Next generation sequencing reveals disparate population frequencies among cytochrome P450 genes: clinical pharmacogenomics of the CYP2 family

William T. Budd*, Greg Meyers, Jeri R. Dilts, Katherine O'Hanlon, John R. Woody, David G. Bostwick, John R. Drury and Thomas Reynolds

American International Biotechnology,
601 Biotech Drive,
Richmond, VA 23235, USA
Fax: 804-305-7648
Email: wbudd@aibiotech.com
Email: gmeyers@aibiotech.com
Email: jdilts@aibiotech.com
Email: kohanlon@aibiotech.com
Email: jwoody@aibiotech.com
Email: dbostwick@aibiotech.com
Email: jdrury@aibiotech.com
Email: treynolds@aibiotech.com
*Corresponding author

Abstract: 85% of medications prescribed are metabolised by a CYP450 superfamily member. This family contains SNPs linked to medication response. 30,000 participants were evaluated to determine potential differences in ethnic distributions of nucleotide polymorphisms. Next generation sequencing with the Ion Torrent PGM was used to genotype medication response genes. Variations in these key enzymes were common leading to a variety of responses to medication therapy. Our study showed there exists a large range of genetic variation within/between various ethnic groups. With exception of CYP2C9, the wild type genotype is not most common. Each gene showed a unique pattern of distribution that significantly differed within and across ethnic groups. These findings call into question the concept of a 'normal' patient. Our results highlight the need for and applicability of pharmacogenomic testing. Genetic determination of patient response groups can help tailor therapies and increase the likelihood of success.

Keywords: personalised medicine; pharmacogenomics; translational research; cytochrome P450; NGS; next generation sequencing; precision medicine; ethnic variability; medication metabolism; ion torrent; CYP2D6; VKORC; CYP2C9; CYP2C19.

Reference to this paper should be made as follows: Budd, W.T., Meyers, G., Dilts, J.R., O'Hanlon, K., Woody, J.R., Bostwick, D.G., Drury, J.R. and Reynolds, T. (2016) 'Next generation sequencing reveals disparate population frequencies among cytochrome P450 genes: clinical pharmacogenomics of the CYP2 family', *Int. J. Computational Biology and Drug Design*, Vol. 9, Nos. 1/2, pp.54–86.

1 Introduction

Medications are prescribed to patients on the basis of a physician's physical assessment and an expectation that medications respond as reported. These expectations are based on the results of clinical trials. However, patients often display a large range in the anticipated response to therapy that can be caused by a combination of genetic and non-genetic factors. Studies show that the average medication administered at recommended dosages is only effective for 30–60% of patients (Meyer et al., 2013). Inter-individual variations in responses to treatments can lead to therapeutic failure or development of life threatening adverse reactions (Yiannakopoulou, 2013). Discovery of single nucleotide polymorphisms (SNPs) affecting drug metabolism has the potential for the first time to truly create the concept of personalised medicine and minimise potential variations in medication effect. Variations in genes affecting drug metabolism or transportation are associated with adverse drug reactions (ADRs).

Incidences of ADRs have dramatically increased over the last 10 years. As the number of healthcare facility admissions increases, so does the number of ADRs. From a patient perspective, a negative reaction can cause them to lose trust in their healthcare provider, and increases their likelihood of poor compliance to all therapeutic regimens. ADRs increase the length of stay in the hospital, thus increasing the cost of healthcare delivery impacting all members of society. Medications are evaluated using a clinical trial and typically studied in controlled environments in relatively low numbers of patients. In these settings, adverse reactions are likely underrepresented as confounding variables are often inadequate (Moses et al., 2013).

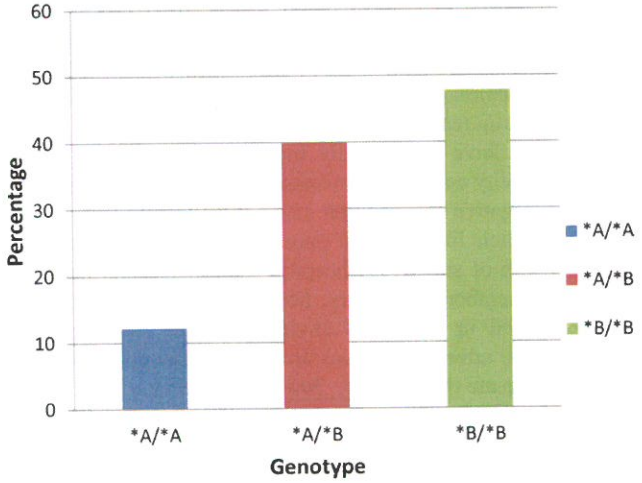
Crucial to understanding the impact on drug response is understanding of ethnic and inter-ethnic genetic differences in genes affecting medication metabolism (Li et al., 2011). Until recently, the cost of evaluating pharmacologically relevant variants was cost prohibitive. The pharmacogenomic resource for enhanced decisions in care & treatment (PREDICT) project at Vanderbilt University estimated 64.8% of patients were exposed to at least one medication with a known genetic association in a five year period (Schildcrout et al., 2012). More than 10% of patients were exposed to at least four medications with known pharmacogenomic interactions. Most pharmacogenomics studies to date have been conducted in limited populations and often lack sufficient numbers to achieve significance. Recent studies have shown that allele frequencies substantially vary across and within populations (Adeyemo and Rotimi, 2010). This variability can impact a medication's anticipated actions.

Of clinical importance, the CYP450 superfamily of enzymes is a major source of variability in a medications pharmacokinetics and response. The CYP 1, 2 and 3 families of enzymes are responsible for the majority of drug and xenobiotic metabolism. An estimated 70–80% of all prescribed medications are biotransformed by a member of the CYP450 super family. The function of each enzyme can be affected by genetic polymorphisms. Distinct pharmacogenetic phenotypes are a result of this polymorphism causing patients to metabolise medications differently. A priori knowledge of a patient's metaboliser status can help physicians tailor therapeutic regimens specifically to their patient reducing the chance of an ADR and increasing the likelihood of therapeutic success.

Next generation sequencing (NGS) has increased the amount of data generated per run at a lower cost than traditional technologies (Xiong et al., 2010). NGS technology has increased our ability to study human genomics and provides a powerful new tool for

genomic based medicine. As a consequence of this change, genetic factors underlying patient response to medications and therapies for the first time can be determined. This leap in technology has enabled medical professionals to better use genetic information for truly targeted and personalised medicine.

Figure 1 Position –1639 of the VKORC1 gene was sequenced for all participant samples (34,251). The B/B genotype (WT) was most frequently observed with less than 50% of participants being classified as B/B. The remaining participants likely possess at least one copy of a mutant gene and are likely to have some degree of reduced enzymatic activity (see online version for colours)



The focus of this work is detection of clinically actionable SNPs by NGS of the Cytochrome P450 2 (CYP2) family of enzymes. The CYP2 family is the largest family of CYP450 enzymes with the most family members. Genes included in this work are the CYP2D6, CYP2C9, CYP2C19, and VKORC genes. The specific genes and SNPs evaluated in this study are described in Supplementary Table S1. These variations were chosen as they are linked to medication response variability thus they are considered clinically actionable. Many of these SNPs have been hypothesised to vary in proportion across members of various ethnic populations. Our study compared a large number (~30,000) of anonymised, patient samples submitted for pharmacogenomic testing to a clinical lab and was composed of several ethnic groups in the USA. This study includes a large number of participant samples from African American, White, Hispanic, and Asian ethnicities and aims to determine if the genotypes of the CYP450 genes vary between various ethnic groups in the USA. The number of samples for each experiment varied slightly as not every participant was evaluated for each potential gene. To our knowledge, this study is the largest and most comprehensive of its kind conducted in the USA. This study includes all clinically actionable SNPs for each gene. This is a retrospective analysis of samples evaluated based on a physician's order. The average age of the patient in this work is 66 years of age (median = 68). The distribution of patient ages is better described in Supplementary Figure 1. This study shows the range of genetic variation and predicted phenotypes within persons that subscribe to a particular ethnic orientation differs dramatically from one ethnic group to the next.

2 Materials and methods

2.1 Clinical samples

Samples sent to American International Biotechnology for genetic testing were used for this study. Each participant consented to participation in the clinical study. All samples are anonymous to lab personnel and researchers participating in the experiment have no knowledge of patient's age or ethnicity. Each study participant was asked to include their age in years and self-select race/ethnic identification. Participants could select African-American, Asian, White, Hispanic, or describe in their own terms their ethnicity. For this study, data was presented for whites, African Americans, Asians, and Hispanics living in the USA. Patients that reported other ethnicity and respondents that did not list an ethnicity were not considered in this analysis. Study groups with less than 50 respondents were not presented (Pacific Islanders, Native Americans). Buccal swabs (2) were received by the lab from physician offices after buccal sampling.

2.2 DNA extraction

Buccal cells were eluted from polystyrene flock swabs (HydraFlock 25-3306-H, Puritan Medical, Guilford, ME) in PBS by vortexing. DNA was extracted via a modified method utilising the QIAamp mini kit extraction (Qiagen, Cat#51306). Briefly, cell lysis was achieved by Proteinase K and Buffer AL at 56°C. DNA was eluted from the column with Qiagen AE buffer at 65°C. DNA concentration was determined by NanoDrop 8000 (Thermo Fisher Scientific) and samples were normalised to 10 ng/µl with molecular grade DNAse/RNAse free H2O. Samples were held at 4°C for up to two weeks.

2.3 Library preparation

Bar-coded DNA libraries were generated using Ion AmpliSeq™ Library Kit 2.0 – 96LV (Cat. No. 4480441). See Ion AmpliSeq™ Library Preparation for exact details (Publication Part Number MAN0006735 Revision A.0). Two separate primer pools were utilised in the initial amplification reaction. The pools were combined before proceeding with the FuPa reaction. Ion Xpress™ Barcode Adapters 1–16 Kit (Cat. No. 4471250) and Barcode Adapters 17–32 Kit (Cat. No. 4474009) were utilised for sample barcoding and adaptor ligation. AmPure Agencourt XP (Cat. No. A63882, Beckman) magnetic PCR cleanup beads were utilised as indicated in the Ion AmpliSeq™ Library Preparation User Guide.

2.4 Ion torrent sequencing

Sample libraries were normalised by Ion Library Equaliser™ Kit (Cat. No. 4482298, Life Technologies), yielding a final sample library of 100 μl at 100 pM concentration. Emulsion PCR (emPCR) was performed on the Ion OneTouch™ 2 Instrument (Cat. No. 4474778) as indicated in Ion PGM™ Template OT2 200 Kit (Publication Number MAN0007220. Revision 5.0). Reagent kit Ion PGM™ Template OT2 200 Kit (Cat. No. 4480974). Briefly, normalised 100 pM sample libraries were pooled and loaded with OT2 kit reagents with ISP beads. Samples were processed for enrichment on the Ion OneTouch™ ES no later than 16 hours post emPCR run completion. Enrichment of ISPs

was achieved using Reagent kit Ion PGM™ Template OT2 200 Kit (Cat. No. 4480974) and DynaBeads MyOne streptavidin C1 beads (Cat. No. 65001, Life Technologies) according to the manufacturer's protocols (Ion PGM™ Template OT2 200 Kit User Guide).

