CYP2D6: a global analysis of phenotypic and genotypic variation in search of radical cure of *Plasmodium vivax* malaria

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Abstract

CYP2D6: a global analysis of phenotypic and genotypic variation in search of radical cure of *Plasmodium vivax* malaria

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**Background:** Malaria is a debilitating, life-threatening disease caused by *Plasmodium* protozoan parasites. Female Anopheles mosquitoes are the vector for the disease and responsible for disease transmission. In 2013, malaria deaths were estimated to be 584,000, mostly among sub-Saharan African children younger than five years of age. Even though huge number of morbidity and mortality are associated with this disease, malaria is preventable and can be cured. Efforts for increased malaria prevention and radical cure measures are being taken to reduce and eventually eradicate malaria globally.

CYP2D6 is an enzyme encoded by the *cyp2d6* gene. The gene is located in the q arm of chromosome 22 (22q13.1) and is known to be highly polymorphic. The enzyme is responsible for metabolism of 25% of clinically used drugs.

Another enzyme important in malaria treatment is Glucose-6-phosphate dehydrogenase (G6PD) encoded by a highly polymorphic *g6pd* gene. G6PD deficiency is the most common enzyme deficiency in the world, known to be present in about 400 million people worldwide. This deficiency is found mostly in the malaria endemic regions of the world.
Currently, the main treatment for *P. vivax* malaria is with chloroquine and primaquine drugs. The aim of this research is to study the importance of CYP2D6 variants in the *P. vivax* endemic regions of the world and to evaluate whether these mutations are linked with primaquine failure. We will also study G6PD deficiencies in those regions of the world. This data would serve as a step towards the ultimate goal for *P. vivax* malarial treatment, radical cure and elimination.

**Methods:** Data was collected by conducting literature survey and was focused towards geographic distribution of CYP2D6. We searched English-language literature in Pubmed and CYP2D6 databases. Additional information was obtained by searching reference lists of all relevant articles. Search items for CYP2D6 included keyword searches such as geographical distribution, polymorphism, phenotypes, genotypes, malaria, *P. vivax* malaria, primaquine treatment. A similar literature search was also conducted on G6PD deficiency. A detailed CYP2D6 database and malarial database search was conducted for *P. vivax* prevalence data in malaria endemic countries. Finally global malaria report was accessed from WHO malaria report 2014.

**Results:** Preliminary results did indicate that there is a link between primaquine drug metabolism and the poor metabolizer and intermediate metabolizer variants of CYP2D6. However, further studies need to be conducted to establish this role.

**Conclusion:** Globally, the poor metabolizer and intermediate metabolizers of CYP2D6 might hinder primaquine metabolism. Furthermore, G6PD deficiency along with the CYP2D6 mutation might be a major problem in *P. vivax* endemic regions of the world. Rapid point-of care detection tests for these mutations can be developed in the future to prevent relapses from malarial episodes and fatalities from primaquine treatment and seek other effective drugs for radical cure and eradication of malaria.
ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my thesis committee: Dr. Ian Painter, Dr. Carol Sibley and Dr. Gonzalo Domingo for their help, interest, time and support with this research project. I would also like to offer special thanks to Mr. Michael Kalnoky, Dr. Sampa Pal and the entire G6PD team at PATH for their professionalism, willingness to share data, constant support and helpful suggestions. My sincere thanks to all the work you do in global health. My special thanks also goes to the Executive Master of Public Health (eMPH) program staff and faculty at University of Washington. I would also like to thank my family and friends for their continual support throughout the program and believing in me. I wouldn’t have been able to pursue this without your support. I think this is my last academic degree. Maybe.

Last but not the least, I would like to thank my wonderful batch mates of “e13” cohort from the eMPH program. We developed great friendships and everlasting bonding through our various projects and coffee shop outings. I will treasure those memories forever.
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INTRODUCTION

Malaria is a debilitating, life-threatening disease caused by *Plasmodium* protozoan parasites. The disease is transmitted through the bites of infected female Anopheles mosquitoes\(^1\). In 2013, malaria deaths were estimated to be 584,000, mostly among sub-Saharan African children younger than five years of age\(^1\). Even though a huge number of morbidity and mortality are reported every year from this disease, malaria is preventable and can be cured\(^1\). Various efforts for increased malaria prevention and radical cure measures are being taken to reduce and eventually eradicate malaria globally\(^1,2\).

