Priority pharmacogenetics for the African continent:

Focus on Cytochrome P450

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Summary

Countries in Africa have a high burden of communicable disease, and are experiencing an increase in non-communicable diseases due to the effects of globalization, industrialization and urbanization. The costs incurred through adverse drug reactions and non-responsiveness to therapy further aggravate the situation, and the application of pharmacogenetic principles is likely to provide some relief. Having undertaken an extensive evaluation of CYP450 reports in Africa, our objective was to map out areas of need based on regional disease burdens. The data confirms a paucity of CYP450 reports and illustrates large regions for which no population information exists. There is a dire need to address the health problems of Africa, and wide-scale pharmacogenetic profiling of these populations will add significantly to improving patient care on the continent. Priority pharmacogenetics for the African continent gives precedence to the profiling of clinically relevant pharmacogenetic biomarkers, and defines the immediate need in the context of disease burden.

Key words

Developing world; African populations; communicable disease; infectious disease; non-communicable disease; CYP450; adverse drug reaction.

Africa and its burden of disease

Africa is home to an extensive degree of diversity, as typified geographically by the extreme heights of Mount Kilimanjaro, the aridness of the Sahara desert and the richness of tropical rain forests. These diverse environmental conditions are paralleled by considerable linguistic, cultural and genetic diversity amongst its people. Globally, the continent is the most diverse linguistically with over 1,000 languages spoken vigorously by its people [101]. Since modern humans originated in Africa, its populations have had 200,000 years for genetic diversity to accumulate, which is exemplified by low level uniformity in genomic architecture and decreased levels of linkage disequilibrium [1, 2]. This diversity is attributed to centuries of migratory patterns, population admixture and natural selection in the context of diverse environmental conditions [3, 4].

From a health perspective, people in Africa are severely affected by communicable diseases and an increasing prevalence of non-communicable diseases. Although the continent only accounts for 14.5% of the global population, it carries nearly 30% of the global disease burden. Poor sanitation, lack of healthcare services and the impact of climate change are significant contributors to the high levels of mortality and productive years of life lost due to disability. Data obtained from the Institute for Health Metrics and Evaluation [102] reveals important features regarding the burden of disease in Africa (Figure 1). A heat map of the top 20 causes of disability adjusted life years (DALYs) in Africa in 2010, relative to the developing world as a whole, reveals the significant impact of communicable disease, primarily in the form of lower respiratory tract infections, diarrheal disease, malaria, HIV/AIDS and tuberculosis. The remaining DALYs are attributed to non-communicable disease, malnutrition and injury, of which the disorders of lifestyle (cardiovascular and diabetes) feature prominently.

Figure 1: Burden of disease in Africa in 2010 relative to the developing world

A.

	Developing World	Southern Africa	Central Africa	Eastern Africa	Western Africa	Northern Africa
Lower respiratory infections	1	2	2	1	2	9
Diarrhoeal disease	2	3	3	4	3	13
Ischaemic heart disease	3	14	15	17	16	1
Malaria	4	4	1	3	1	20
Stroke	5	7	13	14	13	3
HIV/AIDS	6	1	4	2	7	16
Preterm birth complications	7	5	5	5	5	8
Road injury	8	7	7	11	11	6
COPD	9	13	17	18	18	11
Low back pain	10	17	17	15	17	3
Major depressive disorders	11	11	14	13	15	2
Neonatal encephalopathy	12	7	9	8	9	12
Tuberculosis	13	6	15	7	12	17
Iron deficiency anaemia	14	11	10	12	9	10
Neonatal sepsis	15	18	12	10	4	14
Diabetes	16	19	19	19	19	5
Congenital anomalies	17	15	10	16	14	6
Protein-energy malnutrition	18	10	6	6	6	15
Meningitis	19	15	8	8	7	19
Self harm	20	20	20	20	20	18

B.

A. Heat map of the top 20 causes of Disability Adjusted Life Years (DALYs) in Africa, relative to the developing world. The darker shading indicates diseases that contribute the most to the health burden in a given region. A dataset of the top 20 causes of DALYs was obtained for each country from the 2010 Global Burden of Disease study provided by the Institute for Health Metrics and Evaluation [102], and the contribution of each cause further ranked relative to the developing world as a whole. **B.** Geographic regions of the African continent used for this investigation.

It should be noted that the distribution of disease burden varies across different regions in Africa and that the health priorities tend to differ throughout. This is evident when comparing countries in sub-Saharan Africa to those in the north, where non-communicable disease and, in particular ischemic heart disease, major depressive disorders, stroke and diabetes, are the major contributors to DALYs. Other typical examples are the distinctively high prevalence of malaria between the tropics; and the impact that HIV/AIDS in southern Africa.

It is important to note that although communicable disease is still the leading contributor to disease burden in Africa, there is a shift towards non-communicable disease, as is the case globally [5]. Furthermore, there is a change in the nature of this burden with a decrease in mortality and an increase in the management of chronic disease or disability. This anticipated shift is to a large extent the consequence of globalization, industrialization and urbanization, and the impact that these trends have on lifestyle and dietary habits.

Pharmacogenetics is a field that can make significant improvements to the management of healthcare. Although the cost and logistical implication of screening every person prior to the selection of therapy is a prohibiting factor, particularly in the developing world, there is great value in defining the degree of pharmacogenetic variability in a population. Such information not only assists in defining populations at risk for developing toxicity or experiencing reduced drug response, but also offers insight for policy in improving healthcare guidelines and advising formularies. Given the extent of disease in Africa, and coupled to the fact that it generally constitutes a resource-poor environment, there is a need to adopt innovative and cost effective methods to limit disease burden on the continent. The costs incurred through non-responsiveness to therapy and adverse drug reactions further aggravate the health status in Africa, and the application of pharmacogenetics principles is likely to provide some relief.

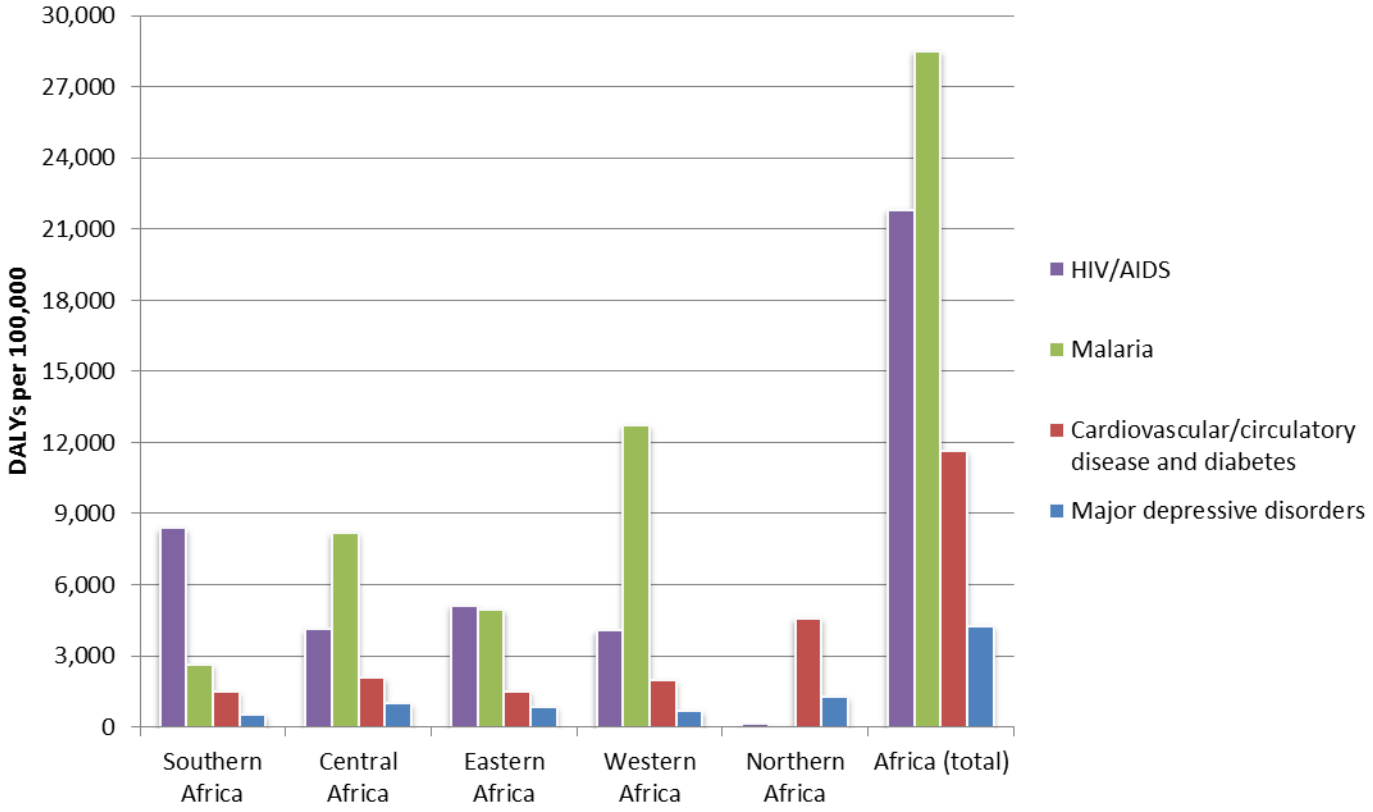
Cytochrome P450 pharmacogenetics in Africa

The cytochrome P450 (CYP450) family of enzymes is involved in the oxidative metabolism of more than 90% of prescribed drugs [6, 7]. The pharmacogenetic impact of CYP450 on phenotype is well described and results in a broad range of catalytic activities, ranging from functionally absent (poor metabolizer, PM) to markedly increased activity (ultra-rapid metabolizer, UM). Variants resulting in decreased enzyme activity are also clinically relevant and are referred to as intermediate metabolism (IM) associated alleles. CYP450 enzymes are highly polymorphic and play a central role in the therapeutic management of various conditions.