Ion Torrent Ion 316™ Chip Kit v2 (Cat. No. 4483188, Life Technologies) were prepared and loaded according to the manufacturer's recommendation (Ion PGM™ Sequencing 200 Kit v2 (Publication Number MAN0007273 Revision 3.0). Chips were manually loaded with enriched ISPs with primed sequencing polymerase (provided in kit) using Rainin® pipette tips SR-L200F. The Ion Torrent PGM was run according to Ion Torrent 316 chip specifications, 500 flows, and use of 18 MΩ water system (multistage system including: Carbon tank, RO membrane, UV irradiation, post-filtering, deionisation, and an Elga water polisher). Standard compressed nitrogen gas supplied to the PGM system. Versions of the Ion Torrent software used are included in Supplementary Table S13.

2.5 Cyp2D6 copy number detection

To determine the presence of Cyp2D6 gene deletion or duplication, all samples were tested utilising a TaqMan® Copy Number Assay (Life Technologies, Cat. No. 4400291, Assay ID: Hs00010001_cn) on the ABI 7500, real time PCR analyser. All samples indicated as non-normal copy number, *5 deletion or XN gene duplication, were confirmed on the Infiniti MicroArray System (AGI, Autogenomics). This copy number assay specifically targets CYP2D6 exon 9 sequences and will not amplify CYP2D7 or CYP2D8 pseudogenes or CYP2D6/CYP2D7 hybrid alleles carrying CYP2D7 exon 9 sequences. The number of copies of the target sequence in each test sample is determined by relative quantitation (RQ) using the comparative C_T ($\Delta\Delta C_T$) through the onboard ABI 7500 software (see Applied Biosystems Manual: TaqMan® Copy Number Assays Protocol). TaqMan® Copy Number Reference Assay, human, RNase P (Cat. No. 4403326, Life Technologies) was used as the reference diploid gene target. TaqMan® Control Genomic DNA, human (Cat. No. 4312660) was used as the reference genomic material. TaqMan® Gene Expression Master Mix (Cat. No. 4369514) was used for all reactions.

Assay procedure follows Applied Biosystems Manual: TaqMan® Copy Number Assays Protocol with the following noted exceptions. The DNA concentration was determined by NanoDrop 8000 (Thermo Fisher Scientific) and samples were normalised to 10 ng/µl with molecular grade DNAse/RNAse free H2O. DNA input for each replicate reaction was 50 ng. A total of 35 PCR cycles was used for reaction parameters. Samples were tested in triplicate and the mean RQ value (relative quantity) for each sample is determined. Potential positive Cyp2D6 CNV sample results were determined by an RQ value <1.0 or >1.75. Each possible positive sample for Cyp2D6 CNV was confirmed by reflexing to a Biofilm chip microarray, INFINITI Cyp450 2D6i Assay (Autogenomics, CA USA).

2.6 Data analysis

A custom data analysis pipeline was created to analyse SNPs of interest listed in Supplementary Table S1. Following sequencing, the BAM file for each patient sample was extracted into the SAM format and reads less than 75 nucleotides in length were

removed. The variant caller designed by Life Technologies and included in Version 3.4.2 of the Torrent Suite was used to identify variants. Specific program versions are detailed in Supplementary Table S13. Unless indicated the default settings for the program were used. The AmpliSeq platform was used and a custom reference/ hotspot file were created. Data filtering was applied to remove samples with less than 5500 mapped reads or less than 20X coverage at all SNPs of interest. Failed samples were re-sequenced and the original data was not included in this analysis. Data from the Ion Torrent variant caller was used to genotype each sample according to the criteria found in dbSNP. A custom genotyper was developed in house using the PERL programming language. Samples were considered mutant if the alternative nucleotide exceeded 80% allelic ratio at the position of interest. Heterozygous calls were made if the alternative nucleotide exceeded 20% and was less than 80%. A wild type call was made if the alternative nucleotide allele ratio was less than 20%. Cutoffs were established during experimental design by using a series of known positive controls purchased from Coriell Biorepository and con-current genotyping using the Infiniti platform from Autogenomics.

2.7 VKORC1 genotyping

A custom AmpliSeq™ primer was used to sequence 178 nucleotides (90 nucleotides on each side of potential SNP) in the upstream region of the VKORC1 gene containing position –1639. Using the allele ratios described in the data analysis sub-section, samples were genotyped as B/B if they were homozygous for the non-alternative nucleotide. Samples were classified A/B is they were heterozygous for the alternative nucleotide and homozygous samples for the alternative nucleotide were called as an A/A genotype. Patients were classified as low sensitivity if they possessed the B/B genotype, intermediate sensitivity if they possessed the A/B genotype and high sensitivity if they had the A/A genotype.

2.8 CYP2C9, CYP2C19 and CYP2D6 genotyping

AmpliSeq™ primers were used to sequence all of the listed variations described. The average length of amplicons was approximately 180 nucleotides with the variant of interest centred in the amplicon. Variants were called using the allele ratios outlined in the data analysis sub-section. Mutations were distributed by assigning a homozygous mutation two hits and heterozygous mutation a single hit. All potential mutant and heterozygous alleles are collected and prioritised using a 'worst case' approach. That is inactive alleles take priority over reduced or normal activity alleles. Priorities of each potential variation are described in Supplementary tables. The top two highest priorities are assigned as the genotype.

2.9 Statistical analysis and preparation of figures

Pan-ethnic analyses use all available data described in the supplement. Ethnic distributions of genotypes and phenotypes are presented for sample groups with over 50 participants and do not include persons that list themselves as other or do not report an ethnicity. All reported statistical analyses were conducted using JMP Pro v 10.0.2. ChiSquare analysis was used to test for statistically significant differences within and across ethnic sub-groups. The Likelihood ChiSquare value and p-values are reported.

Alpha was set at significance 0.05. All distribution figures were prepared using a PERL script to group participants by ethnicity and genotype. Microsoft Excel 2010 was used to prepare figures.

3 Results and discussion

3.1 Vitamin K epoxide reductase 1 (VKORC1) genotype and phenotype variation

Several genetic polymorphisms in the VKORC1 gene have been associated with enzyme activity. In this study, we examined a promoter region polymorphism (VKORC1 –1639 G>A) that previously had been associated with Warfarin sensitivity. Variations within the promoter region have an effect on the activity of the enzyme (Geisen et al., 2005). Promoter variations account for much of the VKORC1 associated Warfarin variability. The overall distribution of VKORC1 genotypes was not random (p-value < 0.0001) (Figure 1). Homozygosity of the G nucleotide at position –1639 (B/B genotype) is the most frequently observed genotype in US pan-ethnic samples (Supplementary Table S2). In this study, the B/B genotype was observed 47.8% of the time. Patients with the B/B genotype have a 3-fold higher rate of VKORC1 transcription and thus often require a higher dose of Warfarin. Person's homozygote for the A nucleotide (A/A genotype) were observed with the least frequency (12.2%). The remaining patients were heterozygous at this position.

A large degree of genotype variation within and between ethnicities was discovered (Figure 2). Asian Americans had a greater likelihood of possessing the A/A haplotype (46%) and therefore as a population tend to be more highly sensitive to Warfarin (Figure 2 and Table 1). Our genetic analysis was performed on over 500 persons of self-described Asian ancestry. In this group, 46% of respondents possessed the high sensitivity haplotype and would require a lower weekly dose of Warfarin. In the same group, 21% of participants likely require higher doses of Warfarin as they possess a low sensitivity haplotype. In our study of the Asian population, there was a sub-group of 70 patients that could be identified as Vietnamese ethnicity (Table 1). The distribution of VKORC1 genotypes differed significantly as compared to the pan-Asian distribution (X^2 (2, N = 70) = 17.6019, p = 0.0002). Whereas the average Asian patient would likely benefit from a lower dosage of Warfarin, patients with Vietnamese ancestry are less sensitive and require higher doses. A recent study compares five populations of Asians living in Taiwan (Supplementary Table S3) and show a large range of variation within this population (Lee et al., 2009). Clearly, great variation exists within the Asian ethnicity with regards to the VKORC1 genotype and the predicted metaboliser status. Likely, there is variation in the individual response to Warfarin therapy. This analysis shows the need to consider pharmacogenomic information when deciding on therapeutic modalities.

Previous studies have concluded that Africans are more genetically diverse than other ethnic populations (Campbell and Tishkoff, 2008). Interestingly, in our study, African Americans had less VKORC1 diversity than other ethnic populations (Figure 2). Approximately 80% of African Americans were classified as low sensitivity to Warfarin indicating that they carried the B/B genotype (Figure 2). African Americans typically require larger doses of Warfarin as compared to their white and Asian counterparts

(Fung et al., 2012; Takahashi et al., 2006). Even though a large majority of African Americans carry the B/B genotype, there were still a large number of African Americans that would likely be more sensitive to Warfarin and if given higher doses at an increased risk of an adverse reaction.

Figure 2 The distribution of genotypes significantly differs across all ethnic groups (X^2 df 14, 2211.22, p-value < 0.0001). African-Americans are the only group in which the majority of participants are genotyped B/B. Persons who self-described themselves as Asian, were more likely to possess the mutant genotype (A/A). The distribution of genotypes significantly differs between Asian-Americans and all other ethnicities (p-value < 0.05) (see online version for colours)

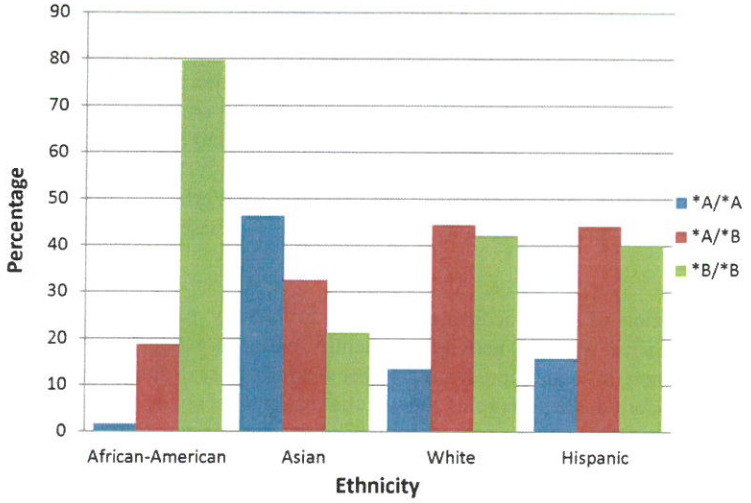


Table 1 Predicted sensitivity of Warfarin based on VKORC1 mutations

Sensitivity	*African–American* (%)	*Asian* (%)	*White* (%)	*Hispanic* (%)	*Vietnamese* (%)
High	1.6	46.3	13.4	15.8	5.7
Intermediate	18.7	32.5	44.4	44.2	27.1
Low	79.7	21.2	42.1	40.0	67.1

White and Hispanic persons were less likely to be highly sensitive to Warfarin but there was still a large range in genetic variation (Figure 2 and Table 1). On the basis of their genotype, approximately half of the patients prescribed Warfarin will not respond in a predictable manner. The use of pharmacogenomics testing can be used to determine dosages that are more likely to achieve the desired outcome while decreasing the overall risk of adverse reactions.