*Plasmodium vivax* (*P. vivax*) is endemic to tropical and subtropical areas of Asia, North and South America, the Middle East, North Africa, and the South Pacific. This protozoan parasite is most common among the four human malaria species (*P. falciparum, malariae, ovale,* and *vivax*)\(^1\). *P. vivax*, is also the most persistent form of malaria common to south and Southeast Asia, South America, and the Mediterranean regions and is known to become dormant in the liver and can only be killed with primaquine (*PQ*)\(^2\). For decades the main treatment for *P. vivax* malaria has been chloroquine and primaquine drugs\(^2\). *PQ* acts against the liver stage of the parasite effecting radical cure, thus decreasing the recurring relapses from the disease\(^1\). However, in recent years, *P. vivax* is becoming increasingly resistant to both chloroquine and primaquine, hence alternate drugs are being explored possible human genetic variation, with the goal of optimizing drug treatment\(^1,3\). Tafenoquine (*TQ*), a similar 8-aminoquinoline drug to *PQ* is being researched as an alternative\(^3\).

CYP2D6 is an enzyme encoded by the *cyp2d6* gene. The gene is located in the q arm of chromosome 22 (22q13.1) and the molecular weight of the protein is 55,769 Da, comprised of 497 amino acids\(^4\). This locus contains two neighboring pseudogenes, *cyp2d7* and *cyp2d8*\(^4\). The
official name for the enzyme is cytochrome P450, family 2, subfamily D, polypeptide 6 (abbreviated as CYP2D6)\textsuperscript{4,5}. The enzyme belongs to the class of mono-oxygenases and catalyzes reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids\textsuperscript{5}. The protein is located on the endoplasmic reticulum and in contrast to many of the cytochrome P450 enzymes is constitutively expressed. CYP2D6 is expressed mainly in liver, kidney, small intestine and brain\textsuperscript{5,6}.

This enzyme is a member of cytochrome P450 superfamily of proteins and is one of the most important enzymes involved in metabolism of clinically used drugs\textsuperscript{6}. Though CYP2D6 constitutes only 2\% of human hepatic Cytochrome P450 enzymes, it has been shown to metabolize 25\% drugs belonging to classes such as antiarrhythmics, antidepressants, neuroleptics and beta-blockers\textsuperscript{6,7}.

The structure of \textit{cyp2d6} locus in humans with its two adjacent pseudogenes makes it a common target for mutations arising from unbalanced recombination. As a result, insertions and deletions involving \textit{cyp2d6} gene are of common occurrence, including deletion of the entire gene in some humans\textsuperscript{3}. Tandem duplication of \textit{cyp2d6} is also known to occur and individuals have been observed to carry as many as 12 active copies of the gene\textsuperscript{1,3}. Also, \textit{cyp2d6} gene is a site of numerous small mutations like SNPs (Single Nucleotide Polymorphisms) and small insertions/deletions leading to frameshifts in the reading frame and stop codons (signaling premature stop to translation)\textsuperscript{5,9}. Currently, more than 100 allelic variants of the gene have been reported\textsuperscript{9}. These genetic variations result in the highly polymorphic expression of the enzyme. These differences are categorized in four basic phenotypes: poor metabolizers (PM; two inactive alleles), intermediate metabolizers (IM; one reduced activity allele and one inactive allele or two reduced alleles), extensive metabolizers (EM; at least one functional allele) and ultrarapid
metabolizers (UM; three or more functional copies)\textsuperscript{5,9}. The highly polymorphic nature of the 
cyp2d6 locus and the functional inequalities associated with its variants are ultimately 
responsible for the extremely variable drug responses\textsuperscript{5,6}.