Several of the most debilitating diseases in Africa are treated with therapies that are subject to CYP450 enzyme metabolism. Well defined examples include (1) HIV/AIDS, where CYP2B6 and CYP2A6 play a role in the metabolism of efavirenz and nevirapine; (2) malaria, where CYP2C8 is important in the metabolism of amodiaquine; (3) cardiovascular/circulatory disease, and diabetes, where numerous therapeutic strategies are subject to CYP2C9 metabolism; and (4) the major depressive disorders, where CYP2C19 and CYP2D6 are central to the metabolism of several of the antidepressants. While the contribution in DALYs of each of these diseases differs from region to region (Figure 2), they make up the greater proportion of disease burden on the continent.

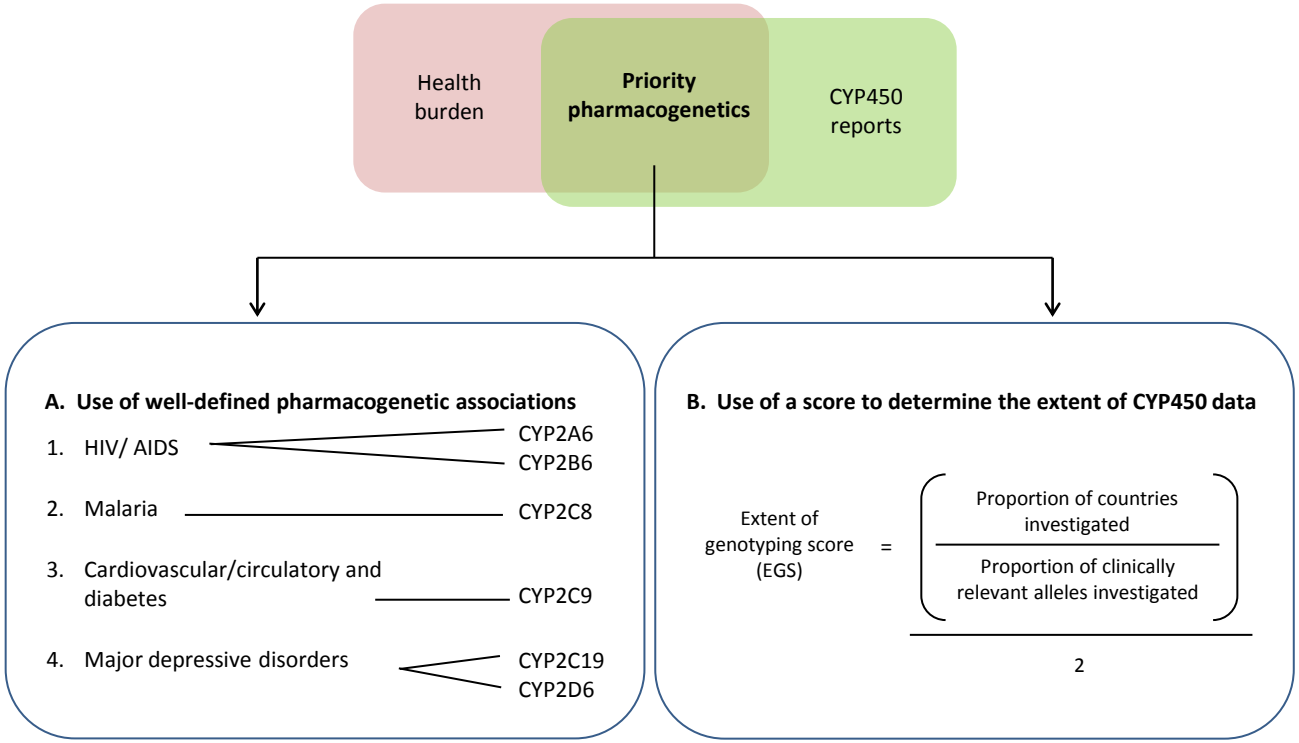
Although well documented in Caucasian and Asian populations, there are limited reports of CYP450 variability in Africans. It was hence our objective to undertake an extensive evaluation of CYP450 reports in African populations and in so doing to map out areas of need for pharmacogenetic profiling on the African continent. We recently reported an analysis of all these studies [8], and illustrated (1) the apparent lack of data, (2) poor genotype and phenotype correlations, (3) the presence of African-specific alleles, and (4) the high degree of variation amongst populations in Africa. By placing these reports in the context of the relative disease burden in Africa, further clinical relevance is provided, which forms the basis for being able to prioritize future studies, as described

Figure 2: Regional burden of disease where the management is subject to CYP450 metabolism



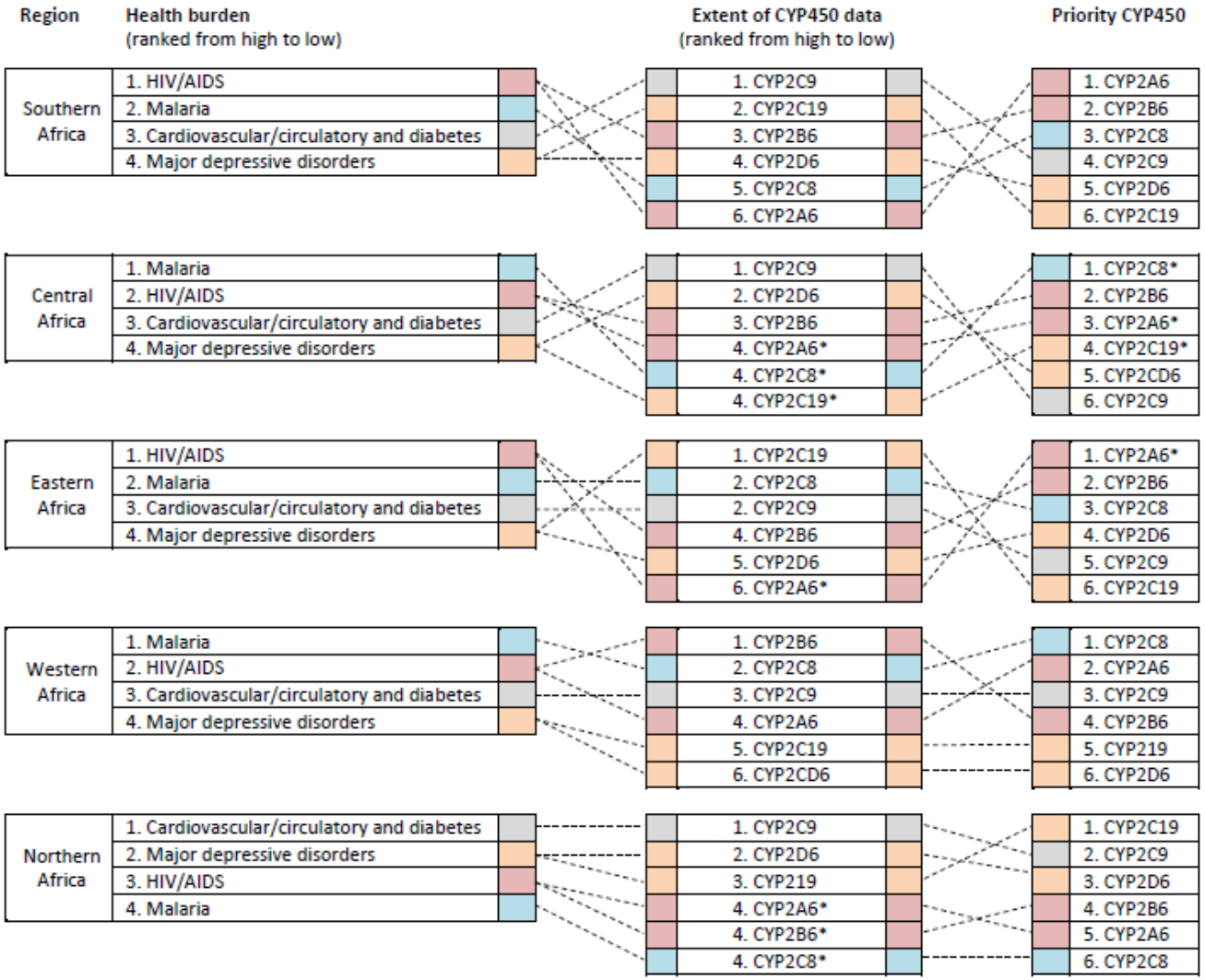
Data obtained from the 2010 Global Burden of Disease study provided by the Institute for Health Metrics and Evaluation [102].