Treatment of arterial and venous thromboembolism and atrial fibrillation is often accomplished with Warfarin (Coumadin), a derivative of Coumarin. Warfarin, a vitamin K antagonist first introduced as a rodenticide, has been a traditional treatment for embolism and is often used in prophylaxis of thromboembolic conditions (Czogalla et al., 2013). Vitamin K is an essential co-factor necessary for the synthesis of clotting factors II, VII, IX and X (Bell and Matschiner, 1972). As the clotting factors are being

synthesised, Vitamin K undergoes reduction into vitamin K_1 2,3epoxide and rapidly reconverted back into Vitamin K. Vitamin K_1 epoxide reductase (VKORC) is responsible for the reconversion of the reduced Vitamin K into the clotting factor precursor. Warfarin inhibits clotting factor formation by inhibition of the VKORC1 subunit.

Even though treatment of thromboembolism with warfarin has been around for many years, it carries a high risk. According to the Quarterly Report from the Institute for Safe Medication Practice, Warfarin was the subject of the second highest number of safety reports. The International Normalisation Ratio (INR) is used to guide therapeutic adjustments to the weekly dose of Warfarin. Patients over treated with the medication (INR > 4.0) are at an increased risk of an adverse event owing to bleeding. Patients who find themselves under dosed (INR < 2.0) are at a continued risk of thromboembolic event. Major bleeding episodes occur in 12% of patients undergoing Warfarin therapy and approximately 2% will die from complications (Li et al., 2006). The risk of bleeding owing to Warfarin administration is 10-fold higher in the first month of exposure (Moyer et al., 2009). Wide variations in therapeutic efficacy are observed owing to inter-individual variables such as age, gender, weight, ethnicity, and diet. Wide pharmacologic variation can have profound impacts on medication activity and subsequently patient health. A study from 2006 showed the median dose of Warfarin needed to achieve a therapeutic INR differed significantly between white, Japanese, and African Americans (Takahashi
et al., 2006).

The utilisation of pharmacogenomics (PG) to identity patients that have genetic mutations that affect VKORC activity can identify potential patient variability and allow physicians to more accurately predict a patients Warfarin dose. Studies comparing the use of PG and standard dosing therapies showed that patients receiving PG based dosing had significant reductions in the percent of patients in the out of range INRs (Anderson et al., 2012). The results of a randomised trial showed that the patients treated using the genotyped dosing protocol had a greater time in therapeutic range, reached therapeutic range faster and became stable on Warfarin more quickly than patients with standard dosing (Pirmohamed et al., 2013).

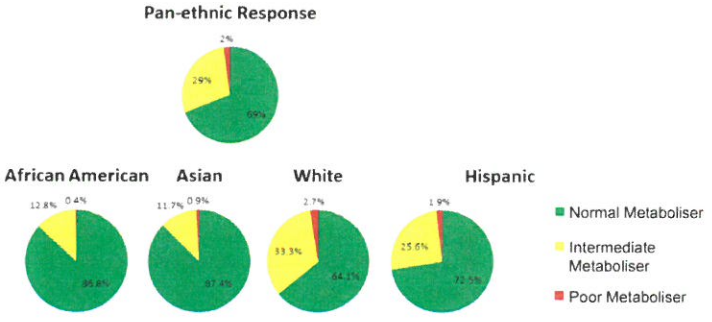
While studies clearly demonstrate that genetic variations in the VKORC1 gene affect the activity of Warfarin. Most studies comparing the utilisation of pharmacogenomics dosing protocols are limited as they often are over represented with white and European participants. African Americans have been largely unrepresented in clinical studies and initial findings have demonstrated conflicting benefits of phamacogenomic based dosing. Further studies evaluating the clinical outcomes and genetic variation within all ethnic populations are needed.

3.2 Cytochrome P450 2C9 (CYP2C9)

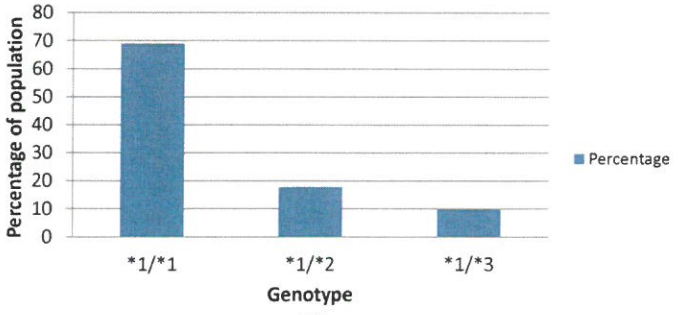
Pan-ethnic analysis showed that approximately 2/3 of patients was predicted to respond normally to medications that use the CYP2C9 enzyme (Figure 3). That is they possessed two copies of the wild type gene and were genotyped as *1/*1 (Figure 3(B)). The remaining patients likely would exhibit metabolic differences in CYP2C9 activity and will show varying degrees of reduced effect from medications that undergo CYP2C9 metabolism (Supplementary Table S4). Although only 2% of the total population

were classified as poor metabolisers, they were more likely to suffer from adverse medication reactions (Hung et al., 2004; Martinez et al., 2004). The observed effect is magnified when one considers the metaboliser status in association with the reported ethnicity. White and Hispanic ethnicities were more likely to be affected by reduced or poor CYP2C9 metabolism (Supplementary Table S5). African American and Asian patients exhibited significant differences in the genotype distribution and the predicted metabolic effects as compared to the pan-ethnic distribution (X^2 df,2 = 14.973, 15.86 p-value <0.001) (Figure 3(C)).

Figure 3 Predicted CYP2C9 metaboliser status varies with ethnicity. (A) CYP2C9 is the only gene included in this study for which a majority of participants were classified as normal metabolisers. In spite of this, there remain approximately 30% of patients that were classified with impaired metabolism (pan-ethnic). The distribution of phenotypes varies with the ethnicity of the participants. African–American and Asians have greater proportions of members classified as normal metabolisers. (B) The three most common genotypes in the pan-ethnic grouping are presented. Participants are more likely to be genotyped as *1/*1 than they are any other genotype. The *1/*1 genotype is considered the wild type sequence and is defined for this study as the absence of any of the examined variations. (C) Respondents were grouped by reported ethnicity. The distribution of genotypes significantly differs across ethnic groups (X^2 df 112, 1708.453, p-value <0.0001). The five genotypes with the highest proportion of occurrence are presented (see online version for colours)

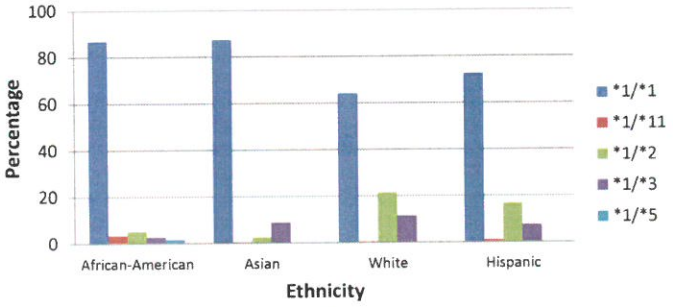


(A)



(B)

Figure 3 Predicted CYP2C9 metaboliser status varies with ethnicity. (A) CYP2C9 is the only gene included in this study for which a majority of participants were classified as normal metabolisers. In spite of this, there remain approximately 30% of patients that were classified with impaired metabolism (pan-ethnic). The distribution of phenotypes varies with the ethnicity of the participants. African–American and Asians have greater proportions of members classified as normal metabolisers. (B) The three most common genotypes in the pan-ethnic grouping are presented. Participants are more likely to be genotyped as *1/*1 than they are any other genotype. The *1/*1 genotype is considered the wild type sequence and is defined for this study as the absence of any of the examined variations. (C) Respondents were grouped by reported ethnicity. The distribution of genotypes significantly differs across ethnic groups (X^2 df 112, 1708.453, p-value <0.0001). The five genotypes with the highest proportion of occurrence are presented (see online version for colours) (continued)



(C)

CYP2C9 is a highly polymorphic gene involved in the metabolism of a number of commonly prescribed medications (Supplementary Table S6). These medications carry FDA boxed/blackbox warnings. Blackbox warnings are used to highlight potentially serious interactions that need to be assessed when evaluating the risks and benefits of medication (US Department of Health Food and Drug Administration, 2011). As previously discussed, there is a great need to consider pharmacogenomic information when prescribing medications such as Warfarin. Not only does the VKORC1 promoter polymorphism affect the metabolism and activity of Warfarin but polymorphisms in CYP2C9 also affect the activity of Warfarin. In January 2010, the FDA amended the blackbox warning for Warfarin to include dose ranges based on pharmacogenomic information for both the VKORC1 and CYP2C9 genes. However, they stopped short of requiring pharmacogenomic testing to be performed. In addition to the medications carrying blackbox warnings, a number of other prescribed and illicit drugs can be affected by the CYP2C9 enzyme (Kirchheiner and Brockmoller, 2005; Yamaori et al., 2013). Patients with impaired CYP2C9 metaboliser status need to use caution when taking any medication that is metabolised through the CYP2C9 pathway. Patients and physicians need to be aware of numerous potential drug to drug interactions as the CYP2C9 enzyme is inhibited by a number of other medications such as Fluconazole and substances such as pomegranate juice (Gschwind et al., 2013; Hanley, 2013; Zgheib et al., 2007).

Celecoxib a COX-2 inhibiter, non-steroidal anti-inflammatory drug (NSAID) is commonly used in the treatment of arthritis. Celecoxib is primarily hepatically metabolised and undergoes methyl hydroxylation by the CYP2C9 enzyme (Prieto-Perez et al., 2013). Allelic variations in the CYP2C9 gene inhibit the enzymatic activity

(CYP2C9 *2 and CYP2C9*3) and the concentration of Celecoxib remains elevated in the blood. The metaboliser status of the patient depends upon the combination of the two alleles (Supplementary Table S4). Flurbiprofen, another NSAID, also carries an FDA blackbox warning. Persons with a poor metaboliser status have lower oral clearance, increased time to reach maximal plasma concentration, and increased elimination half-life (Kumar et al., 2008). NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal (Gabriel et al., 1991). In addition an FDA warning for adverse cardiac events is given declaring NSAIDs may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal (Waxman, 2005). Polymorphic changes in the CYP2C9 gene cause a decreased metabolism of the medication and build up the drug in the system which can lead to adverse outcomes.

3.3 Cytochrome P450 2C19 (CYP2C19)

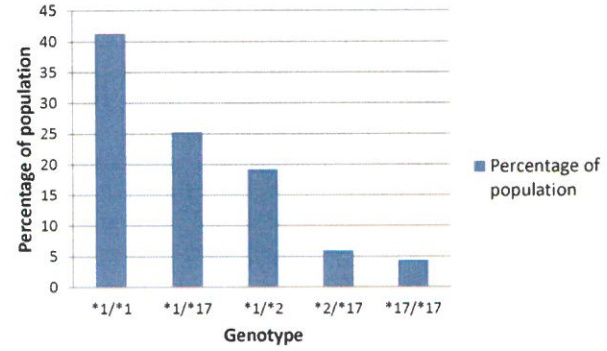
Like most other CYP450 superfamily members, CYP2C19 is extremely polymorphic. There are many known variant alleles associated with impaired medication metabolism. A large number of commonly administered medications including the platelet inhibiter Clopidogrel are metabolised by CYP2C19 (Supplementary Table S7) (Scott et al., 2013). Clopidogrel is commonly used to prevent thromboembolic events such as an acute myocardial infarction (AMI), or cerebrovascular accident (CVA). Patients with acute coronary syndromes are often administered a platelet inhibitor such as Clopidogrel to prevent subsequent coronary events (Spokoyny et al., 2013). Administered as a pro-drug, the anti-platelet agent must undergo hepatic oxidation by the CYP450 enzymes into the active metabolite (Kazui et al., 2010). Substantial inter-individual variability exists in the platelet inhibition ability of Clopidogrel. Traditionally, patients have been denoted as either being a responder or a non-responder to therapy. However, it is now known that there is a large range of responses to standard doses of Clopidogrel and the response is patient specific (Gurbel et al., 2003). It is known that the dichotomous classification mechanism is no longer clinically relevant.

