



Pharmacogenomics for infectious diseases in sub-Saharan Africa: Successes and opportunities



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The rate of mortality in developing countries due to communicable disease remains alarmingly high. The leading contributors to disease burden in these regions are the so-called “big three”, namely HIV/AIDS, TB, and malaria. The global prevalence of these diseases is over 250 million, and it should be noted that 71%, 28% and 88% of these cases, respectively, occur in sub-Saharan Africa alone (World Health Organization). African countries are continuously struggling to contain infectious diseases, and there is a need for governments to commit resources not only for treatment, but also towards research and development aimed at innovative approaches.

Only 25–60% of patients respond positively to drug therapies (Squassina et al., 2010). This is due to variability in phenotypic and environmental factors, and up to 95% of these variations may be determined by genetic factors alone (Ross et al., 2012). A negative response ranges from the occurrence of adverse drug reactions (ADRs) to complete non-responsiveness to treatment. It has been reported that ADRs account for up to 18% of deaths in hospitalized patients in Norway and the UK (Mouton et al., 2015). In another UK based study, 6.5% of hospital admissions were reported to be due to ADRs (Pirmohamed et al., 2004). And in the USA, fatal ADRs occur in 0.32% of patients, ranking it among the six leading causes of death (Squassina et al., 2010). Given the increased mortality and cost associated with ADRs, they are regarded as major public health and economic problems worldwide. Pharmacogenomics is believed to be a key technology which could alleviate the costs associated with ADR-related hospitalizations and improve dosage optimization. It uses genetic information to predict the efficacy and safety of drugs, and aims specifically to improve treatment decision-making.

In the context of HIV treatment, neuropathy, neutropenia and lipodystrophy are major ADRs reported in Africa (Nwokike, 2008). These ADRs are often exacerbated by the prevalence of malnutrition, TB and anaemia in HIV patients (Subbaraman et al., 2007). In urban regions in Kenya, combination antiretroviral therapy (cART) related ADRs were found in 40.6% of patients, and peripheral neuropathy in 20.7% (Hawkins et al., 2007). Efavirenz (EFV) is used as the basis for first-line cART in most parts of Africa. However, this drug of choice often results in multiple side effects, including rash, hepatotoxicity, lipodystrophy and neuropsychiatric toxicity – all of which are directly

linked to elevated EFV plasma concentrations (Hawkins et al., 2007; Abah et al., 2015). While therapeutic concentrations are recommended to be between 1000–4000 ng/ml, concentrations <1000 ng/ml are indicative of a failure in therapy, and plasma concentrations >4000 ng/ml often result in ADRs. This variability is linked to polymorphisms in the *CYP2B6* gene. More specifically, the *c.516G>T* and *c.983T>C* variants have been shown to be predictive of reduced enzyme activity and higher EFV plasma concentrations (Swart et al., 2015). *CYP2B6* genotyping offers an apparent benefit in this setting, and it has been demonstrated that decreasing the daily dose from 600 mg/day to 200 mg/day in patients homozygous for the *CYP2B6**6 minor allele would be adequate to maintain therapeutic levels and reduce ADRs (Nemauro et al., 2012; Dhoro et al., 2015). The predictive accuracy can be further improved in patients heterozygous for *CYP2B6**6 when phenotypic data such as gender and body weight are also considered (Nemauro et al., 2012; Dhoro et al., 2015). The use of novel, cost-effective technologies to detect relevant *CYP2B6* genotypes may therefore become part of routine clinical practice in due course, for which benefits in terms of cost reduction for government and patient compliance are evident.