Figure 3: Prioritizing CYP450 pharmacogenetic studies in Africa



In order to prioritize future CYP450 pharmacogenetic studies in Africa, it is necessary to identify overlapping areas of health burden and the current extent of CYP450 data in these areas. (A) Well-defined pharmacogenetic associations were identified, which include diseases that contribute significantly to the high disease burden in Africa, and for which there is evidence for the influence of CYP450 enzymes on therapeutic management. (B) The extent of genotyping score (EGS) was developed to enable the establishment of a prioritized list of genes to be investigated.

Figure 4: Derivation of CYP450 priority pharmacogenetics for the African continent



* = CYP450 genes for which no allelic frequency data exists.

herein. A schematic illustration of our approach is presented in Figure 3, and discussed in more detail in the sections to follow. Although extensive, this report is not fully comprehensive, as several other less clinically relevant enzymes of the CYP2 family, as well as CYP1 and CYP3, have not been included. Furthermore, other well described pharmacogenetic associations, such as in the case of cancer therapy and the management of pain, have not been included.

A summary of the extent to which CYP450 pharmacogenetic studies have been performed in the various regions in Africa is illustrated in Table 1. The information provided reflects the number of countries in a given region for which frequency data of clinically relevant CYP450 alleles has been reported. Shaded cells, in the form of a heat map, are representative of the number of countries in a region for which there is published CYP450 allelic frequency data (as reviewed in [8]).

Table 1: Regional breakdown of the number of countries and extent to which clinically relevant alleles of CYP450 have been investigated in Africa

Gene	Associated phenotype[a]	Clinically relevant alleles[a]	Southern Africa	Central Africa	Eastern Africa	Western Africa	Northern Africa	Africa (total)
			13[b]	10[b]	9[b]	16[b]	5[b]	53[b]
CYP2A6	PM	*2	0	0	0	1	0	1
		*4	0	0	0	1	0	1
		*5	0	0	0	1	0	1
	IM	*6	0	0	0	1	0	1
		*7	0	0	0	1	0	1
		*9	0	0	0	1	0	1
		*11	0	0	0	1	0	1
		*17	1	1	0	1	0	3
		*10; *12; *18 - *21; *23; *24; *26; *27; *35	0	0	0	0	0	0
		Extent of genotyping score (EGS):	***0.06***	***0.08***	***0.00***	***0.24***	***0.00***	***0.24***
CYP2B6[c]	PM	*28	0	0	0	0	0	0
	IM	*6	3	1	3	6	0	13
		*18	2	0	1	1	0	4
		*19	0	0	0	1	0	1
		*20	2	0	1	1	0	4
		*21	0	0	0	1	0	1
		*8; *11-16	0	0	0	0	0	0
	UM	*4	1	0	1	5	0	7
		*22	0	0	0	1	0	1
		Extent of genotyping score (EGS):	***0.25***	***0.08***	***0.30***	***0.42***	***0.00***	***0.36***
CYP2C8	PM	*5, *7, *11	0	0	0	0	0	0
	IM	*2	2	0	2	3	0	7
		*3	0	0	1	2	0	3
		*4	0	0	1	2	0	3
		*8, *14	0	0	0	0	0	0
		Extent of genotyping score (EGS):	***0.14***	***0.00***	***0.30***	***0.28***	***0.00***	***0.25***
CYP2C9	PM	*3	4	4	2	3	4	17
		*5	3	3	3	3	1	13
		*6	2	1	1	2	0	6
		*8	1	1	0	1	0	0
		*11	2	3	1	2	1	9
		*15, *25	0	0	0	0	0	0
	IM	*2	4	4	2	3	4	17
		*12, *13, *14, *16, *18, *26, *28, *30, *33	0	0	0	0	0	0
		Extent of genotyping score (EGS):	***0.33***	***0.38***	***0.31***	***0.27***	***0.52***	***0.31***
CYP2C19	PM	*2	2	0	4	3	1	10
		*3	2	0	4	3	1	10
		*4-8	0	0	0	0	0	0
	IM	*9	2	0	2	1	0	5
		*27	1	0	0	0	0	1
		*10	0	0	0	0	0	0
	UM	*17	1	0	2	0	0	3
		Extent of genotyping score (EGS):	***0.30***	***0.00***	***0.40***	***0.23***	***0.19***	***0.32***
CYP2D6	PM	*3	3	3	3	3	2	14
		*4	3	3	3	3	3	15
		*5	3	3	3	3	2	14
		*6	2	2	3	3	1	11
		*14	1	0	0	0	0	1
		*40	1	0	0	0	0	1
		*7, *8, *11-13, *15, *18-21, *31, *38, *42, *44, *56A, *62	0	0	0	0	0	0
	IM	*10	3	2	3	3	1	12
		*17	3	3	3	3	1	13
		*29	3	2	2	3	1	11
		*41	2	2	2	2	1	9
		*59	1	0	0	0	0	1
		*9, *49, *50, *54, *55, *69, *72	0	0	0	0	0	0
	UM	Functional duplications	3	3	3	3	1	13

	*53	0	0	0	0	0	0
	Extent of genotyping score (EGS):	***0.28***	***0.28***	***0.29***	***0.22***	***0.43***	***0.31***

Shading is used in the form of a heat map, which corresponds to the number of countries in a given region for which CYP450 data exists (darker shaded cells indicates more countries). The extent of genotyping score (EGS) for each region is based on the equation: (proportion of countries investigated + proportion of clinically relevant alleles investigated)/2. A zero score reflects that no allelic frequency data exists in a given region, while a score of one indicates that all countries in a region and all clinically relevant alleles of a gene have been investigated. No exclusion criteria were used and all reports in populations residing in Africa have been included, as reviewed in [8]. [a] Associated phenotype and clinically relevant alleles listed according to that provided on the CYP450 Allele Nomenclature Database webpage (http://www.cypalleles.ki.se); [b] Indicates the number of countries in each of the respective regions; [c] CYP2B6 activity is substrate specific and hence the assigned activities do not hold true for all drugs, such as the anticancer and immunosuppressant drug cyclophosphamide; IM = intermediate metabolizer associated allele; PM = poor metabolizer associated allele; UM = ultra-rapid metabolizer associated allele.

It is apparent that there is a tremendous dearth in CYP450 reports across the continent, particularly with respect to *CYP2B6*, *CYP2A6* and *CYP2C8*. In fact, even the best described CYP enzymes with pharmacogenetic implications, namely CYP2C9, CYP2C19 and CYP2D6, have been investigated in less than a third of African countries to date. In an attempt to objectively measure the extent of CYP450 data in Africa, the extent of genotyping score (EGS) was developed, which is based on two variables, namely the proportion of countries in a given region for which CYP450 allelic frequency data exists, and the proportion of clinically relevant alleles investigated in the same region. The end result is divided by two to give a score from zero to one, where zero indicates no allelic frequency data in a given region, and one indicates that all countries in a region and all the clinically relevant alleles of a gene have been investigated. The EGS does not account for the number of individuals genotyped in a given region, nor was there any criteria used to exclude studies, and hence this is merely used to give an indication of the great need for pharmacogenetic profiling.

***CYP2B6*, *CYP2A6* and the management of HIV/AIDS**

The global disease burden due to HIV/AIDS has decreased over the years with the introduction of highly active antiretroviral therapy (HAART). HIV/AIDS was however still the fifth leading cause of

global DALYs in 2010 [9]. Inter-individual variability in response to HAART is well documented, including the development of toxicity, resistance to therapy and variable levels of patient compliance [10]. Although the patient's health status and environmental conditions contribute to this varied response, it is believed that genetic variability is a major contributor in 20-95% of cases [11]. The HAART regimen includes the use of nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs). Although cytochrome P450 enzymes play a limited role in metabolizing NRTIs, they do contribute to variability in response to NNRTIs and PIs. The best described enzyme in this context is *CYP2B6*, which plays a pivotal role in phase I metabolism of efavirenz and to a lesser extent nevirapine [12-17]. Decreased metabolism of these NNRTIs results in increased plasma levels and related side effects, including neurotoxicity in the case of efavirenz, and hepatotoxicity when nevirapine is used. Both of these scenarios contribute to reduced patient compliance and the emergence of resistant strains of the virus.

