Drobnik, 2003). There are no definitive genetic markers that account for observed variations despite a number of non-coding variations previously associated with differential responses to statins (de Maat et al., 1999; Marian et al., 2000; Zambon et al., 2001; Schmitz & Drobnik, 2003; Kajinami et al., 2004).

These few transporter and receptor examples highlight the important contribution regulatory polymorphisms will play in defining genetic variability in pharmacokinetics and pharmacodynamics. The identification of allelic differences in key genes may also allow eventual targeting of sequence-specific functional *cis*-regulatory polymorphisms (Fluiter et al., 2003; Miller et al., 2003; Achenbach et al., 2004; Bruno et al., 2004). Alternatively, general classes of *cis*-regulatory variants may be targeted. For instance, aminoglycoside antibiotics have been shown to inhibit proofreading activity by misincorporating an amino acid and thus ameliorating nonsense mutations through increased translational read-through (Howard et al., 1996). Analysis of cystic fibrosis genotypes among over 1000 known variants has thus been used to select targeted therapy in a small, stratified subgroup of the disease population (Wilschanski et al., 2003).

## 6. *Cis*-acting polymorphisms in relevant *trans*-factors

While most genes harbor *cis*-acting changes relevant to their expression, genetic variants regulating gene expression in *trans* are likely to dominate interindividual variability in mRNA expression profiles (Wittkopp et al., 2004). This results from pleiotropic consequences on gene expression by *cis*-acting polymorphisms that alter the function of transcription factors, receptors, and other signaling molecules. We therefore need to consider *cis*-acting polymorphisms within the genes encoding *trans*-factors influencing drug-metabolizing enzymes, transporters and targets, reflecting complex regulatory networks underlying pharmacogenetics phenotypes (Rushmore & Kong, 2002; Akiyama & Gonzalez, 2003). Among the transcription factors, we can distinguish between those determining constitutive expression, on the one hand, and factors mediating enzyme induction, a common cause of temporal changes in drug-metabolizing capacity. Key players in the regulation of genes discussed in this review include members of the nuclear hormone receptor superfamily (pregnane X receptor [PXR], CAR, farnesol X receptor [FXR], HNF4α, peroxisome proliferator-activated receptor alpha [PPAR-α], vitamin D receptor [VDR]), and transcription factors—HNF1α, HNF3, HNF6, CCAAT/enhancer binding proteins [C/EBP], albumin D-site binding protein [DBP]; Akiyama & Gonzalez, 2003). Studies of human polymorphisms in these genes may have important pharmacological implications, but current understanding is immature.

For example, PXR stimulates transcription of a number of drug-metabolizing enzymes (e.g., *CYP3A4*; Lehmann et al., 1998; Goodwin et al., 1999; Schuetz et al., 2001; Tirona et al., 2003), as well as *ABCB1* in intestine (Geick et al., 2001). *CYP3A4 cis*-variations do not adequately account for observed drug phenotypes, thus implicating variability in other factors such as PXR (Lamba et al., 2002). PXR expression correlates with CYP3A subfamily expression (Westlind-Johnsson et al., 2003). In vitro assays of *PXR* variants encoding altered proteins have demonstrated correlated changes in CYP3A4 expression, particularly in response to rifampicin induction of the enzyme (Hustert et al., 2001b; Zhang et al., 2001). However, these changes are infrequent, and a heterozygous carrier of one such polymorphism showed normal CYP3A4 metabolism (Zhang et al., 2001). Three silent mutations in PXR also correlated with changes in its expression level (Zhang et al., 2001). Moreover, PXR is also transcribed from an alternative initiation codon (Bertilsson et al., 1998). A 6-bp deletion in the promoter of this alternative transcript, *hPAR*-2, disrupts a predicted HNF1α binding site and abolishes transcription in a liver cell line, but again no human phenotype was observed (Uno et al., 2003).

HNFs have a large role in the liver-specific enhancement of transcription of many cytochromes (Akiyama & Gonzalez, 2003). *HNF1*α and *HNF4*α polymorphisms have been widely scanned because of their relation with diabetes in many populations (Ryffel, 2001), but associated effects on drug phenotypes are not well investigated. Functional *HNF1*α promoter polymorphisms, including one in a putative HNF4 binding site have been described (Gragnoli et al., 1997). Amino acid changes were recently shown to result in a PTC and decreased protein stability through nonsense-mediate decay (Harries et al., 2004). The documented role of HNFs in the expression of many genes of pharmacological relevance (e.g., HNF4 regulation of CYP3A4; Tirona et al., 2003) warrants further work on the effects of polymorphisms in these *trans*-factors. Because genes are regulated by multiple factors, often with overlapping specificity, characterization of the effects of variation in *trans* factors remains a challenging task. Ideally, for genes of critical pharmacological importance, we will eventually arrive at a multivariate understanding that accounts for variations at the target locus and variations effecting the contextual *trans*-inputs to the locus.

## 7. Conclusion

Accounting for genetic components of variation in all phenotypes must ultimately be done at *cis*-sequences with pleoitropic effects reverberating in *trans* (Fig. 1). The examples presented here demonstrate that as pharmacogenetics proceeds, continuing identification of novel *cis*-

regulatory variants and their functional effects is necessary. High-throughput genotyping for clinical pharmacological applications is increasingly feasible, and screening panels are easily developed, but the identification and selection of relevant alleles lags behind. This process remains difficult for reasons discussed here, including population-specific sources of error, limits of experimental approaches, and the challenges of investigating the many potential regulatory modes, including accounting for epistasis and epigenetics. If well-characterized genes are indicative, a few common polymorphisms within a population will cover the bulk of the genetic variation while many, less frequent polymorphisms will explain the rest. However, in genes that have accumulated many mutations, such as *CYP2D6*, a complete accounting of all functional polymorphisms is needed to permit prospective clinical applications including drug choice and selection of dosage. Because frequent null mutations can combine with less frequent mutations on the sister chromosome (compound heterozygosity), even low-frequency variants may be clinically important. Haplotyping may be effective in identifying key combinations of polymorphisms, but insufficient marker density and assumption of linkage over large sequences may lead to missed functional alleles. The standardization of nomenclature (Table 3) and integration of databases (Table 2) also remain ongoing challenges as the number of marker and functional alleles continues to increase. Appropriate meta-analyses can also provide a bird's-eye view of the most important alleles within specific populations (e.g., Phillips et al., 2001). Although newer drugs may evade cytochrome metabolism, the examples given here demonstrate that many classes of genes must be pursued as potential contributors to interindividual differences in drug response, and efforts to identify their functional *cis*-regulatory variants will have lasting importance.

## Acknowledgments

This work was supported in part by the NIH National Institute on Drug Abuse, DA018744, and an Ohio State University Distinguished University Fellowship.

## References

Achenbach, J. C., Chiuman, W., Cruz, R. P., & Li, Y. (2004). DNAzymes: from creation in vitro to application in vivo. *Curr Pharm Biotechnol 5*(4), 321–336.

Adjei, A. A., Thomae, B. A., Prondzinski, J. L., Eckloff, B. W., Wieben, E. D., & Weinshilboum, R. M. (2003). Human estrogen sulfotransferase (SULT1E1) pharmacogenomics: gene resequencing and functional genomics. *Br J Pharmacol 139*(8), 1373–1382.

Aithal, G. P., Day, C. P., Kesteven, P. J., & Daly, A. K. (1999). Association of polymorphisms in the cytochrome *P*450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. *Lancet 353*(9154), 717–719.

Akiyama, T. E., & Gonzalez, F. J. (2003). Regulation of *P*450 genes by liver-enriched transcription factors and nuclear receptors. *Biochim Biophys Acta 1619*(3), 223–234.

Aklillu, E., Carrillo, J. A., Makonnen, E., Hellman, K., Pitarque, M., Bertilsson, L., et al. (2003). Genetic polymorphism of CYP1A2 in Ethiopians affecting induction and expression: characterization of novel haplotypes with single-nucleotide polymorphisms in intron 1. *Mol Pharmacol 64*(3), 659–669.

Allorge, D., Chevalier, D., Lo-Guidice, J. M., Cauffiez, C., Suard, F., Baumann, P., et al. (2003). Identification of a novel splice-site mutation in the CYP1A2 gene. *Br J Clin Pharmacol 56*(3), 341–344.

Alves, S., Amorim, A., Ferreira, F., & Prata, M. J. (2001). Influence of the variable number of tandem repeats located in the promoter region of the thiopurine methyltransferase gene on enzymatic activity. *Clin Pharmacol Ther 70*(2), 165–174.

Amirimani, B., Ning, B., Deitz, A. C., Weber, B. L., Kadlubar, F. F., & Rebbeck, T. R. (2003). Increased transcriptional activity of the CYP3A4*1B promoter variant. *Environ Mol Mutagen 42*(4), 299–305.