Because this enzyme is involved in both activation and detoxification of so many 
therapeutic medicines, these phenotypic variations raise the risk for potential toxicity to certain 
medications that are substrates for the enzyme or to complete therapeutic failure for certain 
genotypes if the medicine requires activation\textsuperscript{8}. In order to perform its function in radical cure of 
P. vivax, primaquine does require activation, so it is vital to understand the prevalence of 
potential patients who might be unresponsive to primaquine as a result of their being in the poor 
metabolizer category of CYP2D6\textsuperscript{10}.

Another enzyme, Glucose-6-phosphate dehydrogenase (G6PD) is an inherited enzyme 
deficiency found in about 400 million people worldwide\textsuperscript{11,12}. This deficiency is found mostly in 
the malaria endemic regions of the world. The degree of deficiency varies within different 
ethnicities and also within each population\textsuperscript{12}. This enzyme is critical to the function of red blood 
cells (RBCs). G6PD deficiency is an X-linked hereditary genetic defect due to mutations in the 
g6pd gene. The gene has mutant alleles expressed as deficient phenotype and results in decreased 
enzyme activity\textsuperscript{11}. Males being hemizygous for the gene, (since they have 1 X chromosome), can 
have either normal or deficient expression. Females, who have 2 copies of G6PD gene on each X 
chromosome, can have normal or heterozygous expression and very rarely be homozygous 
deficient\textsuperscript{11,12}. In deficient individuals, diminished activity of the enzyme results in decreased 
ability of RBCs to withstand oxidative stress and could result in hemolysis, triggered by drugs 
such as primaquine\textsuperscript{13}. At present, the radical cure for Plasmodium vivax malaria is possible only 
through treatment with primaquine (PQ)\textsuperscript{13}. However, in malarial patients with G6PD deficiency,
this drug can trigger acute hemolytic anemia (AHA), which could be potentially lethal\textsuperscript{13}. The aim of this thesis was to conduct an extensive literature survey and database search to study the importance of CYP2D6 variants in the \textit{P. vivax} endemic regions of the world and to evaluate whether these mutations are linked with PQ failure, and to compare with G6PD deficiencies in those regions of the world.
METHODS

Literature search

We searched electronic databases (PubMed and EMBASE) using terms: “CYP2D6”, “geographical distribution”, “polymorphism”, “phenotypes”, “genotypes”, “malaria”, “P.vivax malaria” and “primaquine treatment”. A similar search was conducted on “G6PD”, and “geographical distribution”, “G6PD”, “deficiency” and geographical distribution”, “G6PD” “deficiency”, “P. vivax malaria” and “primaquine”. We searched the MeSH terms- Titles and abstracts were screened to retrieve relevant articles for further review. Reference lists of all relevant articles were also screened. We also searched in the databases for authors who have published in this field, cited in systematic reviews.

Database search

A detailed database search was conducted to look into the details of P. vivax malaria incidence and prevalence over the years across different countries. Three databases were searched for this purpose. The first database (http://www.cypalleles.ki.se/cyp2d6.htm) was the CYP2D6 allele nomenclature database. This database was searched for details on allele types, enzyme activity (in vitro and in vivo) along with the alleles corresponding to the mutations from four different CYP2D6 phenotypes. The cited research articles were reviewed for details.

A database search was also conducted on the malaria atlas project (http://www.map.ox.ac.uk). This database consists of latest information on malaria, its associated topics and publications. The data are organized on a geographical basis providing detailed information on prevalence and incidence of malaria in endemic countries. This site also
provides detailed interactive maps and modeling approaches about the distribution of the parasites, estimated risk, prevalence and incidence of malaria across different countries.

Additional data on malaria endemic country profiles, total population, number of people living with malaria was taken from global malaria report accessed from WHO malaria report 2014:

(http://www.who.int/malaria/publications/world_malaria_report_2014/en/)\(^ {11} \).

**Analyses**

The literature search data was synthesized into a conceptual diagram predicting the overlapping populations of *P. vivax* malaria cases with CYP2D6 PMs and IMs subgroups (since literature search revealed that those two groups are responsible for relapsing malaria and primaquine failure) along with G6PD deficient population (Figure 4). These overlapping populations are important target groups for future considerations for effectiveness of malarial treatment.