CYP2C19 is the major CYP450 family member that activates the Clopidogrel pro-drug into its active metabolite. CYP2C19 is associated with several genetic polymorphisms that can either increase or abolish enzymatic function (Supplementary Table S8). Less than 50% of all patients were expected to metabolise medications affected by the CYP2C19 enzyme normally (Supplementary Table S9 and Figure 4(A)). There were two variants common in the population that can affect substrate metabolism. The CYP2C19 *2 allele was the most common loss of function variant that negatively affects enzymatic activity. The *2 variant has no enzymatic activity and was present in one copy in 24% of the US population (Figure 4(A)) (Thorn et al., 2013). Approximately, 1/4 of US residents (26%) carried a mutation (*17) that increased the enzymatic activity of the CYP2C19 gene. It is clear that the range of activity of the CYP2C19 enzyme is can vary greatly from patient to patient. As enzymatic activity varies so does medication response.

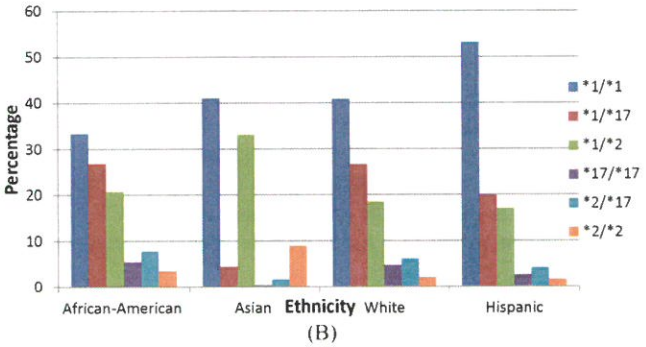
As seen in the other CYP450 superfamily members, the CYP2C19 activity and genotypes varied significantly across ethnic populations (X^2 df 189, 1551.66, p-value <0.0001) (Supplementary Table S9, Figure 4(B) and (C)). African Americans were only 33% likely to carry two wild type copies of the CYP2C19 allele. As such, there is a

greater chance that an African American would not respond "normally" to a medication. That is they are less likely to have the same response that a white recipient would and are more likely to suffer an ADR. Approximately, a third of African American patients being treated with a CYP2C19 substrate would metabolise Clopidogrel quickly and are likely to suffer from increased platelet inhibition giving them a higher risk of uncontrolled bleeding. Another third of patients are likely to inadequately metabolise the medication and not receive the therapeutic effect. The CYP2C19 *2 variation is associated with an increased risk for major cardiovascular events including thrombosis of stents and recurrent myocardial infarctions (Scott et al., 2013). This analysis highlights the need to assess genotype status before beginning therapies, as the therapeutic approach could be modified to more accurately match a patient's genotype.

Figure 4 CYP2C19 genotypes vary within the population and between ethnic groups. (A) 40% of the samples sequenced did not possess a variation in the CYP2C19 (*1/*1). Approximately 35% of participants possessed a gain of function mutation (*17). Approximately 4% of the population are genotyped *17/*17. (B) The genetic distribution significantly differs across ethnicities (X^2 df 189, 1551.66, p-value < 0.0001). African-Americans are less likely than any other ethnic group to be classified as a *1/*1 genotype. Asians have the largest incidence of *1/*2 genotypes. (C) The distributions of metaboliser status vary from one ethnic group to another. For all ethnic groups, with the exception of Hispanics, a normal metaboliser status is not a majority. Each ethnic group has a large dynamic range of predicted enzymatic activity (see online version for colours)

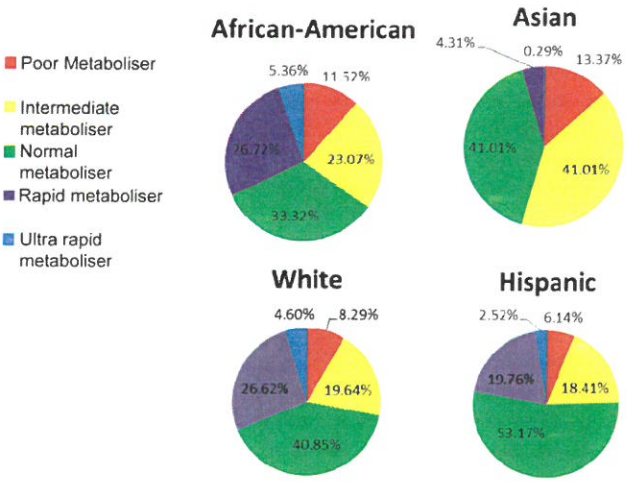


(A)



(B)

Figure 4 CYP2C19 genotypes vary within the population and between ethnic groups. (A) 40% of the samples sequenced did not possess a variation in the CYP2C19 (*1/*1). Approximately 35% of participants possessed a gain of function mutation (*17). Approximately 4% of the population are genotyped *17/*17. (B) The genetic distribution significantly differs across ethnicities (X^2 df 189, 1551.66, p-value < 0.0001). African-Americans are less likely than any other ethnic group to be classified as a *1/*1 genotype. Asians have the largest incidence of *1/*2 genotypes. (C) The distributions of metaboliser status vary from one ethnic group to another. For all ethnic groups, with the exception of Hispanics, a normal metaboliser status is not a majority. Each ethnic group has a large dynamic range of predicted enzymatic activity (see online version for colours) (continued)



(C)

As discussed above for African American patients, similar considerations exist for all ethnicities but the proportions vary. Nearly 55% of Asians were poor or intermediate metabolisers and physicians should consider alternative methods of anti-coagulation if not contraindicated (Scott et al., 2013). The risk for over inhibition of platelets was not as likely in the Asian population as less than 5% of the ethnic group carry the *17 variant (Figure 4(B)). Hispanics and whites were more likely to be some form of a rapid metaboliser and carry one or more *17 variants and therefore were more likely to suffer an adverse bleeding reaction owing to over anti-coagulation.

As discussed previously, there are a large number of other medications that are metabolised by CYP2C19 presented in Supplementary Table S7. Each of these medications needs to be considered carefully in any patient that has a CYP2C19 variant. Another class of medications commonly administered is proton pump inhibitors (PPI). PPIs are used to treat gastroesophageal reflux disease (GERD) and prevent complications from peptic ulcer disease. Treating GERD with PPIs cost the US over $9.3 billion in 2008 (Sandler et al., 2002). Several PPIs (Omeprazole, Esomeprazole, and Lansoprazole) have the ability to inhibit the CYP2C19 enzyme and interfere with the action of Clopidogrel (Supplementary Table S7). In 2008, the American College of Cardiology recommended the use of a PPI in patients undergoing dual anti-platelet therapy with

Clopidogrel and Aspirin (El-Halabi et al., 2013). Omeprazole inhibits approximately 85% of the active metabolite formation through the CYP2C19 enzyme (Ohbuchi et al., 2012). As previously discussed, the range of Clopidogrel response is highly variable within individuals owing to CYP2C19 polymorphism. Any patient that has a genotype that causes CYP2C19 loss of function needs to use extreme caution when taking a combination of Clopidogrel and a PPI. These results again highlight the need to consider PG indicators when choosing appropriate therapeutic modalities.

3.4 Cytochrome P4502D6 (CYP2D6)

Despite having a relatively low hepatic abundance, the CYP2D6 enzyme has a large effect on drug metabolism. It is extremely polymorphic with a large number of allelic and copy number variations affecting its activity (Ingelman-Sundberg, 2005). There have been discovered over 70 variants, some of which increase the activity of the enzyme but most allelic variations result in loss of function. Variants examined in this study are shown in Supplementary Table S10. The CYP2D6 enzyme is of great medical importance, as approximately 25% of all prescribed medications are likely metabolised by this enzyme (Supplementary Table S11). Patients with multiple gene copies of CYP2D6 will metabolise medications at a faster rate (Dorado et al., 2005). Thus, they are likely to not achieve a therapeutic medication level at typical dosages. Patients with one or more inactive alleles are likely not to metabolise substrates at the appropriate rate and are more likely to suffer an ADR (Ingelman-Sundberg, 2005).

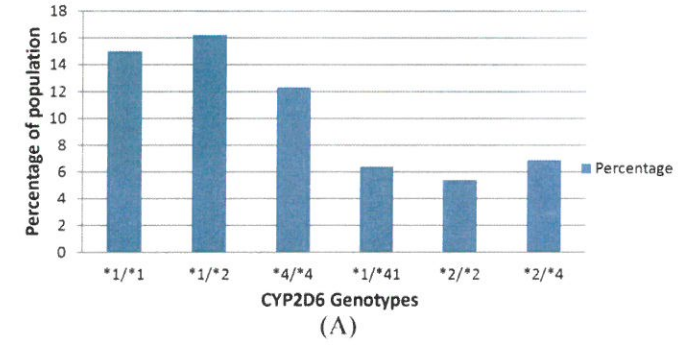
Pan-ethnic distribution of the CYP2D6 genotypes showed a wide variation in the sequence of the genes within the US population (Figure 5(A) and Supplementary Table S12). The allelic variations in the CYP2D6 gene can lead to a variety of metabolic phenotypes. Patients that possess two copies of the wild type gene, two copies of the *2 variant or a combination thereof are likely to metabolise medications at the expected rate. As a whole, approximately 35% of the population would function as normal/ extensive metabolisers, meaning they are CYP2D6 *1/*1, *1/*2, or *2/*2 (Figure 5(C)). All other study participants carried some form of an alternative variation. Three alleles of the CYP2D6 gene were more common than the others; they were the *1 (wild type), the *2 variant that functions equivalently to the wild type and the *4 inactive variant (Figure 5(B) and Supplementary Table S12). Even though these variants are the most common, there remain a large number of potential combinations of alleles. The distribution of CYP2D6 genotypes varied significantly across ethnic populations (X^2 df 609, 8087.88, p-value <0.0001). Clearly, there exists great heterogeneity within and between study groups.