Arranz, M. J., Munro, J., Sham, P., Kirov, G., Murray, R. M., Collier, D. A., et al. (1998). Meta-analysis of studies on genetic variation in 5-HT2A receptors and clozapine response. *Schizophr Res 32*(2), 93–99.

Arteaga, C. L., & Baselga, J. (2004). Tyrosine kinase inhibitors: why does the current process of clinical development not apply to them? *Cancer Cell 5*(6), 525–531.

Bakheet, T., Williams, B. R., & Khabar, K. S. (2003). Ared 2.0: an update of Au-rich element mRNA database. *Nucleic Acids Res 31*(1), 421–423.

Bamberger, C. M., Bamberger, A. M., de Castro, M., & Chrousos, G. P. (1995). Glucocorticoid receptor beta, a potential endogenous inhibitor of glucocorticoid action in humans. *J Clin Invest 95*(6), 2435–2441.

Bargetzi, M. J., Aoyama, T., Gonzalez, F. J., & Meyer, U. A. (1989). Lidocaine metabolism in human liver microsomes by cytochrome *P*450IIIA4. *Clin Pharmacol Ther 46*(5), 521–527.

Battersby, S., Ogilvie, A. D., Blackwood, D. H., Shen, S., Muqit, M. M., Muir, W. J., et al. (1999). Presence of multiple functional polyadenylation signals and a single nucleotide polymorphism in the 3′ untranslated region of the human serotonin transporter gene. *J Neurochem 72*(4), 1384–1388.

Bell, D. A., Badawi, A. F., Lang, N. P., Ilett, K. F., Kadlubar, F. F., & Hirvonen, A. (1995). Polymorphism in the *N*-acetyltransferase 1 (NAT1) polyadenylation signal: association of NAT1*10 allele with higher n-acetylation activity in bladder and colon tissue. *Cancer Res 55*(22), 5226–5229.

Bertilsson, G., Heidrich, J., Svensson, K., Asman, M., Jendeberg, L., Sydow-Backman, M., et al. (1998). Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc Natl Acad Sci U S A 95*(21), 12208–12213.

Blanchard, R. L., Freimuth, R. R., Buck, J., Weinshilboum, R. M., & Coughtrie, M. W. (2004). A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics 14*(3), 199–211.

Blaisdell, J., Jorge-Nebert, L. F., Coulter, S., Ferguson, S. S., Lee, S. J., Chanas, B., et al. (2004). Discovery of new potentially defective alleles of human CYP2C9. *Pharmacogenetics 14*(8), 527–537.

Bonham Carter, S. M., Rein, G., Glover, V., Sandler, M., & Caldwell, J. (1983). Human platelet phenolsulphotransferase m and p: substrate specificities and correlation with in vivo sulphoconjugation of paracetamol and salicylamide. *Br J Clin Pharmacol 15*(3), 323–330.

Bosma, P. J., Chowdhury, J. R., Bakker, C., Gantla, S., de Boer, A., Oostra, B. A., et al. (1995). The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med 333*(18), 1171–1175.

Botma, G. J., Verhoeven, A. J., & Jansen, H. (2001). Hepatic lipase promoter activity is reduced by the C-480T and G-216A substitutions present in the common LIPC gene variant, and is increased by upstream stimulatory factor. *Atherosclerosis 154*(3), 625–632.

Bray, N. J., Buckland, P. R., Owen, M. J., & O'Donovan, M. C. (2003). *Cis*-acting variation in the expression of a high proportion of genes in human brain. *Hum Genet 113*(2), 149–153.

Bray, N. J., Buckland, P. R., Hall, H., Owen, M. J., & O'Donovan, M. C. (2004). The serotonin-2a receptor gene locus does not contain common polymorphism affecting mRNA levels in adult brain. *Mol Psychiatry 9*(1), 109–114.

Broly, F., Marez, D., Lo Guidice, J. M., Sabbagh, N., Legrand, M., Boone, P., et al. (1995). A nonsense mutation in the cytochrome *P*450 CYP2D6 gene identified in a Caucasian with an enzyme deficiency. *Hum Genet 96*(5), 601–603.

Bruhn, C., Brockmoller, J., Cascorbi, I., Roots, I., & Borchert, H. H. (1999). Correlation between genotype and phenotype of the human arylamine *N*-acetyltransferase type 1 (NAT1). *Biochem Pharmacol 58*(11), 1759–1764.

Bruno, I. G., Jin, W., & Cote, G. J. (2004). Correction of aberrant FGFR1 alternative RNA splicing through targeting of intronic regulatory elements. *Hum Mol Genet 13*(20), 2409–2420.

Butcher, N. J., Boukouvala, S., Sim, E., & Minchin, R. F. (2002). Pharmacogenetics of the arylamine *N*-acetyltransferases. *Pharmacogenomics J 2*(1), 30–42.

Bylund, J., Bylund, M., & Oliw, E. H. (2001). cDNA cloning and expression of CYP4F12, a novel human cytochrome *P*450. *Biochem Biophys Res Commun 280*(3), 892–897.

Calado, R. T., Falcao, R. P., Garcia, A. B., Gabellini, S. M., Zago, M. A., & Franco, R. F. (2002). Influence of functional MDR1 gene polymorphisms on p-glycoprotein activity in CD34+hematopoietic stem cells. *Haematologica 87*(6), 564–568.

Cartegni, L., Wang, J., Zhu, Z., Zhang, M. Q., & Krainer, A. R. (2003). Esefinder: a web resource to identify exonic splicing enhancers. *Nucleic Acids Res 31*(13), 3568–3571.

Cauffiez, C., Klinzig, F., Rat, E., Tournel, G., Allorge, D., Chevalier, D., et al. (2004). Functional characterization of genetic polymorphisms identified in the human cytochrome *P*450 4F12 (CYP4F12) promoter region. *Biochem Pharmacol 67*(12), 2231–2238.

Chasman, D. I., Posada, D., Subrahmanyan, L., Cook, N. R., Stanton Jr., V. P., & Ridker, P. M. (2004). Pharmacogenetic study of statin therapy and cholesterol reduction. *JAMA 291*(23), 2821–2827.

Chen, Y., Carlini, D. B., Baines, J. F., Parsch, J., Braverman, J. M., Tanda, S., et al. (1999). RNA secondary structure and compensatory evolution. *Genes Genet Syst 74*(6), 271–286.

Chevalier, D., Allorge, D., Lo-Guidice, J. M., Cauffiez, C., Lepetit, C., Migot-Nabias, F., et al. (2002). Sequence analysis, frequency and ethnic distribution of VNTR polymorphism in the 5′ -untranslated region of the human prostacyclin synthase gene (CYP8A1). *Prostaglandins Other Lipid Mediat 70*(1–2), 31–37.

Chida, M., Yokoi, T., Fukui, T., Kinoshita, M., Yokota, J., & Kamataki, T. (1999). Detection of three genetic polymorphisms in the 5′ -flanking region and intron 1 of human CYP1A2 in the Japanese population. *Jpn J Cancer Res 90*(9), 899–902.

Cigler, T., LaForge, K. S., McHugh, P. F., Kapadia, S. U., Leal, S. M., & Kreek, M. J. (2001). Novel and previously reported single-nucleotide polymorphisms in the human 5-HT(1B) receptor gene: no association with cocaine or alcohol abuse or dependence. *Am J Med Genet 105*(6), 489–497.

Collie-Duguid, E. S., Etienne, M. C., Milano, G., & McLeod, H. L. (2000). Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics 10*(3), 217–223.

Conde, L., Vaquerizas, J. M., Santoyo, J., Al-Shahrour, F., Ruiz-Llorente, S., Robledo, M., et al. (2004). PupaSNP finder: a web tool for finding SNPS with putative effect at transcriptional level. *Nucleic Acids Res 32*(Web Server issue), W242–W248.

Conne, B., Stutz, A., & Vassalli, J. D. (2000). The 3′ untranslated region of messenger RNA: a molecular 'hotspot' for pathology? *Nat Med 6*(6), 637–641.

Cooper, T. A., & Mattox, W. (1997). The regulation of splice-site selection, and its role in human disease. *Am J Hum Genet 61*(2), 259–266.

Danielson, P. B. (2002). The cytochrome *P*450 superfamily: biochemistry, evolution and drug metabolism in humans. *Curr Drug Metab 3*(6), 561–597.

