Published in final edited form as:

Trends Mol Med. 2011 May ; 17(5): 244–251. doi:10.1016/j.molmed.2011.01.007.

Copy Number Variants in pharmacogenetic genes

Yijing He, Janelle M. Hoskins, and Howard L. McLeod*

UNC Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina, Chapel Hill, NC, USA

Abstract

Variation in drug efficacy and toxicity remains an important clinical concern. Presently, single nucleotide polymorphisms (SNP) only explain a portion of this problem, even in situations where the pharmacological trait is clearly heritable. The Human CNV Project identified copy number variations (CNVs) across approximately 12% of the human genome, and these CNVs were considered causes of diseases. Although the contribution of CNVs to the pathogenesis of many common diseases is questionable, CNVs play a clear role in drug related genes by altering drug metabolizing and drug response. Here we provide a comprehensive review of the clinical relevance of CNVs to drug efficacy, toxicity, disease prevalence in world populations and discuss the implication of using CNVs as diagnosis in clinical intervention.

Keywords

pharmacogenetics; copy number variants; drug efficacy; drug toxicity

Pharmacogenetics

Pharmacogenetics aims to study the gene variants associated with drug metabolism enzymes, transporters, drug receptors and relieve the burden of sickness caused by interindividual differences in drug response or vulnerabilities to drug toxicity [1, 2]. Since the completion of the Human Genome Project in 2000, there has been a burst in the number of pharmacogenetic research studies published, with some of the findings now being introduced into clinical practice. To practice pharmacogenetics testing in the clinic, the United States Food and Drug Administration (FDA) approved the first DNA microarray molecular diagnostic test in 2004, the AmpliChip CYP450 (Roche Molecular Diagnostics, Alameda, CA, USA), for the analysis of single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) of *CYP2D6* and *CYP2C19* genes which are closely related with the drug therapy of depression, gastroesophageal reflux and cardiovascular diseases. [3]. The FDA also issued “Draft Guidelines for Industry: Pharmacogenomic Data Submission”, making the submission of pharmacogenomic data mandatory for new drug applications from drug companies [4]. Since 2008, the FDA has issued a list of valid pharmacogenetic

© 2011 Elsevier Ltd. All rights reserved.

*Corresponding author: McLeod, HL (hmcLeod@unc.edu), UNC Institute for Pharmacogenomics and Individualized Therapy, University of North Carolina – Chapel Hill, Campus Box 7361, Rm 1096 Genetic Medicine Building, 120 Mason Farm Rd, Chapel Hill, NC 27599-7360, USA Phone: +1-919-966-0512, Fax: +1-919-966-5863.

Conflicts of interest

Dr McLeod is a consultant to Medco Health Solutions, Gentris corporation, and Myriad Genetics.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

biomarkers in the context of approved drug labels that are updated every year [5], which accelerates the translation of pharmacogenetics from bench to bedside.

At present, pharmacogenetics can be applied to the treatment of patients with psychiatric illnesses, cancer, cardiovascular disease, pain, HIV infection, and microorganism infection, to enhance drug efficacy and decrease drug toxicities [6–9]. According to a survey of 1200 drug labels for the years 1945–2005, 121 drugs labels contained pharmacogenetic information [10]. Of those, 69 labels referred to human genomic biomarkers (e.g. atomoxetine, fluoxetine and codeine are labeled with *CYP2D6* variants associated drug efficacy and toxicity change) [10]. Over 20 of these markers were either required or recommended by the FDA to be tested in patients before starting drug therapy [11]. For example, tramadol is an orally administered synthetic analogue of codeine and its product label warns that patients with cytochrome P450 2D6 (*CYP2D6*) gene (*CYP2D6*) duplications are at increased risk of respiratory depression [12].

What is a copy number variant?

Human DNA has one copy of autosomal regions on each chromosome. However, as discovered by the Human Genome Project, many genetic regions display a variation in the number of copies (more or less than two copies totally). Alleles containing 0–13 gene copies have been reported across the human population [13]. These genetic variants are termed copy number variants (CNVs) and are defined as DNA segments ranging in size from one kilobase to several megabases among individuals due to deletion, insertion, inversion, duplication or complex recombination [14] (Figure 1). Many groups have addressed the presence of CNV in the human genome and their associations with common human diseases including neuropsychiatric, autoimmune, infectious and cardiovascular diseases [15, 16], while others found evidence that CNVs are not associated with common diseases [17]. While the contribution of CNVs to the pathogenesis of common diseases is questionable, CNVs in some pharmacogenetic genes play a clear role in drug efficacy and toxicity [18]. Inspired by the increasing interest in CNVs, here we review our current knowledge of CNVs in relation to drug efficacy and toxicity, their prevalence in ethnically diverse populations, and the potential utilization of CNV knowledge in the clinical setting.

CNVs associated with drug efficacy in the clinic

CYP2D6

CYP2D6 is predominantly expressed in human liver and it metabolizes over 25% of drugs currently used in the clinic [19]. *CYP2D6* is highly polymorphic and its variants account for the majority of the variation in enzyme activity that is observed within and between populations. To date, more than 75 *CYP2D6* alleles have been documented in the Human Cytochrome P450 (CYP) Allele Nomenclature database (<http://www.cypalleles.ki.se/cyp2d6.htm>). Alleles are comprised of a combination of polymorphisms, including SNPs, insertion/deletions and/or gene conversions, which influence *CYP2D6* enzyme activity. These alleles include normal activity alleles (*1, *2, *33, *35), reduced activity alleles (*9, *10, *17, *29, *41, *59), nonfunctional alleles, which encode enzymes with no activity or produce no enzyme at all (*3, *4, *5–*8, *11–*16, *18–*21, *36, *38, *40, *42, *44, *56, *62), and as yet functionally undetermined alleles (*22–*28, *30–*32, *34, *37, *39, *43, *45–*55, *70, *71, *73, *74) [20]. The functional polymorphisms of the nonfunctional and reduced activity alleles introduce splice defects, non-synonymous amino acid changes or frameshifts, which alter enzyme activity. In addition, CNVs of the normal, nonfunctional and reduced activity *CYP2D6* alleles are observed and these can also alter *in vivo* *CYP2D6* enzyme activity. For example, more than two copies of *CYP2D6* normal alleles (e.g. *1×N, *2×N, *33×N, *35×N, 13>N>2, Figure 2)

