Forensic Science International: Genetics 33 (2018) 48–58

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Research paper

Predicted activity of *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* using full-gene haplotypes and their association with the *CYP2D6*-inferred metabolizer phenotype

Frank R. Wendt[a,b,*], Antti Sajantila[c], Bruce Budowle[a,b,d]

[a] Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[b] Graduate School of Biomedical Sciences, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA
[c] Department of Forensic Medicine, University of Helsinki, P.O. Box 40, 00014 Helsinki, Finland
[d] Center of Excellence in Genomic Medicine (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Keywords:
UGT2B7
ABCB1
OPRM1
COMT
CYP2D6
Comprehensive pharmacogenetics
Molecular autopsy

ABSTRACT

The pharmacogene, *CYP2D6*, is commonly used to infer metabolizer phenotype of many marketed drugs and endogenous toxins in ante- and post-mortem patients but only represents the efficiency of phase 1 metabolism. Downstream metabolic enzymes encoded by *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* also have been implicated in variable individual response to drugs due to their activity at different stages of the tramadol ADME (absorption, distribution, metabolism, and excretion) process. While commonly studied as single genes using targeted genotyping approaches, a more comprehensive tramadol metabolism profile has not been evaluated. 1000 Genomes Project data for *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* were used to characterize full-gene haplotypes and their effect on protein function using in-house excel-based workbooks, PopART, and TreeView. Population genetic summary statistics and intergenic analyses associated these haplotypes with full-gene *CYP2D6*-inferred metabolizer phenotype. The findings suggest that *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* may contribute to predicted metabolizer phenotype as opposed to relying solely on *CYP2D6*.

1. Introduction

The cytochrome p450 family 2, subfamily D, polypeptide 6 (CYP2D6) enzyme is responsible for phase I metabolism of approximately 30% of marketed drugs and endogenous toxins [1,2]. *CYP2D6* is a highly variable pharmacogene with well documented allele distributions that vary by demography [3–6]. Constellations of individual single nucleotide (SNPs) or insertion/deletion (INDELs) polymorphisms in *CYP2D6* define star (*) alleles (i.e. a haplotype [operationally defined by a set of SNPs]) which may be used to predict the metabolizer phenotype (e.g. poor [PM], intermediate [IM], extensive/normal [EM/NM] and ultrarapid [UM]) of an individual using their *CYP2D6* diplotype (i.e., combination of two *CYP2D6* * alleles) information and associated activity scores. These data have demonstrated value for guiding individualized prescription medication practices and even post-mortem investigations [7–10].

The *CYP2D6*-inferred metabolizer phenotype describes only one phase of the tramadol (T) ADME (absorption, distribution, metabolism, and excretion) and response process and does not explain all genotypic contribution of an individual's phenotypic expression [11]. Numerous polymorphisms in the downstream metabolic enzymes uridine diphosphate glucuronosyltransferase, family 1, polypeptide B7 (UGT2B7), adenosine triphosphate (ATP) binding cassette, subfamily B, number 1 (ABCB1), opioid receptor mu 1 (OPRM1), and catechol-O-methyltransferase (COMT) also have been implicated in idiosyncratic response to drugs. These ADME proteins are less well characterized and typically are interrogated in single-gene studies that associate relatively few SNPs/INDELs to rate of drug metabolism and/or enzyme activity [12–17]. It has been demonstrated that combinatorial pharmacogenetic profiles (i.e., data from multiple genes) improved patient outcomes in response to antidepressants [18,19] and opiates [20]. Therefore, a higher confidence in predicting a metabolizer phenotype may be realized if information from multiple enzymes in an ADME pathway, such as CYP2D6, UGT2B7, ABCB1, OPRM1, and COMT, are included in the analysis. For example, a *CYP2D6*4/CYP2D6*4* homozygote is considered a PM and may be prescribed a higher dose of pro-drug (e.g., T) to reach the therapeutic window. However, that same individual may harbor an *ABCB1* diplotype which confers decreased efflux of O-desmethyltramadol (M1, the primary active metabolite of T) across the blood brain barrier, enabling a relatively large concentration of M1 to

* Corresponding author at: Center for Human Identification, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., CBH-250, Fort Worth, TX 76107, USA.
E-mail address: Frank.Wendt@my.unthsc.edu (F.R. Wendt).

https://doi.org/10.1016/j.fsigen.2017.11.012
Received 25 September 2017; Received in revised form 1 November 2017; Accepted 22 November 2017
Available online 26 November 2017
1872-4973/

reach OPRM1 and stimulate analgesia propagation. Ultimately, a patient with this pair of diplotypes at *CYP2D6* and *ABCB1* should experience the desired, and safe, degree of pain relief, but relying solely on *CYP2D6* information for this patient would support increasing the tramadol dose which potentially could cause hyperalgesia.

While combinatorial studies have been performed, they rely on targeted genotyping approaches to interrogate *a priori* SNPs and/or INDELs [13,15,20–24]. Consequently, novel polymorphism(s) cannot be identified that refine estimates of enzyme activity [25]. Massively parallel sequencing (MPS) of the full gene region increases the potential to discover polymorphisms that are currently excluded from phenotype predictions [26].

Herein, the SNP and INDEL variant effect prediction data presented by Wendt et al. [27] are expanded upon using the phased data of the 1000 Genomes Project [28]. Full-gene haplotypes of *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* were characterized in self-reported healthy individuals. When compared to *CYP2D6*-predicted metabolizer phenotype for the same individuals [25], it was demonstrated that NMs by *CYP2D6* genotyping may possess poorly active downstream metabolic enzymes. Logistic regression suggests that phenotype predictions using *CYP2D6*-inferences alone do not explain all phenotypic variability as there may be contribution from polymorphisms in *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT*.

2. Materials and methods

Polymorphisms in the *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT* gene regions, including introns, exons, 5′ and 3′ untranslated regions (UTRs), and promoters, were downloaded from Phase 3 of the 1000 Genomes Project and analyzed individually in 5 super- and 26 sub-populations (Table S1) according to Wendt et al. [27]. Haplotypes for each gene were produced according to Table 1 and individual haplotypes are listed in Table S2. Certain polymorphisms characterized were removed from haplotype formation to simplify downstream analyses but capture meaningful levels of variation within each gene. Those excluded variants differ for each gene based on gene size, number of polymorphic sites within each gene, and the consensus variant effect prediction of each polymorphism. In general, polymorphisms that were not scored by Sort Intolerant From Tolerant (SIFT) [34–39], Polymorphisms Phenotyping v2 (PolyPhen-v2) [34,40,41], Protein Variant Effect Analyzer (PROVEAN) [42–44], or Human Splicing Finder (HSF) [45], were removed. Private mutations (SNPs or INDELs observed once in the 1000 Genomes Project) were included/excluded on a gene-by-gene basis. *ABCB1* was divided into four haplotype blocks based on Sai et al. [30,31]. Herein, haplotype block *ABCB1-Block-1* has been extended to include untranslated exon 1 (Fig. 1).

Using in-house Excel-based workbooks, haplotypes were aligned to the hg19 and hg38 reference genomes. Haplotypes were named with the following nomenclature format: reference sequence (genome name)-community recognized star allele (if known)-list of polymorphism rs numbers, if known, and the base at each position. Note that within text haplotypes were referenced using numeric identifiers relative to their frequency in the global population of all 2504 1000 Genomes Project individuals (Table S2).

Population genetic summary statistics for five super- and 26 sub-populations, including haplotype and diplotype frequencies (analogous to allele and genotype frequencies), observed (H_o) and expected (H_e) heterozygosities, pairwise genetic distances, and tests for detection of departures from Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium were performed using Genetic Data Analysis (GDA) [46] and the RStudio® package ggplot2 [47]. TreeView Version 1.6.6 Build 7601 [48,49] was used to create phylogenetic trees; haplotype network analyses were performed using Population Analysis with Reticulate Trees (PopART) using the ancestral parsimony setting [50].

Enzyme activity was predicted using commonly typed and previously described polymorphisms for each gene [13,17,29–31,51–53].

Table 1
Haplotype production approach for *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT*. Private mutations are defined as those observed once in the global population (all 2504 1000 Genomes Project individuals).

Gene	Total Polymorphisms	Full-Gene Haplotypes	Processing Notes	Polymorphisms Removed	Polymorphisms Included	Final Haplotypes
UGT2B7	613	887	Removal of private mutations except those predicted damaging or most likely damaging [27,29]	246	367	641
ABCB1	5986	> 3000	Removal of all unscored polymorphisms [27]; gene broken into haplotype blocks [30,31]	5310	676 Total (51 Block 3; 511 Block 2; 106 Block 1; 8 Block -1)	98 Block 3; 754 Block 2; 208 Block 1; 9 Block -1
OPRM1	6831	> 3000	Removal of all unscored polymorphisms [27]	6627	204	527
COMT	1007	2131	Removal of all unscored polymorphisms [27]	924	83	377

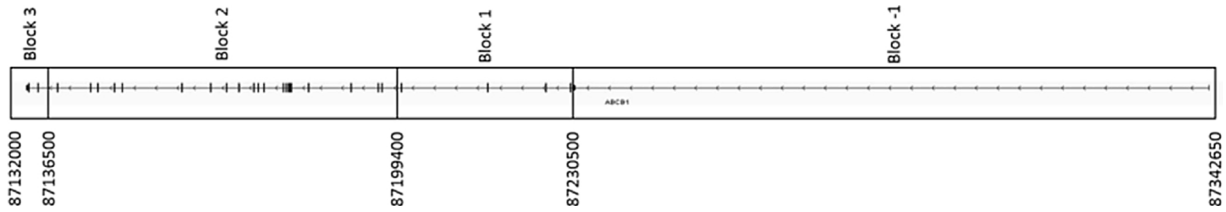

Fig. 1. *ABCB1* haplotype blocks. Image modified from Integrative Genomics Viewer [32,33] indicated chromosome 7 coordinates are relative to the hg19 reference genome.

