



Research Paper

African Genetic Diversity: Implications for Cytochrome P450-mediated Drug Metabolism and Drug Development



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ABSTRACT

Genetic diversity is greater in Africa than in other continental populations. Genetic variability in genes encoding drug metabolizing enzymes may contribute to the high numbers of adverse drug reactions reported in Africa. We reviewed publications (1995–April 2016) reporting frequencies of known cytochrome P450 (CYP) variants in African populations. Using principal components analysis (PCA) we identified CYP alleles of potential clinical relevance with a marked difference in distribution in Africa, compared with Asian and Caucasian populations. These were CYP2B6*6, CYP2C8*2, CYP2D6*3, CYP2D6*17, CYP2D6*29, CYP3A5*6, and CYP3A5*7. We show clearly that there is greater diversity in CYP distribution in Africa than in other continental populations and identify a need for optimization of drug therapy and drug development there. Further pharmacogenetic studies are required to confirm the CYP distributions we identified using PCA, to discover uniquely African alleles and to identify populations at a potentially increased risk of drug-induced adverse events or drug inefficacy.

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1. Introduction

Modern humans have inhabited Africa for longer than any other geographic region (>200,000 years; Tishkoff et al., 2009); the processes of random mutation, meiotic recombination, and genetic drift have therefore led to the accumulation of a relatively large pool of genetic variation in Africa compared with elsewhere. Approximately 100,000 years ago, a limited number of African subgroups migrated from Africa to other continents; many genotypes are therefore found only in Africa (Tishkoff et al., 2009; Fujikara et al., 2015; Kozyra et al., 2016; Wright et al., 2016). Based on a study calculating worldwide disease burden for 21 regions, the African continent has 15.5% of the global population but carries approximately 25% of the global disease burden (Murray et al., 2012). Furthermore, the African population carries a high burden of adverse drug reactions owing partly to the use of old, poorly-optimized drugs for the treatment of parasitic infections (Prentis et al., 1988; Ampadu et al., 2016), and also possibly owing to high levels of genetic diversity resulting in a greater proportion of patients having adverse reactions to therapeutic drugs.

Drug metabolism involves a complex interplay between multiple metabolic enzymes, often with overlapping substrate specificity. One

significant class of enzymes involved in therapeutic drug metabolism is the cytochrome P450 (CYP) family. CYP1, CYP2, and CYP3 are the key sub-families involved in phase I drug metabolism. Single nucleotide polymorphisms (SNPs) in CYP genes have been extensively reported (Fujikara et al., 2015) with over 400 allelic variants recorded across the CYP1, CYP2, and CYP3 sub-families (The human cytochrome P450 allele nomenclature database <http://cypalleles.ki.se/> accessed 28 October 2016). Variations in gene sequence and protein structure give rise to alleles conferring no enzyme function, decreased enzyme function, normal enzyme function or increased enzyme function. Individuals who carry allelic variants may demonstrate one of the following metabolic phenotypes: poor metabolizers (PM; individuals with a combination of no function or decreased function alleles, and little to no enzyme activity); intermediate metabolizers (IM; individuals with a combination of normal and decreased function alleles conferring decreased enzyme activity); normal metabolizers (NM; individuals with fully functional enzyme activity) or rapid and ultra-rapid metabolizers (UM; individuals with two increased function alleles or more than two normal function alleles) (Caudle et al., 2016; Gaedigk et al., 2016). Genotype information can be used to guide appropriate therapeutic drug doses to reduce the risk of drug-induced adverse reactions in PMs or drug resistance in UMs for drugs with inactive metabolites, or of drug-induced adverse reactions in UMs and drug resistance in PMs for pro-drugs that require metabolic activation (Ingeman-Sundberg et al., 2007).

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The majority of pharmacogenetic studies are performed in Asian and Caucasian populations; data generated in African-American populations are sometimes extrapolated to represent the population of the African continent. However, the ancestry of African-Americans is predominantly from Niger-Kordofanian (~71%), European (~13%), and other African (~8%) populations (Tishkoff et al., 2009). It is therefore unlikely that the African-American population will be representative of the many different populations present in Africa. Raising awareness of the greater genetic variability in Africa and its relevance to drug metabolism and efficacy, and the requirement for further pharmacogenetic and clinical studies in African populations, has the potential to result in modifications to drug regimens that could reduce the risk of adverse drug reactions and the overall disease burden.

Here, we provide a comprehensive and up-to-date review of the frequencies of known *CYP* variants in African populations. In addition, we use principal components analysis to compare these with data from Asian and Caucasian populations. Such analyses can identify *CYP* variants demonstrating a marked difference in distribution in Africa, and African regions or populations that may be at a higher risk of drug toxicity or inefficacy. The findings of our study may help to support the future pharmacogenetic profiling of Africa which may be of relevance to both clinical therapeutics and drug research and development.