Pharmacogenomics studies have also influenced our understanding of TB treatment in Africa. Both pulmonary and extra-pulmonary TB are treated with a combination of drugs including isoniazid, rifampicin, pyrazinamide and ethambutol for an initial two months, followed by isoniazid and rifampicin for a further four months. ADRs including isoniazid hepatotoxicity and peripheral neuropathy have been documented, and polymorphisms in *NAT2*, *CYP2E1*, *GSTMI*, and *GSTTII* have been associated with isoniazid dependent hepatotoxicity (Ramachandran and Swaminathan, 2012). Genetic testing for these polymorphisms can identify fast, intermediate and slow acetylators. Slow acetylators are at risk of hepatotoxicity, while fast acetylators may be at risk of developing drug resistance and treatment failure under current treatment regimens in South Africa (Wilkins et al., 2011). Polymorphisms in the *SLCO1B1* gene are common in South Africa and are often associated with increased rifampicin clearance and higher recommended treatment dosing (Chigutsa et al., 2011).

Artemisinin combination therapies, including mefloquine, lumefantrine, amodiaquine and chlorproguanil are used for the treatment of malaria. Various enzymes (*CYP2A6*, *CYP2C8*, *CYP3A5*, *UGT1A9*, *UGT2B7* and *ABCB1*) play a role in the metabolism and transport of the above-mentioned drugs (Roederer et al., 2011). Amodiaquine is predominantly metabolized by *CYP2C8* and a decrease in enzyme activity increases the bio-availability of the drug, which in

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turn results in ADRs such as hepatotoxicity and agranulocytosis. The *CYP2C8*2*3* and **4* alleles are associated with decreased enzyme activity in several African populations (Alessandrini and Pepper, 2014). However, it has been confirmed that the *CYP2C8*2* allele is the most frequent variant to cause ADRs in this region (Cavaco et al., 2005; Kudzi et al., 2009). This same allele is found to be associated with drug-resistance in malaria patients (Paganotti et al., 2011). Although it may not be economically feasible to offer pharmacogenetically informed anti-malarial treatment, determining variant allele frequencies in a population group will be helpful in devising population based amodiaquine therapy (Gil, 2012). Table 1 summarizes ADRs often reported in patients being managed for HIV, TB and malaria, as well as important ADME genes involved in determining drug response.

Several reports demonstrate the potential benefits of applying pharmacogenomics in clinical practice. In addition to the routine use of drug resistance testing for the discussed infectious diseases, the adoption of pharmacogenomics practices to decrease the burden of disease is believed to offer cost savings, reduce the occurrence of ADRs and limit the emergence of drug resistance. However, pharmacogenomics is yet to live up to its expectations in both the developed and developing worlds. The reasons for this are manifold and require both practical and clinical considerations. In many parts of sub-Saharan Africa, access to basic healthcare services is limited, and hence implementation of pharmacogenomics into routine clinical practice may seem a step too far. Even in the more developed centers, the necessary facilities, infrastructure and expertise will need considerable attention and committed resources to achieve reliable and consistent pharmacogenomics service offerings.

From a clinical point of view, pharmacogenomics testing and corresponding guidelines have recently emerged for several drugs and conditions. The Clinical Pharmacogenetics Implementation Consortium (CPIC, cpicpgx.org) is prominent in this area with the vision of assisting the translation of clinically relevant and evidence based pharmacogenetic testing into routine clinical practice. Of the over 33 guidelines provided, only four focus on drugs for infectious diseases (abacavir, atazanavir, PEG-interferon alpha-2a and 2b). The primary reason for this is that there is still a paucity of clinically relevant data, and hence it has to date not been possible to demonstrate concrete association and clinical value. This is further complicated by the fact that many people in developing countries suffer

multiple acute and chronic comorbidities, and are hence managed with several medications simultaneously. This further complicates interpretation and the predictive power that pharmacogenomics may provide.