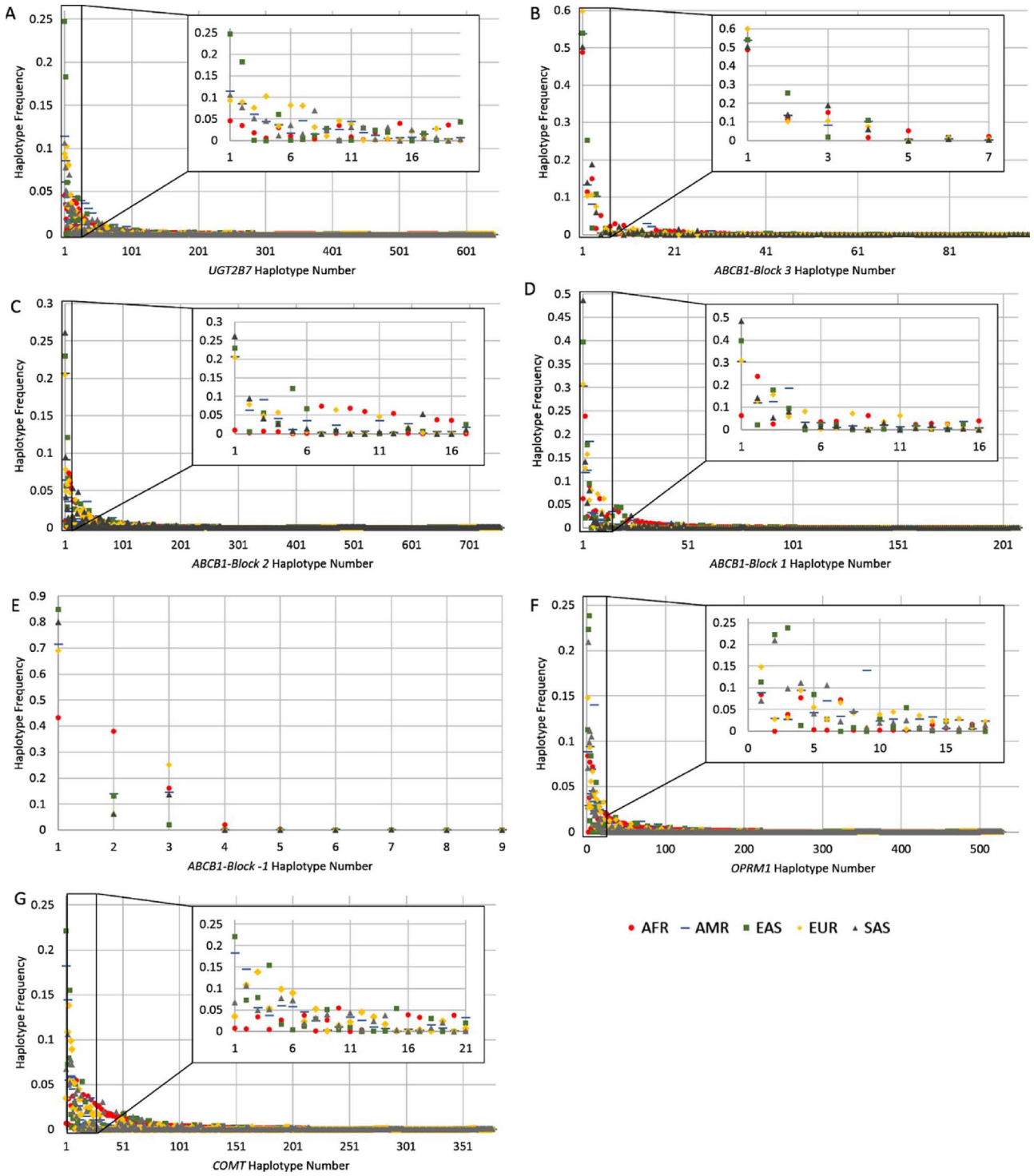

Fig. 2. Haplotype frequencies for *UGT2B7* (A), *ABCB1-Block 3* (B), *ABCB1-Block 2* (C), *ABCB1-Block 1* (D), *ABCB1-Block -1* (E), *OPRM1* (F), and *COMT* (G) in five super-populations (African [AFR; circles], Admixed American [AMR; horizontal lines], East Asian [EAS; squares], European [EUR; diamonds], and South Asian [SAS; triangles]).

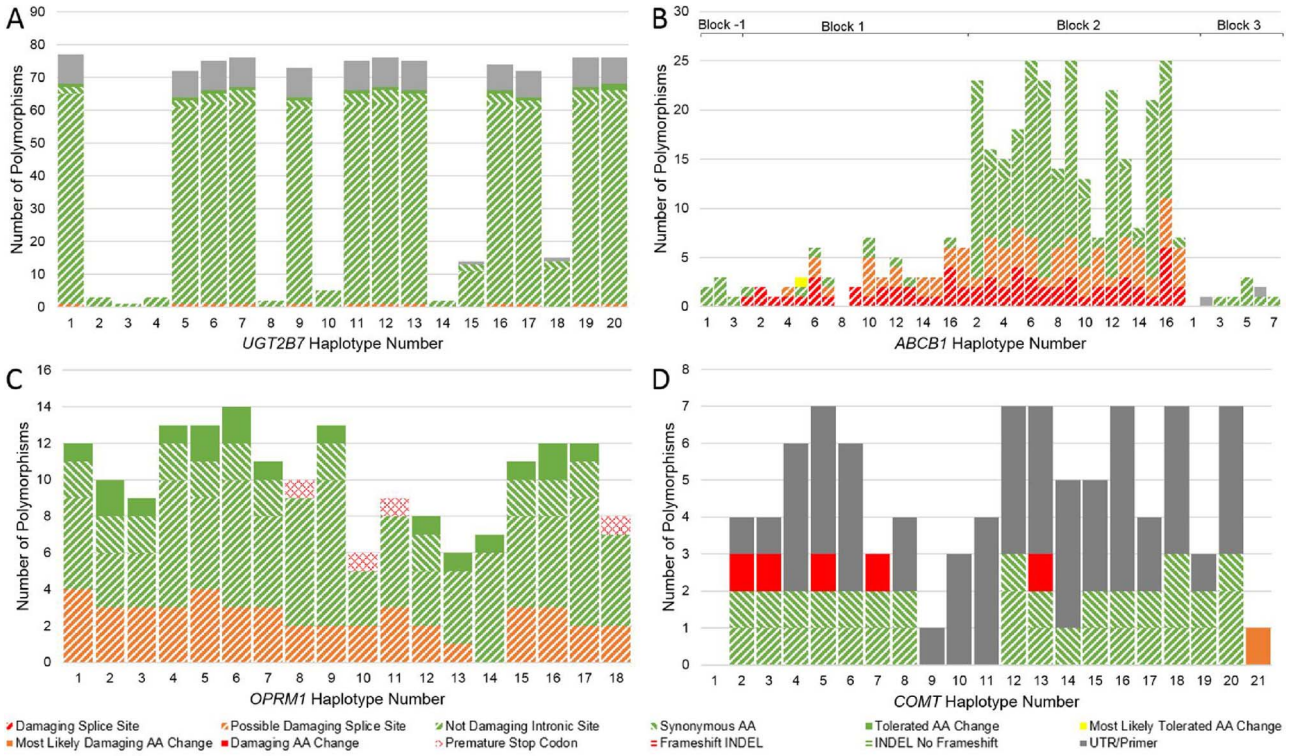

Fig. 3. Haplotype composition of 19, 3, 16, 17, 7, 18, and 21 haplotypes in *UGT2B7* (A), *ABCB1*-Block -1 (B), *ABCB1*-Block 1 (B), *ABCB1*-Block 2 (B), *ABCB1*-Block 3 (B), *OPRM1* (C), and *COMT* (D), respectively, with global frequencies ≥ 1%. Variant effect predictions presented by Wendt et al. [27] using Sort Intolerant From Tolerant [34–39], Polymorphism Phenotyping v2 [34,40,41], Protein Variant Effect Analyzer [42–44], and Human Splicing Finder [45].

Due to lack of empirical data for each polymorphism, additional damaging or most likely damaging polymorphisms in a gene were assumed to completely eliminate enzyme function. Logistic regression was used to explore possible relationships between the well-characterized *CYP2D6*-inferred metabolizer phenotype, represented as an activity score (a qualitative measure of phenotype derived from the activity conferred by each * allele an individual carries [54]) and the predicted activity of *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT*. These data were then used to interpret the potential utility of a combinatorial pharmacogenetic profile.