2. Methods

2.1. Search Strategy and Selection Criteria

Embase, Ovid MEDLINE and BIOSIS Previews were searched for publications (1995–April 2016) reporting *CYP* allele frequencies in African populations using the search terms 'genetic polymorphism' or 'DNA polymorphism' or 'genetic variability' and 'drug metabolizing enzyme' and 'Africa'. Following the selection of relevant publications from the initial search, PubMed was used to perform additional targeted searches to provide data on specific *CYP* alleles and/or populations for which data were minimal or absent in the initial search. Our review focused on

reports describing the frequencies of the key subfamilies involved in phase I drug metabolism (*CYP1*, *CYP2* and *CYP3*). Criteria used to include or exclude studies are summarized in Fig. 1.

2.2. Principal Components Analysis

Principal components analysis (PCA) is a statistical technique that systematically identifies underlying variables, or principal components, that best differentiate a set of data. In total, 17 *CYP* variants were selected for PCA (Supplemental Table 1, Table 1). These variants have been previously studied in a number of global populations and have a known association with significant functional changes in metabolic activity. Additional targeted searches of PubMed were performed to obtain *CYP* frequency data for Asian and Caucasian populations for comparison with African data. Search terms included a specific *CYP* variant (e.g. *CYP2B6*6*) and a specific population (e.g. Asians or Caucasians). Studies reporting *CYP* frequencies were selected only if they included >50 participants, in order to exclude misleading frequency data. A minimum of ten studies were selected for Asian populations and Caucasian populations; once sufficient data had been obtained, searches were terminated. Search results were reviewed in order of publication date (as displayed in PubMed). To ensure that data for Asian and Caucasian populations were obtained for a minimum of 50% of the 17 *CYP* variants, frequencies from multiple studies of a specific population were pooled if required. When more than one study investigated the same variant in the same population, a weighted mean of the frequencies was calculated, accounting for study participant number. For an Asian or Caucasian population to be included in the PCA, data for 50% (i.e. at least 9 out of 17) of the selected variants must have been available. As a limited number of variants have been studied in African populations, it was not always possible to obtain frequency data for 50% of the selected *CYP* variants for individual populations; data for 40% (i.e. at least 7 out of 17) of the selected variants were therefore required for the inclusion of an African population in the PCA. The selection of a lower cut-off value for African populations allowed the

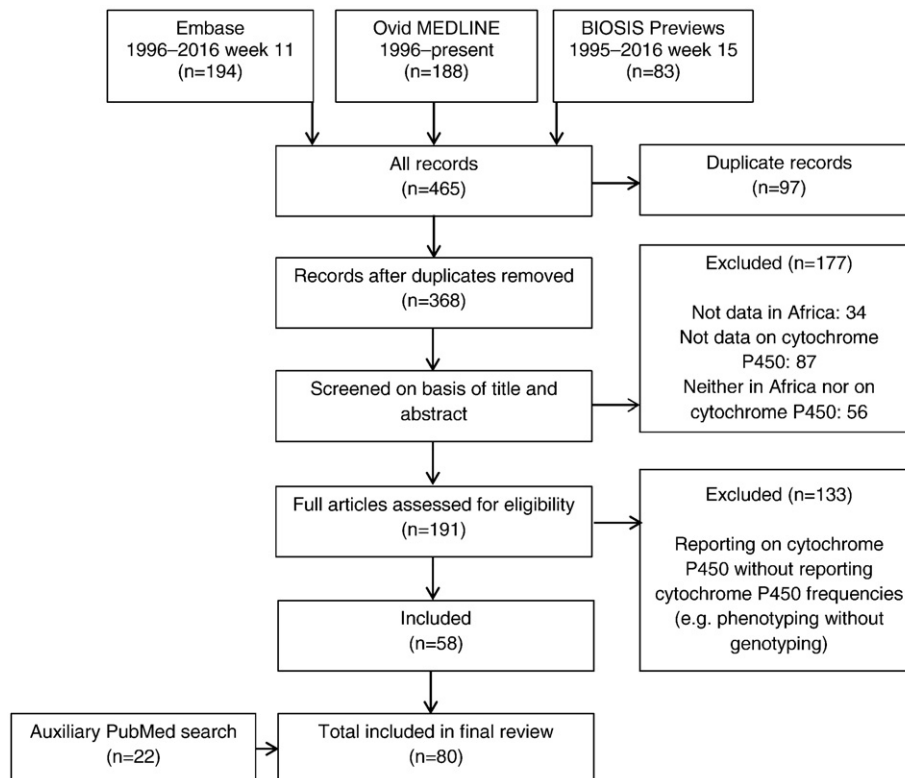


Fig. 1. Flow diagram of study selection.

Table 1
Highest and lowest frequencies (%) reported for 17 CYP allelic variants.