The CYP2B6 enzyme is expressed in several organs and tissues, including the liver, nasal mucosa, trachea, lung and brain [18]. Over 30 variants of the *CYP2B6* gene have been described, and of the clinically relevant alleles, one has been confirmed to be associated with a PM phenotype (*28), 15 with an IM phenotype and two with UM activity [103]. The enzyme, however, does display substrate specificity [18], which tends to complicate interpretation. The most investigated variants are those linked to the IM associated allele *CYP2B6**6 (primarily 516G>T), which has been demonstrated in several reports to be present at higher frequencies in African (median of 37%) [8] than in Caucasian (14-27%) and Asian populations (10-21%) [18]. This was first illustrated by Klein *et al*. (2005) [19], where it was revealed that *CYP2B6**6 was present in 32% of African-Americans and 47% of individuals in a Ghanaian population. These findings were also the first to provide a reasonable explanation for the previously reported 20% decrease in clearance of efavirenz in HIV patients of African origin [20]. It has subsequently been demonstrated in several studies that the presence of IM

and/or PM associated alleles of *CYP2B6* are reliable predictors of increased plasma concentrations of efavirenz and nevirapine [21-23].

Although CYP2B6 is responsible for the primary metabolism of efavirenz, variability in response to the drug is also mediated via CYP2A6. The latter enzyme has been reported to oxidize efavirenz and, together with CYP2B6, may prove to be a good predictor of response to the drug [24, 25]. *CYP2A6* is predominantly expressed in the liver, and 23 clinically relevant genetic variants of this enzyme have been described, five of which are associated with a PM phenotype and 18 with an IM phenotype [104]. When assessing the allelic frequencies of 11 *CYP2A6* variants in 105 Ghanaians [26], it was observed that the PM associated allele *4A, and the IM associated allele *9, were present at frequencies of 1.9% and 5.7%, respectively. The remaining alleles investigated (*CYP2A6*2*, **5*, **6*, **7*, **8* and **11*) were all absent in this cohort. In 65 HIV infected individuals of the same population, the African-specific IM associated allele *CYP2A6*17* was reported at a frequency of 12%, while *CYP2A6*9* was present in 5% of individuals [27].

Given the fact that variants of *CYP2B6* and *CYP2A6* may be used to inform efavirenz dosing guidelines, it is surprising that these enzymes have not been adequately profiled in African populations (Table 1). *CYP2B6* has been investigated to a greater extent, particularly the IM associated allele *6, which has been the primary allele investigated over the years. Variants of this allele have been reported in 13 African countries, six of which are on the western coastline of Africa (Ghana, Guinea, Ivory Coast, Nigeria, Senegal and Sierra Leone). Furthermore, each of the eight populations investigated in this region are of Bantu/Niger-Congo origin. Notably, the mean frequency for the presence of *CYP2B6**6 is 44%, which is higher than the 33% reported for the remaining African countries [8]. Therefore, given the risk of NNRTI toxicity and the high frequency of this allele alone, we believe it reasonable to suggest that clinicians and health authorities should consider the role of IM and PM associated alleles of *CYP2B6* when using efavirenz and nevirapine in populations

that reside in western Africa. To complement this, there would be great value in investigating the true extent of variation with respect to loss of function alleles and to work towards developing population or regional specific pharmacogenetic tests.

Both of the studies reporting *CYP2A6* variability in Africans used Ghanaians as the study population. This highlights the necessity and the opportunity afforded to investigate the degree of variability of this enzyme in other African populations. Moreover, based on the handful of *CYP2A6* genotyping reports in populations of African origin, there appears to be a distinctive population specific distribution of allelic frequencies. *CYP2A6*17*, for example, has been reported to be present in neither Caucasian nor Asian populations, while it is present in African and African-American populations at a frequency of 9.5-25% [24, 25, 28-30]. When comparing joint frequencies of PM and IM associated alleles, the same authors have demonstrated that populations of African origin have twice the allelic frequency of non-functional alleles (approximately 20%) than in Caucasians, while this is at least half that observed in Asian populations. Another important allele with a population specific distribution is *CYP2A6*4*, which is characterized by a *CYP2A6* whole gene deletion and hence is associated with a PM phenotype. While *CYP2A6*4* has been reported at a frequency of 1.2-1.9% in Africans, African-Americans and Caucasians, it has been observed in up to 24% of individuals in Asian populations [26, 31].

The lack of *CYP2A6* profiling in several African populations, coupled with the highly valuable information derived from the handful of studies mentioned above, provides for an exciting opportunity to investigate the degree of variability in these populations. Such information would provide several benefits, including (1) determination of the extent of altered metabolism in various regions on the continent, (2) the discovery of novel variants that may further elucidate the variability in response to antiretrovirals, and (3) the development of improved dosing guidelines by health authorities.

CYP2C8 and the management of malaria

Despite the fact that progress has been made towards the worldwide eradication of malaria in the past two decades, disease incidence and death due to this disease are still a major health burden in the developing world. Today, approximately half of the world's population is at risk of contracting malaria, and according to the World Health Statistics 2013 report [105], it can be estimated that there are over 200 million people living with malaria globally. In Africa, malaria is the largest contributor to DALYs, and it predominates as the highest health burden in central and western Africa (Figure 2). The disease is ranked third in eastern Africa, fourth in southern Africa, but has a negligible contribution to DALYs in northern Africa (Figure 1A).

The treatment of uncomplicated malaria, as recommended by the WHO, follows one of four regimens that all include artemisinin. In one of these combinations, amodiaquine is recommended, which is metabolized almost exclusively by CYP2C8. As expected, decreased activity of CYP2C8 will result in an accumulation of amodiaquine, resulting in hepatotoxicity and agranulocytosis. It has also been reported recently [32] and confirmed by an independent group [33] that decreased CYP2C8 activity may result in a selective pressure resulting in the development of resistance to therapy by the *Plasmodium* parasite.

The frequency of three clinically relevant alleles of *CYP2C8* has to date been reported in seven African populations (Table 1). These alleles (*CYP2C8*2*, **3* and **4*) are associated with decreased enzyme activity, and are present at frequencies of up to 24% in the populations investigated [8]. Although there is limited global data on the allelic distribution of *CYP2C8*, it is apparent that distinctive inter-ethnic differences do exist, particularly with respect to *CYP2C8*2* and **3*; where the former is often referred to as the African-specific allele and the latter as Caucasian-specific. *CYP2C8*2* is present at frequencies of 10-24% in African populations and at less than 1% in

Caucasians. In contrast, while *CYP2C8*3* is present in 0-2.1% in Africans, it can be as frequent as 17% in Caucasians.

When investigating the extent of *CYP2C8* profiling in Africa (Table 1), it should be noted that no pharmacogenetic data exists for populations residing in central Africa, even though malaria is the highest contributor to burden of disease in most countries in this region (Figure 2). This indicates the absolute necessity for *CYP2C8* genotyping studies in this region. Furthermore, in eastern Africa, where malaria is ranked second in terms of its contribution to DALYs, only two countries have been investigated. In western Africa, where malaria is the highest contributor to disease burden, three countries have allelic frequency data for *CYP2C8*, namely Burkina Faso [34, 35], Ghana [36-38] and Senegal [32]. The fact that *CYP2C8* data exists for populations in a mere seven African countries, does not allow for sufficient inference of the distribution of alleles across the continent. Likewise, it is difficult to estimate the impact the enzyme may have in areas where malaria is a major problem, as is the case in central Africa where no published *CYP2C8* pharmacogenetic data exists. It should also be noted that no populations have been investigated for any of the PM associated alleles of this enzyme, namely *CYP2C8*5*, **7* and **11*, which may well have additional pharmacogenetic implications.

CYP2C9 and the management of cardiovascular/circulatory disease and diabetes

Cardiovascular/circulatory disease is the highest contributor to the health burden due to non-communicable disease in the developing world. Furthermore, in light of the rapid rate of globalization, industrialization and urbanization in Africa, an increase in the incidence of cardiovascular disease is underway. Leading risk factors for cardiovascular disease are diabetes, hypertension and obesity. In the developing world, ischemic heart disease and stroke are the primary cardiovascular/circulatory diseases that contribute to DALYs. In 2010, these were ranked third and fifth, respectively, in terms of their health burden [5]. The impact of these diseases is most

pronounced in northern Africa, where ischemic heart disease is the highest ranked cause of DALYs, with stroke occupying third position (Figure 1A). Diabetes also features prominently in this region and is the fifth highest contributor to disease burden. While cardiovascular/circulatory disease and diabetes may not appear to be as great a health concern as communicable diseases in Africa, it is acknowledged that they will be major contributors to DALYs in years to come.

Altered drug response in the management of cardiovascular disease is related to variability in the pharmacogenetic make-up of a patient [39]. In this context, CYP2C9 is probably the best described enzyme with pharmacogenetic implications. It is involved in the metabolism of the anticoagulant warfarin (together with VKORC1); the angiotensin II blocker, losartan; and several hypoglycemic agents, including glyburide, glimepiride and glipizide. CYP2C9 is abundantly expressed in the liver and more than 50 alleles have been assigned to date [106], of which three are associated with a PM phenotype and more than 10 with intermediate metabolism.