3. Results and discussion

3.1. UGT2B7, ABCB1, OPRM1, and COMT

A total of 641, 98, 754, 208, 9, 527, and 377 string sequences were observed for *UGT2B7*, *ABCB1-Block 3*, *ABCB1-Block 2*, *ABCB1-Block 1*, *ABCB1-Block -1*, *OPRM1*, and *COMT*, respectively (Table 1 and Fig. 2). *ABCB1-Block 3* haplotype 1, *ABCB1-Block 2* haplotype 191, *ABCB1-Block 1* haplotype 8, *ABCB1-Block -1* haplotype 3, and *COMT* haplotype 1, respectively, were identical to the hg19/hg38 reference genomes. No *UGT2B7* and *OPRM1* haplotypes were identical to the hg19/hg38 reference sequences. A majority of haplotypes were observed once in the global population so the average global frequency of haplotypes for each gene was quite low (0.00156 ± 0.00690 for *UGT2B7*, 0.0102 ± 0.0566 for *ABCB1-Block 3*, 0.00133 ± 0.00699 for *ABCB1-Block 2*, 0.00481 ± 0.0243 for *ABCB1-Block 1*, 0.111 ± 0.222 for *ABCB1-Block -1*, 0.00190 ± 0.00873 for *OPRM1*, and 0.00265 ± 0.00900 for *COMT*). *UGT2B7* haplotypes 1–20, *ABCB1-Block 3* haplotypes 1–7, *ABCB1-Block 2* haplotypes 1–17, *ABCB1-Block 1* haplotypes 1–16, *ABCB1-Block -1* haplotypes 1–3, *OPRM1* haplotypes 1–18, and *COMT* haplotypes 1–21 had global alleles frequencies ≥ 1% (Fig. 2), with average frequencies of 0.0284 ± 0.0278 for *UGT2B7*, 0.127 ± 0.186 for *ABCB1-Block 3*, 0.0293 ± 0.0371 for *ABCB1-Block 2*, 0.0516 ± 0.0748 for *ABCB1-Block 1*, 0.331 ± 0.229 for *ABCB1-Block -1*, 0.0371 ± 0.0311 for *OPRM1*, and 0.0298 ± 0.0255 for *COMT*.

Variant compositions for the most common haplotypes of each gene and for all haplotypes are displayed in Figs. 3 and S1, respectively. Empirical data are not present for the large number of haplotypes observed so for the descriptive purposes of this work, the presence of one damaging, or most likely damaging [27,34–45], polymorphism in the haplotype is considered sufficient to decrease enzyme function. The average number of polymorphisms per haplotype was 59.8 ± 27.6 for *UGT2B7*, 3.56 ± 1.01 for *ABCB1-Block -1*, 4.50 ± 1.97 for *ABCB1-Block 1*, 16.5 ± 7.00 for *ABCB1-Block 2*, 3.08 ± 1.21 for *ABCB1-Block 3*, 11.3 ± 2.62 for *OPRM1*, and 4.89 ± 1.99 for *COMT*. Due to limited studies of the polymorphic nature of these four genes and inclusion of additional interrogated regions, none of the observed sequences herein were identical to previously reported star (*) alleles (a haplotype of polymorphisms along the length of the gene region) for *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT*. It should be noted that a substantial number of SNPs/INDELs found in each haplotype (Figs. 3 and S1) are found in intronic or 5′ and 3′ untranslated regions and may have no individual impact on protein function but my play roles in regulating splice variation, rate of transcription, or have epistatic effects.

Network analysis was performed to determine the relatedness of two sets of haplotypes for each gene of interest: (1) haplotypes having > 1% global haplotype frequency (Fig. 4), and (2) haplotypes observed more than once in the 1000 Genomes Project dataset (Fig. S2). Networks for *UGT2B7*, *ABCB1-Block 3*, *ABCB1-Block 2*, and *ABCB1-Block-1* haplotypes (Fig. S2) appear to have more clearly defined haplotype relationships, less looping (multiple haplotypes may have multiple relationships with nearby haplotypes), and/or less reticulation (the degree of "webbing" in the network) than those of *OPRM1* and *COMT*. This observation is possibly attributable to the relatively few number of polymorphisms separating *OPRM1* and *COMT* haplotypes or be an artifact of deleting private mutations which may sufficiently

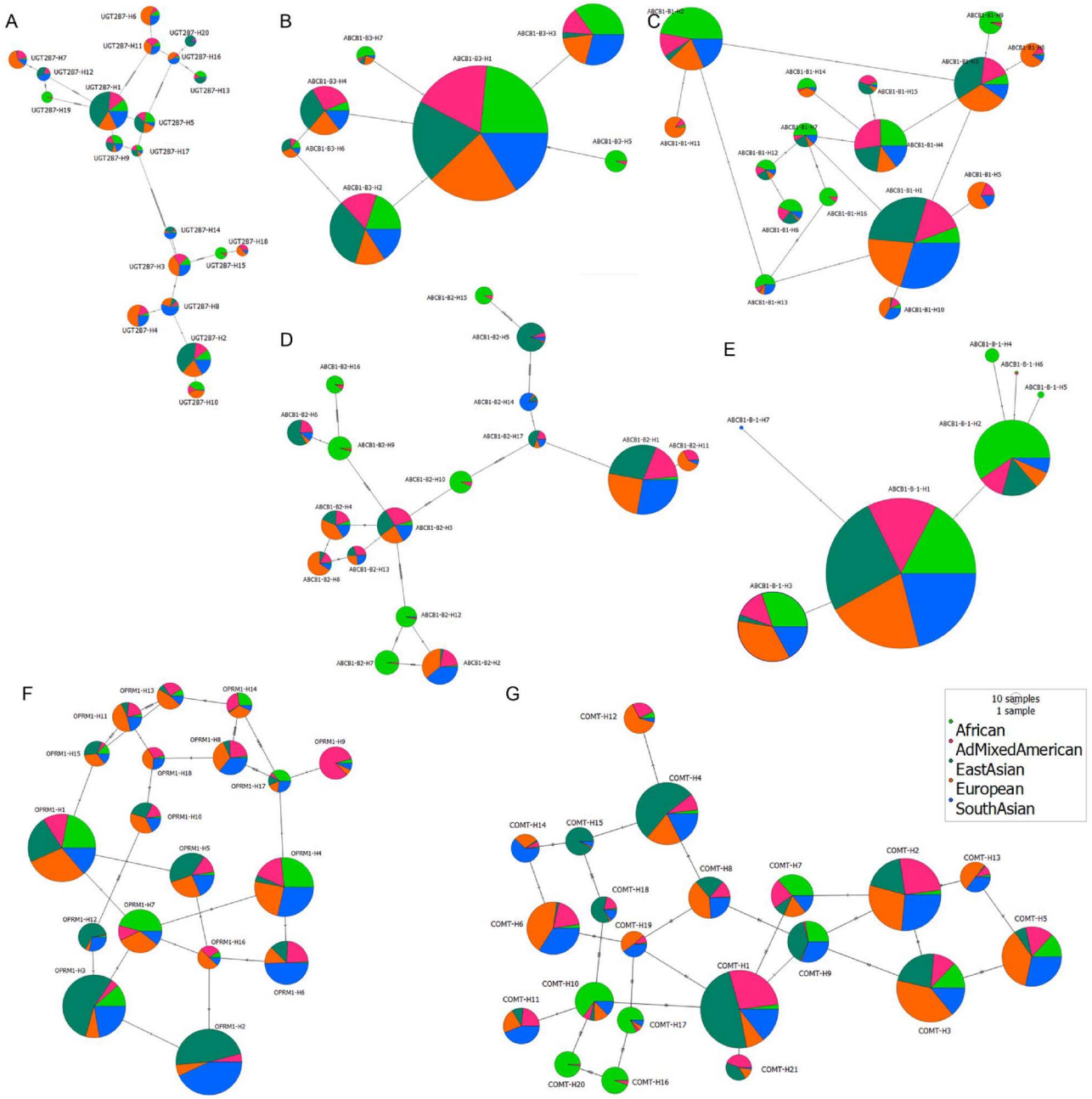

Fig. 4. Network analysis of *UGT2B7* haplotypes 1–20 (A), *ABCB1*-Block 3 haplotypes 1–7 (B), *ABCB1*-Block 2 haplotypes 1–17 (C), *ABCB1*-Block 1 haplotypes 1–16 (D), *ABCB1*-Block -1 haplotypes 1–9 (E), *OPRM1* haplotypes 1–18 (F), and *COMT* haplotypes 1–21 (G). The size of each circle is proportional to the global frequency of each haplotype, segments within each circle are proportional to the super-population haplotype frequency, and lines connecting circles are dashed with the number of mutations separating two haplotypes.

differentiate the relationships between haplotypes; alternatively, the substantial reticulation in the *OPRM1* and *COMT* haplotype networks might also suggest some degree of recombination between the regions of interest. Most major haplotypes in all four genes were observed in all five super-populations while many minor haplotypes were unique to one super-population, namely African (i.e., *UGT2B7* haplotypes stemming from *UGT2B7*-H19). This observation may be due to population-specificity and/or sampling effects.

There were 1414, 225, 1530, 567, 17, 1219, and 1267 unique *UGT2B7*, *ABCB1-Block 3*, *ABCB1-Block 2*, *ABCB1-Block 1*, *ABCB1-Block -1*, *OPRM1*, and *COMT* diplotypes, respectively, observed across 2504 individuals. The average global diplotype frequencies were $7.07 \times 10^{-4} \pm 0.00151$ for *UGT2B7*, 0.00444 ± 0.0234 for *ABCB1-Block 3*, $6.534 \times 10^{-4} \pm 0.00149$ for *ABCB1-Block 2*, 0.00176 ± 0.00685 for *ABCB1-Block 1*, 0.0588 ± 0.125 for *ABCB1-Block -1*, $8.20 \times 10^{-4} \pm 0.00211$ for *OPRM1*, and $7.96 \times 10^{-4} \pm 0.00142$ for *COMT*. Population-specific diplotype frequencies are displayed in Fig. S5. The average observed diplotype heterozygosity was 0.850 ± 0.129, 0.745 ± 0.172, 0.690 ± 0.224, 0.753 ± 0.170 0.687 ± 0.191 for the African (AFR), Ad Mixed American (AMR), East Asian (EAS), European (EUR), and South Asian (SAS) super-populations, respectively. Prior to Bonferroni correction ($p < 0.05$), *UGT2B7*, *OPRM1*, and *COMT* deviated significantly from HWE expectations in all five, two (AFR and EAS), and one (AMR) super-populations, respectively. After Bonferroni correction ($p < 0.00714$), *UGT2B7* and *OPRM1* deviated significantly from HWE expectations in four (AMR, EAS, EUR, and SAS) and one (EAS) super-populations, respectively, out of the five total super-populations (Fig. 5).