CYP variant	Highest reported frequency (%) (population)	Lowest reported frequency (%) (population)
CYP2B6*6	69 (Uganda)	9 (South Africa MA)
CYP2C8*2	22 (Senegal)	11 (Uganda)
CYP2C8*3	5 (Mozambique)	0 (Tanzania)
CYP2C8*4	0.6 (Tanzania)	0 (Ghana)
CYP2C9*2	19 (Morocco)	0 (multiple populations) ^a
CYP2C9*3	35 (Libya)	0 (multiple populations) ^b
CYP2C19*2	33 (Igbo)	6 (Ghana)
CYP2C19*3	7 (South Africa MA)	0 (multiple populations) ^c
CYP2D6*3	9 (San)	0 (multiple populations) ^d
CYP2D6*4	9 (San)	0 (DRC, Kenya, Ethiopia, Kikuyu, South Africa Bantu, Tanzania, Yoruba)
CYP2D6*9	0 (All populations)	0 (All populations)
CYP2D6*10	19 (South Africa Venda)	0 (DRC, Kenya, South Africa Bantu, Zimbabwe Shona)
CYP2D6*17	34 (Shona)	0 (DRC)
CYP2D6*29	29 (Igbo)	2 (San)
CYP3A5*3	92 (Morocco)	4 (Uganda)
CYP3A5*6	33 (North Sudan, Malawi Ngoni)	4 (Morocco)
CYP3A5*7	23 (Gambia)	0 (North Sudan, Ethiopia)

Data are from Supplementary Table 1, Table 2.

CYP, cytochrome P450; DRC, Democratic Republic of Congo; MA, mixed ancestry.

^a Benin, Central African Republic, Democratic Republic of Congo, Ghana, Mozambique, Namibia, Senegal, South Africa.

^b Benin, Central African Republic, Democratic Republic of Congo, Ghana, Kenya (Luo), Namibia, Nigeria, Senegal, South Africa, Sudan, Tanzania.

^c African-American, Benin, Ghana, Hausa, Igbo, Kikuyu, Luo, Maasai, San, Shona, South Africa, (Venda, Xhosa), Yoruba.

^d Central African Republic, Democratic Republic of Congo, Ethiopia, Ghana, Kenya, Namibia, Yoruba, Senegal, South Africa (Bantu, Black, Venda).

inclusion of more populations in order to gain the best accuracy. PCA was performed using SIMCA version 14.1 (MKS Data Analytics Solutions, San José, CA, USA).

3. Results

The initial literature search returned a total of 368 publications, 58 of which reported CYP frequencies in African populations. An additional 22 publications were obtained from the PubMed search (Fig. 1). CYP frequencies obtained for African populations are summarized in Supplemental Tables 1 and 2. Clinically relevant CYP substrates are detailed in Supplemental Tables 1 and 3.

Of the 74 papers cited as the basis of population-specific CYP allele frequencies in Supplemental Table 1 (Table 1), only 12 utilized genotyping methodologies capable of detecting novel sequence variations (Bains et al., 2013; Xiong et al., 2011; Chen et al., 2008; Klein et al., 2005; Park et al., 2008; Lee et al., 2006; Leathart et al., 1998; Gaedigk and Coetsee, 2008; Dickmann et al., 2001; Drögemöller et al., 2010; Wright et al., 2010; Dodgen et al., 2013). Of these, four were in Asian populations (two Chinese [Xiong et al., 2011; Chen et al., 2008]; two Korean [Park et al., 2008; Lee et al., 2006]) and eight involved Africans or African-Americans (Bains et al., 2013; Klein et al., 2005; Gaedigk and Coetsee, 2008; Leathart et al., 1998; Dickmann et al., 2001; Drögemöller et al., 2010; Wright et al., 2010; Dodgen et al., 2013).

The analysis in SIMCA used three principal components; PC1 and PC2 described most of the variance in the data set and were therefore further analyzed. PCA demonstrated distinct clustering of African, Asian and Caucasian populations (Fig. 2), with the exception of Xhosa (South Africa; positioned outside of the three clusters). The lack of available CYP frequency data for admixed populations in South Africa prevented further analysis of this result. While Caucasian and Asian populations demonstrated tight clustering, a considerably greater variability in CYP frequencies was observed across Africa as indicated by the widespread distribution of the African populations on the PCA plot

(Fig. 2). The following CYP variants showed a marked difference in distribution in African populations compared with other global populations: CYP2B6*6, CYP2C8*2, CYP2D6*3, CYP2D6*17, CYP2D6*29, CYP3A5*6 and CYP3A5*7 (Fig. 2).

Our literature review also identified reports of polymorphisms in CYP2A6 (*17) and CYP2B6 (*33–*37) that have not been reported in populations outside of Africa (Supplemental Table 1, Table 2). In addition, African populations with the highest and lowest frequencies for certain CYP alleles are presented in Table 1. Of the 17 CYP variants selected for PCA, the greatest differences in reported frequencies were observed for CYP2B6*6 (highest reported frequency: Uganda, 69%; lowest reported frequency: South Africa mixed ancestry, 9%), CYP2C9*3 (Libya, 35%; Ghana, 0%), CYP2D6*17 (Shona, 34%; Democratic Republic of Congo, 0%) and CYP3A5*3 (Morocco, 92%; Uganda, 4%). Frequencies were very similar among African populations for CYP2D6*9 (0% in all populations investigated) and CYP2C8*4 (Tanzania, 0.6%; Ghana, 0%).

4. Discussion

Our study provides a comprehensive review and statistical analysis of CYP frequency data in global populations including Africa. We have demonstrated that genetic variation is greater in African populations than in Asian and Caucasian populations, and therefore that Africa cannot be treated as a single entity in drug research and development. PCA identified allelic variants showing a marked difference in distribution in African populations which may be of clinical significance and are discussed below. Moreover, our literature review identified reports of polymorphisms in CYP genes that have not been described in populations outside of Africa.