Day, D. A., & Tuite, M. F. (1998). Post-transcriptional gene regulatory mechanisms in eukaryotes: an overview. *J Endocrinol 157*(3), 361–371.

de Leon, J. H., Vatsis, K. P., & Weber, W. W. (2000). Characterization of naturally occurring and recombinant human *N*-acetyltransferase variants encoded by NAT1. *Mol Pharmacol 58*(2), 288–299.

de Maat, M. P., Jukema, J. W., Ye, S., Zwinderman, A. H., Moghaddam, P. H., Beekman, M., et al. (1999). Effect of the stromelysin-1 promoter on efficacy of pravastatin in coronary atherosclerosis and restenosis. *Am J Cardiol 83*(6), 852–856.

De Morais, S. M., Wilkinson, G. R., Blaisdell, J., Meyer, U. A., Nakamura, K., & Goldstein, J. A. (1994a). Identification of a new genetic defect responsible for the polymorphism of (*S*)-mephenytoin metabolism in Japanese. *Mol Pharmacol 46*(4), 594–598.

De Morais, S. M., Wilkinson, G. R., Blaisdell, J., Nakamura, K., Meyer, U. A., & Goldstein, J. A. (1994b). The major genetic defect responsible for the polymorphism of *S*-mephenytoin metabolism in humans. *J Biol Chem 269*(22), 15419–15422.

Delbruck, S. J., Wendel, B., Grunewald, I., Sander, T., Morris-Rosendahl, D., Crocq, M. A., et al. (1997). A novel allelic variant of the human serotonin transporter gene regulatory polymorphism. *Cytogenet Cell Genet 79*(3–4), 214–220.

Derijk, R. H., Schaaf, M. J., Turner, G., Datson, N. A., Vreugdenhil, E., Cidlowski, J., et al. (2001). A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *J Rheumatol 28*(11), 2383–2388.

Ding, C., Maier, E., Roscher, A. A., Braun, A., & Cantor, C. R. (2004). Simultaneous quantitative and allele-specific expression analysis with real competitive PCR. *BMC Genet 5*(1), 8.

Di Paola, R., Frittitta, L., Miscio, G., Bozzali, M., Baratta, R., Centra, M., et al. (2002). A variation in 3′ UTR of HPTP1B increases specific gene expression and associates with insulin resistance. *Am J Hum Genet 70*(3), 806–812.

Duan, J., Wainwright, M. S., Comeron, J. M., Saitou, N., Sanders, A. R., Gelernter, J., et al. (2003). Synonymous mutations in the human dopamine receptor d2 (*Drd2*) affect mRNA stability and synthesis of the receptor. *Hum Mol Genet 12*(3), 205–216.

Duester, G., Farres, J., Felder, M. R., Holmes, R. S., Hoog, J. O., Pares, X., et al. (1999). Recommended nomenclature for the vertebrate alcohol dehydrogenase gene family. *Biochem Pharmacol 58*(3), 381–395.

Duester, G., Mic, F., & Molotkov, A. (2003). Cytosolic retinoid dehydrogenases govern ubiquitous metabolism of retinol to retinaldehyde followed by tissue-specific metabolism to retinoic acid. *Chem Biol Interact 201–210*, 143–144.

Edenberg, H. J., Jerome, R. E., & Li, M. (1999). Polymorphism of the human alcohol dehydrogenase 4 (ADH4) promoter affects gene expression. *Pharmacogenetics 9*(1), 25–30.

Eichhammer, P., Langguth, B., Wiegand, R., Kharraz, A., Frick, U., & Hajak, G. (2003). Allelic variation in the serotonin transporter promoter affects neuromodulatory effects of a selective serotonin transporter reuptake inhibitor (SSRI). *Psychopharmacology (Berl) 166*(3), 294–297.

Ekins, S., Vandenbranden, M., Ring, B. J., Gillespie, J. S., Yang, T. J., Gelboin, H. V., et al. (1998). Further characterization of the expression in liver and catalytic activity of CYP2B6. *J Pharmacol Exp Ther 286*(3), 1253–1259.

Enard, W., Khaitovich, P., Klose, J., Zollner, S., Heissig, F., Giavalisco, P., et al. (2002). Intra- and interspecific variation in primate gene expression patterns. *Science 296*(5566), 340–343.

Evans, W. E., & Relling, M. V. (1999). Pharmacogenomics: translating functional genomics into rational therapeutics. *Science 286*(5439), 487–491.

Evert, B., Griese, E. U., & Eichelbaum, M. (1994). A missense mutation in exon 6 of the CYP2D6 gene leading to a histidine

324 to proline exchange is associated with the poor metabolizer phenotype of sparteine. *Naunyn Schmiedebergs Arch Pharmacol 350*(4), 434–439.

Fang, J. L., & Lazarus, P. (2004). Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: significantly decreased glucuronidating activity against benzo(*a*)pyrene-7,8-dihydrodiol(−) in liver microsomes from subjects with the UGT1A1*28 variant. *Cancer Epidemiol Biomark Prev 13*(1), 102–109.

Felix, C. A., Walker, A. H., Lange, B. J., Williams, T. M., Winick, N. J., Cheung, N. K., et al. (1998). Association of CYP3A4 genotype with treatment-related leukemia. *Proc Natl Acad Sci U S A 95*(22), 13176–13181.

Ferguson, R. J., De Morais, S. M., Benhamou, S., Bouchardy, C., Blaisdell, J., Ibeanu, G., et al. (1998). A new genetic defect in human CYP2C19: mutation of the initiation codon is responsible for poor metabolism of *S*-mephenytoin. *J Pharmacol Exp Ther 284*(1), 356–361.

Fessing, M. Y., Krynetski, E. Y., Zambetti, G. P., & Evans, W. E. (1998). Functional characterization of the human thiopurine *S*-methyltransferase (TPMT) gene promoter. *Eur J Biochem 256*(3), 510–517.

Floyd, M. D., Gervasini, G., Masica, A. L., Mayo, G., George Jr., A. L., Bhat, K., et al. (2003). Genotype-phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women. *Pharmacogenetics 13*(10), 595–606.

Fluiter, K., Housman, D., Ten Asbroek, A. L., & Baas, F. (2003). Killing cancer by targeting genes that cancer cells have lost: allele-specific inhibition, a novel approach to the treatment of genetic disorders. *Cell Mol Life Sci 60*(5), 834–843.

Freimuth, R. R., Eckloff, B., Wieben, E. D., & Weinshilboum, R. M. (2001). Human sulfotransferase SULT1C1 pharmacogenetics: gene resequencing and functional genomic studies. *Pharmacogenetics 11*(9), 747–756.

Freimuth, R. R., Wiepert, M., Chute, C. G., Wieben, E. D., & Weinshilboum, R. M. (2004). Human cytosolic sulfotransferase database mining: identification of seven novel genes and pseudogenes. *Pharmacogenomics J 4*(1), 54–65.

Frisch, A., Finkel, B., Michaelovsky, E., Sigal, M., Laor, N., & Weizman, R. (2000). A rare short allele of the serotonin transporter promoter region (5-HTTLPR) found in an aggressive schizophrenic patient of Jewish Libyan origin. *Psychiatr Genet 10*(4), 179–183.

Fukushima-Uesaka, H., Saito, Y., Watanabe, H., Shiseki, K., Saeki, M., Nakamura, T., et al. (2004). Haplotypes of CYP3A4 and their close linkage with CYP3A5 haplotypes in a Japanese population. *Hum Mutat 23*(1), 100.

Gaedigk, A., Blum, M., Gaedigk, R., Eichelbaum, M., & Meyer, U. A. (1991). Deletion of the entire cytochrome *P*450 CYP2D6 gene as a cause of impaired drug metabolism in poor metabolizers of the debrisoquine/sparteine polymorphism. *Am J Hum Genet 48*(5), 943–950.

Gaedigk, A., Ryder, D. L., Bradford, L. D., & Leeder, J. S. (2003). CYP2D6 poor metabolizer status can be ruled out by a single genotyping assay for the −1584G promoter polymorphism. *Clin Chem 49*(6 Pt 1), 1008–1011.

Gao, B., Hagenbuch, B., Kullak-Ublick, G. A., Benke, D., Aguzzi, A., & Meier, P. J. (2000). Organic anion-transporting polypeptides mediate transport of opioid peptides across blood-brain barrier. *J Pharmacol Exp Ther 294*(1), 73–79.

Geick, A., Eichelbaum, M., & Burk, O. (2001). Nuclear receptor response elements mediate induction of intestinal MDR1 by rifampin. *J Biol Chem 276*(18), 14581–14587.

Gelernter, J., Cubells, J. F., Kidd, J. R., Pakstis, A. J., & Kidd, K. K. (1999). Population studies of polymorphisms of the serotonin transporter protein gene. *Am J Med Genet 88*(1), 61–66.

Gerra, G., Garofano, L., Santoro, G., Bosari, S., Pellegrini, C., Zaimovic, A., et al. (2004). Association between low-activity serotonin transporter genotype and heroin dependence: behavioral and personality correlates. *Am J Med Genet 126B*(1), 37–42.