Decreased enzymatic activity of CYP2C9 is well described, and the most frequently investigated alleles are *CYP2C9*2* and **3*. In Caucasian populations, the frequency of these alleles ranges from 8% to over 20% [40]. However, in Asians, the same alleles are mostly absent or present at frequencies of not more than 3% [41, 42]. A similar range in distribution of allelic frequencies is observed across the African continent, but with the noteworthy finding that populations in northern Africa appear to harbor *CYP2C9*2* and **3* alleles at higher frequencies with a mean of 11.8%, whereas the mean allelic frequency in sub-Saharan Africa is 0.6% [8], similar to that in African-Americans [42, 43]. This indicates clear inter-ethnic differences in the distribution of *CYP2C9*2* and **3*, as the populations in northern Africa are mostly of Afro-Asiatic origin, and the majority of those who reside in sub-Saharan Africa are of Bantu/Niger-Congo origin. This is particularly interesting to note, given the distinctively contrasting health burden in northern Africa, where non-communicable disease (primarily ischemic heart disease, stroke and diabetes) is prominent. Since CYP2C9 plays a role in the metabolism of

arachidonic acid, a modulator of vascular tone and cardiovascular homeostasis, the increased frequency of defective alleles may be postulated to contribute to the high levels of cardiovascular disease in northern Africa, a concept supported previously by other groups [44, 45]. Based on a handful of studies reported to date, *CYP2C9*8* may also be regarded as a contributor to decreased drug clearance in populations of African origin, where it is present at allelic frequencies ranging from 1-12% [8, 46, 47]. When investigating the variants that contribute to variability in warfarin metabolism, it has been reported that this allele, together with CYP2C9*5, *6 and *11, may contribute to up to 30% of the variability in Africa-Americans [48].

CYP2C9 pharmacogenetics has been investigated in 20 African countries, making it the most studied CYP450 enzyme on the continent. Four of the five countries in northern Africa have *CYP2C9*2* and **3* data, and only Algeria has been investigated for any of the other clinically relevant alleles (*CYP2C9*5* and **11*), albeit in a small sample size of 30 [49]. Western Africa is the least profiled region, followed by southern, eastern and central Africa. Considering that *CYP2C9*2* and **3* are present at low frequencies in populations of Bantu/Niger-Congo origin, it is recommended that the pharmacogenetics of this enzyme be investigated in more detail in the sub-Saharan region. This is supported by the recent report by Perera and colleagues [50], in which a novel and independent predictor of warfarin metabolism in Africa Americans was identified by means of genome wide association studies. The single nucleotide polymorphism, which is present in the CYP2C cluster, was significantly associated with the need for a dose reduction of warfarin.

***CYP2C19*, *CYP2D6* and the management of major depressive disorders**

The health burden of depression increased by 61% from 1990 to 2010, and with over 350 million people being affected by one or other major depressive disorder, it is today regarded as the leading cause of disability worldwide [5]. On the African continent, major depressive disorders are of primary concern in northern Africa, where they were ranked as the second highest contributor of DALYs in

2010 (Figure 1A). Relative to the top 20 DALYs in the developing world, these disorders were also ranked in the top 15 in the remaining four African regions.

Treatment of depression requires a multifaceted approach and several options are available for antidepressant pharmacotherapy. These include the tricyclic antidepressants (TCAs), such as amitriptyline and clomipramine; the selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, sertraline and citalopram; and the norepinephrine and dopamine reuptake inhibitors (NDRIs), such as bupropion. Several antidepressants used to treat these disorders are either metabolized exclusively by CYP2C19 or CYP2D6, or by a combination of these enzymes. The relevance of these enzymes is exemplified by the recent publication of guidelines for their use in defining TCA dosage regimens [51].

CYP2C19 is expressed mostly in the liver and duodenum [52, 53], and over 30 allelic variants have been assigned to date. Of the clinically relevant alleles, seven are associated with PM, three with IM, and one has an UM phenotype [107]. *CYP2C19*2* and **3* are PM alleles and both have been investigated in numerous African populations (Table 1). The former allele is present in Africans at frequencies ranging from 6-33%, while *CYP2C19*3* is reported at frequencies of 6% or less [8]. This is consistent with studies reported in African-Americans [54]. To our knowledge, studies for the remaining five PM associated alleles (*CYP2C19*4* to **8*) have not yet been reported in populations of African origin. Of the three IM associated alleles, *CYP2C19*9* and *CYP2C19*27* have only been reported in five African countries. The recently reported UM associated allele, *CYP2C19*17*, has been investigated in three African populations and is present at frequencies of 10-17% [55-57], which is consistent with that seen in Caucasian populations, but is far higher than the <1% in Asians [54].

CYP2D6 is subject to greater variability than *CYP2C19*, and is the best described CYP450 enzyme to date. Even though it only constitutes about 4% of the total amount of expressed CYP450, the enzyme

metabolizes approximately 25% of commonly prescribed drugs [58]. *CYP2D6* is the most highly polymorphic CYP450 gene, and over 100 alleles have been assigned [108]. To date, this variability has only been investigated in 15 of the 53 countries in Africa (Table 1), and there seems to be sufficient evidence to suggest that a unique and population-specific distribution of *CYP2D6* alleles exists on the continent [8].

Historically, the PM associated alleles *CYP2D6*3*, **4*, **5* and **6* have been the most extensively investigated, and of these, *CYP2D6*4* and **5* are present most frequently, while *CYP2D6*3* and **6* are seldom observed [59]. *CYP2D6*5* is characterized by a deletion of the entire gene, and is present at similar frequencies of less than 10% in all populations, with the exception of a handful of African populations, where the allele may be as frequent as 19% [8]. In contrast, *CYP2D6*4*, which is present in approximately 20% of Caucasians, is less frequent in all African [8], African-American (<8%), and Asian populations (0-2.7%) [59].

Population-specific distributions are evident when investigating alleles associated with a *CYP2D6* IM phenotype. *CYP2D6*10* is reported to be present in 11- 70% of Asian populations [41, 60], while it is seldom higher than 10% in populations of African origin [8, 59]. *CYP2D6*17* and *CYP2D6*29*, among others, are accepted as being African-specific alleles, and are present at frequencies of up to 34% and 29%, respectively . In contrast, these alleles are either absent or occur at frequencies of less than 1% in Asian and Caucasian populations [59]. The reduced function allele *CYP2D6*41* has also been investigated in Africa, and, on average, is harbored by 10% of African individuals [8, 59].

CYP2D6 ultra-rapid metabolism is characterized by multiplication of functional alleles, such as *CYP2D6*1xN* and *CYP2D6*2xN*, where the 'N' denotes the presence of a multiplication. *CYP2D6*2xN* is more frequently reported in African populations and, furthermore, appears to be present at higher frequencies in populations of Afro-Asiatic origin, such as in Algeria (28%) and Ethiopia (10-16%) [56,

61-63]. These duplications are less frequent in populations of Bantu/Niger-Congo origin, such as the Yoruba in Nigeria (4%), Gabonese (4%) and Ghanaians (1.6%) [49, 64, 65]. The notion that *CYP2D6*2xN* is an Afro-Asiatic specific allele is further supported by the fact that these alleles also occur commonly in Saudi-Arabia (20%), and to a lesser extent in African-Americans, Europeans and Asians (<2.5%) [41, 66, 67].

Neither *CYP2C19*, nor *CYP2D6* have been investigated adequately in African populations. Pharmacogenetic variability of *CYP2C19* is yet to be studied in central Africa, and only the Egyptian population has been investigated in northern Africa (Table 1). Regions in which the most countries have been profiled are eastern Africa (four out of nine) and western Africa (three out of sixteen). Notably, seven of the 11 clinically relevant alleles of *CYP2C19* have yet to be investigated in African populations. With regard to *CYP2D6*, it is apparent that there are a greater number of reports across the continent, with three countries in each of the regions having been investigated to date. However, given that several novel alleles have been reported in recent years, there are still in excess of 20 clinically relevant alleles for which there is no frequency data in any of the African countries.

Priority pharmacogenetics and cytochrome P450

Priority pharmacogenetics is an expression we introduce to describe the need and urgency for pharmacogenetic profiling. In this context, we use it to identify the need for CYP450 studies in populations residing on the African continent. By correlating data available in the public domain on pharmacogenetic studies with the burden of disease in Africa, we wish to highlight several areas of need and prioritize CYP450 pharmacogenetics accordingly. As outlined in Figure 4, a rank was assigned with regard to health burden for each region of Africa based on the contribution to DALYs (Figure 2), and is listed in order from highest to lowest. Similarly, the amount of published CYP450 data was ranked from highest to lowest, and is based on the extent of genotyping scores (Table 1) for each of the clinically relevant CYP450 enzymes discussed. By correlating these rankings, it was

possible to derive a priority listing of CYP450 studies that need to be performed, as indicated in the far-right column of Figure 4.