have been shown to elevate CYP2D6 enzyme activity [21]. Individuals who inherit these alleles have more rapid metabolism of CYP2D6 substrates and are classified as ultrarapid-metabolizers (UMs) [21]. CNVs are also found for nonfunctional ($*4\times N$, $*36\times N$) and reduced activity alleles ($*10\times N$, $*17\times N$), but individuals who inherit these alleles do not have increased metabolism of CYP2D6 substrates [22]. It is noteworthy that *CYP2D6**36 is absent in white populations but in Asian and African American populations it exists on each chromosome in three forms: 1. *CYP2D6**36 $\times$ 2 (2 copies of nonfunctional allele); 2. *CYP2D6**36+**10* (1 copy of nonfunctional allele in a tandem arrangement with **10*); and 3. *CYP2D6**36 alone (Figure 2) [23]. Only the first situation is considered a CNV. In addition to gaining extra copies of the *CYP2D6* gene, *CYP2D6**5 is a loss of gene copy that produces no CYP2D6 protein [24]. Individuals with two nonfunctional *CYP2D6* alleles, including *CYP2D6**5, have no CYP2D6 activity, in turn have impaired metabolism of CYP2D6 substrates and are classified as poor-metabolizers (PMs). If two normal activity alleles are inherited, subjects have normal metabolism of CYP2D6 substrates and are classified as extensive metabolisers (EMs) [21]. *CYP2D6* CNV is a significant cause of CYP2D6 phenotype diversity in the clinic.

In Caucasian and Asian populations, the frequency of duplicated normal activity alleles, *CYP2D6**1 $\times$ 2 and *2 $\times$ 2, is approximately 0.5–1.5% [25], whereas in African populations, the frequency is approximately 1.6–3.3% [26, 27]. The frequency of *CYP2D6**5 is similar in Caucasian, Asian and African populations (2–7%) [28]. Although the frequency of duplication of *CYP2D6* normal alleles is quite low, the clinical impact of the corresponding phenotype cannot be ignored.

Nortriptyline, a tricyclic antidepressant, is prescribed for the treatment of depression and acts by inhibiting the reuptake of norepinephrine [29]. Taken orally, nortriptyline is well absorbed from the gastrointestinal tract and metabolized in the liver by CYP2D6 [30]. Dalen and colleagues found that the plasma exposure and clearance of nortriptyline was clearly related to the number of copies of the *CYP2D6* gene of subjects (ranging from 0 to 13) [31]. Subjects with 13 copies of functional *CYP2D6* gene displayed a 17-fold higher oral clearance and 36-fold lower plasma concentrations of nortriptyline than those carrying no copies of the functional gene [31]. Interestingly, a recent genome-wide study showed that CNVs in two intergenic regions (chromosome 1 and 10) were associated with nortriptyline response in unipolar depression patients of European ancestry [32]. However, *CYP2D6* CNVs were not shown to be associated with the nortriptyline response. Further studies are required to validate the associations between CNVs and nortriptyline efficacy for *CYP2D6* and new CNV candidates in the clinic.

Tamoxifen is a tissue selective estrogen receptor (ER) modulator, which serves as an antagonist of ERs in breast tissue; however, it is an agonist in endometrial tissue [33]. It is prescribed to treat and prevent hormone-dependent breast cancer in premenopausal and postmenopausal women [34]. Since the 1990s, tamoxifen has been approved by the FDA as the standard anti-estrogen treatment for metastatic breast cancer and adjuvant treatment of primary breast cancer for both female and male patients [35]. Tamoxifen is a prodrug and undergoes complex metabolism *in vivo* and *in vitro*, primarily catalysed by CYPs [36]. CYP2D6 is considered the key enzyme that converts N-desmethyltamoxifen (NDM), a primary tamoxifen metabolite, to endoxifen. Endoxifen has 10-fold higher affinity for ERs than tamoxifen, and is therefore considered the active form of tamoxifen *in vivo* [37]. Indeed, endoxifen/NDM plasma concentration ratios are significantly higher in UMs who carried multiple copies of *CYP2D6* functional alleles than in EMs and PMs who carried two or less copies of *CYP2D6* alleles [38]. It is possible that UM patients may derive more therapeutic benefit from tamoxifen than other patients; however, this has not yet been tested. The recent Italian Tamoxifen Prevention Trial genotyped 47 breast cancer patients using the

AmpliChip CYP450 chip [39]. Subjects who carried more than two functional *CYP2D6* alleles ($*1/*1 \times N$, $*1/*2A \times N$ and $*1 \times N/*2A$) were classified as UMs and those with one or two nonfunctional alleles ($*3/*5$, $*41/*5$ and $*4A/*5$) were classified as PMs. Although the small sample size limited the statistical power of the study, PMs had a higher risk of developing breast cancer than other subjects [39]. In addition, a larger German study genotyped 492 ER⁺ breast cancer patients who had received tamoxifen treatment. In this study patients with more than two *CYP2D6* functional genes ($*1/*1 \times N$, $*2/*1 \times N$ and $*1/*2 \times N$) were also classified as UMs. A Kaplan-Meier survival analyses displayed highest benefit from tamoxifen treatment in UM patients. Meanwhile, PM patients who carried no copies of *CYP2D6* functional alleles had poorer outcomes than EMs (hazard ratio, 2.77; 95% confidence interval 1.31–5.89) [40]. Due to the low frequency of *CYP2D6* CNV in Caucasians, small sample size is a common limitation in these studies, which contributes to low statistical power in data analysis. Clinical trials with larger sample sizes are required to further validate these findings.