Glatt, H., & Meinl, W. (2004). Pharmacogenetics of soluble sulfotransferases (SULTs). *Naunyn Schmiedebergs Arch Pharmacol 369*(1), 55–68.

Goodwin, B., Hodgson, E., & Liddle, C. (1999). The orphan human pregnane X receptor mediates the transcriptional activation of CYP3A4 by rifampicin through a distal enhancer module. *Mol Pharmacol 56*(6), 1329–1339.

Gorski, J. C., Hall, S. D., Jones, D. R., VandenBranden, M., & Wrighton, S. A. (1994). Regioselective biotransformation of midazolam by members of the human cytochrome *P*450 3A (CYP3A) subfamily. *Biochem Pharmacol 47*(9), 1643–1653.

Gragnoli, C., Lindner, T., Cockburn, B. N., Kaisaki, P. J., Gragnoli, F., Marozzi, G., et al. (1997). Maturity-onset diabetes of the young due to a mutation in the hepatocyte nuclear factor-4 alpha binding site in the promoter of the hepatocyte nuclear factor-1 alpha gene. *Diabetes 46*(10), 1648–1651.

Grewal, S. I., & Moazed, D. (2003). Heterochromatin and epigenetic control of gene expression. *Science 301*(5634), 798–802.

Guengerich, F. P. (2004). Cytochrome *P*450: what have we learned and what are the future issues? *Drug Metab Rev 36*(2), 159–197.

Guillemette, C. (2003). Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J 3*(3), 136–158.

Guitton, J., Buronfosse, T., Desage, M., Lepape, A., Brazier, J. L., & Beaune, P. (1997). Possible involvement of multiple cytochrome *P*450s in fentanyl and sufentanil metabolism as opposed to alfentanil. *Biochem Pharmacol 53*(11), 1613–1619.

Haehner, B. D., Gorski, J. C., Vandenbranden, M., Wrighton, S. A., Janardan, S. K., Watkins, P. B., et al. (1996). Bimodal distribution of renal cytochrome *P*450 3A activity in humans. *Mol Pharmacol 50*(1), 52–59.

Hagenbuch, B., & Meier, P. J. (2004). Organic anion transporting polypeptides of the OATP/SLC21 family: phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. *Pflugers Arch 447*(5), 653–665.

Hamid, Q. A., Wenzel, S. E., Hauk, P. J., Tsicopoulos, A., Wallaert, B., Lafitte, J. J., et al. (1999). Increased glucocorticoid receptor beta in airway cells of glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med 159*(5 Pt 1), 1600–1604.

Hariri, A. R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., et al. (2002). Serotonin transporter genetic variation and the response of the human amygdala. *Science 297*(5580), 400–403.

Harries, L. W., Hattersley, A. T., & Ellard, S. (2004). Messenger RNA transcripts of the hepatocyte nuclear factor-1alpha gene containing premature termination codons are subject to nonsense-mediated decay. *Diabetes 53*(2), 500–504.

Hashizume, T., Imaoka, S., Hiroi, T., Terauchi, Y., Fujii, T., Miyazaki, H., et al. (2001). cDNA cloning and expression of a novel cytochrome *P*450 (CYP4F12) from human small intestine. *Biochem Biophys Res Commun 280*(4), 1135–1141.

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., et al. (1996). Allelic variation of human serotonin transporter gene expression. *J Neurochem 66*(6), 2621–2624.

Hein, D. W., Doll, M. A., Fretland, A. J., Leff, M. A., Webb, S. J., Xiao, G. H., et al. (2000). Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol Biomark Prev 9*(1), 29–42.

Heinz, A., Jones, D. W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., et al. (2000). A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. *Biol Psychiatry 47*(7), 643–649.

Hirota, T., Ieiri, I., Takane, H., Maegawa, S., Hosokawa, M., Kobayashi, K., et al. (2004). Allelic expression imbalance of the human CYP3A4 gene and individual phenotypic status. *Hum Mol Genet 13*(23), 2959–2969.

Ho, P. C., Abbott, F. S., Zanger, U. M., & Chang, T. K. (2003). Influence of CYP2C9 genotypes on the formation of a hepatotoxic metabolite of valproic acid in human liver microsomes. *Pharmacogenomics J 3*(6), 335–342.

Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H. P., Brockmoller, J., Johne, A., et al. (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with p-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U S A* *97*(7), 3473–3478.

Horie, N., Aiba, H., Oguro, K., Hojo, H., & Takeishi, K. (1995). Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′ -terminal regulatory region of the human gene for thymidylate synthase. *Cell Struct Funct* *20*(3), 191–197.

Howard, M., Frizzell, R. A., & Bedwell, D. M. (1996). Aminoglycoside antibiotics restore cftr function by overcoming premature stop mutations. *Nat Med* *2*(4), 467–469.

Hranilovic, D., Stefulj, J., Schwab, S., Borrmann-Hassenbach, M., Albus, M., Jernej, B., et al. (2004). Serotonin transporter promoter and intron 2 polymorphisms: relationship between allelic variants and gene expression. *Biol Psychiatry* *55*(11), 1090–1094.

Hu, Y., Hakkola, J., Oscarson, M., & Ingelman-Sundberg, M. (1999). Structural and functional characterization of the 5′ -flanking region of the rat and human cytochrome *P*450 2E1 genes: identification of a polymorphic repeat in the human gene. *Biochem Biophys Res Commun* *263*(2), 286–293.

Hustert, E., Haberl, M., Burk, O., Wolbold, R., He, Y. Q., Klein, K., et al. (2001a). The genetic determinants of the CYP3A5 polymorphism. *Pharmacogenetics* *11*(9), 773–779.

Hustert, E., Zibat, A., Presecan-Siedel, E., Eiselt, R., Mueller, R., Fuss, C., et al. (2001b). Natural protein variants of pregnane X receptor with altered transactivation activity toward CYP3A4. *Drug Metab Dispos* *29*(11), 1454–1459.

Ibeanu, G. C., Blaisdell, J., Ferguson, R. J., Ghanayem, B. I., Brosen, K., Benhamou, S., et al. (1999). A novel transversion in the intron 5 donor splice junction of CYP2C19 and a sequence polymorphism in exon 3 contribute to the poor metabolizer phenotype for the anticonvulsant drug *S*-mephenytoin. *J Pharmacol Exp Ther* *290*(2), 635–640.

Iida, A., Saito, S., Sekine, A., Mishima, C., Kondo, K., Kitamura, Y., et al. (2001a). Catalog of 258 single-nucleotide polymorphisms (SNPS) in genes encoding three organic anion transporters, three organic anion-transporting polypeptides, and three NADH:ubiquinone oxidoreductase flavoproteins. *J Hum Genet* *46*(11), 668–683.

Iida, A., Sekine, A., Saito, S., Kitamura, Y., Kitamoto, T., Osawa, S., et al. (2001b). Catalog of 320 single nucleotide polymorphisms (SNPS) in 20 quinone oxidoreductase and sulfotransferase genes. *J Hum Genet* *46*(4), 225–240.

Iida, A., Saito, S., Sekine, A., Kondo, K., Mishima, C., Kitamura, Y., et al. (2002). Thirteen single-nucleotide polymorphisms (SNPS) in the alcohol dehydrogenase 4 (ADH4) gene locus. *J Hum Genet* *47*(2), 74–76.

Imaoka, S., Yamada, T., Hiroi, T., Hayashi, K., Sakaki, T., Yabusaki, Y., et al. (1996). Multiple forms of human *P*450 expressed in saccharomyces cerevisiae. Systematic characterization and comparison with those of the rat. *Biochem Pharmacol* *51*(8), 1041–1050.

Ingelman-Sundberg, M. (2001). Implications of polymorphic cytochrome *P*450-dependent drug metabolism for drug development. *Drug Metab Dispos* *29*(4 Pt 2), 570–573.

Ioannidis, J. P. (2003). Genetic associations: false or true? *Trends Mol Med* *9*(4), 135–138.

Iyer, L., Hall, D., Das, S., Mortell, M. A., Ramirez, J., Kim, S., et al. (1999). Phenotype-genotype correlation of in vitro sn-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin Pharmacol Ther* *65*(5), 576–582.

Iyer, L., Das, S., Janisch, L., Wen, M., Ramirez, J., Karrison, T., et al. (2002). UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J* *2*(1), 43–47.

Ji, Y., Xu, X., & Stormo, G. D. (2004). A graph theoretical approach for predicting common RNA secondary structure motifs including pseudoknots in unaligned sequences. *Bioinformatics* *20*(10), 1591–1602.