Given the extent to which HIV/AIDS and malaria contribute to the health burden in sub-Saharan Africa, it might have been expected that pharmacogenetic studies would have focused on CYP450 enzymes that are relevant to the management of these diseases. However, this has not been the case. For example, in southern Africa, the region that suffers the highest HIV/AIDS health burden (Figure 2), the pharmacogenetics of CYP2A6 and CYP2B6 has been poorly investigated (EGS of 0.06 and 0.25, see Table 1). In central Africa, malaria contributes approximately double the disease burden of HIV/AIDS (Figure 2), yet the impact of *CYP2C8* variability on amodiaquine metabolism has not been investigated in any of the central African populations. Furthermore, in the context of HIV/AIDS therapy in this same region, *CYP2A6* and *CYP2B6* have received the least attention of the enzymes investigated. Finally, malaria has its greatest impact in western Africa, and in the context of amodiaquine metabolism, *CYP2C8* has only been investigated in three of the 16 countries in this region, and only three of the eight clinically relevant alleles have been genotyped, resulting in an EGS of 0.28 (Table 1).

The situation in northern Africa is strikingly different, where communicable diseases constitute the greatest burden. In fact, the joint contribution of cardiovascular/circulatory disease, diabetes and major depressive disorders to DALYs is two to three times that of any other region in sub-Saharan Africa (Figure 2). Pharmacogenetic studies in northern Africa have paralleled that of the disease burden, in that *CYP2C9* has been most investigated (which addresses drug metabolism for cardiovascular/circulatory disease and diabetes), followed by *CYP2C19* and *CYP2D6* (which address major depressive disorders). No pharmacogenetic data exists for *CYP2A6*, *CYP2B6* and *CYP2C8*, and this may be expected given the low to negligible impact of HIV/AIDS and malaria in this region.

From a priority point of view, it is evident that immediate attention should be given to investigating the pharmacogenetics of CYP2A6, CYP2B6 and CYP2C8 in sub-Saharan Africa. The order in which these enzymes are to be prioritized is dependent on the health burden and the extent to which genotyping has been done (Figure 4). Bearing in mind that the burden of non-communicable disease is rapidly increasing as a result of industrialization, urbanization and globalization; continued investigation of the enzymes influencing drug response in the management of these diseases should be seen as a priority across the African continent. With regard to northern Africa, although pharmacogenetic investigations reflect the disease burden in this region, the extent of pharmacogenetic profiling of clinically relevant alleles is nonetheless inadequate, as portrayed in the EGS of 0.53 for *CYP2C9*; 019 for *CYP2C19*; and 0.43 for *CYP2D6* (Table 1). Priority should hence be given to intensifying studies on *CYP2C19*, since it is the least profiled in this context, followed by *CYP2C9* and *CYP2D6*, which would enable further understanding of drug tolerance in the context of cardiovascular/circulatory disease and diabetes, and major depressive disorders, respectively.

Concluding remarks

Our evaluation of the extent to which CYP450 has been investigated reveals, and confirms, a paucity of CYP450 allelic frequency data for populations residing on the African continent. In several regions, no pharmacogenetic data exists, and even where there is data, the limited extent to which clinically relevant alleles have been investigated is alarming. Only in northern and western Africa is it possible to see a parallel between the CYP450 enzymes investigated and disease burden. Reasons for the lack of data are likely to be manifold, with limited resources, expertise and infrastructure being of central importance. This has led us to propose the need to prioritize CYP450 profiling in the various regions identified. There is a dire need to address the health problems of Africa, and wide-scale pharmacogenetic profiling of these populations will contribute to improving patient care on the continent. Having said this, several aspects should be considered when deciding on the manner in which pharmacogenetic studies are to be performed. The ultimate goal would be to profile a critical

mass of individuals in each population in order to determine the true extent of CYP450 variation across the continent. Furthermore, the local populations and the research community would benefit from study designs that extend well beyond simply screening for previously defined alleles. This includes whole gene sequencing in combination with a variety of substrate-based phenotypic studies in order to fully elucidate the activity and functional properties of multiple CYP450 enzymes. In so doing, the extent of CYP450 variation will be determined, novel alleles will be discovered, and the outcome of well-defined genotype-phenotype relationships will enable one to measure of contribution of environmental factors, such as diet and the impact of climate, on drug response. Comprehensive data of this nature may also be used to extrapolate or give indication to variants that may be important in other populations with African ancestry around the world, such as in African Americans.

Given the high degree of genetic diversity in African populations, there is an expectation that genome-wide sequencing will reveal a multitude of previously undescribed variants and functional characteristics of CYP450. This may also be the case in populations unique to the African continent, including and amongst others, the San population of 100,000 individuals that is dispersed throughout Southern Africa, and the mixed ancestry populations in the Western Cape region of South Africa (referred to as the Cape Coloureds), for which a unique genomic architecture in *CYP2D6* has already been indicated [68].

The logistics of implementing pharmacogenetics in clinical practice has proven to be a challenge worldwide, and it is expected that this would be an even greater challenge in the larger part of Africa, where the extent of healthcare and laboratory services is limited. In order to realize the true benefits of pharmacogenetic practices, novel and innovative approaches will be needed, such as the introduction of simplified, low cost medical devices that can be used to screen for population specific variants at the point-of-care.

Future perspective

By comprehensively evaluating the extent to which CYP450 has been investigated in Africa, and by introducing for the first time the notion of “priority pharmacogenetics”, our intention is to highlight an urgent need for region-specific pharmacogenetic profiling in Africa. In so doing, numerous opportunities will be identified for relevant research endeavors, and it is hoped this may prompt the establishment of an extensive collaborative effort across the continent, in which multiple groups will have the opportunity to not only to assist in enriching CYP450 data, but also to build capacity in pharmacogenetic research. We further envisage several additional benefits to such an initiative, of which the most important include (1) the discovery of novel, clinically relevant and population specific variants, given the known genotype-phenotype discordance observed for several of the CYP450 enzymes in Africa populations [8]; (2) further elucidation of the effects of specific variations (existing and novel) that alter drug response, thereby refining pharmacogenetic associations that could inform decisions made by health authorities in various African countries; and (3) reducing adverse drug reactions and the impact that non-response has on the burden of disease and the management of healthcare on the continent, including the emergence of resistant microorganisms.

Executive summary

Africa and its burden of disease

- Africa is characterized by great diversity, which is exemplified geographically and in the variability observed amongst its people (linguistically and genetically)
- The burden of disease in Africa is similar to that of the developing world as a whole, with the burden of communicable disease being the primary cause of mortality and life years lost due to disability
- The extent and nature of disease burden varies from region to region in Africa, and hence the health priorities are not consistent throughout

Cytochrome P450 pharmacogenetics in Africa

- CYP450 enzymes play a significant role in metabolizing pharmacotherapeutic agents used to treat many of the top 20 contributors to disease burden, including HIV/AIDS, malaria, ischemic heart disease, stroke, diabetes and major depressive disorders
- The genotyping score (EGS) for each CYP450 enzyme reveals the extent of inadequate profiling on the continent

***CYP2B6*, *CYP2A6* and the management of HIV/AIDS**

- The pharmacogenetics of *CYP2B6* and *CYP2A6*, which metabolize key NNRTIs antiretrovirals like efavirenz and nevirapine, has been inadequately profiled in African populations
- *CYP2B6* has been best profiled in western Africa and it appears that the frequency of the decreased metabolism allele *CYP2B6**6 may be higher in this region than in the rest of Africa

***CYP2C8* and the management of malaria**

- Decreased enzyme activity of *CYP2C8* results in an accumulation of the antimalarial amodiaquine, the associated adverse drug reactions, and a selective pressure resulting in resistance to drug therapy by the *Plasmodium* parasite
- Malaria is the major contributor to disease burden in central Africa, yet there are no reports of *CYP2C8* profiling in this region

***CYP2C9* and the management of cardiovascular/circulatory disease and diabetes**

- *CYP2C9* plays a central role in the metabolism of the anticoagulant warfarin, the angiotensin II blocker losartan, and several hypoglycemic agents, such as glyburide, glimepiride and glipizide
- Although *CYP2C9* has been investigated in more countries than any other CYP450 enzyme, the extent to which clinically relevant alleles have been studied is poor

***CYP2C19*, *CYP2D6* and the management of major depressive disorders**

- *CYP2C19* and *CYP2D6* metabolize, either exclusively or in combination, numerous antidepressants, including amitriptyline, fluoxetine and bupropion
- There are still more than 20 clinically relevant alleles of *CYP2C19* and *CYP2D6* for which no data exists in African populations

Priority pharmacogenetics in view of Cytochrome P450

- By taking into account disease burden and the extent of CYP450 pharmacogenetic data in Africa, we were able to derive a regional-specific and prioritized list of CYP450 enzymes that require investigation.