Metoprolol is a selective beta1-adrenergic antagonist, which is prescribed to decrease heart rate in patients with acute myocardial infarction (AMI), hypertension or dilated cardiomyopathy [41]. It is extensively metabolized in human liver and predominantly by *CYP2D6* [42]. In a pharmacokinetics study, subjects with three copies of *CYP2D6* functional alleles, classified as UMs, showed significantly lower metoprolol plasma concentrations and higher drug clearances after a single dose of 100 mg metoprolol compared with EMs and PMs ($P < 0.0001$) [43]. For multi-doses and long-term (42 days) drug administration, metoprolol plasma concentrations (normalized for daily dose) and metoprolol/a-hydroxymetoprolol ratios at steady state were markedly lower in UMs than EMs and PMs ($P < 0.0001$), which suggested that UMs are at potential risk of treatment failure [42]. In another study, 187 AMI patients received metoprolol and heart rate (HR) was recorded at administration and discharge. PM patients achieved pronounced HR reduction, while UM patients ($*1/*1 \times N$) failed the treatment [44]. Pre-screening for *CYP2D6* CNVs may help physicians evaluate the potential benefit that patients can receive from metoprolol treatment.

In summary, associations between *CYP2D6* CNVs and therapeutic response to drugs were found in wide range of drug classes including antidepressants, endocrine therapy agents, and beta-blocking agents. Pro-drugs that depend on an activation process via *CYP2D6* may demonstrate a better response in individuals with multiple copies of functional *CYP2D6* genes (UMs). Individuals with two copies of nonfunctional *CYP2D6* alleles (PMs) may benefit from low metabolizing efficiency of drugs exempt from activation process; however, they could be at increased risk of toxicities.

CNV associations with drug toxicity in the clinic

CYP2D6

Codeine is a widely used analgesic and antitussive agent in the clinic. It is activated by *CYP2D6* to a strong central nervous system (CNS) sedative, morphine. Kirchheiner and colleagues found that after a single 30 mg dose of codeine, the plasma concentrations of morphine are significantly higher in *CYP2D6* UMs than EMs [45]. High plasma concentrations of morphine can lead to several fatal effects including hypotension and respiratory depression, especially in pediatric patients. A healthy 2-year-old boy recently died after elective adenotonsillectomy because of the commonly prescribed pain-killer, codeine [46]. Postmortem genotyping revealed that the boy had a duplication of a functional *CYP2D6* gene, which most likely made him a UM of codeine and the subsequent elevated morphine exposure was considered the likely cause of respiratory depression and death in this tragic case [46]. There was a similar case where an infant died with high morphine

concentrations in his blood. The morphine was transferred via breastmilk from a mother with a UM genotype who was taking codeine for postpartum pain [47]. Breastfed infants are at increased risk of potentially life-threatening CNS suppression in such cases [48]. Codeine and its analogues have been labeled with *CYP2D6* CNVs markers by FDA.

Glutathione S-transferases (GSTs)

Glutathione S-transferases (GSTs) are a superfamily of enzymes that catalyze the detoxification of carcinogens, therapeutic chemicals and environmental toxins. GSTs catalyze the reaction of glutathione (GSH) with an acceptor molecule to form a sulfur-substituted glutathione. GST enzymes are encoded by eight distinct loci including alpha, kappa, mu, omega, pi, sigma, theta, and zeta [49]. The GST Mu-1 and Theta-1 enzymes are encoded by the *GSTM1* and *GSTT1* genes, respectively. Complete gene deletions (loss of functional gene copy) of *GSTM1* and *GSTT1* are relatively common. About 50% and 30% of Caucasians are homozygous for *GSTM1* and *GSTT1* gene deletions respectively, compared with >22% and >14% in Asians, and >27% and >37% in Africans [50, 51]. In addition to gene deletions, a multiplex real-time PCR method was developed to detect *GSTM1* and *GSTT1* gene duplications [52]. However, *GSTT1* duplications have not been observed, and a *GSTM1* duplication has only been detected in one of 1320 Caucasian samples. No further functional studies on *GSTM1/GSTT1* gene duplications have been reported.

Consistent with the important roles that *GSTM1* and *GSTT1* play in the detoxification of exogenous compounds, carriers of gene deletions have elevated risks of various cancers (e.g. colorectal cancer, acute myeloid leukemia), especially in Caucasians [53, 54], and drug-related toxicities. Cho and colleagues investigated the associations between *GSTM1* and *GSTT1* gene deletions and toxicity from the standard chemotherapy regimen for lymphoma, R-CHOP (rituximab with cyclophosphamide, doxorubicin, vincristine and prednisone), in 94 diffuse large B-cell lymphoma (DLBCL) patients [55]. R-CHOP caused more grade III-IV toxicities, including leukocytopenia (odds ratio (OR)=3.12), fever (OR=5.27), and mucositis (OR=4.61), in patients homozygous for *GSTT1* gene deletion, than other patients. Patients with a *GSTM1/GSTT1* double-deletion genotype had an even higher risk of grade III-IV thrombocytopenia than other patients (OR=7.75). Loss of either or both *GSTM1* and *GSTT1* genes might be expected to increase toxicity along with improved efficacy of chemotherapy, however no difference was observed in this study. *GSTM1* and *GSTT1* gene deletions are likely to predict R-CHOP induced toxicity in DLBCL patients.