In addition to SNPs that affect the enzymatic activity, the CYP2D6 gene is subject to copy number variation. Loss of one copy (*5 variant) of the gene was seen in approximately 3% of the entire population (Figure 6). As discussed previously, gene loss causes patients to behave as a poor metaboliser thus they are more likely to suffer from ADRs. Even though deletions of CYP2D6 are seen in all ethnic groups, it is more common in Asians. The CYP2D6 is also subject to duplication, extra copies of functional alleles can lead to rapid metabolism of CYP2D6 substrates. Persons of Hispanic descent have the highest incidence of gene duplication. It is clear that there exists a large

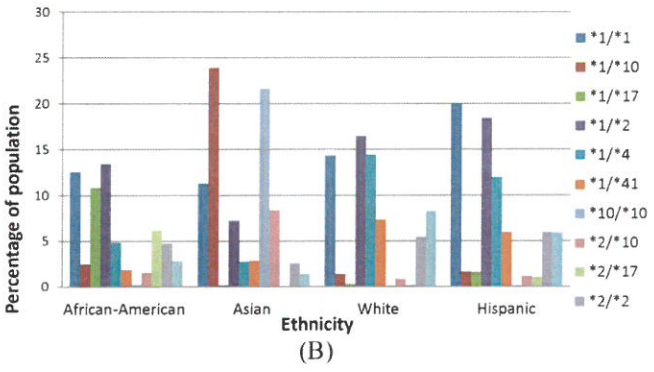
variation of both nucleotide and structural changes of the CYP2D6 gene across ethnic groups in the USA. Physicians should be aware of the frequency of variants as it is likely to have an effect on the metabolism of various substrates.

The *10 variation results in an enzyme with reduced activity, persons that carry one active allele and one partially active allele are classified as intermediate metabolisers. Our study showed that while the *10 variation is rare in most ethnic groups, Asian descendants were a notable exception. 30% of Asians are either *1/*10 or *2/*10 and classified as intermediate metabolisers (Figure 5(C) and Figure 7). A large number of study participants were homozygous for the *10 variation and therefore were classified as poor metabolisers. As illustrated in Figure 5(C), Asian Americans were more likely than any other group to be classified as a poor metaboliser.

Figure 5 CYP2D6 Genotypes vary within the population and vary between ethnic groups. (A) Genotypes exceeding 5% in proportion are displayed. The distribution of allele variants is displayed without regard to ethnicity. (B) The genetic distribution differs across all ethnicities (X^2 df 609, 8087.88, p-value < 0.0001). Pairwise comparisons showed, unless otherwise indicated, all relationships were statistically different from one another ($p < 0.0001$). (B) The distributions of metaboliser status vary from one ethnic group to another. Notably, Asian–Americans show a larger number of poor metabolisers (~33%) than any other ethnic group represented in this study, while Hispanics have a larger number of extensive metabolisers (see online version for colours)

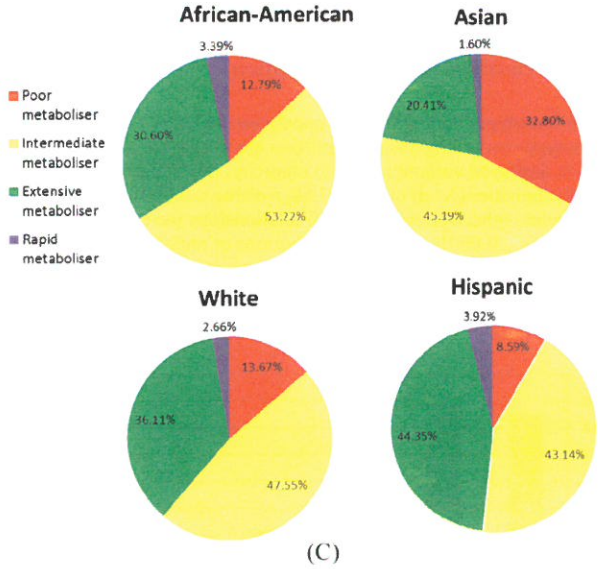


(A)



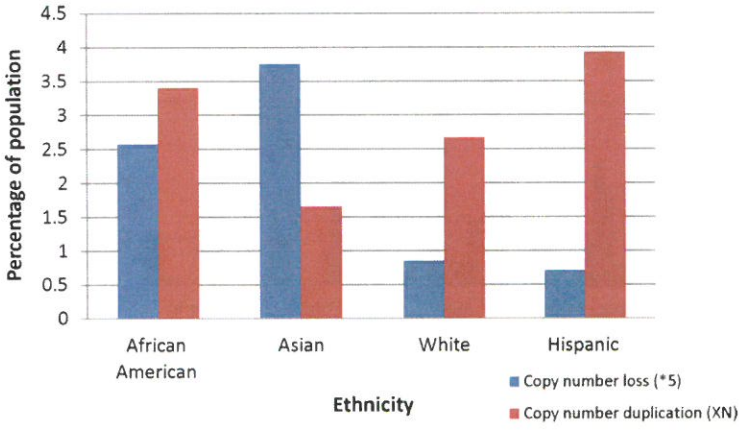
(B)

Figure 5 CYP2D6 Genotypes vary within the population and vary between ethnic groups. (A) Genotypes exceeding 5% in proportion are displayed. The distribution of allele variants is displayed without regard to ethnicity. (B) The genetic distribution differs across all ethnicities (X^2 df 609, 8087.88, p-value < 0.0001). Pairwise comparisons showed, unless otherwise indicated, all relationships were statistically different from one another ($p < 0.0001$). (B) The distributions of metaboliser status vary from one ethnic group to another. Notably, Asian–Americans show a larger number of poor metabolisers (~33%) than any other ethnic group represented in this study, while Hispanics have a larger number of extensive metabolisers (see online version for colours) (continued)



(C)

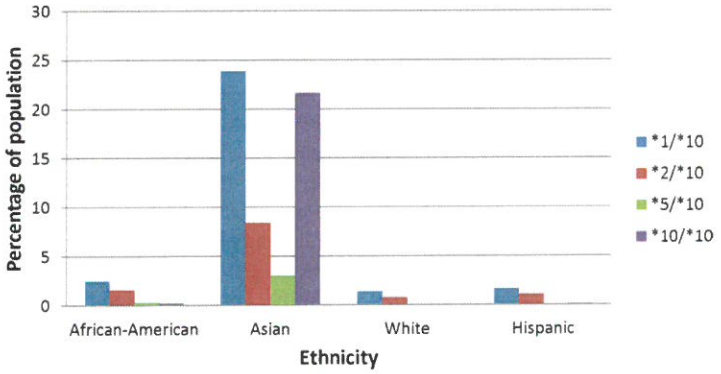
Figure 6 CYP2D6 copy number variation within ethnic groups. Copy number variation of the CYP2D6 gene can affect metabolic rates of drug metabolism. Approximately 5% of respondents had a CYP2D6 copy number variation, either a duplication (XN) or deletion (*5). The percentage of each event was identified and is displayed by ethnic group (see online version for colours)



Many different medications are under the control of the CYP2D6 enzyme. A large number of anti-depressants are metabolised by the CYP2D6 enzyme and carries FDA black box warnings for possible genetic interactions (Supplementary Table S11). Anti-depressant efficacy and tolerance vary widely among persons with clinical depression (Perlis, 2014). Physicians should be aware that there is a strong likelihood of variable effects that can be caused by the genetic polymorphisms discussed. Specifically, the FDA warns physicians to consider genetic status by inclusion of the black box warnings on a large number of anti-depressants and recommends the use of genetic testing.

Many patients undergoing treatment for clinical depression are prescribed selective serotonin reuptake inhibitors (SSRIs), so called second generation antidepressants. The majority of second generation antidepressants undergo biotransformation/metabolism by the CYP2D6 enzyme (Spina and de Leon, 2014). A recent study evaluating the effectiveness of PG guided therapies in an outpatient setting found that guided dosing of psychoactive medications resulted in a greater reduction of symptoms as compared to the group of patients that underwent non-guided intervention (Hall-Flavin et al., 2013). It was observed that the physicians changed medications more frequently for the patients in the guided group than they did for the patients being treated in an unguided manner. Physicians treating patients in the guided group were able to adjust their medications to fit their patient's metaboliser status. Patients with major depressive disorders often need hospitalisations for long periods of time to control their symptoms. Patients classified as poor CYP2D6 metabolisers experience longer hospital admissions for major depressive disorder because the incidence of medication related side effects is greater and there is often reduced anti-depressant efficacy (Ruano et al., 2013).

Figure 7 The *10 variation of the CYP2D6 gene gives rise to a partially active allele. Asian–Americans have an increased likelihood of possessing a *10 variant as compared to the other ethnic groups represented in this study. All other ethnic groups had a low frequency of the *10 variant (see online version for colours)



It is predicted that over 40% of all variance in pharmacologic treatment with an anti-depressant is associated with common genetic polymorphisms. Interestingly, only 40% of patients being treated with an antidepressant will experience complete resolution of their depressive symptoms (Hall-Flavin et al., 2013). Many of the patients that are unsuccessfully treated will remain depressed and are unlikely to be successfully treated even if the medication is changed. Identifying potential patients that are likely to not

experience remission of their symptoms with traditional pharmacological interventions is a high priority for physicians.

Beta adrenergic blockers are commonly administered to patients suffering from cardiovascular disease and hypertension. Several beta blockers carry FDA black box warnings related to the CYP2D6 gene (Supplementary Table S11). Particularly, patients with loss of function alleles that are classified as intermediate or poor metabolisers are at higher risk for development of an ADR (Rau et al., 2002). Beta blockers cause the heart rate and force of contraction to decrease as the plasma concentration of the beta blocker increases. Patients that are poor CYP2D6 metabolisers have higher 3–10 times the plasma concentrations of the beta blocker and thus are at an increased risk of side effects (Kertai et al., 2013). Patients classified as poor metabolisers were observed to have significantly lower heart rates and more likely to be hypotensive.

This study was an observational, retrospective analysis describing the ethnic distribution of clinically relevant SNPs of persons living in the USA. All participants in this study submitted samples to the lab for genetic testing and the reasons for sample submission are not known. It is reasonable to hypothesise that study participants were likely prescribed one or medications that interact with a member of the CYP450 superfamily. As the study was not comprised of a random sampling of the population, there exists a potential sampling bias in this study. To prevent analytical bias, researchers were blinded to participant's names, ages and ethnicities.

4 Conclusion

Physicians prescribe medications to patients with the expectation that a given pharmaceutic will respond as expected. Experience has shown that response to medications is highly variable and therapeutic failures are not uncommon. Until recently, physicians did not have the ability to predict a patient's response to a particular medication. The advent of pharmacogenomics has now made it possible to detect and predict an individual's genetic impact on drug response. The CYP450 superfamily is responsible for the metabolism of many prescribed and illicit drugs. Identification of specific phenotypes is crucial as up to 85% of all prescribed medications will undergo metabolism by a member of the CYP450 superfamily. Most persons will be exposed to a medication with a known genetic interaction during their lifetime and many patients are commonly prescribed medications that compete with these metabolic enzymes. The impact of pharmacogenomic evaluation has the potential to help millions of people by increasing the incidence of therapeutic success and reducing the incidence of ADRs.

Our study examined the distribution of genetic polymorphisms and the predicted effect for several key members of the CYP2C family. This study used NGS with a mean coverage at all SNPs of interest of 1144.25 (median = 899). This depth of coverage increases the confidence of the findings. While other studies have shown that the genes of the CYP450 family are polymorphic, most suffer from limited sample sizes or were composed of limited ethnic groups. This study examined over 30,000 subjects from various ethnic groups living in the USA using NGS technology. To our knowledge, this is the largest study of its kind in the USA. Our work revealed that genetic variation of key medication metabolising enzymes is common within and between ethnic populations.

This calls into question the concept of a normal patient and showed that the range of genotypes is highly variable. Results from this study demonstrate that physicians have the ability to personalise therapeutic strategies by identifying genetic metabolotypes increasing the efficacy of prescribed medications while minimising adverse reactions.

Acknowledgements

The authors acknowledge the efforts of all employees at American International Biotechnology.