Kagimoto, M., Heim, M., Kagimoto, K., Zeugin, T., & Meyer, U. A. (1990). Multiple mutations of the human cytochrome *P*450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes. *J Biol Chem* *265*(28), 17209–17214.

Kajinami, K., Brousseau, M. E., Ordovas, J. M., & Schaefer, E. J. (2004). Interactions between common genetic polymorphisms in ABCG5/G8 and CYP7A1 on LDL cholesterol-lowering response to atorvastatin. *Atherosclerosis* *175*(2), 287–293.

Kamatani, N., Sekine, A., Kitamoto, T., Iida, A., Saito, S., Kogame, A., et al. (2004). Large-scale single-nucleotide polymorphism (SNP) and haplotype analyses, using dense SNP maps, of 199 drug-related genes in 752 subjects: the analysis of the association between uncommon SNPS within haplotype blocks and the haplotypes constructed with haplotype-tagging SNPS. *Am J Hum Genet* *75*(2), 190–203.

Kidd, R. S., Curry, T. B., Gallagher, S., Edeki, T., Blaisdell, J., & Goldstein, J. A. (2001). Identification of a null allele of CYP2C9 in an African-American exhibiting toxicity to phenytoin. *Pharmacogenetics* *11*(9), 803–808.

King, L. M., Ma, J., Srettabunjong, S., Graves, J., Bradbury, J. A., Li, L., et al. (2002). Cloning of CYP2J2 gene and identification of functional polymorphisms. *Mol Pharmacol* *61*(4), 840–852.

Kivisto, K. T., Griese, E. U., Fritz, P., Linder, A., Hakkola, J., Raunio, H., et al. (1996). Expression of cytochrome *P*450 3A enzymes in human lung: a combined RT-PCR and immunohistochemical analysis of normal tissue and lung tumours. *Naunyn Schmiedebergs Arch Pharmacol* *353*(2), 207–212.

Kiyotani, K., Yamazaki, H., Fujieda, M., Iwano, S., Matsumura, K., Satarug, S., et al. (2003). Decreased coumarin 7-hydroxylase activities and CYP2A6 expression levels in humans caused by genetic polymorphism in CYP2A6 promoter region (CYP2A6*9). *Pharmacogenetics* *13*(11), 689–695.

Knight, J. C., Keating, B. J., Rockett, K. A., & Kwiatkowski, D. P. (2003). In vivo characterization of regulatory polymorphisms by allele-specific quantification of RNA polymerase loading. *Nat Genet* *33*(4), 469–475.

Kohle, C., Mohrle, B., Munzel, P. A., Schwab, M., Wernet, D., Badary, O. A., et al. (2003). Frequent co-occurrence of the tata box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians. *Biochem Pharmacol* *65*(9), 1521–1527.

Kuehl, P., Zhang, J., Lin, Y., Lamba, J., Assem, M., Schuetz, J., et al. (2001). Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* *27*(4), 383–391.

Kullak-Ublick, G. A., Beuers, U., Fahney, C., Hagenbuch, B., Meier, P. J., & Paumgartner, G. (1997). Identification and functional characterization of the promoter region of the human organic anion transporting polypeptide gene. *Hepatology* *26*(4), 991–997.

Kupfer, A., & Preisig, R. (1984). Pharmacogenetics of mephenytoin: a new drug hydroxylation polymorphism in man. *Eur J Clin Pharmacol* *26*(6), 753–759.

Lamba, J. K., Lin, Y. S., Schuetz, E. G., & Thummel, K. E. (2002). Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev* *54*(10), 1271–1294.

Lamba, V., Lamba, J., Yasuda, K., Strom, S., Davila, J., Hancock, M. L., et al. (2003). Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and car (constitutive androstane receptor) expression. *J Pharmacol Exp Ther* *307*(3), 906–922.

Lamba, J. K., Lamba, V., Yasuda, K., Lin, Y. S., Assem, M., Thompson, E., et al. (2004). Expression of car splice variants in human tissues and their functional consequences. *J Pharmacol Exp Ther* *311*(2), 811–821.

Lang, T., Klein, K., Fischer, J., Nussler, A. K., Neuhaus, P., Hofmann, U., et al. (2001). Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics* *11*(5), 399–415.

Lang, T., Klein, K., Richter, T., Zibat, A., Kerb, R., Eichelbaum, M., et al. (2004). Multiple novel nonsynonymous CYP2B6 gene polymorphisms in Caucasians: demonstration of phenotypic null alleles. *J Pharmacol Exp Ther* *311*(1), 34–43.

Lee, C. R., Goldstein, J. A., & Pieper, J. A. (2002). Cytochrome *P*450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* *12*(3), 251–263.

Lehmann, J. M., McKee, D. D., Watson, M. A., Willson, T. M., Moore, J. T., & Kliewer, S. A. (1998). The human orphan nuclear receptor PXR is activated by compounds that regulate CYP3A4 gene expression and cause drug interactions. *J Clin Invest* *102*(5), 1016–1023.

Lesch, K. P., Balling, U., Gross, J., Strauss, K., Wolozin, B. L., Murphy, D. L., et al. (1994). Organization of the human serotonin transporter gene. *J Neural Transm Gen Sect* *95*(2), 157–162.

Liu, R., McEachin, R. C., & States, D. J. (2003). Computationally identifying novel NF-kappa b-regulated immune genes in the human genome. *Genome Res* *13*(4), 654–661.

Lo, H. S., Wang, Z., Hu, Y., Yang, H. H., Gere, S., Buetow, K. H., et al. (2003). Allelic variation in gene expression is common in the human genome. *Genome Res* *13*(8), 1855–1862.

Loktionov, A. (2004). Common gene polymorphisms, cancer progression and prognosis. *Cancer Lett* *208*(1), 1–33.

Lotrich, F. E., Pollock, B. G., & Ferrell, R. E. (2003). Serotonin transporter promoter polymorphism in African Americans: allele frequencies and implications for treatment. *Am J Pharmacogenomics* *3*(2), 145–147.

Lovlie, R., Daly, A. K., Matre, G. E., Molven, A., & Steen, V. M. (2001). Polymorphisms in CYP2D6 duplication-negative individuals with the ultrarapid metabolizer phenotype: a role for the CYP2D6*35 allele in ultrarapid metabolism? *Pharmacogenetics* *11*(1), 45–55.

Mackenzie, P. I., Owens, I. S., Burchell, B., Bock, K. W., Bairoch, A., Belanger, A., et al. (1997). The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* *7*(4), 255–269.

Mandola, M. V., Stoehlmacher, J., Muller-Weeks, S., Cesarone, G., Yu, M. C., Lenz, H. J., et al. (2003). A novel single nucleotide polymorphism within the 5′ tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and alters transcriptional activity. *Cancer Res* *63*(11), 2898–2904.

Mandola, M. V., Stoehlmacher, J., Zhang, W., Groshen, S., Yu, M. C., Iqbal, S., et al. (2004). A 6 bp polymorphism in the thymidylate synthase gene causes message instability and is associated with decreased intratumoral TS mRNA levels. *Pharmacogenetics* *14*(5), 319–327.

Marez, D., Sabbagh, N., Legrand, M., Lo-Guidice, J. M., Boone, P., & Broly, F. (1995). A novel CYP2D6 allele with an abolished splice recognition site associated with the poor metabolizer phenotype. *Pharmacogenetics* *5*(5), 305–311.

Marez, D., Legrand, M., Sabbagh, N., Lo-Guidice, J. M., Boone, P., & Broly, F. (1996). An additional allelic variant of the CYP2D6 gene causing impaired metabolism of sparteine. *Hum Genet* *97*(5), 668–670.

Marez-Allorge, D., Ellis, S. W., Lo Guidice, J. M., Tucker, G. T., & Broly, F. (1999). A rare G2061 insertion affecting the open reading frame of CYP2D6 and responsible for the poor metabolizer phenotype. *Pharmacogenetics* *9*(3), 393–396.

Marian, A. J., Safavi, F., Ferlic, L., Dunn, J. K., Gotto, A. M., & Ballantyne, C. M. (2000). Interactions between angiotensin-I converting enzyme insertion/deletion polymorphism and response of plasma lipids and coronary atherosclerosis to treatment with fluvastatin: the lipoprotein and coronary atherosclerosis study. *J Am Coll Cardiol* *35*(1), 89–95.

Marinaki, A. M., Arenas, M., Khan, Z. H., Lewis, C. M., Shobowale-Bakre el, M., Escuredo, E., et al. (2003). Genetic determinants of the thiopurine methyltransferase intermediate activity phenotype in British Asians and Caucasians. *Pharmacogenetics* *13*(2), 97–105.

Marsh, S., McKay, J. A., Cassidy, J., & McLeod, H. L. (2001). Polymorphism in the thymidylate synthase promoter enhancer region in colorectal cancer. *Int J Oncol* *19*(2), 383–386.