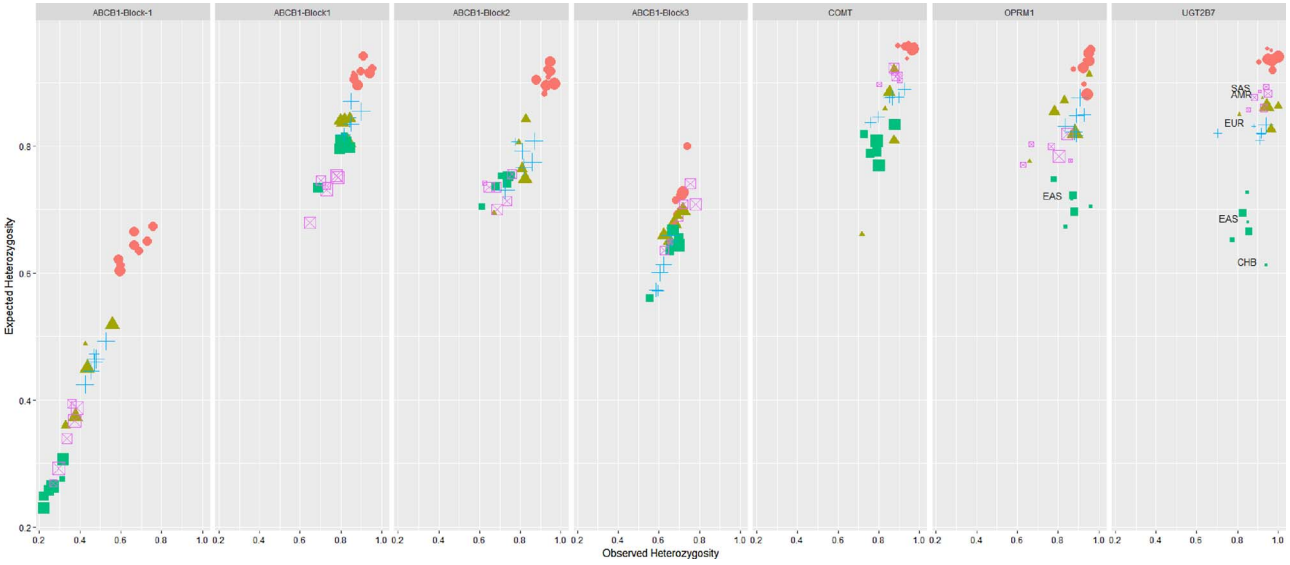

Fig. 5. Observed and expected heterozygosity of *ABCB1*-Block -1, *ABCB1*-Block 1, *ABCB1*-Block 2, *ABCB1*-Block 3, *COMT*, *OPRM1*, and *UGT2B7* haplotypes in five super-populations (African [AFR] in solid circles; Admixed American [AMR] in solid triangles; East Asian [EAS] in squares; European [EUR] in plus signs; South Asian [SAS] in "X"-filled squares) and the 26 sub-populations within each super-population. The size of each data point represents the Hardy-Weinberg Equilibrium p-value for each population; labeled populations indicate significance after Bonferroni correction ($p < 0.00714$).

3.2. Intergenic analyses

Unrooted neighbor-joining trees (Fig. S4) of super- and sub-populations using each gene individually (*ABCB1* is a combination of all four haplotype blocks) tend to show separation more so of the AFR and EAS populations while the AMR, EAS, and SAS populations cluster closer together. Considering all five genes (Fig. 6) the same super-population trend is seen. Generally, the sub-populations within each super-population were grouped closely together; however, the Gujarati Indian from Houston, Texas (GIH) and the Peruvians from Lima, Peru (PEL) populations plot separately from the group of AMR, EUR, and SAS sub-populations.

Intergenic pairwise LD was tested using full-gene haplotypes for *CYP2D6* [25], *UGT2B7*, *ABCB1-Block 3*, *ABCB1-Block 2*, *ABCB1-Block 1*, *ABCB1-Block -1*, *OPRM1*, and *COMT* to identify associations between metabolically relevant genes. Prior to Bonferroni correction ($p < 0.05$) and after removal of significant associations between *ABCB1* haplotype blocks, there were ten, 16, eight, five, and ten significant pairwise LDs in the AFR, AMR, EAS, EUR, and SAS super-populations, respectively (Fig. 7Figs. 7 and S5). After Bonferroni correction ($p < 0.00179$), there were six, five, one, two, and one significant pairwise LDs in the AFR, AMR, EAS, EUR, and SAS super-populations, respectively, most of which contain *CYP2D6* and an additional downstream metabolic enzyme. The AFR super-population exhibited more LDs than any other super-population (though the significant correlations are weak [average Pearson's $r = 0.0181$]) and those increased LDs are detected in the AFR sub-populations as well. These data are contrary to the expectations of lower LD in AFR populations compared with other population groups [55] but were observed with the individual SNP data as well so this observation is not surprising [27]. However, the effect may be artifactual and possibly explained by the highly polymorphic nature of these genes in the AFR population which results in an overall low frequency of each haplotype (Fig. 2). Consequently, a large number of diplotypes may be observed only once in the AFR super-population, making the comprised haplotypes appear to be in LD due to scant observations of each haplotype. When compressed to minimize the impact of rare diplotypes using the "collapse less-frequent alleles" function in GDA, significant LDs were observed between *CYP2D6* and *UGT2B7*, *ABCB1-Block -1, ABCB1-Block 1, ABCB1-Block 2, ABCB1-Block 3*, *OPRM1* and *COMT*, with Pearson's r-values ranging from −0.0562 to 0.0610 for AFR and −0.0903 to 0.129 for AMR. Though not observed across the whole ADME process, there were some significant LDs between *CYP2D6* and other downstream enzymes in the EAS, EUR, and SAS populations as well. Of particular interest are the significant pairwise LDs between *CYP2D6/UGT2B7* (−0.0562 [AFR] to 0.0934 [AMR]) and *CYP2D6/COMT* (−0.0902 [EUR] to 0.129 [AMR]) in all five super-populations, which may represent associations between their functional impact. The *COMT* locus is found in a one megabase (Mb) region of chromosome 22 with a relatively high average recombination rate (2.40 ± 1.56 centimorgans/Mb) which may artificially inflate the LD pattern involving this locus [55–59]. These empirical data have not yet been explored and more research is needed to support whether an effect is real.

Using previously identified genotype-phenotype data [13,17,29–31,51–53] and additional polymorphisms characterized by Wendt et al. [27], the activities of UGT2B7, ABCB1, OPRM1 and COMT were predicted for each 1000 Genomes Project individual. When grouped by *CYP2D6*-inferred metabolizer phenotype as a global cohort (2504 self-reported healthy individuals), there was no association detected between metabolizer phenotype and the diplotype-predicted activity of the selected downstream metabolically-relevant enzymes. Positive and negative correlations were observed between *COMT* ($p = 0.0223$) and *UGT2B7* ($p = 0.0389$) and *CYP2D6* activities, respectively; however the variance at *CYP2D6* activity score of 3 is quite large and may have influenced the significance of this relationship (Fig. 8A shaded regions). CYP2D6 activity score of 3 was only detected in one Toscani in Italia individual who carries one normally active and one increased activity *CYP2D6* * allele (*CYP2D6*1/*53). On the super-population level, there were more obvious trends, again between *UGT2B7* and *COMT* activities and the *CYP2D6* activity score. Two super-populations showed significant associations between *CYP2D6* and other enzyme activity: AMR and *UGT2B7* ($p = 0.0340$), and EAS and *OPRM1* ($p = 0.0361$). The remaining super-populations and genes did not exhibit significant associations between the *CYP2D6*-inferred metabolizer phenotype and diplotype-predicted downstream metabolic activity. Variant effect predictions [34–45] suggested that all 1000 Genomes Project self-reported healthy individuals possess an *ABCB1* diplotype that confers abnormal transporter activity. This observation may be misleading due to inaccuracies of the variant effect prediction programs used [27]. The functional consequences of individual *ABCB1*