Tacrine was the first drug for the treatment of mild to moderate dementia of Alzheimer's disease [56], which is the most common form of dementia characterized for a progressive and fatal neurodegenerative disorder [57]. Tacrine-induced hepatotoxicity is primarily caused by one of its metabolites, which is de-activated by *GSTM1* and *GSTT1* [58]. A study in 141 Caucasian Alzheimer's disease patients found that patients with the *GSTM1* and *GSTT1* double-deletion had alanine aminotransferase (ALT) levels that were three times the upper limit of normal (relative hazard: 2.84, 95% confidence interval:1.51–5.35, P=0.001), which is a marker of tacrine-induced hepatotoxicity [59]. In addition, a patient's susceptibility to tacrine-induced hepatotoxicity could not be predicted by either *GSTM1* or *GSTT1* deletion alone suggesting redundancy in the enzymes [59]. Due to the high prevalence of *GSTM1* and *GSTT1* gene deletions in Caucasians, over 10% of patients have a *GSTM1* and *GSTT1* double deletion and are therefore at increased risk of tacrine-induced hepatotoxicity. A nitric oxide donor-tacrine hybrid compound may be an alternative therapy for these patients to achieve both drug efficacy and low risk of hepatotoxicity [60].

In summary, many phase I and II drug metabolism enzymes are involved in the detoxification of drugs used in clinic. CNVs can lead to the over-activation or loss of

detoxification of a drug which may cause increased drug toxicities in patients. Precautions should be adopted based on individual genotyping information.

Prevalence of CNVs in drug related gene in human populations

Understanding the prevalence of major genetic variations related to drug efficacy and toxicity is critical for both health providers and patients. Both the Ministry of Health in each nation and physicians in clinics can use this knowledge to maximize benefit and minimize harm for patients prior to and during drug therapy [61], which is widely accepted as the base for personalized genomic medicine [62].

The frequency of CNVs is shown in Table 1. These data can help each population to select the most appropriate drugs and proper doses for specific treatments. For example, in the United States, over 50% and 30% of Caucasian populations carry either *GSTM1* or *GSTT1* gene deletions [50], which leads to increased susceptibility to colorectal cancer, acute myeloid leukemia and chemotherapy-induced toxicities [53]. Using the CNVs frequency data, physicians will be aware of the probability of achieving benefit or developing a drug-induced toxicity in his/her patients and thus precautions can be taken. Meanwhile, multi-copies of *CYP2D6* functional genes are more prevalent in Europe, Saudi Arab, and Ethiopia than in Asia and the U.S. (Caucasian). In former regions, more people may respond to tamoxifen treatment, and less codeine induced CNS suppression will be observed in the latter. The data of CNVs global prevalence can help to avoid translating a regional drug problem for a specific population into a common problem for all other populations [63].

Which drugs should be labeled with CNV markers?

Since 2008, the FDA has released a list of valid genomic biomarkers in the context of approved drug labels [5]. The term “valid biomarkers” has been described in the previously released “Guidance for Industry: Pharmacogenomic Data Submissions” [4] as a “biomarker that is measured in an analytical test system with well established performance characteristics and for which there is an established scientific framework or body of evidence that elucidates the physiologic, toxicological, pharmacologic, or clinical significance of the test results.” Drugs whose therapeutic response or toxicity is affected by these markers were recorded in this list including one CNV marker: *CYP2D6**2×2. The package insert for codeine sulfate and other drugs containing codeine as an ingredient, such as Fiorinal® with codeine (butalbital, aspirin, caffeine, and codeine phosphate) and Fioricet® with codeine (butalbital, acetaminophen, caffeine, and codeine phosphate) warns of elevated risk of codeine-induced toxicity in individuals with more copies of *CYP2D6* functional genes. However, none of the other drugs (tamoxifen, tacrine, etc) or other CNVs (*CYP2D6**5, *GSTM1*, *GSTT1* deletions) discussed in this review are included in this list yet. Meanwhile, an FDA-approved analytical test system for *GSTM1* and *GSTT1* deletions is not available at this time. The only approved genotyping platform for CNV markers diagnosis in clinic is the AmpliChip CYP450 chip [3], which detects *CYP2D6* CNVs. There is still a gap between the knowledge of drug-related CNVs and implementation of drug label changes. The association of pharmacogenetic CNVs with drugs and clinical indications are summarized in Table 2.

Concluding remarks

The translation of CNV knowledge into clinic intervention remains a big challenge, with a growing number of pharmacogenetics studies providing robust evidence for the impact of CNV on the efficacy and toxicity of drug therapy [18]. However, few comparative effectiveness studies have been conducted in this field, which has limited the expansion of genetic testing of CNVs into primary health care system. In addition, investigations on the

cost versus benefits of genetic testing have been questioned. Additionally, health providers and insurance companies quite understandably are reluctant to reimburse genetic tests for low frequency variants to identify only a few patients who may benefit. That includes *CYP2D6* CNVs and over 10 other clinically important pharmacogenetic variants (*CYP2C19**3, *CYP2C9**2, *3, *CYP2D6**3, *6, *DPYD**2A, *TPMT**2, *3, *4, etc) that are considered rare. These variants exist in every population and may cause severe drug-induced toxicities or reduce the therapeutic benefit that a patient derives from a drug. As a result of this economic reality, diffusion of genetic tests for drug treatment has been slow. Unless we take a coordinated approach to CNV translational research, by adding policy and economics members to the investigation team, we will never see the promise of genome-guided therapy.

Box 1

Currently available methodologies for CNV detection
Many PCR based methods have been developed for the detection of CNVs. However, the low-throughput and low reproductively rate limit the use of these methods in the clinic. Recently, new methods have been developed based on microarray hybridization technology.

Conventional methods

Long distance and multiplex PCR

This is the first method developed for the detection of *CYP2D6* CNV. Long PCR is used to amplify a > 4000 bp fragment of the *CYP2D6* gene. Specific primers for *CYP2D6**5 have been designed. Two separate PCR reactions for either wild-type or mutated allele primers are run for each sample using the long PCR product of *CYP2D6* gene. Following up with gel electrophoresis, the *CYP2D6**5 can be identified [64].