4.1. CYP2B6*6

CYP2B6 is involved in the metabolism of numerous therapeutic drugs including antiretrovirals (efavirenz, nevirapine), chemotherapeutics (cyclophosphamide), antidepressants (bupropion), and analgesics (ketamine, methadone; Flockhart Table™ <http://medicine.iupui.edu/CLINPHARM/ddis/main-table> accessed 28 October 2016). CYP2B6*6 is a decreased function allele and has been reported at very high frequencies of 66–68% in Uganda and Zimbabwe (Supplemental Table 1, Table 2), which is approximately tenfold higher than the reported frequency in South Africa (mixed ancestry; 9%; Supplemental Table 1, Table 2). Frequencies reported in Caucasian populations are considerably lower (21–29%; Supplemental Table 1, Table 1). Sub-Saharan Africa accounts for two-thirds of the global total of new HIV infections, with 25.6 million individuals living with HIV in 2015 (World Health Organization HIV/AIDS Fact Sheet <http://www.who.int/mediacentre/factsheets/fs360/en/> accessed 28 October 2016). Many African patients who are prescribed antiretrovirals may be carriers of CYP2B6*6 and are therefore potentially at a greater risk of drug-induced adverse events than patients from other continents. Furthermore, nevirapine and efavirenz are the two most common drugs reported in all reports of drug-induced adverse events in Africa (Table 2; Ampadu et al., 2016). To reduce the frequency of adverse events reported for these (and potentially other) drugs, prospective genotyping of patients should be performed so that appropriate dose adjustments can be made, particularly given the very high frequencies of CYP2B6*6 (sometimes >50%) observed in some African populations. In resource-limited regions where prospective genotyping may not be feasible, more frequent therapeutic monitoring should be performed in patients who are prescribed CYP2B6 substrates such as efavirenz or nevirapine, and a dose adjustment could be considered according to a patient's response.

4.2. CYP2C8*2

This variant has been reported at frequencies of 10–22% in populations from Uganda, Burkina Faso, Tanzania, Zanzibar, Madagascar,