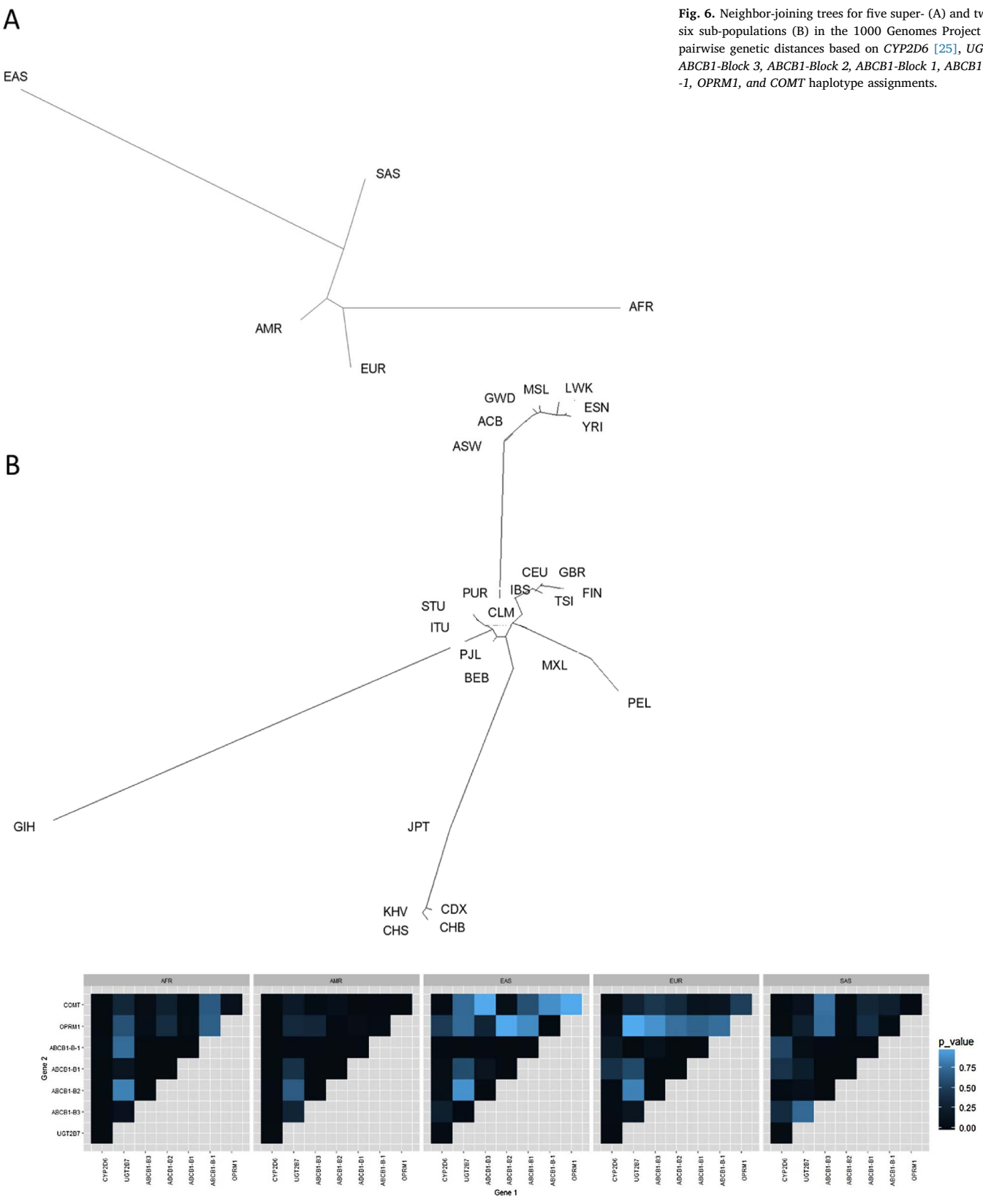

Fig. 6. Neighbor-joining trees for five super- (A) and twenty-six sub-populations (B) in the 1000 Genomes Project using pairwise genetic distances based on *CYP2D6* [25], *UGT2B7*, *ABCB1-Block 3, ABCB1-Block 2, ABCB1-Block 1, ABCB1-Block -1, OPRM1, and COMT* haplotype assignments.

Fig. 7. Heat maps of pairwise linkage disequilibrium p-values using *CYP2D6* [25], *UGT2B7*, *ABCB1-Block 3*, *ABCB1-Block 2*, *ABCB1-Block 1, ABCB1-Block -1*, *OPRM1*, and *COMT* diplotype in the African (AFR), Admixed American (AMR), East Asian (EAS), European (EUR), and South Asian (SAS) super-populations.

polymorphisms, the combined impact of multiple *ABCB1* polymorphisms, and the interaction between the effects of multiple polymorphisms in different genes are unavailable for comparison in this study but eventually will be needed to be empirically evaluated in affected, or drug-exposed, populations. If these observations are correct, the relative abundance of these splice-altering polymorphisms suggests that decreased ATP-dependent efflux efficiency may be the norm for self-reported healthy individuals. For example, rs2235027 has an alternate

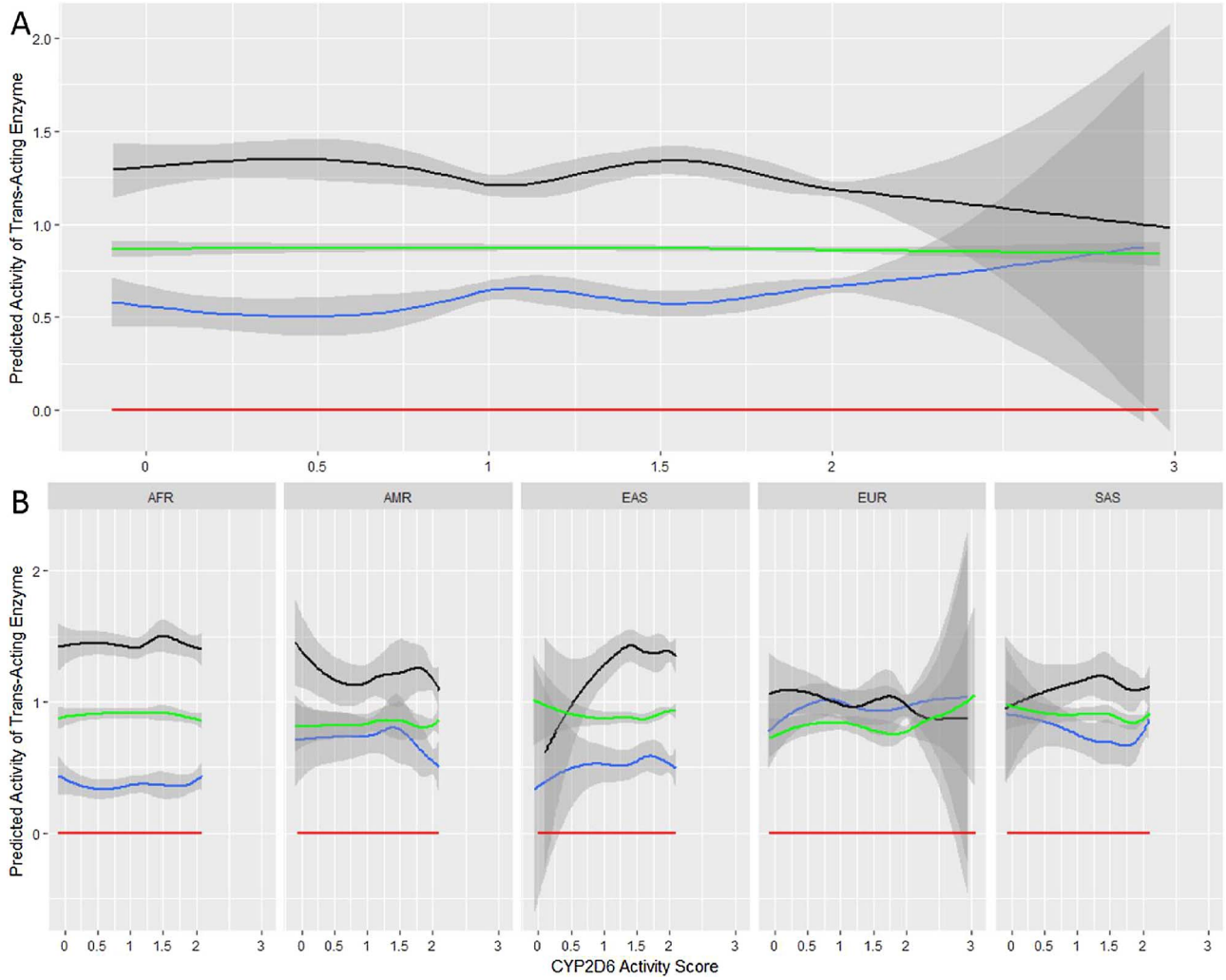

Fig. 8. Regression analysis between CYP2D6 metabolizer phenotype [25] and predicted activity of downstream metabolic enzymes *UGT2B7* (blue), *ABCB1* (red), *OPRM1* (green), and *COMT* (black) in the global population of 2504 1000 Genomes Project individuals (A) and by super-populations (B). Predicted activity of each trans-acting metabolic enzyme is based on the sum of predicted haplotype activities and ranges from zero to two (inactive to normally active, respectively). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

allele frequency of 0.517, 0.516, 0.383, 0.509, and 0.397 in the AFR, AMR, EAS, EUR, and SAS super-populations, respectively [27]. It can be hypothesized that affected, or drug-exposed, individuals possess additional polymorphisms, or are enriched for those identified here, that further alter transporter function and play a role in the idiosyncratic drug response phenotype [60–63]. Also epistatic interactions between multiple *ABCB1* SNPs/INDELs have been demonstrated to influence antiepileptic drug resistance [64]. Possibly a similar phenomenon is observed in self-reported healthy individuals who have either 1) not been exposed to a drug with which the epistasis-associated phenotype is observed or 2) are expressing a low level phenotype below level of personal discomfort and reporting.