TaqMan Real-Time PCR

This is a PCR quantification method. TaqMan probes emit a specific report fluorophore (usually a short-wavelength colored dye, such as green) during the elongation process of PCR reactions. The fluorophore can be detected by a corresponding detection system and compared with the signal of reference genes, such as albumin, to calculate the amount of specific genes in each sample. This is a low-through put detection method for CNVs [65].

Microarrays hybridization-based methods

Array comparative genomic hybridization (aCGH)

This is a genome-wide screening technique for CNVs [66] with higher resolution than chromosome-based comparative genomic hybridization [67], which allows detection of copy number changes of 5–10 kb of DNA sequence.

Roche AmpliChip CYP450 system

This is an oligonucleotide microarray hybridization method for genotyping 27 *CYP2D6* variants (including CNVs) and two *CYP2C19* variants. It has been developed by Roche based on Affymetrix microarray technology [68]. A logarithmic scale is used to predict *CYP2D6* and *CYP2C19* phenotypes [69]. This is the first FDA approved pharmacogenetics test for clinic use.

Genome-wide association SNP microarrays

These arrays cover 300K to more than 1 million genetic markers including thousands of probes for the detection of CNVs [70].

Affymetrix DMET plus microarray

This is a novel genotyping tool customized for pharmacogenetics research. It covers 225 essential pharmacogenetics genes and 1936 common or rare variants including five CNVs belongs to *CYP2D6*, *CYP2A6*, *UGT2B17*, *GSTM1* and *GSTT1* [71].

Acknowledgments

The authors are supported by the NIH Pharmacogenetics Research Network (U01 GM63340), the University Cancer Research Fund, and UL1RR025747 from the National Center for Research Resources. This work is part of the Pharmacogenetics for Every Nation Initiative. (www.pgeni.org).

References

1. Meyer UA. Pharmacogenetics and adverse drug reactions. *Lancet*. 2000; 356:1667–1671. [PubMed: 11089838]
2. Eichelbaum M, et al. Pharmacogenomics and individualized drug therapy. *Annu Rev Med*. 2006; 57:119–137. [PubMed: 16409140]
3. de Leon J, et al. The AmpliChip CYP450 genotyping test: Integrating a new clinical tool. *Mol Diagn Ther*. 2006; 10:135–151. [PubMed: 16771600]
4. U.S.FDA. Guidance for Industry: Pharmacogenomic Data Submissions. 2005.
5. Table of Valid Genomic Biomarkers in the Context of Approved Drug Labels. 2008.
6. Normanno N, et al. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol*. 2009; 6:519–527. [PubMed: 19636327]
7. Lonjou C, et al. A European study of HLA-B in Stevens-Johnson syndrome and toxic epidermal necrolysis related to five high-risk drugs. *Pharmacogenet Genomics*. 2008; 18:99–107. [PubMed: 18192896]
8. Schwarz UI, et al. Genetic determinants of response to warfarin during initial anticoagulation. *N Engl J Med*. 2008; 358:999–1008. [PubMed: 18322281]
9. Roy PD, et al. Pharmacogenomics of anti-TB drugs-related hepatotoxicity. *Pharmacogenomics*. 2008; 9:311–321. [PubMed: 18303967]
10. Frueh FW, et al. Pharmacogenomic biomarker information in drug labels approved by the United States food and drug administration: prevalence of related drug use. *Pharmacotherapy*. 2008; 28:992–998. [PubMed: 18657016]
11. Administration, U.F.a.D. Table of valid genomic biomarkers in the context of approved drug labels. 2006.
12. Stamer UM, et al. Respiratory depression with tramadol in a patient with renal impairment and CYP2D6 gene duplication. *Anesth Analg*. 2008; 107:926–929. [PubMed: 18713907]
13. Weinshilboum R. Inheritance and drug response. *N Engl J Med*. 2003; 348:529–537. [PubMed: 12571261]
14. Redon R, et al. Global variation in copy number in the human genome. *Nature*. 2006; 444:444–454. [PubMed: 17122850]
15. Schoumans J, et al. Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). *J Med Genet*. 2005; 42:699–705. [PubMed: 16141005]
16. McCarroll SA, et al. Common deletion polymorphisms in the human genome. *Nat Genet*. 2006; 38:86–92. [PubMed: 16468122]
17. Craddock N, et al. Genome-wide association study of CNVs in 16,000 cases of eight common diseases and 3,000 shared controls. *Nature*. 2010; 464:713–720. [PubMed: 20360734]
18. Johansson I, Ingelman-Sundberg M. CNVs of human genes and their implication in pharmacogenetics. *Cytogenet Genome Res*. 2008; 123:195–204. [PubMed: 19287156]
19. Zhou SF. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clin Pharmacokinet*. 2009; 48:689–723. [PubMed: 19817501]