References

Adeyemo, A. and Rotimi, C. (2010) 'Genetic variants associated with complex human diseases show wide variation across multiple populations', *Public Health Genomics*, Vol. 13, No. 2, pp.72–79.

Anderson, J.L., Horne, B.D., Stevens, S.M., Woller, S.C., Samuelson, K.M., Mansfield, J.W., Robinson, M., Barton, S., Brunisholz, K., Mower, C.P., Huntinghouse, J.A., Rollo, J.S., Siler, D., Bair, T.L., Knight, S., Muhlestein, J.B. and Carlquist, J.F. (2012) 'A randomized and clinical effectiveness trial comparing two pharmacogenetic algorithms and standard care for individualizing warfarin dosing (CoumaGen-II)', *Circulation*, Vol. 125, No. 16, pp.1997–2005.

Bell, R.G. and Matschiner, J.T. (1972) 'Warfarin and the inhibition of vitamin K activity by an oxide metabolite', *Nature*, Vol. 237, No. 5349, pp.32–33.

Campbell, M.C. and Tishkoff, S.A. (2008) 'African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping', *Annual Review of Genomics and Human Genetics*, Vol. 9, pp.403–433.

Czogalla, K.J., Biswas, A., Wendeln, A.C., Westhofen, P., Muller, C.R., Watzka, M. and Oldenburg, J. (2013) 'Human VKORC1 mutations cause variable degrees of 4-hydroxycoumarin resistance and affect putative warfarin binding interfaces', *Blood*, Vol. 122, No. 15, pp.2743–2750.

Dorado, P., Caceres, M., Pozo-Guisado, E., Wong, M.L., Licinio, J. and Llerena, A. (2005) 'Development of a PCR-based strategy for CYP2D6 genotyping including gene multiplication of worldwide potential use', *BioTechniques*, Vol. 39, No. 10 Suppl., pp.S571–4.

El-Halabi, M.M., Zgheib, N., Mansour, N.M., Malli, A., Ghaith, O.A., Mahfouz, R., Alam, S. and Sharara, A.I. (2013) 'CYP2C19 genetic polymorphism, rabeprazole and esomeprazole have no effect on the antiplatelet action of clopidogrel', *Journal of Cardiovascular Pharmacology*, Vol. 62, No. 1, pp.41–49.

Fung, E., Patsopoulos, N.A., Belknap, S.M., O'Rourke, D.J., Robb, J.F., Anderson, J.L., Shworak, N.W. and Moore, J.H. (2012) 'Effect of genetic variants, especially CYP2C9 and VKORC1, on the pharmacology of warfarin', *Seminars in Thrombosis and Hemostasis*, Vol. 38, No. 8, pp.893–904.

Gabriel, S.E., Jaakkimainen, L. and Bombardier, C. (1991) 'Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis', *Annals of Internal Medicine*, Vol. 115, No. 10, pp.787–796.

Geisen, C., Watzka, M., Sittinger, K., Steffens, M., Daugela, L., Seifried, E., Muller, C.R., Wienker, T.F. and Oldenburg, J. (2005) 'VKORC1 haplotypes and their impact on the inter-individual and inter-ethnical variability of oral anticoagulation', *Thrombosis and Haemostasis*, Vol. 94, No. 4, pp.773–779.

Gschwind, L., Rollason, V., Boehlen, F., Rebsamen, M., Combescure, C., Grunenwald, M., Matthey, A., Bonnabry, P., Dayer, P. and Desmeules, J.A. (2013) 'Impact of CYP2C9 polymorphisms on the vulnerability to pharmacokinetic drug-drug interactions during acenocoumarol treatment', *Pharmacogenomics*, Vol. 14, No. 7, pp.745–753.

Gurbel, P.A., Bliden, K.P., Hiatt, B.L. and O'Connor, C.M. (2003) 'Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity', *Circulation*, Vol. 107, No. 23, pp.2908–2913.

Hall-Flavin, D.K., Winner, J.G., Allen, J.D., Carhart, J.M., Proctor, B., Snyder, K.A., Drews, M.S., Eisterhold, L.L., Geske, J. and Mrazek, D.A. (2013) 'Utility of integrated pharmacogenomic testing to support the treatment of major depressive disorder in a psychiatric outpatient setting', *Pharmacogenetics and Genomics*, Vol. 23, No. 10, pp.535–548.

Hanley, M. (2013) *Inhibition of Cytochrome P450 Enzymes by Fruit Juices: In vitro Effects, Clinical Relevance, and Therapeutic Implications*, Sackler School of Graduate Biomedical Sciences, Tufts University, http://pqdtopen.proquest.com/doc/1269176048.html?FMT=ABS

Hung, C.C., Lin, C.J., Chen, C.C., Chang, C.J. and Liou, H.H. (2004) 'Dosage recommendation of phenytoin for patients with epilepsy with different CYP2C9/CYP2C19 polymorphisms', *Therapeutic Drug Monitoring*, Vol. 26, No. 5, pp.534–540.

Ingelman-Sundberg, M. (2005) 'Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity', *The Pharmacogenomics Journal*, Vol. 5, No. 1, pp.6–13.

Kazui, M., Nishiya, Y., Ishizuka, T., Hagihara, K., Farid, N.A., Okazaki, O., Ikeda, T. and Kurihara, A. (2010) 'Identification of the human cytochrome P450 enzymes involved in the two oxidative steps in the bioactivation of clopidogrel to its pharmacologically active metabolite', *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, Vol. 38, No. 1, pp.92–99.

Kertai, M.D., Esper, S.A., Akushevich, I., Voora, D., Ginsburg, G.S., Stafford-Smith, M., Grichnik, K., Newman, M.F., Fontes, M.L., Smith, P., Podgoreanu, M.V., Mathew, J.P. and Cardiothoracic Anesthesia Research Endeavors (CARE) Group (2013) 'Preoperative CYP2D6 metabolism-dependent beta-blocker use and mortality after coronary artery bypass grafting surgery', *The Journal of Thoracic and Cardiovascular Surgery*, Vol. 147, No. 4, pp.1368–1375.

Kirchheiner, J. and Brockmoller, J. (2005) 'Clinical consequences of cytochrome P450 2C9 polymorphisms', *Clinical Pharmacology and Therapeutics*, Vol. 77, No. 1, pp.1–16.

Kumar, V., Brundage, R.C., Oetting, W.S., Leppik, I.E. and Tracy, T.S. (2008) 'Differential genotype dependent inhibition of CYP2C9 in humans', *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, Vol. 36, No. 7, pp.1242–1248.

Lee, M.T., Chen, C.H., Chuang, H.P., Lu, L.S., Chou, C.H., Chen, Y.T., Liu, C.Y., Wen, M.S., Lu, J.J., Chang, C.F., Wu, J.Y. and Chen, Y.T. (2009) 'VKORC1 haplotypes in five East-Asian populations and Indians', *Pharmacogenomics*, Vol. 10, No. 10, pp.1609–1616.

Li, J., Zhang, L., Zhou, H., Stoneking, M. and Tang, K. (2011) 'Global patterns of genetic diversity and signals of natural selection for human ADME genes', *Human Molecular Genetics*, Vol. 20, No. 3, pp.528–540.

Li, T., Lange, L.A., Li, X., Susswein, L., Bryant, B., Malone, R., Lange, E.M., Huang, T.Y., Stafford, D.W. and Evans, J.P (2006) 'Polymorphisms in the VKORC1 gene are strongly associated with warfarin dosage requirements in patients receiving anticoagulation', *Journal of Medical Genetics*, Vol. 43, No. 9, pp.740–744.

Martinez, C., Blanco, G., Ladero, J.M., Garcia-Martin, E., Taxonera, C., Gamito, F.G., Diaz-Rubio, M. and Agundez, J.A. (2004) 'Genetic predisposition to acute gastrointestinal bleeding after NSAIDs use', *British Journal of Pharmacology*, Vol. 141, No. 2, pp.205–208.

Meyer, U.A., Zanger, U.M. and Schwab, M. (2013) 'Omics and drug response', *Annual Review of Pharmacology and Toxicology*, Vol. 53, pp.475–502.

Moses, C., Celi, L.A. and Marshall, J. (2013) 'Pharmacovigilance: an active surveillance system to proactively identify risks for adverse events', *Population Health Management*, Vol. 16, No. 3, pp.147–149.

Moyer, T.P., O'Kane, D.J., Baudhuin, L.M., Wiley, C.L., Fortini, A., Fisher, P.K., Dupras, D.M., Chaudhry, R., Thapa, P., Zinsmeister, A.R. and Heit, J.A. (2009) 'Warfarin sensitivity genotyping: a review of the literature and summary of patient experience', *Mayo Clinic Proceedings*, Vol. 84, No. 12, pp.1079–1094.

Ohbuchi, M., Noguchi, K., Kawamura, A. and Usui, T. (2012) 'Different effects of proton pump inhibitors and famotidine on the clopidogrel metabolic activation by recombinant CYP2B6, CYP2C19 and CYP3A4', *Xenobiotica: The Fate of Foreign Compounds in Biological Systems*, Vol. 42, No. 7, pp.633–640.

Perlis, R.H. (2014) 'Pharmacogenomic testing and personalized treatment of depression', *Clinical Chemistry*, Vol. 60, No. 1, pp.53–59.

Pirmohamed, M., Burnside, G., Eriksson, N., Jorgensen, A.L., Toh, C.H., Nicholson, T., Kesteven, P., Christersson, C., Wahlstrom, B., Stafberg, C., Zhang, J.E., Leathart, J.B., Kohnke, H., Maitland-van der Zee, A.H., Williamson, P.R., Daly, A.K., Avery, P., Kamali, F., Wadelius, M. and EU-PACT Group (2013) 'A randomized trial of genotype-guided dosing of warfarin', *The New England Journal of Medicine*, Vol. 369, No. 24, pp.2294–2303.

Prieto-Perez, R., Ochoa, D., Cabaleiro, T., Roman, M., Sanchez-Rojas, S.D., Talegon, M. and Abad-Santos, F. (2013) 'Evaluation of the relationship between polymorphisms in CYP2C8 and CYP2C9 and the pharmacokinetics of celecoxib', *Journal of Clinical Pharmacology*, Vol. 53, No. 12, pp.1261–1267.

Rau, T., Heide, R., Bergmann, K., Wuttke, H., Werner, U., Feifel, N. and Eschenhagen, T. (2002) 'Effect of the CYP2D6 genotype on metoprolol metabolism persists during long-term treatment', *Pharmacogenetics*, Vol. 12, No. 6, pp.465–472.

Ruano, G., Szarek, B.L., Villagra, D., Gorowski, K., Kocherla, M., Seip, R.L., Goethe, J.W. and Schwartz, H.I. (2013) 'Length of psychiatric hospitalization is correlated with CYP2D6 functional status in inpatients with major depressive disorder', *Biomarkers in Medicine*, Vol. 7, No. 3, pp.429–439.

Sandler, R.S., Everhart, J.E., Donowitz, M., Adams, E., Cronin, K., Goodman, C., Gemmen, E., Shah, S., Avdic, A. and Rubin, R. (2002) 'The burden of selected digestive diseases in the United States', *Gastroenterology*, Vol. 122, No. 5, pp.1500–1511.