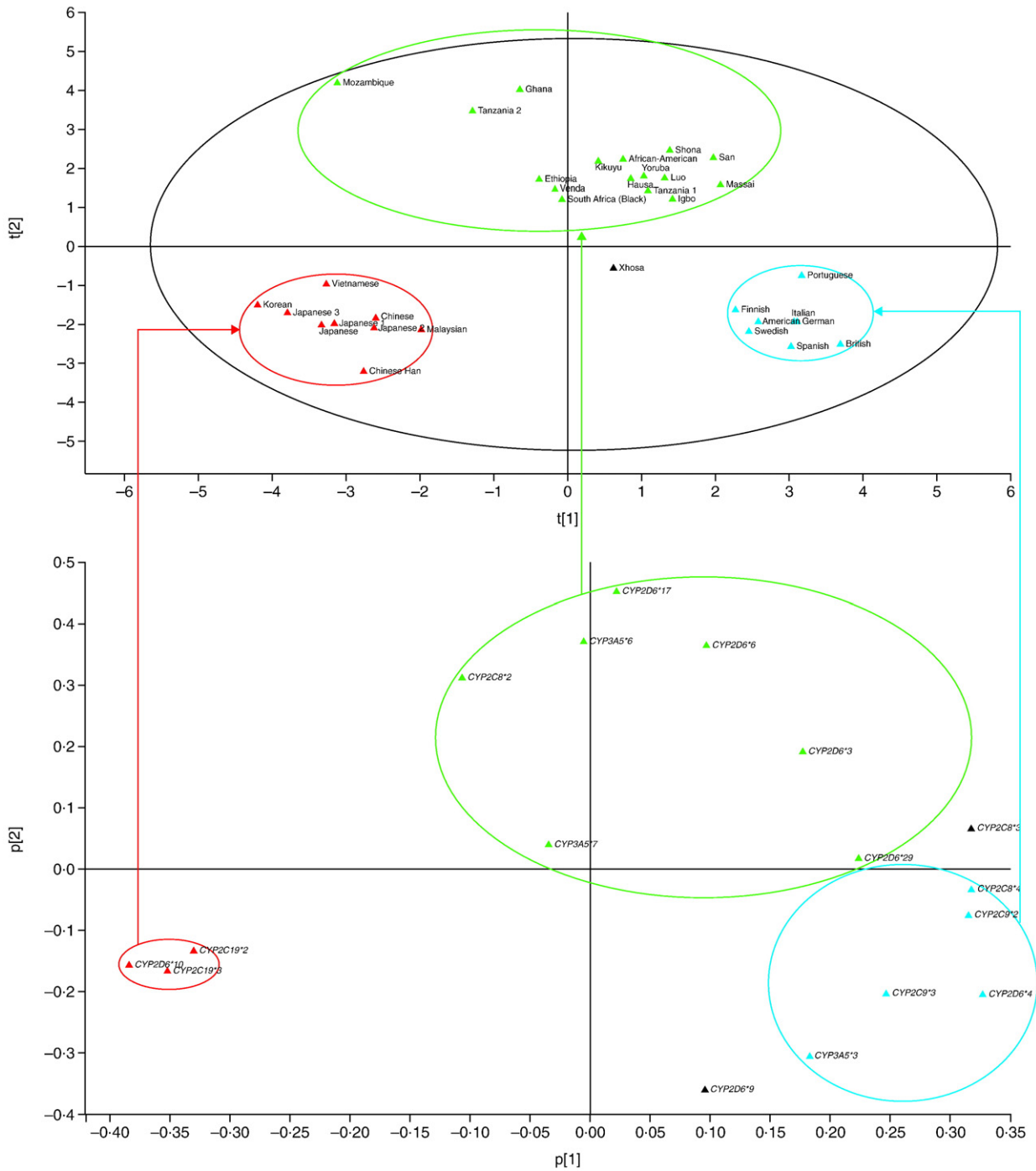


Fig. 2. Pharmacogenetics PCA cluster of global populations according to different CYP allele frequencies. PCA analysis identifies X-variables (CYP variants) that most differentiate Y-objects (African/Asian/Caucasian populations). In this analysis, the software projects the populations into clusters based on shared characteristics with respect to the frequency and/or absence of the CYP variants analyzed. Green ovals indicate African populations. Blue ovals indicate Caucasian populations. Red ovals indicate Asian populations. Kikuyu, Luo and Maasai are regional populations of Kenya. Hausa, Igbo and Yoruba are regional populations of Nigeria. San and Shona are regional populations of Zimbabwe. Xhosa is a regional population of South Africa. CYP, cytochrome P450; PCA, principal components analysis.

Mozambique, Ghana, and Senegal (Supplemental Table 1, Table 2), whereas lower frequencies (0–1.6%) have been reported in Asian and Caucasian populations (Supplemental Table 1, Table 1). CYP2C8 is involved in the metabolism of antimalarial drugs (e.g. amodiaquine; Li et al., 2002), the anti-cancer drug paclitaxel, and the anti-tuberculosis drug rifampicin (Flockhart Table™ <http://medicine.iupui.edu/CLINPHARM/ddis/main-table> accessed 28 October 2016). The *2 variant is a decreased function allele. In 275 individuals from Burkina Faso with malaria, a sixfold reduction in the intrinsic clearance of amodiaquine was observed in carriers of the mutant allele compared with individuals

who were homozygous for the wild-type allele (Parikh et al., 2007). Additionally, our literature review identified a number of CYP2C8 variants for which frequency data were not available in many African populations (Supplemental Table 1, Table 1). Malaria is the disease with the highest health burden in central and western Africa (Alessandrini and Pepper, 2014); therefore, it is concerning that there are few reports of CYP2C8 profiling in these regions. This highlights the requirement for further data collection in African populations to identify those at a potentially increased risk of drug-induced adverse events or drug inefficacy.

Table 2

The 25 most common drugs (international non-proprietary names) reported in all drug adverse events in Africa.

Drug	Total count of adverse events
Nevirapine ^a	1195
Efavirenz ^a	1099
Sulfamethoxazole and trimethoprim ^{b,c}	1068
Lamivudine	859
Stavudine	713
Zidovudine ^a	690
Ribavirin	682
Diclofenac ^a	679
Lamivudine and zidovudine ^b	634
Ciprofloxacin ^c	631
Peginterferon alfa-2a	623
Tenofovir	595
Lamivudine, nevirapine, and zidovudine ^b	565
Ethambutol, isoniazid, pyrazinamide, and rifampicin ^{a,b,d}	548
Carbamazepine ^a	546
Isoniazid ^d	523
Amoxicillin ^a	512
Insulin glargine	499
Paracetamol ^a	492
Amodiaquine and artesunate ^b	460
Ceftriaxone ^a	457
Acetylsalicylic acid ^d	441
Valproic acid ^a	439
Docetaxel ^{a,d}	426
Rifampicin ^{a,d}	423

Data are from VigiBase® (June 2016). VigiBase® is the World Health Organization Global Individual Case Safety Reports database, containing reports of adverse reactions received from member countries since 1968. Data in this table are from 33 countries in Africa (Angola, Benin, Botswana, Burkina Faso, Cameroon, Cape Verde, The Democratic Republic of the Congo, Côte d'Ivoire, Egypt, Eritrea, Ethiopia, Ghana, Guinea, Kenya, Liberia, Madagascar, Mali, Mauritius, Morocco, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, South Africa, United Republic of Tanzania, Togo, Tunisia, Uganda, Zambia and Zimbabwe). CYP, cytochrome P450.

^a Metabolized by CYP enzymes.

^b Combination therapies.

^c CYP inhibitor.

^d CYP inducer.