20. Hoskins JM, et al. CYP2D6 and tamoxifen: DNA matters in breast cancer. *Nat Rev Cancer*. 2009; 9:576–586. [PubMed: 19629072]
21. Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J*. 2005; 5:6–13. [PubMed: 15492763]
22. Ishiguro A, et al. Metabolic activity of dextromethorphan O-demethylation in healthy Japanese volunteers carrying duplicated CYP2D6 genes: duplicated allele of CYP2D6*10 does not increase CYP2D6 metabolic activity. *Clin Chim Acta*. 2004; 344:201–204. [PubMed: 15149890]
23. Gaedigk A, et al. CYP2D6*36 gene arrangements within the cyp2d6 locus: association of CYP2D6*36 with poor metabolizer status. *Drug Metab Dispos*. 2006; 34:563–569. [PubMed: 16415111]
24. Steen VM, et al. Detection of the poor metabolizer-associated CYP2D6(D) gene deletion allele by long-PCR technology. *Pharmacogenetics*. 1995; 5:215–223. [PubMed: 8528268]
25. Sistiö J, et al. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenet Genomics*. 2007; 17:93–101. [PubMed: 17301689]
26. Fuselli S, et al. Evolution of detoxifying systems: the role of environment and population history in shaping genetic diversity at human CYP2D6 locus. *Pharmacogenet Genomics*. 2010; 20:485–499. [PubMed: 20520586]
27. Griese EU, et al. Analysis of the CYP2D6 gene mutations and their consequences for enzyme function in a West African population. *Pharmacogenetics*. 1999; 9:715–723. [PubMed: 10634134]
28. Bradford LD. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*. 2002; 3:229–243. [PubMed: 11972444]
29. Nortriptyline. 2008. <http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0000732>
30. Yue QY, et al. Pharmacokinetics of nortriptyline and its 10-hydroxy metabolite in Chinese subjects of different CYP2D6 genotypes. *Clin Pharmacol Ther*. 1998; 64:384–390. [PubMed: 9797795]
31. Dalen P, et al. 10-Hydroxylation of nortriptyline in white persons with 0, 1, 2, 3, and 13 functional CYP2D6 genes. *Clin Pharmacol Ther*. 1998; 63:444–452. [PubMed: 9585799]
32. Uher R, et al. Genome-wide pharmacogenetics of antidepressant response in the GENDEP project. *Am J Psychiatry*. 2010; 167:555–564. [PubMed: 20360315]
33. Wood CE, et al. Endometrial profile of tamoxifen and low-dose estradiol combination therapy. *Clin Cancer Res*. 2010; 16:946–956. [PubMed: 20103679]
34. Jordan VC. Tamoxifen (ICI46,474) as a targeted therapy to treat and prevent breast cancer. *Br J Pharmacol*. 2006; 147(Suppl 1):S269–276. [PubMed: 16402113]
35. Ingle JN. Pharmacogenomics of tamoxifen and aromatase inhibitors. *Cancer*. 2008; 112:695–699. [PubMed: 18072234]
36. Desta Z, et al. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther*. 2004; 310:1062–1075. [PubMed: 15159443]
37. Johnson MD, et al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat*. 2004; 85:151–159. [PubMed: 15111773]
38. Borges S, et al. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther*. 2006; 80:61–74. [PubMed: 16815318]
39. Serrano D, et al. Efficacy of tamoxifen based on cytochrome P450 2D6, CYP2C19 and SULT1A1 genotype in the Italian Tamoxifen Prevention Trial. *Pharmacogenomics J*. 2010
40. Schroth W, et al. CYP2D6 polymorphisms as predictors of outcome in breast cancer patients treated with tamoxifen: expanded polymorphism coverage improves risk stratification. *Clin Cancer Res*. 2010; 16:4468–4477. [PubMed: 20515869]
41. Prakash A, Markham A. Metoprolol: a review of its use in chronic heart failure. *Drugs*. 2000; 60:647–678. [PubMed: 11030472]

42. Fux R, et al. Impact of CYP2D6 genotype on adverse effects during treatment with metoprolol: a prospective clinical study. *Clin Pharmacol Ther.* 2005; 78:378–387. [PubMed: 16198657]
43. Kirchheiner J, et al. Impact of the ultrarapid metabolizer genotype of cytochrome P450 2D6 on metoprolol pharmacokinetics and pharmacodynamics. *Clin Pharmacol Ther.* 2004; 76:302–312. [PubMed: 15470329]
44. Goryachkina K, et al. CYP2D6 is a major determinant of metoprolol disposition and effects in hospitalized Russian patients treated for acute myocardial infarction. *Eur J Clin Pharmacol.* 2008; 64:1163–1173. [PubMed: 18648788]
45. Kirchheiner J, et al. Pharmacokinetics of codeine and its metabolite morphine in ultra-rapid metabolizers due to CYP2D6 duplication. *Pharmacogenomics J.* 2006
46. Ciszowski C, et al. Codeine, ultrarapid-metabolism genotype, and postoperative death. *N Engl J Med.* 2009; 361:827–828. [PubMed: 19692698]
47. Madadi P, et al. Safety of codeine during breastfeeding: fatal morphine poisoning in the breastfed neonate of a mother prescribed codeine. *Can Fam Physician.* 2007; 53:33–35. [PubMed: 17872605]
48. Madadi P, et al. Pharmacogenetics of neonatal opioid toxicity following maternal use of codeine during breastfeeding: a case-control study. *Clin Pharmacol Ther.* 2009; 85:31–35. [PubMed: 18719619]
49. Hayes JD, et al. Glutathione transferases. *Annu Rev Pharmacol Toxicol.* 2005; 45:51–88. [PubMed: 15822171]
50. Garte S, et al. Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev.* 2001; 10:1239–1248. [PubMed: 11751440]
51. Piacentini S, et al. GSTT1 and GSTM1 gene polymorphisms in European and African populations. *Mol Biol Rep.* 2010
52. Timofeeva M, et al. A multiplex real-time PCR method for detection of GSTM1 and GSTT1 copy numbers. *Clin Biochem.* 2009; 42:500–509. [PubMed: 19161997]
53. Economopoulos KP, Sergeantis TN. GSTM1, GSTT1, GSTP1, GSTA1 and colorectal cancer risk: a comprehensive meta-analysis. *Eur J Cancer.* 2010; 46:1617–1631. [PubMed: 20207535]
54. Das P, et al. Meta-analysis study of glutathione-S-transferases (GSTM1, GSTP1, and GSTT1) gene polymorphisms and risk of acute myeloid leukemia. *Leuk Lymphoma.* 2009; 50:1345–1351. [PubMed: 19811334]
55. Cho HJ, et al. Glutathione-S-transferase genotypes influence the risk of chemotherapy-related toxicities and prognosis in Korean patients with diffuse large B-cell lymphoma. *Cancer Genet Cytogenet.* 2010; 198:40–46. [PubMed: 20303013]
56. Farlow M, et al. A controlled trial of tacrine in Alzheimer's disease. The Tacrine Study Group. *Jama.* 1992; 268:2523–2529. [PubMed: 1404819]
57. Kawas CH. Clinical practice. Early Alzheimer's disease. *N Engl J Med.* 2003; 349:1056–1063. [PubMed: 12968090]
58. Patocka J, et al. Possible role of hydroxylated metabolites of tacrine in drug toxicity and therapy of Alzheimer's disease. *Curr Drug Metab.* 2008; 9:332–335. [PubMed: 18473751]
59. Simon T, et al. Combined glutathione-S-transferase M1 and T1 genetic polymorphism and tacrine hepatotoxicity. *Clin Pharmacol Ther.* 2000; 67:432–437. [PubMed: 10801254]
60. Fang L, et al. NO-donating tacrine hybrid compounds improve scopolamine-induced cognition impairment and show less hepatotoxicity. *J Med Chem.* 2008; 51:7666–7669. [PubMed: 19053746]
61. Roederer MW, McLeod HL. Applying the genome to national drug formulary policy in the developing world. *Pharmacogenomics.* 2010; 11:633–636. [PubMed: 20415551]
62. Collins, FS. *The Language of Life: DNA and the Revolution in Personalized Medicine.* HarperCollins Publisher; 2009.
63. Cavaco I, et al. CYP2C8 polymorphism frequencies among malaria patients in Zanzibar. *Eur J Clin Pharmacol.* 2005; 61:15–18. [PubMed: 15785959]
64. Stuvén T, et al. Rapid detection of CYP2D6 null alleles by long distance- and multiplex-polymerase chain reaction. *Pharmacogenetics.* 1996; 6:417–421. [PubMed: 8946473]