Schildcrout, J.S., Denny, J.C., Bowton, E., Gregg, W., Pulley, J.M., Basford, M.A., Cowan, J.D., Xu, H., Ramirez, A.H., Crawford, D.C., Ritchie, M.D., Peterson, J.F., Masys, D.R., Wilke, R.A. and Roden, D.M. (2012) 'Optimizing drug outcomes through pharmacogenetics: a case for preemptive genotyping', *Clinical Pharmacology and Therapeutics*, Vol. 92, No. 2, pp.235–242.

Scott, S.A., Sangkuhl, K., Stein, C.M., Hulot, J.S., Mega, J.L., Roden, D.M., Klein, T.E., Sabatine, M.S., Johnson, J.A., Shuldiner, A.R. and Clinical Pharmacogenetics Implementation Consortium (2013) 'Clinical pharmacogenetics implementation consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update', *Clinical Pharmacology and Therapeutics*, Vol. 94, No. 3, pp.317–323.

Spina, E. and de Leon, J. (2014) 'Clinically relevant interactions between newer antidepressants and second-generation antipsychotics', *Expert Opinion on Drug Metabolism & Toxicology*, Vol. 10, No. 5, pp.721–726.

Spokoyny, I., Barazangi, N., Jaramillo, V., Rose, J., Chen, C., Wong, C. and Tong, D. (2013) 'Reduced clopidogrel metabolism in a multiethnic population: prevalence and rates of recurrent cerebrovascular events', *Journal of Stroke and Cerebrovascular Diseases: The Official Journal of National Stroke Association*, Vol. 23, No. 4, pp.694–698.

Takahashi, H., Wilkinson, G.R., Nutescu, E.A., Morita, T., Ritchie, M.D., Scordo, M.G., Pengo, V., Barban, M., Padrini, R., Ieiri, I., Otsubo, K., Kashima, T., Kimura, S., Kijima, S. and Echizen, H. (2006) 'Different contributions of polymorphisms in VKORC1 and CYP2C9 to intra- and inter-population differences in maintenance dose of warfarin in Japanese, Caucasians and African-Americans', *Pharmacogenetics and Genomics*, Vol. 16, No. 2, pp.101–110.

Thorn, C.F., Klein, T.E. and Altman, R.B. (2013) 'PharmGKB: the pharmacogenomics knowledge base', *Methods in molecular biology (Clifton, N.J.)*, Vol. 1015, pp.311–320.

US Department of Health Food and Drug Administration (2011) *Guidance for Industry
Warnings and Precautions, Contraindications, and Boxed Warning Sections of Labeling for Human Prescription Drug and Biological Products-Content and Format*, Available: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm075096.pdf (Accessed 20 November, 2013).

Waxman, H.A. (2005) 'The lessons of Vioxx – drug safety and sales', *The New England Journal of Medicine*, Vol. 352, No. 25, pp.2576–2578.

Xiong, M., Zhao, Z., Arnold, J. and Yu, F. (2010) 'Next-generation sequencing', *Journal of Biomedicine & Biotechnology*, Vol. 2010, p.370710.

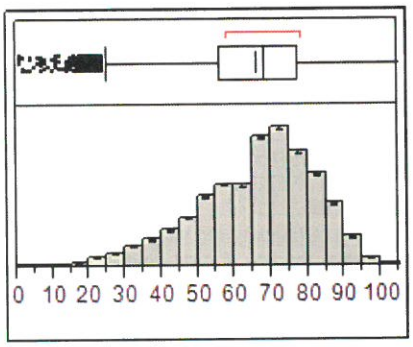
Yamaori, S., Kushihara, M., Koeda, K., Yamamoto, I. and Watanabe, K. (2013) 'Significance of CYP2C9 genetic polymorphism in inhibitory effect of Î'9-tetrahydrocannabinol on CYP2C9 activity', *Forensic Toxicology*, Vol. 31, No. 1, pp.70–75.

Yiannakopoulou, E. (2013) 'Pharmacogenomics of acetylsalicylic acid and other nonsteroidal anti-inflammatory agents: clinical implications', *European Journal of Clinical Pharmacology*, Vol. 69, No. 7, pp.1369–1373.

Zgheib, N.K., Frye, R.F., Tracy, T.S., Romkes, M. and Branch, R.A. (2007) 'Evaluation of flurbiprofen urinary ratios as in vivo indices for CYP2C9 activity', *British Journal of Clinical Pharmacology*, Vol. 63, No. 4, pp.477–487.

Supplementary figure

Figure S1 Distribution of participant ages. In addition to reported ethnicity, participants in this study were asked to provide their age. The distribution of patient's ages that participated in pharmacogenomic testing was asymmetrically distributed and skewed towards the negative. This demonstrated that average age of the patients in this study tended to be older (mean = 65.8, SD = 16.01) (see online version for colours)



Supplementary tables

Table S1 Nucleotide polymorphisms included in this study

dbSNP ID	*Gene*	*WT*	*Mut*
rs12248560	CYP2C19*17	C	T
rs4244285	CYP2C19*2	C	T
rs4244285	CYP2C19*2	G	A
rs4986893	CYP2C19*3	G	A
rs28399504	CYP2C19*4	A	G
rs72558186	CYP2C19*7	T	A
rs17884712	CYP2C19*9	G	A
rs41291556	CYP2C19*8	T	C
rs72552267	CYP2C19*6	G	A
rs1799853	CYP2C9*2	C	T
rs28371686	CYP2C9*5	C	G
rs1057910	CYP2C9*3	A	C
rs28371685	CYP2C9*11	C	T
rs56165452	CYP2C9*4	T	C
rs9332131	CYP2C9*6	A	–
rs1065852	CYP2D6*10	C	T
rs5030862	CYP2D6*12	G	A
rs28371706	CYP2D6*17	C	T
rs16947	CYP2D6*2	C	T
rs5030867	CYP2D6*7	A	C
rs28371725	CYP2D6*41	G	A
rs61736512	CYP2D6*29	G	A
rs5030655	CYP2D6*6	T	–
rs5030865	CYP2D6*8	G	T
rs5030865	CYP2D6*14	G	A
rs35742686	CYP2D6*3	A	–
rs5030656	CYP2D6*9	AAG	–
rs3892097	CYP2D6*4	G	A
rs9923231	VKORC1	G	A

Table S2 VKORC1 genotypes

VKORC1 Genotype	*African–American*	*Asian*	*White*	*Hispanic*	*Native American*	*Other*	*Pacific Islander*	*Unreported*	*Total*
*A/*A	50	251	2160	477	2	126	3	1118	4187
*A/*B	577	176	7144	1336	7	334	13	4094	13,681
*B/*B	2457	115	6767	1210	9	349	8	5468	16,383
Total	3084	542	16,071	3023	18	809	24	10,680	34,251

Table S3 Distribution of VKORC1 genotypes among Asian ethnic sub-groups

Ethnicity	*Number*	*VKORC1 A/A* (%)	*VKORC1 A/B* (%)	*VKORC1 B/B* (%)
Indian	46	0	30	70
Taiwanese	75	82	16	2
Indonesian	51	62	38	0
Phillipino	49	70	28	2
Thai	51	61	37	2
Han Chinese	235	80	17	3
Vietnamese	49	76	22	2

Source: Lee et al. (2009)

Table S4 Nucleotide polymorphisms of CYP2C9

CYP2C9 Allele	*Nucleotide change*	*Metaboliser status*	*Priority*
*1	Wild Type (None)	Normal metaboliser	7
*2	430C->T	Reduced activity	6
*3	1075 A->C	Minimal activity	5
*4	1076 T->C	Reduced activity	4
*5	1080 C->G	Reduced activity	3
*6	818 delA	Null	2
*11	1003 C->T	Reduced activity	1

Table S5 CYP2C9 genotypes

CYP2C9 genotype	*African-American*	*Asian*	*White*	*Hispanic*	*Native American*	*Other*	*Pacific Islander*	*Unreported*
*1/*1	2728	577	10,511	2210	16	553	12	7589
*1/*11	99	2	54	27	0	5	0	86
*1/*2	163	17	3523	511	2	130	7	1913
*1/*3	84	57	1866	230	1	117	5	1066
*1/*5	53	1	10	9	0	2	0	46
*1/*6	4	0	0	3	0	0	0	2
*11/*11	0	0	0	0	0	0	0	1
*2/*11	1	0	14	1	0	0	0	6
*2/*2	2	3	125	18	0	3	0	65
*2/*3	5	1	279	33	0	11	0	168
*2/*5	1	0	0	0	0	1	0	0
*2/*6	1	0	0	0	0	1	0	0
*3/*11	0	0	8	2	0	0	0	0
*3/*3	0	2	8	1	0	1	0	5
*3/*5	1	0	1	2	0	0	0	2
*3/*6	0	0	0	0	0	0	0	1
*6/*11	0	0	0	1	0	0	0	0
	3142	660	16,399	3048	19	824	24	10,950

Table S6 FDA boxed warnings associated with CYP2C9

Medication	*Prescribed conditions*	*Caution conditions*
Celecoxib	Rheumatology	CYP2C9 poor metabolisers
Flurbiprofen	Rheumatology	CYP2C9 poor metabolisers
Warfarin	Cardiology or Hematology	CYP2C9 intermediate or poor metabolisers

Table S7 FDA boxed warnings associated with CYP2C19

Medication	*Prescribed conditions*	*Caution conditions*
Clopidogrel	Cardiology	CYP2C19 intermediate or poor metabolisers
Prasugrel	Cardiology	CYP2C19 poor metabolisers
Ticagrelor	Cardiology	CYP2C19 poor metabolisers
Dexlansoprazole	Gastroenterology	CYP2C19 poor metabolisers
Esomeprazole	Gastroenterology	CYP2C19 poor metabolisers
Lansoprazole	Gastroenterology	CYP2C19 poor metaboliser
Omeprazole	Gastroenterology	CYP2C19 poor metabolisers
Pantoprazole	Gastroenterology	CYP2C19 poor metabolisers
Rabeprazole	Gastroenterology	CYP2C19 poor metabolisers
Voriconazole	Infectious diseases	CYP219 intermediate or poor metabolisers
Clobazam	Neurology	CYP2C19 poor metabolisers
Citalopram	Psychiatry	CYP2C19 poor metabolisers
Diazepam	Psychiatry	CYP2C19 poor metabolisers
Carisoprodol	Rheumatology	CYP2C19 poor metabolisers

Table S8 Nucleotide polymorphisms of CYP2C19

CYP2C19 variant	*Nucleotide change*	*Metaboliser status*	*Priority*
*1	Wild Type (None)	Normal	10
*2	19154 G>A	No activity	9
*3	17948 G>A	No activity	8
*4	1A>G	No activity	7
*6	13748 G>A	No activity	5
*7	19294 T>A	No activity	4
*8	12711 T>C	No activity	3
*9	12784 G>A	No activity	2
*10	19153 C>T	No activity	1
*17	–806 C>T	Increased activity	9