4.3. CYP2D6*3, CYP2D6*17 and CYP2D6*29

CYP2D6 is involved in the metabolism of >25% of registered drugs; the major classes are antidepressants, β -blockers, antipsychotics, and antiarrhythmics (Flockhart Table™ <http://medicine.iupui.edu/CLINPHARM/ddis/main-table> accessed 28 October 2016). *17 and *29 are decreased function alleles; *17 has been reported at a frequency of 34% in a Zimbabwean (Shona) population (Supplemental Table 1, Table 2), compared with very low frequencies (0–0.9%) in Asian and Caucasian populations (Supplemental Table 1, Table 1). *29 has been reported at a frequency of 20% in a Tanzanian population compared with a low frequency of 0% in Asian and Caucasian populations (Supplemental Table 1, Table 1). The CYP2D6*3 variant confers no enzyme activity and has been reported at frequencies of 0–9% in African populations (9% in a Zimbabwe San population) compared with frequencies of 0–3.5% in Asian and Caucasian populations (Supplemental Table 1, Table 1). As CYP2D6 is the main enzyme involved in converting the anti-cancer drug tamoxifen into its most potent anti-estrogenic metabolites, endoxifen and 4-hydroxytamoxifen, polymorphisms in the CYP2D6 gene may influence tamoxifen metabolism (Dean, 2016). High plasma levels of endoxifen require the presence of fully functional CYP2D6 alleles; in PMs endoxifen levels are decreased (Dean, 2016). The *3 non-functional allele, and the *17 (Masimirembwa et al., 1996) and *29 decreased function alleles of CYP2D6 may therefore potentially be associated with poor treatment outcomes for patients with breast cancer who are treated with tamoxifen in some African populations, but

further clinical studies are required to establish if treatment outcomes are affected.

To date, 109 allelic variants of CYP2D6 have been identified (The human cytochrome P450 allele nomenclature database <http://cypalleles.ki.se/> accessed 28 October 2016) and the phenotypic effects of many of these are well-characterized. Dosing guidelines have been developed by the Clinical Pharmacogenetics Implementation Consortium (CPIC) for many drug substrates of CYP2D6; for example codeine (Crews et al., 2012), fluvoxamine (Hicks et al., 2015), nortriptyline (Hicks et al., 2013; Hicks et al., 2016), and more recently, the antiemetic drugs ondansetron and tropisetron (Bell et al., 2016). Patients with an IM phenotype are recommended to receive a 25% reduction in dose, whereas those with PM or UM phenotypes are recommended to consider an alternative drug. CPIC guidelines and dosage guidelines published by the Royal Dutch Association for the Advancement of Pharmacy – Pharmacogenetics Working Group are freely available and can be accessed via The Pharmacogenomic Knowledgebase (PharmGKB; <https://www.pharmgkb.org/view/dosing-guidelines.do?source=CPIC> accessed 15 December 2016). These guidelines need to be publicized and practiced to a greater extent, especially in regions of Africa demonstrating high frequencies of the *3, *17 and *29 genotypes, or other clinically relevant CYP2D6 polymorphisms. The use of prospective genotyping to identify African patients with such genotypes would help to increase treatment efficacy and to reduce the risk of adverse events in response to drugs for which there are dosage guidelines available. In the absence of genotype data, close therapeutic drug monitoring should be carried out by clinicians prescribing drugs known to be affected by well-characterized CYP polymorphisms.

Genotype–phenotype discordance has been observed in some studies investigating CYP2D6 in African populations. In two South African populations ($n = 96$ and $n = 98$, both San Bushmen), 19% of individuals were identified as having a CYP2D6 PM phenotype when debrisoquine was used as the probe drug (Sommers et al., 1988), whereas only 4.1% were identified as having a PM phenotype when metoprolol was used (Sommers et al., 1989). A study in a Nigerian population reported a 9% prevalence of PM phenotypes of debrisoquine, whereas a later study reported no PMs of the same drug in the same study group (Mbanefo et al., 1980; Iyuni et al., 1986). Such inconsistencies have not been observed in studies of Caucasian and Asian populations, in which the prevalence of PMs is reported to be 5–10% and 0–1%, respectively, regardless of the probe drug used (Mizutani, 2003). Factors contributing to genotype–phenotype discordance in African populations may include insufficiently large study populations, poor participant compliance, lack of comprehensive laboratory methods, effects of other metabolic enzymes, environmental factors (e.g. diet), and undiscovered allelic variants.

4.4. CYP3A5*6 and CYP3A5*7

CYP3A5 is involved in the metabolism of antimalarials, antiretrovirals, and immunosuppressants (Flockhart Table™ <http://medicine.iupui.edu/CLINPHARM/ddis/main-table> accessed 28 October 2016). The functional consequences of the *6 and *7 alleles on metabolic phenotype are not fully understood; hence the CPIC guideline for the immunosuppressant drug tacrolimus recommends therapeutic drug monitoring in patients with an IM phenotype (*1/*6 or *1/*7) or a PM phenotype (*6/*6 or *7/*7) (Birdwell et al., 2015). Considering the higher frequencies of the *6 and *7 alleles reported in Africa compared with other global populations (33% in Malawi and Sudan for *6; 22.9% in Gambia for *7; 0% in Caucasians for both; Supplemental Table 1, Table 2), further investigation into the effect of these polymorphisms on metabolic activity is required.