65. Schaeffeler E, et al. CYP2D6 genotyping strategy based on gene copy number determination by TaqMan real-time PCR. *Hum Mutat.* 2003; 22:476–485. [PubMed: 14635107]
66. Shinawi M, Cheung SW. The array CGH and its clinical applications. *Drug Discov Today.* 2008; 13:760–770. [PubMed: 18617013]
67. Pinkel D, et al. High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet.* 1998; 20:207–211. [PubMed: 9771718]
68. Heller T, et al. AmpliChip CYP450 GeneChip: a new gene chip that allows rapid and accurate CYP2D6 genotyping. *Ther Drug Monit.* 2006; 28:673–677. [PubMed: 17038884]
69. Rebsamen MC, et al. The AmpliChip CYP450 test: cytochrome P450 2D6 genotype assessment and phenotype prediction. *Pharmacogenomics J.* 2009; 9:34–41. [PubMed: 18591960]
70. The International HapMap Project. *Nature.* 2003; 426:789–796. [PubMed: 14685227]
71. Deeken J. The Affymetrix DMET platform and pharmacogenetics in drug development. *Curr Opin Mol Ther.* 2009; 11:260–268. [PubMed: 19479659]
72. Gaedigk A, et al. Unique CYP2D6 activity distribution and genotype-phenotype discordance in black Americans. *Clin Pharmacol Ther.* 2002; 72:76–89. [PubMed: 12152006]
73. Lavender NA, et al. Examination of polymorphic glutathione S-transferase (GST) genes, tobacco smoking and prostate cancer risk among men of African descent: a case-control study. *BMC Cancer.* 2009; 9:397. [PubMed: 19917083]
74. Sosa-Macias M, et al. Influence of CYP2D6 deletion, multiplication, -1584C-->G, 31G-->A and 2988G-->a gene polymorphisms on dextromethorphan metabolism among mexican tepehuanos and mestizos. *Pharmacology.* 2010; 86:30–36. [PubMed: 20588073]
75. Lopez M, et al. CYP2D6 genotype and phenotype determination in a Mexican Mestizo population. *Eur J Clin Pharmacol.* 2005; 61:749–754. [PubMed: 16249913]
76. Perez-Morales R, et al. Polymorphism of CYP1A1*2C, GSTM1*0, and GSTT1*0 in a Mexican Mestizo population: a similitude analysis. *Hum Biol.* 2008; 80:457–465. [PubMed: 19317600]
77. Aklillu E, et al. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. *J Pharmacol Exp Ther.* 1996; 278:441–446. [PubMed: 8764380]
78. Dash B, et al. Determinants of the variability of aflatoxin-albumin adduct levels in Ghanaians. *J Toxicol Environ Health A.* 2007; 70:58–66. [PubMed: 17162498]
79. Ji L, et al. Genetic polymorphisms of CYP2D6 in Chinese mainland. *Chin Med J (Engl).* 2002; 115:1780–1784. [PubMed: 12622923]
80. Liu L, et al. Genetic polymorphisms of glutathione S-transferase and risk of vitiligo in the Chinese population. *J Invest Dermatol.* 2009; 129:2646–2652. [PubMed: 19571817]
81. Nishida Y, et al. CYP2D6 genotypes in a Japanese population: low frequencies of CYP2D6 gene duplication but high frequency of CYP2D6*10. *Pharmacogenetics.* 2000; 10:567–570. [PubMed: 10975611]
82. Kubota T, et al. Frequencies of CYP2D6 mutant alleles in a normal Japanese population and metabolic activity of dextromethorphan O-demethylation in different CYP2D6 genotypes. *Br J Clin Pharmacol.* 2000; 50:31–34. [PubMed: 10886115]
83. Fujihara J, et al. Cytochrome P450 1A1, glutathione S-transferases M1 and T1 polymorphisms in Ovambos and Mongolians. *Leg Med (Tokyo).* 2009; 11(Suppl 1):S408–410. [PubMed: 19264525]
84. Lee SY, et al. Sequence-based CYP2D6 genotyping in the Korean population. *Ther Drug Monit.* 2006; 28:382–387. [PubMed: 16778723]
85. Uhm YK, et al. Association of glutathione S-transferase gene polymorphisms (GSTM1 and GSTT1) of vitiligo in Korean population. *Life Sci.* 2007; 81:223–227. [PubMed: 17568619]
86. Abbas A, et al. GSTM1, GSTT1, GSTP1 and CYP1A1 genetic polymorphisms and susceptibility to esophageal cancer in a French population: different pattern of squamous cell carcinoma and adenocarcinoma. *World J Gastroenterol.* 2004; 10:3389–3393. [PubMed: 15526353]
87. Sachse C, et al. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Hum Genet.* 1997; 60:284–295. [PubMed: 9012401]
88. McLellan RA, et al. Frequent occurrence of CYP2D6 gene duplication in Saudi Arabians. *Pharmacogenetics.* 1997; 7:187–191. [PubMed: 9241658]