4. Conclusions

Full-gene haplotypes of four genes encoding trans-acting T-metabolism proteins, *UGT2B7*, *ABCB1*, *OPRM1*, and *COMT*, were defined and characterized using substantially more polymorphic sites than previously employed in pharmacogenetic studies. In doing so, a large number of haplotypes were observed. The data presented demonstrate significant LDs between full-gene haplotypes of *CYP2D6* and those of *UGT2B7* and *COMT*; however, the functional effects of these findings need to be determined empirically. The relatively low frequency of each haplotype and associated diplotype may confound LD estimates simply because each haplotype was only observed in combination with one other haplotype. This study also proposed an extended *ABCB1-Block -1*, which included distal untranslated exon 1, and did not substantially increase acquired information over the truncated *Block -1* reported by Sai et al. [30,31]. Most individual haplotypes identified in this study were quite rare; however, relatively common haplotypes (≥1% global frequency) were identified which contain at least one damaging, or most likely damaging, polymorphism. It should be noted that copy number variation and *CYP2D6/CYP2D7* gene conversion do occur in some individuals, primary UMs and may alter the presented LD and regression patterns [65]. These events were not considered herein for determining of CYP2D6 activity [11] due to the limitations of short read sequences that comprise 1000 Genomes Project data [66,67]. It is likely that ongoing developments in longer read sequencing technologies will provide more confident interpretation of structural variation from existing short-read sequences [68–71].

The variant effects of many polymorphisms included in these haplotype definitions have not been empirically evaluated by the pharmacogenetics/pharmacogenomics community. There are obvious limitations to using an algorithmic approach to variant effect [72]; however, the predicted implications on phenotype should not be overlooked, instead they can be used to narrow the pool of potentially

causal variants/haplotypes to explore empirically. The inclusion of only self-reported healthy individuals in the 1000 Genomes Project means that additional functionally-relevant haplotypes may be selected against being represented in this dataset. This limiting factor may impact the analyses performed above. It is likely that additional polymorphisms and/or specific haplotypes may be enriched, or selected for, in affected, or T-exposed, cohorts [73–75]. As such, there potentially are additional damaging haplotypes in these affected groups that have not been observed herein so a full-gene interrogation of affected cohorts may provide greater resolution to damaging haplotype population distribution. This possibility lends support to utilizing a comprehensive genotyping approach, such as relatively long-read MPS or continuous-read nanopore technology in pharmacogenetic/pharmacogenomic interrogations [70,71,76].

Though limited to a large cohort of self-reported healthy individuals, associations between individual genes have been identified which may be clinically significant. Though slight, there is a relationship between the *CYP2D6*-inferred metabolizer phenotype and the diplotype-predicted activities of UGT2B7, ABCB1, OPRM1, and COMT. This association highlights the need for comprehensive functional evaluation of the impact of polymorphisms in all five genes, and/or combinations of two, three, or four of these genes, on drug metabolism in the same individuals. It is reasonable to hypothesize that empirical evaluation of these targets will reveal the advantage of combinatorial pharmacogenetic profiles in regards to increased patient efficacy and even assisting with medico-legal accident reconstruction [18–20]. Currently, these data remain relatively scarce in the literature. The data presented herein provide a basis to interrogate the highly polymorphic T-metabolism pathway, defining full-gene haplotypes for, and characterizing the association between, five pharmacogenes that can be utilized in clinical pharmacogenetic evaluations and post-mortem molecular autopsy using gene-targeted MPS. It is likely that these data can be expanded upon, by interrogating additional ADME gene haplotypes, for broad applicability for predicting metabolizer phenotype following exposure to other opioid drugs.

Conflicts of interest

The authors report no conflict of interest.

Acknowledgement

The authors would like to thank Dr. August Woerner from the Center for Human Identification at the University of North Texas Health Science Center for his generous feedback and guidance regarding statistical analyses and data interpretation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2017.11.012.

References

[1] M. Ingelman-Sundberg, S.C. Sim, A. Gomez, C. Rodriguez-Antona, Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects, Pharmacol. Ther. 116 (December (3)) (2007) 496–526 Epub 2007 Oct 9. Review. PubMed PMID: 18001838.

[2] M. Ingelman-Sundberg, Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity, Pharmacogenom. J. 5 (1) (2005) 6–13 Review. PubMed PMID: 15492763.

[3] J.B. Leathart, S.J. London, A. Steward, J.D. Adams, J.R. Idle, A.K. Daly, CYP2D6 phenotype-genotype relationships in African-Americans and Caucasians in Los Angeles, Pharmacogenetics 8 (December (6)) (1998) 529–541 PubMed PMID: 9918137.

[4] L.D. Bradford, CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants, Pharmacogenomics 3 (March (2)) (2002) 229–243 Review. PubMed PMID: 11972444.

[5] J. Sistonen, S. Fuselli, J.U. Palo, N. Chauhan, H. Padh, A. Sajantila, Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales, Pharmacogenet. Genom. 19 (February (2)) (2009) 170–179, http://dx.doi.org/10.1097/FPC.0b013e32831ebb30 PubMed PMID: 19151603.

[6] A. Gaedigk, K. Sangkuhl, M. Whirl-Carrillo, T. Klein, J.S. Leeder, Prediction of CYP2D6 phenotype from genotype across world populations, Genet. Med. 19 (January (1)) (2017) 69–76, http://dx.doi.org/10.1038/gim.2016.80 Epub 2016 Jul 7. PubMed PMID: 27388693; PubMed Central PMCID: PMC5292679.

[7] A. Levo, A. Koski, I. Ojanperä, E. Vuori, A. Sajantila, Post-mortem SNP analysis of CYP2D6 gene reveals correlation between genotype and opioid drug (tramadol) metabolite ratios in blood, Forensic Sci. Int. 135 (July (1)) (2003) 9–15 PubMed PMID: 12893130.

[8] G. Koren, J. Cairns, D. Chitayat, A. Gaedigk, S.J. Leeder, Pharmacogenetics of morphine poisoning in a breastfed neonate of a codeine-prescribed mother, Lancet 368 (August (9536)) (2006) 704 PubMed PMID: 16920476.

[9] A. Koski, I. Ojanperä, J. Sistonen, E. Vuori, A. Sajantila, A fatal doxepin poisoning associated with a defective CYP2D6 genotype, Am. J. Forensic Med. Pathol. 28 (September (3)) (2007) 259–261 PubMed PMID: 17721180.

[10] J. Sistonen, P. Madadi, C.J. Ross, M. Yazdanpanah, J.W. Lee, M.L. Landsmeer, et al., Prediction of codeine toxicity in infants and their mothers using a novel combination of maternal genetic markers, Clin. Pharmacol. Ther. 91 (April (4)) (2012) 692–699, http://dx.doi.org/10.1038/clpt.2011.280 Epub 2012 Mar 7. PubMed PMID: 22398969.

[11] H.S. Smith, Opioid metabolism, Mayo Clin. Proc. 84 (July (7)) (2009) 613–624, http://dx.doi.org/10.1016/S0025-6196(11)60750-7 Review. PubMed PMID: 19567715; PubMed Central PMCID: PMC2704133.

[12] M. Holthe, T.N. Rakvåg, P. Klepstad, J.R. Idle, S. Kaasa, H.E. Krokan, et al., Sequence variations in the UDP-glucuronosyltransferase 2B7 (UGT2B7) gene: identification of 10 novel single nucleotide polymorphisms (SNPs) and analysis of their relevance to morphine glucuronidation in cancer patients, Pharmacogenom. J. 3 (1) (2003) 17–26 Erratum in: Pharmacogenomics J. 2003;3(4):248. PubMed PMID: 12629580.

[13] D. Campa, A. Gioia, A. Tomei, P. Poli, R. Barale, Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief, Clin. Pharmacol. Ther. 83 (April (4)) (2008) 559–566 Epub 2007 Sep 26. PubMed PMID: 17898703.

[14] J.Y. Chung, J.Y. Cho, K.S. Yu, J.R. Kim, K.S. Lim, D.R. Sohn, et al., Pharmacokinetic and pharmacodynamic interaction of lorazepam and valproic acid in relation to UGT2B7 genetic polymorphism in healthy subjects, Clin. Pharmacol. Ther. 83 (April (4)) (2008) 595–600 Epub 2007 Aug 8. PubMed PMID: 17687269.

[15] K. Fujita, Y. Ando, W. Yamamoto, T. Miya, H. Endo, Y. Sunakawa, et al., Association of UGT2B7 and ABCB1 genotypes with morphine-induced adverse drug reactions in Japanese patients with cancer, Cancer Chemother. Pharmacol. 65 (January (2)) (2010) 251–258, http://dx.doi.org/10.1007/s00280-009-1029-2 PubMed PMID: 19466410.

[16] L.M. Hodges, S.M. Markova, L.W. Chinn, J.M. Gow, D.L. Kroetz, T.E. Klein, et al., Very important pharmacogene summary: ABCB1 (MDR1, P-glycoprotein), Pharmacogenet. Genom. 21 (March (3)) (2011) 152–161, http://dx.doi.org/10.1097/FPC.0b013e3283385a1c PubMed PMID: 20216335; PubMed Central PMCID: PMC3098758.

[17] R.C. Crist, W.H. Berrettini, Pharmacogenetics of OPRM1, Pharmacol. Biochem. Behav. 123 (August) (2014) 25–33, http://dx.doi.org/10.1016/j.pbb.2013.10.018 Epub 2013 Nov 5. Review. PubMed PMID: 24201053; PubMed Central PMCID: PMC4010567.

[18] C.A. Altar, J. Carhart, J.D. Allen, D. Hall-Flavin, J. Winner, B. Dechairo, Clinical utility of combinatorial pharmacogenomics-guided antidepressant therapy: evidence from three clinical studies, Mol. Neuropsychiatry 1 (October (3)) (2015) 145–155, http://dx.doi.org/10.1159/000430915 Epub 2015 Jul 31. PubMed PMID: 27606312; PubMed Central PMCID: PMC4996033.