The opportunity of today lies in our ability to unlock genomic information that will allow for improved prediction of drug response. Over 300 proteins are involved in the absorption, distribution, metabolism and excretion (ADME) of drugs, and only a few of these have been investigated in terms of genomic architecture and how variation may influence drug response. This is in part due to the limitations of past technology and our ability to process and interpret large multivariate datasets. Next generation sequencing, and more specifically whole genome sequencing (WGS) and whole exome sequencing (WES) are promising new approaches that exploit the genome for this very purpose. As a case in point, Mizzi et al. identified 16,487 novel variants in 231 ADME related genes using WGS (Mizzi et al., 2014). These variants may have functional implications because of their presence in exons and regulatory regions. Another study utilized WES to identify genetic determinants of clopidogrel response variability in cardiac patients (Price et al., 2012). Similarly, *CYP3A4* defective variants were identified with WES that provide basis for paclitaxel treatment modifications (Apellániz-Ruiz et al., 2015). Although WES is becoming a more cost-effective, accessible and attractive strategy for many research groups, it is limited by its exclusion of the increasingly appreciated untranslated regions of the genome. In the context of pharmacogenomics, targeted genomic sequencing of the ADME genes may be a more rational approach to consider. An increasing number of research institutions are gearing up to store and process “big data”, and genomic data is likely to be the catalyst for most of these endeavors. In order to make the most of these opportunities, it will be important to design well-controlled clinical studies with rational endpoints and detailed phenotypic descriptions. It is only with this in mind that we will be able to unravel the intricacies that complicate our ability to explain and improve the predictive power of pharmacogenomics. The overwhelming task of analyzing and interpreting large multivariate datasets in such instances should be addressed from the outset, and the necessary capacity should be built to allow for meaningful interpretation of data. While cautiously optimistic, few can argue that next generation sequencing technologies will impact significantly on the field of pharmacogenomics and more broadly, precision medicine.

Table 1
Adverse drug reactions reported in HIV, TB and malaria patients.

Infectious disease	Drug	Important ADME genes	ADRs
HIV/AIDS	Efavirenz ^a	<i>CYP2B6</i> , <i>CYP2A6</i> , <i>ABCB1</i> , <i>NR1I3</i> , <i>UGT2B7</i> , <i>CYP3A5</i> , <i>CYP3A4</i>	CNS toxicity ^a , drug hypersensitivity rash ^{b, c} , elevated ALT and AST levels ^b , Stevens-Johnson syndrome, drug induced hepatitis, neuropsychiatric effects (depression, delusions), abnormal dreams, dizziness, drowsiness, nausea, headache, fatigue, neural tube defects, gynaecomastia ^c
TB	Isoniazid	<i>NAT2</i> , <i>CYP2E1</i> , <i>GSTMI</i> , <i>GSTTI</i> , <i>SLCOB1B1</i>	Peripheral neuropathy, convulsions, skin rash, psychosis, hepatitis, arthralgia, sleepiness lethargy, anaemia ^d
	Rifampicin		Gastrointestinal (abdominal pain, nausea, vomiting), osteomalacia, pseudomembranous colitis, hepatitis, pseudo-adrenal crisis, generalised cutaneous reactions, acute renal failure thrombocytopenic purpura, haemolytic anaemia, flu like symptoms ^d
	Pyrazinamide		Arthralgia, cutaneous reactions hepatitis, sideroblastic anaemia gastrointestinal ^d
	Ethambutol		Retrolbulbar neuritis, generalised cutaneous reactions arthralgia, peripheral neuropathy, hepatitis (very rare), visual impairment ^d
Malaria	Mefloquine, lumefantrine, amodiaquine, chlorproguanil	<i>CYP2C8</i> , <i>CYP3A4</i> , <i>CYP2C19</i> , <i>CYP2B6</i> , <i>CYP2A6</i> , <i>UGT1A9</i> , <i>UGT2B7</i>	Nausea, anorexia, vomiting, bitterness in mouth, giddiness, dizziness ^e

ADME = absorption, distribution, metabolism and excretion; ADRs = adverse drug reactions; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CNS = central nervous system.

^a Whirl-Carrillo et al. (2012).

^b Max and Sherer (2000).

^c Orrell (2011).

^d Zaleskis (2005).

^e Belhekar et al. (2012).

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