Table S9 CYP2C19 genotypes

CYP2C19 Genotype	*African–American*	*Asian*	*White*	*Hispanic*	*Native American*	*Other*	*Pacific Islander*	*Unreported*
*1/*1	1094	276	7174	1733	9	344	14	4729
*1/*10	0	0	2	2	0	0	0	1
*1/*17	878	29	4674	644	1	208	6	2961
*1/*2	680	223	3255	555	5	196	4	2226
*1/*3	0	48	10	3	0	7	0	26
*1/*4	0	0	42	15	0	2	0	15
*1/*6	0	0	7	2	0	0	0	5
*1/*8	1	3	57	8	0	0	0	36
*1/*9	51	0	5	4	0	3	0	24
*17/*17	176	2	808	82	1	38	0	503
*2/*10	2	0	0	0	0	0	0	0
*2/*17	254	11	1064	135	2	42	0	678
*2/*2	112	60	363	53	1	28	0	283
*2/*3	0	19	4	2	0	2	0	12
*2/*4	1	0	9	5	0	1	0	9
*2/*6	1	0	1	2	0	0	0	1
*2/*8	0	0	13	1	0	0	0	4
*2/*9	6	0	2	2	0	0	0	6
*3/*17	1	1	4	0	0	0	0	2
*3/*3	0	0	0	0	0	1	0	1
*3/*4	0	1	0	0	0	0	0	0
*4/*17	1	0	37	4	0	0	0	19
*5/*17	0	0	0	0	0	0	0	1
*6/*17	0	0	0	0	0	0	0	1
*8/*17	0	0	27	3	0	1	0	8
*8/*9	0	0	0	0	0	0	0	1
*9/*17	23	0	2	4	0	2	0	8
*9/*9	2	0	0	0	0	0	0	2
Total	3283	673	17,560	3259	19	875	24	11,562

Table S10 Nucleotide polymorphisms of CYP2D6

CYP2D6 variant	*Nucleotide change*	*Metaboliser status*	*Priority*
*1	Wild type (None)	Normal	15
*2	2850 c > T	Normal	14
*3	2549 del A	Inactive	7
*4	1846 G > A	Inactive	8
*5	Deletion	Inactive	1
*6	1707 del T	Inactive	6
*7	2935 A > C	Inactive	5

Table S10 Nucleotide polymorphisms of CYP2D6 (continued)

CYP2D6 variant	*Nucleotide change*	*Metaboliser status*	*Priority*
*8	1785 G > T	Inactive	4
*9	2615_2617 del AAG	Partially active	13
*10	100 C > T	Partially active	12
*12	124 G > A	Inactive	3
*14	1785 G > A	Inactive	2
*17	1023 C > A	Partially active	11
*29	1659 G > A	Partially active	10
*41	2988 G > A	Partially active	9
XN	Gene duplication (*1, *2, *4, *10, *41)	Increased activity	N/A

Table S11 FDA boxed warnings for medications with CYP2D6 warnings

Medication	*Prescribed condition*	*Caution conditions*
Codeine	Anesthesiology	CYP2D6 poor metabolisers
Carvedilol	Cardiology	CYP2D6 poor metabolisers
Metoprolol	Cardiology	CYP2D6 poor metabolisers
Propafenone	Cardiology	CYP2D6 poor metabolisers
Propranolol	Cardiology	CYP2D6 poor metabolisers
Quinidine	Cardiology	CYP2D6 poor metabolisers
Cevimeline	Dermatology	CYP2D6 poor metabolisers
Terbinafine	Infectious diseases	CYP2D6 poor metabolisers
Dextromethorphan and Quinidine	Neurology	CYP2D6 poor metabolisers
Drospirenone and Ethinyl Estradiol	Neurology	CYP2D6 poor metabolisers
Galantamine	Neurology	CYP2D6 poor metabolisers
Tetrabenazine	Neurology	CYP2D6 poor metabolisers
Amitriptyline	Psychiatry	CYP2D6 poor metabolisers
Aripiprazole	Psychiatry	CYP2D6 poor metabolisers
Atomoxetine	Psychiatry	CYP2D6 poor metabolisers
Citalopram (2)	Psychiatry	CYP2D6 poor metabolisers
Clomipramine	Psychiatry	CYP2D6 poor metabolisers
Clozapine	Psychiatry	CYP2D6 poor metabolisers
Desipramine	Psychiatry	CYP2D6 poor metabolisers
Doxepin	Psychiatry	CYP2D6 poor metabolisers
Fluoxetine	Psychiatry	CYP2D6 poor metabolisers
Fluvoxamine	Psychiatry	CYP2D6 poor metabolisers
Iloperidone	Psychiatry	CYP2D6 poor metabolisers
Imipramine	Psychiatry	CYP2D6 poor metabolisers
Modafinil	Psychiatry	CYP2D6 poor metabolisers
Nefazodone	Psychiatry	CYP2D6 poor metabolisers

Table S11 FDA boxed warnings for medications with CYP2D6 warnings (continued)

Medication	*Prescribed condition*	*Caution conditions*
Nortriptyline	Psychiatry	CYP2D6 poor metabolisers
Paroxetine	Psychiatry	CYP2D6 poor metabolisers
Perphenazine	Psychiatry	CYP2D6 poor metabolisers
Pimozide	Psychiatry	CYP2D6 poor metabolisers
Protriptyline	Psychiatry	CYP2D6 poor metabolisers
Risperidone	Psychiatry	CYP2D6 poor metabolisers
Thioridazine	Psychiatry	CYP2D6 poor metabolisers
Trimipramine	Psychiatry	CYP2D6 poor metabolisers
Venlafaxine	Psychiatry	CYP2D6 poor metabolisers
Tramadol and Acetaminophen	Rheumatology	CYP2D6 poor metabolisers
Tolterodine	Urology	CYP2D6 poor metabolisers

Table S12 CYP2D6 genotypes

Genotype	*African–American*	*Asian*	*White*	*Hisp*	*Native*	*Other*	*Pacific Islander*	*Unreport*
*1/*1	409	75	2485	649	2	145	4	1761
*1/*10	81	159	239	53	0	36	0	256
*1/*17	352	1	46	51	0	20	0	246
*1/*2	438	48	2861	595	5	146	3	1883
*1/*29	128	4	14	40	0	9	0	106
*1/*3	6	1	192	24	0	4	0	80
*1/*4	157	18	2507	385	2	86	6	1384
*1/*41	61	19	1280	193	0	60	1	742
*1/*5	89	10	244	63	0	11	0	181
*1/*6	6	1	137	12	0	5	0	83
*1/*7	0	1	7	1	0	1	0	9
*1/*8	0	0	1	0	0	0	0	0
*1/*9	8	0	288	58	0	8	0	143
*1/*XN	25	6	213	30	0	17	1	141
*10/*10	7	144	15	3	0	11	0	72
*10/*14	0	4	0	1	0	0	0	1
*10/*17	52	1	7	8	0	2	0	29
*10/*29	19	0	2	3	0	1	0	14
*10/*41	3	22	65	7	0	6	0	45
*17/*17	127	0	4	7	1	6	0	68
*17/*29	77	1	2	4	0	1	0	47
*17/*41	23	0	18	5	0	4	0	22
*2/*10	51	56	140	36	0	17	1	100

Table S12 CYP2D6 genotypes (continued)

Genotype	*African–American*	*Asian*	*White*	*Hisp*	*Native*	*Other*	*Pacific Islander*	*Unreport*
*2/*12	1	0	0	1	0	0	0	2
*2/*14	0	3	0	0	0	0	0	1
*2/*17	201	0	27	33	0	9	0	150
*2/*2	155	17	942	192	0	41	1	635
*2/*29	132	2	17	26	0	1	0	77
*2/*3	5	0	126	11	0	1	0	63
*2/*4	91	9	1437	190	1	45	2	753
*2/*41	46	16	846	109	2	40	0	445
*2/*5	38	1	152	29	0	5	0	97
*2/*6	5	1	83	13	0	1	0	46
*2/*7	0	2	8	0	0	1	0	6
*2/*8	0	0	0	0	0	2	0	0
*2/*9	8	1	147	23	1	4	1	99
*2/*XN	86	5	249	97	1	24	0	172
*29/*29	24	0	2	0	0	1	0	13
*29/*41	10	1	3	3	0	1	0	6
*3/*10	1	0	7	0	0	0	0	2
*3/*14	0	0	0	0	0	0	0	1
*3/*17	1	0	3	0	0	1	0	1
*3/*29	0	0	1	0	0	0	0	0
*3/*3	0	0	9	0	0	1	0	3
*3/*4	4	0	96	3	0	2	0	48
*3/*41	3	0	48	3	0	1	0	17
*3/*5	0	0	14	1	0	0	0	5
*3/*6	0	0	6	0	0	0	0	5
*3/*7	0	0	1	0	0	0	0	1
*3/*9	0	0	15	0	0	0	0	5
*4/*10	21	4	97	16	0	8	0	65
*4/*12	1	0	1	0	0	0	0	1
*4/*14	1	0	0	0	0	1	0	1
*4/*17	90	1	26	18	0	2	0	72
*4/*29	44	0	7	11	0	2	0	27
*4/*4	42	1	764	66	2	23	0	383
*4/*41	19	1	638	61	1	22	0	316
*4/*5	13	0	102	12	0	5	0	69
*4/*6	1	0	78	2	0	2	0	31
*4/*7	0	1	2	1	0	0	0	4
*4/*8	0	0	1	0	0	0	0	0

Table S12 CYP2D6 genotypes (continued)

Genotype	*African–American*	*Asian*	*White*	*Hisp*	*Native*	*Other*	*Pacific Islander*	*Unreport*
*4/*9	1	1	164	27	1	1	1	74
*4/*XN	0	0	2	0	0	0	0	0
*41/*41	7	3	200	15	0	12	2	109
*5/*10	10	20	12	2	0	2	0	17
*5/*17	28	1	5	6	0	2	0	22
*5/*29	14	0	4	2	0	0	0	15
*5/*41	4	1	68	8	0	3	0	34
*5/*5	25	3	31	5	0	3	0	30
*5/*6	1	0	12	0	0	1	0	1
*5/*7	2	0	0	0	0	0	0	0
*5/*9	0	0	16	0	0	1	0	7
*6/*10	0	0	4	1	0	0	0	11
*6/*12	1	0	0	0	0	0	0	0
*6/*17	1	0	0	2	0	0	0	0
*6/*29	0	0	0	0	0	0	0	1
*6/*41	0	0	34	4	0	2	0	19
*6/*6	0	0	1	3	0	0	0	1
*6/*9	0	0	10	0	0	0	0	7
*7/*10	0	0	1	0	0	0	0	0
*7/*41	0	0	1	0	0	0	0	1
*7/*5	0	0	0	0	0	0	0	1
*7/*9	0	0	1	0	0	0	0	2
*9/*10	2	0	10	2	0	0	0	7
*9/*17	4	0	2	0	0	0	0	2
*9/*29	1	0	1	1	0	0	0	3
*9/*41	1	0	76	8	0	1	0	34
*9/*9	1	0	25	1	0	0	0	10
Total	3265	666	17,402	3236	19	870	23	11,443

Table S13 Analysis software used from ion torrent

Software	*Version identifier*
Torrent suite	3.4.2
ion-alignment	3.4.3-1
ion-analysis	3.4.9-1
ion-dbreports	3.4.31-1
ion-docs	3.4.6-1
ion-gpu	3.0.0-1

Table S13 Analysis software used from ion torrent (continued)

Software	*Version identifier*
ion-onetouchupdater	3.4.6-1
ion-pgmupdates	3.4.7
ion-pipeline	3.4.20-1
ion-plugins	3.4.21-1
ion-publishers	3.4.4-1
ion-referencelibrary	2.2.0
ion-rsmts	3.4.1-1
variantCaller	v3.4.51874