Martin, E. R., Monks, S. A., Warren, L. L., & Kaplan, N. L. (2000). A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am J Hum Genet* *67*(1), 146–154.

Matsumura, K., Saito, T., Takahashi, Y., Ozeki, T., Kiyotani, K., Fujieda, M., et al. (2004). Identification of a novel polymorphic enhancer of the human CYP3A4 gene. *Mol Pharmacol* *65*(2), 326–334.

McCarver, D. G., Byun, R., Hines, R. N., Hichme, M., & Wegenek, W. (1998). A genetic polymorphism in the regulatory sequences of human CYP2E1: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. *Toxicol Appl Pharmacol* *152*(1), 276–281.

McLeod, H. L., & Siva, C. (2002). The thiopurine *S*-methyltransferase gene locus—implications for clinical pharmacogenomics. *Pharmacogenomics* *3*(1), 89–98.

McLeod, H. L., Collie-Duguid, E. S., Vreken, P., Johnson, M. R., Wei, X., Sapone, A., et al. (1998). Nomenclature for human DPYD alleles. *Pharmacogenetics* *8*(6), 455–459.

Michaelovsky, E., Frisch, A., Rockah, R., Peleg, L., Magal, N., Shohat, M., et al. (1999). A novel allele in the promoter region of the human serotonin transporter gene. *Mol Psychiatry* *4*(1), 97–99.

Miller, V. M., Xia, H., Marrs, G. L., Gouvion, C. M., Lee, G., Davidson, B. L., et al. (2003). Allele-specific silencing of dominant disease genes. *Proc Natl Acad Sci U S A* *100*(12), 7195–7200.

Morley, M., Molony, C. M., Weber, T. M., Devlin, J. L., Ewens, K. G., Spielman, R. S., et al. (2004). Genetic analysis of genome-wide variation in human gene expression. *Nature* *430*(7001), 743–747.

Mundo, E., Walker, M., Cate, T., Macciardi, F., & Kennedy, J. L. (2001). The role of serotonin transporter protein gene in antidepressant-induced mania in bipolar disorder: preliminary findings. *Arch Gen Psychiatry* *58*(6), 539–544.

Murayama, N., Soyama, A., Saito, Y., Nakajima, Y., Komamura, K., Ueno, K., et al. (2004). Six novel nonsynonymous CYP1A2 gene polymorphisms: catalytic activities of the naturally occurring variant enzymes. *J Pharmacol Exp Ther* *308*(1), 300–306.

Murray, G. I., Pritchard, S., Melvin, W. T., & Burke, M. D. (1995). Cytochrome *P*450 CYP3A5 in the human anterior pituitary gland. *FEBS Lett* *364*(1), 79–82.

Nakajima, M., Yokoi, T., Mizutani, M., Kinoshita, M., Funayama, M., & Kamataki, T. (1999). Genetic polymorphism in the 5′ -flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. *J Biochem (Tokyo)* *125*(4), 803–808.

Nakamura, K., Goto, F., Ray, W. A., McAllister, C. B., Jacqz, E., Wilkinson, G. R., et al. (1985). Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin Pharmacol Ther* *38*(4), 402–408.

Nakamura, M., Ueno, S., Sano, A., & Tanabe, H. (2000). The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry* *5*(1), 32–38.

Nelson, D. R., Zeldin, D. C., Hoffman, S. M., Maltais, L. J., Wain, H. M., & Nebert, D. W. (2004). Comparison of cytochrome *P*450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics* *14*(1), 1–18.

Ng, P. C., & Henikoff, S. (2002). Accounting for human polymorphisms predicted to affect protein function. *Genome Res* *12*(3), 436–446.

Niemi, M., Schaeffeler, E., Lang, T., Fromm, M. F., Neuvonen, M., Kyrklund, C., et al. (2004). High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). *Pharmacogenetics* *14*(7), 429–440.

Nordmark, A., Lundgren, S., Ask, B., Granath, F., & Rane, A. (2002). The effect of the CYP1A2*1F mutation on CYP1A2 inducibility in pregnant women. *Br J Clin Pharmacol* *54*(5), 504–510.

Ohno, M., Yamaguchi, I., Yamamoto, I., Fukuda, T., Yokota, S., Maekura, R., et al. (2000). Slow *N*-acetyltransferase 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. *Int J Tuberc Lung Dis* *4*(3), 256–261.

O'Reilly, R. L., Bogue, L., & Singh, S. M. (1994). Pharmacogenetic response to antidepressants in a multicase family with affective disorder. *Biol Psychiatry* *36*(7), 467–471.

Oscarson, M., McLellan, R. A., Asp, V., Ledesma, M., Ruiz, M. L., Sinues, B., et al. (2002). Characterization of a novel CYP2A7/CYP2A6 hybrid allele (CYP2A6*12) that causes reduced CYP2A6 activity. *Hum Mutat* *20*(4), 275–283.

Page, G. P., George, V., Go, R. C., Page, P. Z., & Allison, D. B. (2003). "Are we there yet?": deciding when one has demonstrated specific genetic causation in complex diseases and quantitative traits. *Am J Hum Genet* *73*(4), 711–719.

Pai, H. V., Kommaddi, R. P., Chinta, S. J., Mori, T., Boyd, M. R., & Ravindranath, V. (2004). A frameshift mutation and alternate splicing in human brain generate a functional form of the pseudogene cytochrome *P*4502D7 that demethylates codeine to morphine. *J Biol Chem* *279*(26), 27383–27389.

Pastinen, T., & Hudson, T. J. (2004). *Cis*-acting regulatory variation in the human genome. *Science* *306*(5696), 647–650.

Payton, M. A., & Sim, E. (1998). Genotyping human arylamine *N*-acetyltransferase type 1 (NAT1): the identification of two novel allelic variants. *Biochem Pharmacol* *55*(3), 361–366.

Pelkonen, O., Rautio, A., Raunio, H., & Pasanen, M. (2000). CYP2A6: a human coumarin 7-hydroxylase. *Toxicology* *144*(1–3), 139–147.

Phillips, K. A., Veenstra, D. L., Oren, E., Lee, J. K., & Sadee, W. (2001). Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. *JAMA* *286*(18), 2270–2279.

Pinsonneault, J., & Sadee, W. (2003). Pharmacogenomics of multigenic diseases: sex-specific differences in disease and treatment outcome. *AAPS PharmSci* *5*(4), E29.

Pinsonneault, J. K., Nielsen, C. U., & Sadee, W. (2004). Genetic variants of the human H+/dipeptide transporter PepT2: analysis of haplotype functions. *J Pharmacol Exp Ther* *311*(3), 1088–1096.

Pitarque, M., von Richter, O., Oke, B., Berkkan, H., Oscarson, M., & Ingelman-Sundberg, M. (2001). Identification of a single nucleotide polymorphism in the TATA box of the CYP2A6 gene: impairment of its promoter activity. *Biochem Biophys Res Commun* *284*(2), 455–460.

Pitarque, M., von Richter, O., Rodriguez-Antona, C., Wang, J., Oscarson, M., & Ingelman-Sundberg, M. (2004). A nicotine C-oxidase gene (CYP2A6) polymorphism important for promoter activity. *Hum Mutat* *23*(3), 258–266.

Pollock, B. G., Ferrell, R. E., Mulsant, B. H., Mazumdar, S., Miller, M., Sweet, R. A., et al. (2000). Allelic variation in the serotonin transporter promoter affects onset of paroxetine treatment response in late-life depression. *Neuropsychopharmacology* *23*(5), 587–590.

Raimundo, S., Fischer, J., Eichelbaum, M., Griese, E. U., Schwab, M., & Zanger, U. M. (2000). Elucidation of the genetic basis of the common 'intermediate metabolizer' phenotype for drug oxidation by CYP2D6. *Pharmacogenetics* *10*(7), 577–581.

Raimundo, S., Toscano, C., Klein, K., Fischer, J., Griese, E. U., Eichelbaum, M., et al. (2004). A novel intronic mutation, 2988G>A, with high predictivity for impaired function of cytochrome *P*450 2D6 in white subjects. *Clin Pharmacol Ther* *76*(2), 128–138.

Ratain, M. J., Rosner, G., Allen, S. L., Costanza, M., Van Echo, D. A., Henderson, I. C., et al. (1995). Population pharmacodynamic study of amonafide: a cancer and leukemia group B study. *J Clin Oncol* *13*(3), 741–747.

Rausch, J. L., Johnson, M. E., Fei, Y. J., Li, J. Q., Shendarkar, N., Hobby, H. M., et al. (2002). Initial conditions of serotonin transporter kinetics and genotype: influence on SSRI treatment trial outcome. *Biol Psychiatry* *51*(9), 723–732.