4.5. Proposed Actions to be Taken in Africa

Myrand et al. (2008) demonstrated that metabolic activity of polymorphic CYP enzymes is driven by genotype rather than by ethnicity, and that drug or metabolite concentration data from one region may

therefore be extrapolated to other regions provided that a range of genotypes was investigated in the initial region; this would obviate the need for traditional ethnic sensitivity studies. However, the possible contribution to drug metabolism of genotypes that are unique to other regions must be considered before accepting this. 1000 Genomes Project data have shown that African-Americans have many rare pharmacogenetic variants that are not found in Europeans (Kozyra et al., 2016; Wright et al., 2016). Genome sequencing studies have indicated the difficulty of predicting allele frequencies in highly admixed populations (Wright et al., 2016). Furthermore, as the clinical utility of pharmacogenetic information continues to increase, it is likely that pharmacogenetic testing will need to replace colloquial categories of race and ethnicity in support of prescribing decision (Suarez-Kurtz et al., 2014).

Considering the great genetic variability across Africa, it is possible that there may be some as yet unidentified variations in genes coding for metabolizing enzymes that are unique to particular African subpopulations. Such undescribed, clinically relevant variants may also contribute to the high levels of drug-induced adverse events reported in Africa. Our literature review identified polymorphisms in *CYP* genes that to our knowledge have not been reported in populations outside of Africa: *CYP2A6**17 (di Iulio et al., 2009), and *CYP2B6**33–*37 (Radloff et al., 2013); Supplemental Table 1, Table 2). *CYP2A6**17 has been investigated but not found in other global populations; however, the *33–*37 variants of *CYP2B6* have been reported only in a Rwandese population and it is yet to be established if they are present in other global populations. Where novel, Africa-specific alleles have been discovered, there is a need for follow-up studies to identify their potential phenotypic effects. We also identified a number of countries or regions in Africa for which there were no available data on any *CYP* frequencies (Burundi, Cape Verde, Chad, Comoros, Djibouti, Equatorial Guinea, Eritrea, Lesotho, Mauritania, Mauritius, Niger, Somalia, Swaziland, Togo, Western Sahara and Zambia).

The majority of pharmacogenomic studies to date have either analyzed individual candidate genes or a subset of genetic variants using genotyping assays. Current sequencing technologies allow an assessment of the full spectrum of variation present in a given population, and the determination of the effect of rare genetic variation on genes of pharmacogenomic significance. Populations for which data are limited can now be studied on a scale not previously possible. Wright et al. (2016) highlight that sequencing technologies provide the best means for capturing rare variants, and that African populations have the highest number of polymorphic sites in their pharmacogenes. The intricate technological challenges associated with high-throughput sequencing that include highly repetitive DNA sequences, pseudopharmacogenes and detection of copy number variants, may be solved by massively-parallel sequencing methods (Ng et al., 2016). Massively parallel sequencing is a powerful engine for discovery of rare variants in pharmacogenes. In a study interrogating 127 variants in 13 pharmacogenes designated as clinically important by CPIC owing to their potential to alter prescribing decisions, genotypic concordance of exome and genome sequencing was 99.99% when using a clinically-validated pharmacogene microarray performed in a Clinical Laboratory Improvement Amendments–certified laboratory (Yang et al., 2016). It is therefore a reasonable first choice technology with which global populations including Africans may be screened for novel pharmacogenetic variants. Sole reliance on massively parallel, short-read sequencing may, however, prove insufficient despite the optimization of scientific protocols and the technology itself. It will be important to utilize long-read sequencing methods such as Pacific Biosystems (Mardis, 2017) to cross-validate and assess the sensitivity of short-read sequence systems. However, massively parallel sequencing technology's pharmacogenetic performance appears suitable as the first-line equipment for discovery of novel variants, and potentially, with further validation, clinical use within limits that will need to be clearly specified. Such technology has only become available in recent years, is costly and is not widely

available in Africa. Therefore, to further facilitate clinical uptake of sequencing, it will be important to address the data analysis burden and to develop a variant interpretation system that includes drug response prediction.

4.5.1. Recommendations for Pharmacogenetic Profiling in Africa

Pharmacogenetic studies often focus on established clinically relevant polymorphisms, and individuals who do not carry at least one of the defined SNPs are identified as carriers of the wild-type genotype (Čolić et al., 2014). Consequently, new SNPs that may affect therapeutic drug metabolism are not routinely identified. Researchers and clinicians should be encouraged to interrogate existing databases containing reports of unexpected responses to treatment with a given drug in African patients, e.g. VigiBase® (Shankar, 2016), with a view to linking genotype data to the reported phenotypes. A registry of unexplained adverse events in African patients would be very valuable because it could allow further pharmacogenetic investigations to be undertaken, greatly facilitating the identification of clinically relevant events that could have pharmacogenetic underpinnings. Recent research demonstrates burgeoning interest and improving infrastructure for serious adverse drug event reporting in Africa, but pharmacogenetics has not yet been integrated into case investigations (Ampadu et al., 2016). Region-specific pharmacogenetic profiling of a list of prioritized *CYP* alleles in Africa, influenced by disease burden and the extent of available data for that region, could be carried out (Alessandrini and Pepper, 2014). The integration of existing data from resources such as the 1000 Genomes project and the exome sequencing project (Kozyra et al., 2016) into future studies may help to ensure adequate profiling of clinically relevant *CYP* alleles in all regions of Africa.