Concluding remarks

- A paucity of CYP450 allelic frequency data on the continent was confirmed, and there are several regions for which no pharmacogenetic data exists
- The greatest discrepancy between disease burden and clinically relevant CYP450 pharmacogenetic studies is in sub-Saharan Africa
- Wide-scale pharmacogenetic profiling of African populations will contribute significantly to improving patient care on the continent

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References

1. Tishkoff SA, Kidd KK: Implications of biogeography of human populations for 'race' and medicine. *Nature genetics* 36(11 Suppl), S21-27 (2004).
2. Tishkoff SA, Verrelli BC: Role of evolutionary history on haplotype block structure in the human genome: implications for disease mapping. *Current opinion in genetics & development* 13(6), 569-575 (2003).
3. Sirugo G, Hennig BJ, Adeyemo AA *et al.*: Genetic studies of African populations: an overview on disease susceptibility and response to vaccines and therapeutics. *Human genetics* 123(6), 557-598 (2008).
4. Tishkoff SA, Reed FA, Friedlaender FR *et al.*: The genetic structure and history of Africans and African Americans. *Science* 324(5930), 1035-1044 (2009).
5. Murray CJ, Vos T, Lozano R *et al.*: Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380(9859), 2197-2223 (2012).
6. Zhou SF: Polymorphism of human cytochrome P450 2D6 and its clinical significance: part II. *Clinical pharmacokinetics* 48(12), 761-804 (2009).
7. Zhou SF: Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clinical pharmacokinetics* 48(11), 689-723 (2009).
8. Alessandrini M, Asfaha S, Dodgen TM, Warnich L, Pepper MS: Cytochrome P450 pharmacogenetics in African populations. *Drug metabolism reviews* 45(2), 253-275 (2013).
9. Ortblad KF, Lozano R, Murray CJ: The burden of HIV: insights from the GBD 2010. *AIDS*, (2013).
10. Mahungu TW, Johnson MA, Owen A, Back DJ: The impact of pharmacogenetics on HIV therapy. *International journal of STD & AIDS* 20(3), 145-151 (2009).
11. Kalow W, Tang BK, Endrenyi L: Hypothesis: comparisons of inter- and intra-individual variations can substitute for twin studies in drug research. *Pharmacogenetics* 8(4), 283-289 (1998).
12. Jamshidi Y, Moreton M, Mckeown DA *et al.*: Tribal ethnicity and CYP2B6 genetics in Ugandan and Zimbabwean populations in the UK: implications for efavirenz dosing in HIV infection. *The Journal of antimicrobial chemotherapy* 65(12), 2614-2619 (2010).
13. Penzak SR, Kabuye G, Mugyenyi P *et al.*: Cytochrome P450 2B6 (CYP2B6) G516T influences nevirapine plasma concentrations in HIV-infected patients in Uganda. *HIV medicine* 8(2), 86-91 (2007).
14. Tsuchiya K, Gatanaga H, Tachikawa N *et al.*: Homozygous CYP2B6 *6 (Q172H and K262R) correlates with high plasma efavirenz concentrations in HIV-1 patients treated with standard efavirenz-containing regimens. *Biochemical and biophysical research communications* 319(4), 1322-1326 (2004).
15. Wang J, Sonnerborg A, Rane A *et al.*: Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenetics and genomics* 16(3), 191-198 (2006).
16. Rotger M, Telenti A: Optimizing efavirenz treatment: CYP2B6 genotyping or therapeutic drug monitoring? *European journal of clinical pharmacology* 64(4), 335-336 (2008).
17. Haas DW, Mootsikapun P, Ruxrungtham K, Podzamczer D: Clinical perspectives on human genetic screening to prevent nevirapine toxicity. *Personalized medicine* 9(7), 773-782 (2012).
18. Zanger UM, Klein K: Pharmacogenetics of cytochrome P450 2B6 (CYP2B6): advances on polymorphisms, mechanisms, and clinical relevance. *Frontiers in genetics* 4, 24 (2013).
19. Klein K, Lang T, Saussele T *et al.*: Genetic variability of CYP2B6 in populations of African and Asian origin: allele frequencies, novel functional variants, and possible implications for anti-HIV therapy with efavirenz. *Pharmacogenetics and genomics* 15(12), 861-873 (2005).

20. Csajka C, Marzolini C, Fattinger K *et al.*: Population pharmacokinetics and effects of efavirenz in patients with human immunodeficiency virus infection. *Clinical pharmacology and therapeutics* 73(1), 20-30 (2003).
21. Heil SG, Van Der Ende ME, Schenk PW *et al.*: Associations between ABCB1, CYP2A6, CYP2B6, CYP2D6, and CYP3A5 alleles in relation to efavirenz and nevirapine pharmacokinetics in HIV-infected individuals. *Therapeutic drug monitoring* 34(2), 153-159 (2012).
22. Rotger M, Tegude H, Colombo S *et al.*: Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clinical pharmacology and therapeutics* 81(4), 557-566 (2007).
23. Zanger UM, Klein K, Saussele T, Blievernicht J, Hofmann MH, Schwab M: Polymorphic CYP2B6: molecular mechanisms and emerging clinical significance. *Pharmacogenomics* 8(7), 743-759 (2007).
24. Di Iulio J, Fayet A, Arab-Alameddine M *et al.*: In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. *Pharmacogenetics and genomics* 19(4), 300-309 (2009).
25. Kwara A, Lartey M, Sagoe KW, Kenu E, Court MH: CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS* 23(16), 2101-2106 (2009).
26. Gyamfi MA, Fujieda M, Kiyotani K, Yamazaki H, Kamataki T: High prevalence of cytochrome P450 2A6*1A alleles in a black African population of Ghana. *European journal of clinical pharmacology* 60(12), 855-857 (2005).
27. Kwara A, Lartey M, Sagoe KW, Rzek NL, Court MH: CYP2B6 (c.516G-->T) and CYP2A6 (*9B and/or *17) polymorphisms are independent predictors of efavirenz plasma concentrations in HIV-infected patients. *British journal of clinical pharmacology* 67(4), 427-436 (2009).
28. Fukami T, Nakajima M, Yoshida R *et al.*: A novel polymorphism of human CYP2A6 gene CYP2A6*17 has an amino acid substitution (V365M) that decreases enzymatic activity in vitro and in vivo. *Clinical pharmacology and therapeutics* 76(6), 519-527 (2004).
29. Nakajima M, Yoshida R, Fukami T, Mcleod HL, Yokoi T: Novel human CYP2A6 alleles confound gene deletion analysis. *FEBS letters* 569(1-3), 75-81 (2004).
30. Swart MS, M.; Wonkam, A.; Kannemeyer, L.; Chin'ombe, N.; Dandara, C. : CYP1A2, CYP2A6, CYP2B6, CYP3A4 and CYP3A5 polymorphisms in two Bantu-speakiing populations from Cameroon and South Africa: Implications for global pharmacogenetics. *Curr Pharmacogen and Pers Med* 10, 11 (2012).
31. Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF: Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics* 14(9), 615-626 (2004).
32. Paganotti GM, Gramolelli S, Tabacchi F *et al.*: Distribution of human CYP2C8*2 allele in three different African populations. *Malaria journal* 11, 125 (2012).
33. Cavaco I, Martensson A, Froberg G, Msellem M, Bjorkman A, Gil JP: CYP2C8 status of patients with malaria influences selection of Plasmodium falciparum pfmdr1 alleles after amodiaquine-artesunate treatment. *The Journal of infectious diseases* 207(4), 687-688 (2013).
34. Paganotti GM, Gallo BC, Verra F *et al.*: Human genetic variation is associated with Plasmodium falciparum drug resistance. *The Journal of infectious diseases* 204(11), 1772-1778 (2011).
35. Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL: Amodiaquine metabolism is impaired by common polymorphisms in CYP2C8: implications for malaria treatment in Africa. *Clinical pharmacology and therapeutics* 82(2), 197-203 (2007).
36. Adjei GO, Kristensen K, Goka BQ *et al.*: Effect of concomitant artesunate administration and cytochrome P4502C8 polymorphisms on the pharmacokinetics of amodiaquine in Ghanaian children with uncomplicated malaria. *Antimicrobial agents and chemotherapy* 52(12), 4400-4406 (2008).