Reist, C., Mazzanti, C., Vu, R., Tran, D., & Goldman, D. (2001). Serotonin transporter promoter polymorphism is associated with attenuated prolactin response to fenfluramine. *Am J Med Genet* *105*(4), 363–368.

Reiter, C., & Weinshilboum, R. (1982). Platelet phenol sulfotransferase activity: correlation with sulfate conjugation of acetaminophen. *Clin Pharmacol Ther* *32*(5), 612–621.

Rendic, S. (2002). Summary of information on human CYP enzymes: human *P*450 metabolism data. *Drug Metab Rev* *34*(1–2), 83–448.

Rockman, M. V., & Wray, G. A. (2002). Abundant raw material for *cis*-regulatory evolution in humans. *Mol Biol Evol* *19*(11), 1991–2004.

Rushmore, T. H., & Kong, A. N. (2002). Pharmacogenomics, regulation and signaling pathways of phase i and ii drug metabolizing enzymes. *Curr Drug Metab* *3*(5), 481–490.

Ryffel, G. U. (2001). Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. *J Mol Endocrinol* *27*(1), 11–29.

Sachse, C., Brockmoller, J., Bauer, S., Reum, T., & Roots, I. (1996). A rare insertion of t226 in exon 1 of CYP2D6 causes a frameshift and is associated with the poor metabolizer phenotype: CYP2D6*15. *Pharmacogenetics* *6*(3), 269–272.

Sachse, C., Brockmoller, J., Bauer, S., & Roots, I. (1999). Functional significance of a c→a polymorphism in intron 1 of the cytochrome *P*450 CYP1A2 gene tested with caffeine. *Br J Clin Pharmacol* *47*(4), 445–449.

Saeki, M., Saito, Y., Nakamura, T., Murayama, N., Kim, S. R., Ozawa, S., et al. (2003). Single nucleotide polymorphisms and haplotype frequencies of CYP3A5 in a Japanese population. *Hum Mutat* *21*(6), 653.

Saito, S., Iida, A., Sekine, A., Kawauchi, S., Higuchi, S., Ogawa, C., et al. (2003). Catalog of 680 variations among eight cytochrome *P*450 (CYP) genes, nine esterase genes, and two other genes in the Japanese population. *J Hum Genet* *48*(5), 249–270.

Sakaeda, T., Nakamura, T., Horinouchi, M., Kakumoto, M., Ohmoto, N., Sakai, T., et al. (2001). MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res* *18*(10), 1400–1404.

Saxena, R., Shaw, G. L., Relling, M. V., Frame, J. N., Moir, D. T., Evans, W. E., et al. (1994). Identification of a new variant CYP2D6 allele with a single base deletion in exon 3 and its association with the poor metabolizer phenotype. *Hum Mol Genet* *3*(6), 923–926.

Schaaf, M. J., & Cidlowski, J. A. (2002). Auuua motifs in the 3′ UTR of human glucocorticoid receptor alpha and beta mRNA destabilize mRNA and decrease receptor protein expression. *Steroids* *67*(7), 627–636.

Scherl, E., & Frissora, C. L. (2003). Irritable bowel syndrome genophenomics: correlation of serotonin-transporter polymorphisms and alosetron response. *Pharmacogenomics J* *3*(2), 64–66.

Schmitz, G., & Drobnik, W. (2003). Pharmacogenomics and pharmacogenetics of cholesterol-lowering therapy. *Clin Chem Lab Med* *41*(4), 581–589.

Schuetz, E. G., Schuetz, J. D., Grogan, W. M., Naray-Fejes-Toth, A., Fejes-Toth, G., Raucy, J., et al. (1992). Expression of cytochrome *P*450 3A in amphibian, rat, and human kidney. *Arch Biochem Biophys* *294*(1), 206–214.

Schuetz, E. G., Strom, S., Yasuda, K., Lecureur, V., Assem, M., Brimer, C., et al. (2001). Disrupted bile acid homeostasis reveals an unexpected interaction among nuclear hormone receptors, transporters, and cytochrome *P*450. *J Biol Chem* *276*(42), 39411–39418.

Schwarz, U. I. (2003). Clinical relevance of genetic polymorphisms in the human CYP2C9 gene. *Eur J Clin Invest* *33*(Suppl 2), 23–30.

Sheets, M. D., Ogg, S. C., & Wickens, M. P. (1990). Point mutations in aauaaa and the poly (a) addition site: effects on the accuracy and efficiency of cleavage and polyadenylation in vitro. *Nucleic Acids Res* *18*(19), 5799–5805.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., & Guengerich, F. P. (1994). Interindividual variations in human liver cytochrome *P*-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* *270*(1), 414–423.

Shimoda, K., Someya, T., Morita, S., Hirokane, G., Yokono, A., Takahashi, S., et al. (2002). Lack of impact of CYP1A2 genetic polymorphism (C/A polymorphism at position 734 in intron 1 and G/A polymorphism at position-2964 in the 5′-flanking region of CYP1A2) on the

plasma concentration of haloperidol in smoking male Japanese with schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* *26*(2), 261–265.

Sierakowska, H., Sambade, M. J., Agrawal, S., & Kole, R. (1996). Repair of thalassemic human beta-globin mRNA in mammalian cells by antisense oligonucleotides. *Proc Natl Acad Sci U S A* *93*(23), 12840–12844.

Singer-Sam, J., Chapman, V., LeBon, J. M., & Riggs, A. D. (1992). Parental imprinting studied by allele-specific primer extension after PCR: paternal X chromosome-linked genes are transcribed prior to preferential paternal X chromosome inactivation. *Proc Natl Acad Sci U S A* *89*(21), 10469–10473.

Sordella, R., Bell, D. W., Haber, D. A., & Settleman, J. (2004). Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* *305*(5687), 1163–1167.

Sousa, A. R., Lane, S. J., Cidlowski, J. A., Staynov, D. Z., & Lee, T. H. (2000). Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. *J Allergy Clin Immunol* *105*(5), 943–950.

Speicker, M., Darius, H., Hankeln, T., Soufi, M., Sattler, A., Schaefer, J. R., et al. (2004). Risk of coronary artery disease associated with polymorphism of a cytochrome *P*450 epoxygenase, CYP2J2. *Circulation* *110*(15), 2132–2136.

Spielman, R. S., & Ewens, W. J. (1996). The TDT and other family-based tests for linkage disequilibrium and association. *Am J Hum Genet* *59*(5), 983–989.

Spire-Vayron de la Moureyre, C., Debuysere, H., Mastain, B., Vinner, E., Marez, D., Lo Guidice, J. M., et al. (1998). Genotypic and phenotypic analysis of the polymorphic thiopurine *S*-methyltransferase gene (TPMT) in a European population. *Br J Pharmacol* *125*(4), 879–887.

Spire-Vayron de la Moureyre, C., Debuysere, H., Fazio, F., Sergent, E., Bernard, C., Sabbagh, N., et al. (1999). Characterization of a variable number tandem repeat region in the thiopurine *S*-methyltransferase gene promoter. *Pharmacogenetics* *9*(2), 189–198.

Spurdle, A. B., Goodwin, B., Hodgson, E., Hopper, J. L., Chen, X., Purdie, D. M., et al. (2002). The CYP3A4*1B polymorphism has no functional significance and is not associated with risk of breast or ovarian cancer. *Pharmacogenetics* *12*(5), 355–366.

Stamatoyannopoulos, J. A. (2004). The genomics of gene expression. *Genomics* *84*(3), 449–457.

Sun, H. F., Chang, Y. T., Fann, C. S., Chang, C. J., Chen, Y. H., Hsu, Y. P., et al. (2002). Association study of novel human serotonin 5-HT(1B) polymorphisms with alcohol dependence in Taiwanese Han. *Biol Psychiatry* *51*(11), 896–901.

Sun, J., He, Z. G., Cheng, G., Wang, S. J., Hao, X. H., & Zou, M. J. (2004). Multidrug resistance p-glycoprotein: crucial significance in drug disposition and interaction. *Med Sci Monit* *10*(1), RA5–RA14.

Takane, H., Kobayashi, D., Hirota, T., Kigawa, J., Terakawa, N., Otsubo, K., et al. (2004). Haplotype-oriented genetic analysis and functional assessment of promoter variants in the mdr1 (*ABCB1*) gene. *J Pharmacol Exp Ther* *311*(3), 1179–1187.

Tebo, J., Der, S., Frevel, M., Khabar, K. S., Williams, B. R., & Hamilton, T. A. (2003). Heterogeneity in control of mRNA stability by Au-rich elements. *J Biol Chem* *278*(14), 12085–12093.