[19] C.A. Altar, J.M. Carhart, J.D. Allen, D.K. Hall-Flavin, B.M. Dechairo, J.G. Winner, Clinical validity: combinatorial pharmacogenomics predicts antidepressant responses and healthcare utilizations better than single gene phenotypes, Pharmacogenom. J. 15 (October (5)) (2015) 443–451, http://dx.doi.org/10.1038/tpj.2014.85 Epub 2015 Feb 17. PubMed PMID: 25686762.

[20] S. Bastami, A. Gupta, A.L. Zackrisson, J. Ahlner, A. Osman, S. Uppugunduri, Influence of UGT2B7, OPRM1 and ABCB1 gene polymorphisms on postoperative morphine consumption, Basic Clin. Pharmacol. Toxicol. 115 (November (5)) (2014) 423–431, http://dx.doi.org/10.1111/bcpt.12248 Epub 2014 May 19. PubMed PMID: 24703092.

[21] H.Y. Handoko, D.R. Nyholt, N.K. Hayward, D.A. Nertney, D.E. Hannah, L.C. Windus, et al., Separate and interacting effects within the catechol-O-methyltransferase (COMT) are associated with schizophrenia, Mol. Psychiatry 10 (June (6)) (2005) 589–597 PubMed PMID: 15505638.

[22] O. Bartošová, O. Polanecký, F. Perlík, S. Adámek, O. Slanař, OPRM1 and ABCB1 polymorphisms and their effect on postoperative pain relief with piritramide, Physiol. Res. 64 (Suppl. 4) (2015) S521–S527 PubMed PMID: 26681082.

[23] D.J. Christoffersen, P. Damkier, S. Feddersen, S. Möller, J.L. Thomsen, C. Brasch-Andersen, et al., The ABCB1, rs9282564, AG and TT genotypes and the COMT, rs4680, AA genotype are less frequent in deceased patients with opioid addiction than in living patients with opioid addiction, Basic Clin. Pharmacol. Toxicol. 119 (October (4)) (2016) 381–388, http://dx.doi.org/10.1111/bcpt.12602 Epub 2016 May 19. PubMed PMID: 27061230.

[24] N. Sutiman, J.S. Lim, T.E. Muerdter, O. Singh, Y.B. Cheung, R.C. Ng, et al., Pharmacogenetics of UGT1A4, UGT2B7 and UGT2B15 and their influence on tamoxifen disposition in Asian breast cancer patients, Clin. Pharmacokinet. 55 (October (10)) (2016) 1239–1250, http://dx.doi.org/10.1007/s40262-016-0402-7 PubMed PMID: 27098059.

[25] F.R. Wendt, A. Sajantila, R.S. Moura-Neto, A.E. Woerner, B. Budowle, Full-gene