37. Kudzi W, Dodoo AN, Mills JJ: Characterisation of CYP2C8, CYP2C9 and CYP2C19 polymorphisms in a Ghanaian population. *BMC medical genetics* 10, 124 (2009).
38. Rower S, Bienzle U, Weise A *et al.*: Short communication: high prevalence of the cytochrome P450 2C8*2 mutation in Northern Ghana. *Tropical medicine & international health : TM & IH* 10(12), 1271-1273 (2005).
39. Myburgh R, Hochfeld WE, Dodgen TM, Ker J, Pepper MS: Cardiovascular pharmacogenetics. *Pharmacology & therapeutics* 133(3), 280-290 (2012).
40. Zhou K, Donnelly L, Burch L *et al.*: Loss-of-function CYP2C9 variants improve therapeutic response to sulfonylureas in type 2 diabetes: a Go-DARTS study. *Clinical pharmacology and therapeutics* 87(1), 52-56 (2010).
41. Lim JS, Chen XA, Singh O *et al.*: Impact of CYP2D6, CYP3A5, CYP2C9 and CYP2C19 polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients. *British journal of clinical pharmacology* 71(5), 737-750 (2011).
42. Zhou SF, Zhou ZW, Huang M: Polymorphisms of human cytochrome P450 2C9 and the functional relevance. *Toxicology* 278(2), 165-188 (2010).
43. Johnson JA, Gong L, Whirl-Carrillo M *et al.*: Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clinical pharmacology and therapeutics* 90(4), 625-629 (2011).
44. Delozier TC, Kissling GE, Coulter SJ *et al.*: Detection of human CYP2C8, CYP2C9, and CYP2J2 in cardiovascular tissues. *Drug metabolism and disposition: the biological fate of chemicals* 35(4), 682-688 (2007).
45. Yasar U, Bennet AM, Eliasson E *et al.*: Allelic variants of cytochromes P450 2C modify the risk for acute myocardial infarction. *Pharmacogenetics* 13(12), 715-720 (2003).
46. Perera MA, Gamazon E, Cavallari LH *et al.*: The missing association: sequencing-based discovery of novel SNPs in VKORC1 and CYP2C9 that affect warfarin dose in African Americans. *Clinical pharmacology and therapeutics* 89(3), 408-415 (2011).
47. Scott SA, Jaremko M, Lubitz SA, Kornreich R, Halperin JL, Desnick RJ: CYP2C9*8 is prevalent among African-Americans: implications for pharmacogenetic dosing. *Pharmacogenomics* 10(8), 1243-1255 (2009).
48. Cavallari LH, Langaee TY, Momary KM *et al.*: Genetic and clinical predictors of warfarin dose requirements in African Americans. *Clinical pharmacology and therapeutics* 87(4), 459-464 (2010).
49. Sistonen J, Fuselli S, Palo JU, Chauhan N, Padh H, Sajantila A: Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales. *Pharmacogenetics and genomics* 19(2), 170-179 (2009).
50. Perera MA, Cavallari LH, Limdi NA *et al.*: Genetic variants associated with warfarin dose in African-American individuals: a genome-wide association study. *Lancet* 382(9894), 790-796 (2013).
51. Hicks JK, Swen JJ, Thorn CF *et al.*: Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. *Clinical pharmacology and therapeutics* 93(5), 402-408 (2013).
52. Phillips EJ, Mallal SA: Pharmacogenetics and the potential for the individualization of antiretroviral therapy. *Current opinion in infectious diseases* 21(1), 16-24 (2008).
53. Shimada T, Yamazaki H, Mimura M, Inui Y, Guengerich FP: Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *The Journal of pharmacology and experimental therapeutics* 270(1), 414-423 (1994).
54. Scott SA, Sangkuhl K, Stein CM *et al.*: Clinical pharmacogenetics implementation consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clinical pharmacology and therapeutics* 94(3), 317-323 (2013).
55. Drogemoller BI, Wright GE, Niehaus DJ *et al.*: Characterization of the genetic profile of CYP2C19 in two South African populations. *Pharmacogenomics* 11(8), 1095-1103 (2010).

56. Ikediobi O, Aouizerat B, Xiao Y, Gandhi M, Gebhardt S, Warnich L: Analysis of pharmacogenetic traits in two distinct South African populations. *Human genomics* 5(4), 265-282 (2011).
57. Miura J, Obua C, Abbo C, Kaneko S, Tateishi T: Cytochrome P450 2C19 genetic polymorphisms in Ugandans. *European journal of clinical pharmacology* 65(3), 319-320 (2009).
58. Zanger UM, Raimundo S, Eichelbaum M: Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn-Schmiedeberg's archives of pharmacology* 369(1), 23-37 (2004).
59. Crews KR, Gaedigk A, Dunnenberger HM *et al.*: Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for codeine therapy in the context of cytochrome P450 2D6 (CYP2D6) genotype. *Clinical pharmacology and therapeutics* 91(2), 321-326 (2012).
60. Bradford LD: CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* 3(2), 229-243 (2002).
61. Aklillu E, Herrlin K, Gustafsson LL, Bertilsson L, Ingelman-Sundberg M: Evidence for environmental influence on CYP2D6-catalysed debrisoquine hydroxylation as demonstrated by phenotyping and genotyping of Ethiopians living in Ethiopia or in Sweden. *Pharmacogenetics* 12(5), 375-383 (2002).
62. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M: Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. *The Journal of pharmacology and experimental therapeutics* 278(1), 441-446 (1996).
63. Sistonen J, Sajantila A, Lao O, Corander J, Barbujani G, Fuselli S: CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenetics and genomics* 17(2), 93-101 (2007).
64. Griese EU, Asante-Poku S, Ofori-Adjei D, Mikus G, Eichelbaum M: Analysis of the CYP2D6 gene mutations and their consequences for enzyme function in a West African population. *Pharmacogenetics* 9(6), 715-723 (1999).
65. Panserat S, Sica L, Gerard N, Mathieu H, Jacqz-Aigrain E, Krishnamoorthy R: CYP2D6 polymorphism in a Gabonese population: contribution of the CYP2D6*2 and CYP2D6*17 alleles to the high prevalence of the intermediate metabolic phenotype. *British journal of clinical pharmacology* 47(1), 121-124 (1999).
66. Leathart JB, London SJ, Steward A, Adams JD, Idle JR, Daly AK: CYP2D6 phenotype-genotype relationships in African-Americans and Caucasians in Los Angeles. *Pharmacogenetics* 8(6), 529-541 (1998).
67. Masimirembwa CM, Hasler JA: Genetic polymorphism of drug metabolising enzymes in African populations: implications for the use of neuroleptics and antidepressants. *Brain research bulletin* 44(5), 561-571 (1997).
68. Gaedigk A, Coetsee C: The CYP2D6 gene locus in South African Coloureds: unique allele distributions, novel alleles and gene arrangements. *European journal of clinical pharmacology* 64(5), 465-475 (2008).

Internet references

101. www.ethnologue.org, accessed 5 August 2013
102. www.healthmetricsandevaluation.org/gbd, accessed 10 August 2013
103. www.cypalleles.ki.se/*CYP2B6*.htm, accessed 12 September 2013
104. www.cypalleles.ki.se/*CYP2A6*.htm, accessed 12 September 2013
105. www.who.int/gho/publications/world_health_statistics/2013/en

106. www.cypalleles.ki.se/*CYP2C9*.htm, accessed 12 September 2013
107. www.cypalleles.ki.se/*CYP2C19*.htm, accessed 12 September 2013
108. www.cypalleles.ki.se/*CYP2D6*.htm; accessed 12 September 2013

Reference annotations

** Of considerable interest

1. Murray CJ, Vos T, Lozano R *et al.*: Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet* 380(9859), 2197-2223 (2012).
    - An extensive overview of the burden of disease globally.
2. Alessandrini M, Asfaha S, Dodgen TM, Warnich L, Pepper MS: Cytochrome P450 pharmacogenetics in African populations. *Drug metabolism reviews* 45(2), 253-275 (2013).
    - An up to date review of all CYP450 genotypic and phenotypic reports in populations in Africa, which highlights the distribution of clinically relevant alleles and the apparent genotype-phenotype discordance.
3. Sistonen J, Fuselli S, Palo JU, Chauhan N, Padh H, Sajantila A: Pharmacogenetic variation at *CYP2C9*, *CYP2C19*, and *CYP2D6* at global and microgeographic scales. *Pharmacogenetics and genomics* 19(2), 170-179 (2009).
    - Allows the reader to contextualise the variation of relevant CYP450 pharmacogentics in Africa to the rest of the world.

* Of interest

1. Mahungu TW, Johnson MA, Owen A, Back DJ: The impact of pharmacogenetics on HIV therapy. *International journal of STD & AIDS* 20(3), 145-151 (2009).
    - Describes the role of pharmacogeneitcs in treating HIV/AIDS.
2. Parikh S, Ouedraogo JB, Goldstein JA, Rosenthal PJ, Kroetz DL: Amodiaquine metabolism is impaired by common polymorphisms in *CYP2C8*: implications for malaria treatment in Africa. *Clinical pharmacology and therapeutics* 82(2), 197-203 (2007).
    - Report that defines the implication of *CYP2C8* metabolism of amodiaquine in treating malaria.
3. Zhou SF, Zhou ZW, Huang M: Polymorphisms of human cytochrome P450 2C9 and the functional relevance. Toxicology 278(2), 165-188 (2010).
    - Review of *CYP2C9* and its role in pharmacogenetics.
4. Hicks JK, Swen JJ, Thorn CF *et al.*: Clinical Pharmacogenetics Implementation Consortium guideline for *CYP2D6* and *CYP2C19* genotypes and dosing of tricyclic antidepressants. *Clinical pharmacology and therapeutics* 93(5), 402-408 (2013).
    - Recent publication of guidelines for the use of *CYP2C19* and *CYP2D6* in the management of depression.