Tirona, R. G., & Kim, R. B. (2002). Pharmacogenomics of organic anion-transporting polypeptides (OATP). *Adv Drug Deliv Rev* *54*(10), 1343–1352.

Tirona, R. G., Lee, W., Leake, B. F., Lan, L. B., Cline, C. B., Lamba, V., et al. (2003). The orphan nuclear receptor HNF4alpha determines PXR- and CAR-mediated xenobiotic induction of CYP3A4. *Nat Med* *9*(2), 220–224.

Ulrich, C. M., Bigler, J., Velicer, C. M., Greene, E. A., Farin, F. M., & Potter, J. D. (2000). Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. *Cancer Epidemiol Biomark Prev* *9*(12), 1381–1385.

Uno, Y., Sakamoto, Y., Yoshida, K., Hasegawa, T., Hasegawa, Y., Koshino, T., et al. (2003). Characterization of six base pair deletion in the putative HNF1-binding site of human PXR promoter. *J Hum Genet* *48*(11), 594–597.

Vaglenova, J., Martinez, S. E., Porte, S., Duester, G., Farres, J., & Pares, X. (2003). Expression, localization and potential physiological significance of alcohol dehydrogenase in the gastrointestinal tract. *Eur J Biochem* *270*(12), 2652–2662.

Van Kuilenburg, A. B., Meinsma, R., Zoetekouw, L., & Van Gennip, A. H. (2002). High prevalence of the IVS14+ 1 G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. *Pharmacogenetics* *12*(7), 555–558.

Vasilou, V., Bairoch, A., Tipton, K. F., & Nebert, D. W. (1999). Eukaryotic aldehyde dehydrogenase (ALDH) genes: human polymorphisms and recommended nomenclature based on divergent evolution and chromosomal mapping. *Pharmacogenetics* *9*(4), 421–434.

Villafranca, E., Okruzhnov, Y., Dominguez, M. A., Garcia-Foncillas, J., Azinovic, I., Martinez, E., et al. (2001). Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. *J Clin Oncol* *19*(6), 1779–1786.

von Richter, O., Pitarque, M., Rodriguez-Antona, C., Testa, A., Mantovani, R., Oscarson, M., et al. (2004). Polymorphic NF-Y dependent regulation of human nicotine C-oxidase (CYP2A6). *Pharmacogenetics* *14*(6), 369–379.

Vuchetich, J. P., Weinshilboum, R. M., & Price, R. A. (1995). Segregation analysis of human red blood cell thiopurine methyltransferase activity. *Genet Epidemiol* *12*(1), 1–11.

Wang, J., Freeman, D. J., Grundy, S. M., Levine, D. M., Guerra, R., & Cohen, J. C. (1998). Linkage between cholesterol 7alpha-hydroxylase and high plasma low-density lipoprotein cholesterol concentrations. *J Clin Invest* *101*(6), 1283–1291.

Wasserman, W. W., & Sandelin, A. (2004). Applied bioinformatics for the identification of regulatory elements. *Nat Rev Genet* *5*(4), 276–287.

Wei, X., McLeod, H. L., McMurrough, J., Gonzalez, F. J., & Fernandez-Salguero, P. (1996). Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *J Clin Invest* *98*(3), 610–615.

Weinshilboum, R. (1990). Sulfotransferase pharmacogenetics. *Pharmacol Ther* *45*(1), 93–107.

Wenz, H.M. (2004). A novel high-throughput SNP genotyping system utilizing capillary electrophoreisis detection platforms. Applied Biosystems, 850 Lincoln Centre Dr, Foster City, CA 94404, USA.

Westlind, A., Lofberg, L., Tindberg, N., Andersson, T. B., & Ingelman-Sundberg, M. (1999). Interindividual differences in hepatic expression of CYP3A4: relationship to genetic polymorphism in the 5′ -upstream regulatory region. *Biochem Biophys Res Commun* *259*(1), 201–205.

Westlind-Johnsson, A., Malmebo, S., Johansson, A., Otter, C., Andersson, T. B., Johansson, I., et al. (2003). Comparative analysis of CYP3A expression in human liver suggests only a minor role for CYP3A5 in drug metabolism. *Drug Metab Dispos* *31*(6), 755–761.

Whitney, A. R., Diehn, M., Popper, S. J., Alizadeh, A. A., Boldrick, J. C., Relman, D. A., et al. (2003). Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci U S A* *100*(4), 1896–1901.

Wilschanski, M., Yahav, Y., Yaacov, Y., Blau, H., Bentur, L., Rivlin, J., et al. (2003). Gentamicin-induced correction of CFTR function in patients with cystic fibrosis and CFTR stop mutations. *N Engl J Med* *349*(15), 1433–1441.

Wittkopp, P. J., Haerum, B. K., & Clark, A. G. (2004). Evolutionary changes in *cis* and *trans* gene regulation. *Nature* *430*(6995), 85–88.

Wojnowski, L., & Brockmoller, J. (2004). Single nucleotide polymorphism characterization by mRNA expression imbalance assessment. *Pharmacogenetics* *14*(4), 267–269.

Wrighton, S. A., Ring, B. J., Watkins, P. B., & VandenBranden, M. (1989). Identification of a polymorphically expressed member of the human cytochrome *P*-450III family. *Mol Pharmacol* *36*(1), 97–105.

Yamanaka, H., Nakajima, M., Katoh, M., Hara, Y., Tachibana, O., Yamashita, J., et al. (2004). A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics 14*(5), 329–332.

Yamano, S., Nhamburo, P. T., Aoyama, T., Meyer, U. A., Inaba, T., Kalow, W., et al. (1989). cDNA cloning and sequence and cDNA-directed expression of human *P*450 IIB1: identification of a normal and two variant cDNAs derived from the CYP2B locus on chromosome 19 and differential expression of the IIB mRNAs in human liver. *Biochemistry 28*(18), 7340–7348.

Yamazaki, H., Kiyotani, K., Tsubuko, S., Matsunaga, M., Fujieda, M., Saito, T., et al. (2003). Two novel haplotypes of CYP2D6 gene in a Japanese population. *Drug Metab Pharmacokin 18*(4), SN26(269)–SNP 228(271).

Yan, H., & Zhou, W. (2004). Allelic variations in gene expression. *Curr Opin Oncol 16*(1), 39–43.

Yan, L., Zhang, S., Eiff, B., Szumlanski, C. L., Powers, M., O'Brien, J. F., et al. (2000). Thiopurine methyltransferase polymorphic tandem repeat: genotype-phenotype correlation analysis. *Clin Pharmacol Ther 68*(2), 210–219.

Yan, H., Yuan, W., Velculescu, V. E., Vogelstein, B., & Kinzler, K. W. (2002). Allelic variation in human gene expression. *Science 297*(5584), 1143.

Yin, S. J., Chou, C. F., Lai, C. L., Lee, S. L., & Han, C. L. (2003). Human class IV alcohol dehydrogenase: kinetic mechanism, functional roles and medical relevance. *Chem Biol Interact 143–144*, 219–227.

Yoshida, R., Nakajima, N., Nishimura, K., Tokudome, S., Kwon, J. T., & Yokoi, T. (2003). Effects of polymorphism in promoter region of human CYP2A6 gene (CYP2A6*9) on expression level of messenger ribonucleic acid and enzymatic activity in vivo and in vitro. *Chem Pharmacol Ther 74*(1), 69–76.

Zambon, A., Deeb, S. S., Brown, B. G., Hokanson, J. E., & Brunzell, J. D. (2001). Common hepatic lipase gene promoter variant determines clinical response to intensive lipid-lowering treatment. *Circulation 103*(6), 792–798.

Zanger, U. M., Fischer, J., Raimundo, S., Stuven, T., Evert, B. O., Schwab, M., et al. (2001). Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics 11*(7), 573–585.

Zanger, U. M., Raimundo, S., & Eichelbaum, M. (2004). Cytochrome *P*450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch Pharmacol 369*(1), 23–37.

Zhang, J., Kuehl, P., Green, E. D., Touchman, J. W., Watkins, P. B., Daly, A., et al. (2001). The human pregnane X receptor: genomic structure and identification and functional characterization of natural allelic variants. *Pharmacogenetics 11*(7), 555–572.

Zhang, X., Caggana, M., Cutler, T. L., & Ding, X. (2004). Development of a real-time PCR-based method for the measurement of relative allelic expression, and identification of CYP2A13 alleles with decreased expression in human lung. *J Pharmacol Exp Ther 311*(1), 373–381.

Zielinska, E., Niewiarowski, W., & Bodalski, J. (1998). The arylamine *N*-acetyltransferase (NAT2) polymorphism and the risk of adverse reactions to co-trimoxazole in children. *Eur J Clin Pharmacol 54*(9–10), 779–785.

Zuker, M. (2003). Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res 31*(13), 3406–3415.