89. Abu-Amero KK, et al. GSTM1 and GSTT1 deletion genotypes in various spontaneous optic neuropathies in Arabs. *Br J Ophthalmol*. 2009; 93:1101–1104. [PubMed: 19286687]
90. Rao Y, et al. Duplications and defects in the CYP2A6 gene: identification, genotyping, and in vivo effects on smoking. *Mol Pharmacol*. 2000; 58:747–755. [PubMed: 10999944]
91. Lerman C, et al. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. *Clin Pharmacol Ther*. 2010; 87:553–557. [PubMed: 20336063]
92. Farinola N, Piller NB. CYP2A6 polymorphisms: is there a role for pharmacogenomics in preventing coumarin-induced hepatotoxicity in lymphedema patients? *Pharmacogenomics*. 2007; 8:151–158. [PubMed: 17286538]
93. Yang P, et al. Role of the glutathione metabolic pathway in lung cancer treatment and prognosis: a review. *J Clin Oncol*. 2006; 24:1761–1769. [PubMed: 16603718]
94. Lamberti C, et al. Arylamine N-acetyltransferase type 2 and glutathione S-transferases M1 and T1 polymorphisms in familial adenomatous polyposis. *Pharmacogenetics*. 2002; 12:49–54. [PubMed: 11773864]
95. Yang TL, et al. Genome-wide copy-number-variation study identified a susceptibility gene, UGT2B17, for osteoporosis. *Am J Hum Genet*. 2008; 83:663–674. [PubMed: 18992858]

Figure 1.

A diagram for copy number variants in human genome. If the gene recombination event occurs between two genes, a gene duplication or multiplication ($n=2,3..$) or a gene deletion ($n=0$) could happen. A duplication of gene could also carry mutations from the original copy (show in red column).

Figure 2.

A summary of *CYP2D6* CNV structure[18]. (a) Functional *CYP2D6* CNVs are shown in gold. (b) Duplication of *CYP2D6* reduced activity or nonfunctional alleles are shown in dotted background. (c) Three forms of *CYP2D6**36 allele. The first arrangement is considered as a CNV for this allele. (d) Deletion of *CYP2D6* gene.

Table 1

A summary of CNV frequencies in pharmacogenetic genes in different ethnic populations.

Population	CNV frequencies (%)				
	CYP2D6*1×N	CYP2D6*2×N	CYP2D6*5	GSTM1 deletion	GSTT1 deletion
North America					
U.S.A. (Caucasian)	0.2 [72]	0.7 [72]	6.2 [72]	54.3 [50]	27.6 [50]
U.S.A. (African-American)	1.2 [72]	1.6 [72]	4.0 [72]	23.7 [73]	17.5 [73]
Mexico (Mestizo)			1.3–2.7 [74, 75]	33.5 [76]	12.1 [76]
Africa					
Cameroon	3.3 [26]	3.3 [26]		27.8 [51]	46.8 [51]
Ethiopia		10–16 [21, 77]	3.3 [77]	43.8 [51]	37.3 [51]
Ghana		1.6 [27]	6 [27]	19.3 [78]	73.7 [78]
East Asia					
China (Han)	2.2 [25]	0–2 [21]	7.2 [79]	52 [80]	38.7 [80]
Japan	0.5 [81]	0.5 [81]	6.2 [82]	50.8 [83]	45.8 [83]
Korea	0.13 [84]	0.5 [84]	6.1 [84]	51.4 [85]	51.6 [85]
Europe					
France	2.0 [25]		4.0 [25]	49 [86]	26 [86]
Germany	0.51 [87]	1.34 [87]	1.95 [87]	51.6 [50]	19.5 [50]
Middle East					
Saudi Arabia		10.4 [88]	1.0 [88]	8.3 [89]	4.2 [89]

Table 2

Major clinical information for CNVs in pharmacogenetic genes.

	CNV type	Drug affected	Phenotype	Clinical indication	PharmGKB gene category	Reference
<i>CYP2A6</i> *4	Deletion	Nicotine Coumarin	No enzyme activity	Unknown	VIP	[90–92]
<i>CYP2A6</i> *1x2	Duplication	Tegafur	Increased enzyme activity	Unknown	VIP	
<i>CYP2D6</i> *1xN	Multiplication	Anticholinesterases	Increased enzyme activity	Alzheimer Disease	VIP	[19]
<i>CYP2D6</i> *2xN	Multiplication	Antidepressants	Increased enzyme activity	Depression	VIP	
<i>CYP2D6</i> *5	Deletion	Antipsychotics Beta Blocking Agents Opioids	No enzyme activity	Hypertension Breast cancer Codeine dependence	VIP	
<i>GSTM1</i> *0	Deletion	Antineoplastic agent	No enzyme activity	Breast cancer		[93, 94]
<i>GSTT1</i> *0	Deletion	Xenobiotics	No enzyme activity	Non-small cell lung cancer Leukemia Drug toxicity		
<i>SULT1A1</i>	Multiplication	Unclear clinic impact on drugs	Increased enzyme activity	Unknown	VIP	
<i>SULT1A3</i>	Duplication	Unclear clinic impact on drugs	Unknown	Unknown		
<i>UGT2B17</i>	Deletion	Estradiol Testosterone	Decreased enzyme activity	Androgen Excretion		[95]

VIP = very important pharmacogene.