Few investigations of *CYP* variants have been interrogated for ethnic-specific novel variants by pharmacogene resequencing methods. The research focus to date has been mainly on surveys of the allele frequency distributions of known polymorphisms in various national and ethnic subpopulations. Similar to the findings of Gaedigk et al. (2016), only 12 of the 74 papers cited in Supplemental Table 1 (Table 1) utilized genotyping methodologies capable of detecting novel sequence variations. Small clinical trials are poorly powered to permit causal interference of the effect of rare variants on pharmacokinetic variation; large-scale pharmacogenetic epidemiological approaches will therefore be essential for creating a catalog of functionally important rare variants. Advances in methodology, combined with coordinated multi-national collaboration, promises to fill the knowledge gaps surrounding the significance of rare variants in *CYP450*.

Clinical studies performed in Africa are often limited by small study populations (which reduces the chance of identifying pharmacokinetic outliers), use of different probe drugs for a particular *CYP* across different studies, a lack of comprehensive genotyping, and a lack of reproducibility in data obtained from the same populations. The standardization of methods in terms of study population size and probe drug panels used will help to improve the quality and reproducibility of the data generated. Furthermore, the development of local policies will help to address issues such as participant recruitment and sample collection.

4.5.2. Funding and Training for Pharmacogenetic Research in Africa

Africa is a resource-limited setting. It is important that researchers in Africa are provided with the relevant training and facilities to conduct pharmacogenetic studies. Furthermore, the cultural diversity in Africa necessitates additional considerations relating to sample collection, participant understanding of informed consent, and community engagement in order to build trust and help participants to understand the research taking place (Wright et al., 2013). The Africa Genome Variation Project (AGVP; Gurdasani et al., 2015) and The Human Heredity and Health in Africa (H3 Africa) initiative (<http://h3africa.org/> accessed 28 October 2016; Mulder et al., 2016) which has received funding from the Wellcome Trust and the US National

Institutes of Health, have been established with the aims of developing local resources, improving training and research capacity, and increasing pan-African and global research collaborations. The AGVP has assessed genetic diversity among 1481 individuals from 18 ethnolinguistic groups in Sub-Saharan Africa to facilitate further genomic research and to provide a global resource for researchers (Gurdasani et al., 2015).

The pharmaceutical industry could become more involved in raising awareness of the genetic diversity in Africa and the need for more pharmacogenetic data, and could support local clinicians and researchers. We would further propose the establishment of a consortium of pharmaceutical companies that will collaborate with local (African) and global regulatory authorities, other relevant government groups, and expert academic and clinical centers to develop recommendations for the practical use of pharmacogenetics data to optimize the treatment of patients across Africa. The difference in frequency and distribution of some alleles may also influence the outcome of clinical trials and consequently the clinical development of drugs in Africa.

5. Conclusions

In summary, our review of the published literature on *CYP* polymorphisms has clearly demonstrated and confirmed that genetic variation is greater in African populations than in Asian and Caucasian populations. The African continent cannot, therefore, be treated as a single entity in drug research and development, nor can African-American populations be considered an adequate proxy for pharmacogenetic differences across Africa. By use of PCA, we identified allelic variants showing a marked difference in distribution in African populations; however, more research is required to confirm these distributions and to identify populations at a potentially greater risk of drug-induced adverse events or drug inefficacy. We also identified *CYP* alleles that may be unique to Africa or for which data in African populations are limited, and regions where further data need to be collected. Here, we focused on *CYP* alleles, but it is likely that polymorphisms in other enzymes and transporters contribute to the diversity in drug response observed in Africa. Furthermore, the potential effect of genetic variability on drug pharmacodynamics, and the subsequent effect on drug responses in Africa, were not addressed in this study. There are also challenges in translating research findings from the laboratory to the clinical environment. The involvement of clinicians in genomic research will facilitate this translation process, helping to ensure that patients are treated with efficacious doses of therapeutic drugs. The involvement of groups such as H3Africa, AGVP and the proposed consortium of pharmaceutical companies could help to implement these changes, with an aim of reducing the burden of disease and adverse drug reactions in Africa.

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Conflicts of Interest

Iris Rajman is an employee of Novartis Institutes for Biomedical Research, Novartis, Basel, Switzerland. Laura Knapp is an employee of PharmaGenesis London, London, UK which has received funding from Novartis. Thomas Morgan is an employee of Novartis Institutes for Biomedical Research, Cambridge, MA, USA. Collen Masimirembwa is an employee of the African Institute of Biomedical Science and Technology, Harare, Zimbabwe.

Author Contributions

Iris Rajman: study design, data analysis, data interpretation and manuscript preparation.

Laura Knapp: literature search, data collection, data analysis, data interpretation, figures and manuscript preparation.

Thomas Morgan: literature search, data analysis, data interpretation, figures and manuscript preparation.

Collen Masimirembwa: study design, data analysis, data interpretation, figures and manuscript preparation.

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Appendix A. Supplementary Data

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