haplotypes refine CYP2D6 metabolizer phenotype inferences, Int. J. Legal Med. (2017), http://dx.doi.org/10.1007/s00414-017-1709-0 [Epub ahead of print] PubMed PMID: 29075918.
[26] W.H. Koch, Technology platforms for pharmacogenomic diagnostic assays, Nat. Rev. Drug Discov. 3 (September (9)) (2004) 749–761 Review. PubMed PMID: 15340385.
[27] F.R. Wendt, G. Pathak, A. Sajantila, R. Chakraborty, B. Budowle, Global genetic variation of select opiate metabolism genes in self-reported healthy individuals, Pharmacogenom. J. (April) (2017), http://dx.doi.org/10.1038/tpj.2017.13 [Epub ahead of print] PubMed PMID: 28398354).
[28] < http://www.internationalgenome.org/faq/are-1000-genomes-variant-calls-phased/ > . Accessed 01FEB2017.
[29] UGT Nomenclature Committee, UGT Alleles Nomenclature Home Page, (2005) June, < http://www.ugtalleles.ulacal.ca/ > . Accessed 20FEB2017.
[30] K. Sai, M. Itoda, Y. Saito, K. Kurose, N. Katori, N. Kaniwa, et al., Genetic variations and haplotype structures of the ABCB1 gene in a Japanese population: an expanded haplotype block covering the distal promoter region, and associated ethnic differences, Ann. Hum. Genet. 70 (September (Pt 5)) (2006) 605–622 PubMed PMID: 16907707.
[31] K. Sai, N. Kaniwa, M. Itoda, Y. Saito, R. Hasegawa, K. Komamura, et al., Haplotype analysis of ABCB1/MDR1 blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan, Pharmacogenetics 13 (December (12)) (2003) 741–757 PubMed PMID: 14646693.
[32] J.T. Robinson, H. Thorvaldsdóttir, W. Winckler, et al., Integrative genomics viewer, Nat. Biotechnol. 29 (January (1)) (2011) 24–26, http://dx.doi.org/10.1038/nbt.1754 PubMed PMID: 21221095; PubMed Central PMCID: PMC3346182.
[33] H. Thorvaldsdóttir, J.T. Robinson, J.P. Mesirov, Integrative Genomics Viewer (IGV): high performance genomics data visualization and exploration, Brief. Bioinform. 14 (March (2)) (2013) 178–192, http://dx.doi.org/10.1093/bib/bbs017 Epub 2012 Apr 19. PubMed PMID: 22517427; PubMed Central PMCID: PMC3603213.
[34] W. McLaren, L. Gil, S.E. Hunt, H.S. Riat, G.R. Ritchie, A. Thormann, P. Flicek, F. Cunningham, The ensembl variant effect predictor, Genome Biol. 17 (June (1)) (2016) 122, http://dx.doi.org/10.1186/s13059-016-0974-4 PubMed PMID: 27268795; PubMed Central PMCID: PMC4893825.
[35] P. Kumar, S. Henikoff, P.C. Ng, Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm, Nat. Protoc. 4 (7) (2009) 1073–1081, http://dx.doi.org/10.1038/nprot.2009.86 Epub 2009 Jun 25. PubMed PMID: 19561590.
[36] P.C. Ng, S. Henikoff, Predicting the effects of amino acid substitutions on protein function, Annu. Rev. Genom. Hum. Genet. 7 (2006) 61–80 Review. PubMed PMID: 16824020.
[37] P.C. Ng, S. Henikoff, SIFT: predicting amino acid changes that affect protein function, Nucleic Acids Res. 31 (July (13)) (2003) 3812–3814 PubMed PMID: 12824425; PubMed Central PMCID: PMC168916.
[38] P.C. Ng, S. Henikoff, Accounting for human polymorphisms predicted to affect protein function, Genome Res. 12 (March (3)) (2002) 436–446 PubMed PMID: 11875032; PubMed Central PMCID: PMC155281.
[39] P.C. Ng, S. Henikoff, Predicting deleterious amino acid substitutions, Genome Res. 11 (May (5)) (2001) 863–874 PubMed PMID: 11337480; PubMed Central PMCID: PMC311071.
[40] I.A. Adzhubei, S. Schmidt, L. Peshkin, V.E. Ramensky, A. Gerasimova, P. Bork, A.S. Kondrashov, S.R. Sunyaev, A method and server for predicting damaging missense mutations, Nat. Methods 7 (April (4)) (2010) 248–249, http://dx.doi.org/10.1038/nmeth0410-248 PubMed PMID: 20354512; PubMed Central PMCID: PMC2855889.
[41] I. Adzhubei, D.M. Jordan, S.R. Sunyaev, Predicting functional effect of human missense mutations using PolyPhen-2, Curr. Protoc. Hum. Genet. (January) (2013), http://dx.doi.org/10.1002/0471142905.hg0720s76 Chapter 7:Unit7.20. PubMed PMID: 23315928; PubMed Central PMCID: PMC4480630.
[42] Y. Choi, G.E. Sims, S. Murphy, J.R. Miller, A.P. Chan, Predicting the functional effect of amino acid substitutions and indels, PLoS One 7 (January (10)) (2012) e46688, http://dx.doi.org/10.1371/journal.pone.0046688 Epub 2012 Oct 8. PubMed PMID: 23056405; PubMed Central PMCID: PMC3466303.
[43] Y. Choi, A.P. Chan, PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels, Bioinformatics 31 (August (16)) (2015) 2745–2747, http://dx.doi.org/10.1093/bioinformatics/btv195 Epub 2015 Apr 6. PubMed PMID: 25851949; PubMed Central PMCID: PMC4528627.
[44] Y. Choi, A fast computation of pairwise sequence alignment scores between a protein and a set of single-locus variants of another protein, Proceedings of the ACM Conference on Bioinformatics, Computational Biology and Biomedicine (BCB '12), ACM, New York, NY, USA, 2012, pp. 414–417.
[45] F.O. Desmet, D. Hamroun, M. Lalande, G. Collod-Béroud, M. Claustres, C. Béroud, Human Splicing Finder: an online bioinformatics tool to predict splicing signals, Nucleic Acids Res. 37 (May (9)) (2009) e67, http://dx.doi.org/10.1093/nar/gkp215 PubMed PMID: 19339519; PubMed Central PMCID: PMC2685110.
[46] B.S. Weir, Genetic Data Analysis, 2nd ed., Sinauer Associates, Sunderland, Massachusetts, 1996 376 pages.
[47] H. Wickham, ggplot2: Elegant Graphics for Data Analysis, Springer-Verlag, New York, 2009.
[48] R.D.M. Page, TREEVIEW: an application to display phylogenetic trees on personal computers, Comput. Appl. Biosci. 12 (1996) 357–358.
[49] TreeView Version 1.6.6 Build 7601. < http://taxonomy.zoology.gla.ac.uk/rod/treeview/treeview_manual.html#citing_treeview/ > .
[50] Leigh, J., Bryant, D., Steel, M., Population Analysis with Reticulate Trees (PopART). < http://popart.otago.ac.nz/ > .
[51] L. Xin, Z.J. Wang, Bioinformatic analysis of the human mu opioid receptor (OPR) splice and polymorphic variants, AAPS PharmSciTech 4 (4) (2002) E23 PubMed PMID: 12645995; PubMed Central PMCID: PMC2751312.
[52] T.T. Rakvåg, P. Klepstad, C. Baar, T.M. Kvam, O. Dale, S. Kaasa, et al., The Val158Met polymorphism of the human catechol-O-methyltransferase (COMT) gene may influence morphine requirements in cancer pain patients, Pain 116 (July (1–2)) (2005) 73–78 PubMed PMID: 15927391.
[53] H. Takigawa, H. Kowa, K. Nakashima, No associations between five polymorphisms in COMT gene and migraine, Acta Neurol. Scand. 135 (February (2)) (2017) 225–230, http://dx.doi.org/10.1111/ane.12583 Epub 2016 Mar 14. PubMed PMID: 26988620.
[54] A. Gaedigk, S.D. Simon, R.E. Pearce, L.D. Bradford, M.J. Kennedy, J.S. Leeder, The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype, Clin. Pharmacol. Ther. 83 (February (2)) (2008) 234–242 Epub 2007 Oct 31. PubMed PMID: 17971818.
[55] M.C. Campbell, S.A. Tishkoff, African genetic diversity: implications for human demographic history, modern human origins, and complex disease mapping, Annu. Rev. Genom. Hum. Genet. 9 (2008) 403–433, http://dx.doi.org/10.1146/annurev.genom.9.081307.164258 Review. PubMed PMID: 18593304 PubMed Central PMCID: PMC2953791.
[56] D. Serre, R. Nadon, T.J. Hudson, Large-scale recombination rate patterns are conserved among human populations, Genome Res. 15 (November (11)) (2005) 1547–1552 PubMed PMID: 16251464 PubMed Central PMCID: PMC1310642.
[57] K.W. Broman, J.C. Murray, V.C. Sheffield, R.L. White, J.L. Weber, Comprehensive human genetic maps: individual and sex-specific variation in recombination, Am. J. Hum. Genet. 63 (September (3)) (1998) 861–869 PMID: 9718341; PMC: PMC1377399.
[58] C. Dib, S. Fauré, C. Fizames, D. Samson, N. Drouot, A. Vignal, P. Millasseau, S. Marc, J. Hazan, E. Seboun, et al., A comprehensive genetic map of the human genome based on 5,264 microsatellites, Nature 380 (March (6570)) (1996) 152–154 PMID: 8600387.
[59] A. Kong, D.F. Gudbjartsson, J. Sainz, G.M. Jonsdottir, S.A. Gudjonsson, B. Richardsson, S. Sigurdardottir, J. Barnard, B. Hallbeck, G. Masson, et al., A high-resolution recombination map of the human genome, Nat. Genet. 31 (July (3)) (2002) 241–247 PMID: 12053178.
[60] O. Levran, K. O'Hara, E. Peles, D. Li, S. Barral, B. Ray, et al., ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence, Hum. Mol. Genet. 17 (July (14)) (2008) 2219–2227, http://dx.doi.org/10.1093/hmg/ddn122 Epub 2008 Apr 17. PubMed PMID: 18424454; PubMed Central PMCID: PMC2599947.
[61] S. Fuselli, C. de Filippo, S. Mona, J. Sistonen, P. Fariselli, G. Destro-Bisol, et al., Evolution of detoxifying systems: the role of environment and population history in shaping genetic diversity at human CYP2D6 locus, Pharmacogenet. Genom. 20 (August (8)) (2010) 485–499, http://dx.doi.org/10.1097/FPC.0b013e32833bba25 PubMed PMID: 20520586.
[62] C.C. Hung, M.H. Chiou, Y.N. Teng, Y.W. Hsieh, C.L. Huang, H.Y. Lane, Functional impact of ABC variants on interactions between P-glycoprotein and methadone, in: L.E.M. Quintas (Ed.), PLoS One 8 (3) (2013) e59419, http://dx.doi.org/10.1371/journal.pone.0059419.
[63] V. Yuferov, O. Levran, D. Proudnikov, D.A. Nielsen, M.J. Kreek, Search for genetic markers and functional variants involved in the development of opiate and cocaine addiction and treatment, Ann. N. Y. Acad. Sci. 1187 (February) (2010) 184–207, http://dx.doi.org/10.1111/j.1749-6632.2009.05275.x Review. PubMed PMID: 20201854; PubMed Central PMCID: PMC3769182.
[64] M.K. Kim, J.H. Moore, J.K. Kim, K.H. Cho, Y.W. Cho, Y.S. Kim, M.C. Lee, Y.O. Kim, M.H. Shin, Evidence for epistatic interactions in antiepileptic drug resistance, J. Hum. Genet. 56 (January (1)) (2011) 71–76, http://dx.doi.org/10.1038/jhg.2010.151 Epub 2010 Dec 2. PubMed PMID: 21124337.
[65] S.A. McCarroll, T.N. Hadnott, G.H. Perry, P.C. Sabeti, M.C. Zody, J.C. Barrett, S. Dallaire, S.B. Gabriel, C. Lee, M.J. Daly, D.M. Altshuler, International HapMap Consortium, Common deletion polymorphisms in the human genome, Nat. Genet. 38 (January (1)) (2006) 86–92 PubMed PMID: 16468122.
[66] B.I. Drögemöller, G.E. Wright, D.J. Niehaus, R. Emsley, L. Warnich, Next-generation sequencing of pharmacogenes: a critical analysis focusing on schizophrenia treatment, Pharmacogenet. Genom. 23 (December (12)) (2013) 666–674, http://dx.doi.org/10.1097/FPC.0000000000000006 PubMed PMID: 24141736.
[67] G.E. Wright, B. Carleton, M.R. Hayden, C.J. Ross, The global spectrum of protein-coding pharmacogenomic diversity, Pharmacogenom. J. (October) (2016), http://dx.doi.org/10.1038/tpj.2016.77 [Epub ahead of print] PubMed PMID: 27779249.
[68] D.W. Deamer, M. Akeson, Nanopores and nucleic acids: prospects for ultrarapid sequencing, Trends Biotechnol. 18 (April (4)) (2000) 147–151 Review. PubMed PMID: 10740260.
[69] J.J. Kasianowicz, E. Brandin, D. Branton, D.W. Deamer, Characterization of individual polynucleotide molecules using a membrane channel, Proc. Natl. Acad. Sci. U. S. A. 93 (November (24)) (1996) 13770–13773 PubMed PMID: 8943010; PubMed Central PMCID: PMC19421.
[70] R. Ammar, T.A. Paton, D. Torti, A. Shlien, G.D. Bader, Long read nanopore sequencing for detection of HLA and CYP2D6 variants and haplotypes, F1000Research 4 (January) (2015) 17, http://dx.doi.org/10.12688/f1000research.6037.1 eCollection 2015. PubMed PMID: 25901276; PubMed Central PMCID: PMC4392832.
[71] M.R. Lindberg, S.E. Schmedes, F.C. Hewitt, J.L. Haas, K.L. Ternus, D.R. Kadavy, B. Budowle, A comparison and integration of MiSeq and MinION platforms for sequencing single source and mixed mitochondrial genomes, PLoS One 11 (December (12)) (2016) e0167600, http://dx.doi.org/10.1371/journal.pone.0167600 eCollection 2016. PubMed PMID: 27936026; PubMed Central PMCID: PMC5147911.

[72] C. Dong, P. Wei, X. Jian, R. Gibbs, E. Boerwinkle, K. Wang, X. Liu, Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies, Hum. Mol. Genet. 24 (April (8)) (2015) 2125–2137, http://dx.doi.org/10.1093/hmg/ddu733 Epub 2014 Dec 30. PubMed PMID: 25552646; PubMed Central PMCID: PMC4375422.

[73] D. Fallin, A. Cohen, L. Essioux, I. Chumakov, M. Blumenfeld, D. Cohen, et al., Genetic analysis of case/control data using estimated haplotype frequencies: application to APOE locus variation and Alzheimer's disease, Genome Res. 11 (January (1)) (2001) 143–151 PubMed PMID: 11156623; PubMed Central PMCID: PMC311030.

[74] X. Luo, L. Zuo, H. Kranzler, H. Zhang, S. Wang, J. Gelernter, Multiple OPR genes influence personality traits in substance dependent and healthy subjects in two American populations, Am. J. Med. Genet. B Neuropsychiatr. Genet. 147B (October (7)) (2008) 1028–1039, http://dx.doi.org/10.1002/ajmg.b.30701 PubMed PMID: 18213616; PubMed Central PMCID: PMC3162230.

[75] J. Mattei, L.D. Parnell, C.Q. Lai, B. Garcia-Bailo, X. Adiconis, J. Shen, et al., Disparities in allele frequencies and population differentiation for 101 disease-associated single nucleotide polymorphisms between Puerto Ricans and non-Hispanic whites, BMC Genet. 10 (August) (2009) 45, http://dx.doi.org/10.1186/1471-2156-10-45 PubMed PMID: 19682384; PubMed Central PMCID: PMC2734553.

[76] Y. Yang, M.R. Botton, E.R. Scott, S.A. Scott, Sequencing the CYP2D6 gene: from variant allele discovery to clinical pharmacogenetic testing, Pharmacogenomics 18 (May (7)) (2017) 673–685, http://dx.doi.org/10.2217/pgs-2017-0033 Epub 2017 May 4. PubMed PMID: 28470112.