G6PD and CYP2D6 data from these databases were calculated and tabulated from twenty-one *P. vivax* malaria endemic countries and reported (Table 1). These data were used to develop a mathematical model at PATH (Program for Appropriate Technology in Health) where this research was conducted. The model predicts PQ treatment efficacy as a tool to understand who can be treated and will be responsive to the drug. This model is being used to understand these sub-populations in malaria endemic countries for *P. vivax* radical cure and elimination.
RESULTS

**Literature search**

A literature search conducted on CYP2D6 publications is summarized below in figure 1.

*Figure 1: Flowchart on CYP2D6 literature search.*

CYP2D6 metabolism among different regions of the world was based on classification of phenotypes\(^5\). Four phenotypic categories have been described in various studies, namely poor (PM), intermediate (IM), extensive (EM) and ultrarapid (UM) metabolizers\(^5\). Among the phenotypic variants, two decreased-function combination haplotypes or a combination of one-decreased-function variant and one nonfunctional variant were classified as IM\(^4,5\). Whereas UM
was defined as a carrier of an active gene duplication on one chromosome along with a functional variant on the other chromosome$^{4,5}$. PMs were based on no CYP2D6 activity due to complete gene deletion and finally the EMs were classified to have normal gene function and were considered as the wild type$^{6,17}$.

CYP2D6 enzyme activity corresponding to each haplotype was studied based on the database consisting of previously published research papers (http://www.cypalleles.ki.se)$^9$.

The phenotypic variation according to geographical distribution is shown in figure 2 (adapted from Sistonen et al. 2007)$^{17}$. The highest frequency of PM phenotypes was seen among the European population (8%). The second most common metabolic group in North Africa, Oceania, Middle East and America was UM with frequencies of 40%, 26%, 12% and 8% respectively$^{17}$. Decreased function variants, led to IM seen in East Asia (30%), Subsaharan Africa (18%) and Middle East (10%)$^{17}$. The highest frequency was observed among EMs (50% - 90%) with the widest distribution$^{17}$.

Figure 2: Geographic distribution of four phenotypic classes of CYP2D6.
The phenotype-genotype correlation among the various haplotypes of the CYP2D6 alleles was studied from various research publications. The structure of the cyp2d locus makes it a hot spot for mutations due to unbalanced recombination\(^5,7\). As a result, insertions and deletions involving the cyp2d6 gene commonly occur, including deletion of the entire gene, referred to as CYP2D6 *5 allele (PMs)\(^17\). Tandem duplication of cyp2d6 is also known to occur and individuals have been known to carry as many as 12 active copies of the gene (UMs)\(^17\). In addition to large indels, cyp2d6 is also the site of numerous point mutations, insertions/deletions leading to frameshift and premature stop codons\(^17\). Overall, about 104 unique haplotypes have been described for the locus, this number will keep changing with new mutations being discovered\(^3\). cyp2d6 *1A, *2, *39 haplotypes are considered as the wild type with normal enzyme function (EM). Although the majority of cyp2d6 haplotypes have not yet been evaluated for enzymatic activity, a few have been identified as being either deficient (example- *10, *17, *29 and *41) – considered phenotypically as IMs or totally lacking the gene (example- *4, *5)-considered phenotypically as PMs\(^9,10\). Genotyping technique described by Sistonen et al., 2007, identified CYP2D6 variants were represented in various populations across the globe\(^9,17\). Alleles- *2, *4, *10, *17, *29, *39 and *41 were found more common across all the populations\(^17\). Low to null metabolic activity was seen in rare alleles such as *3, *6 and *9. *5 were found in case whole gene deletion\(^9,17\).

The second enzyme that was studied in this research was G6PD. G6PD deficiency, the most common enzyme deficiency in the world, is expressed in red blood cells and develop acute hemolytic anemia (AHA) when exposed to fava beans, certain infections or certain drugs like primaquine\(^11,12,13\). There are extensive studies showing G6PD deficiency and \textit{P. vivax} malaria. PQ drugs stress the red blood cells and trigger AHA and can be lethal in malarial patients with
G6PD deficiency\textsuperscript{12, 13}. In order to prevent AHA it is important to test for G6PD deficiency before administering primaquine to \textit{P. vivax} malarial patients\textsuperscript{12, 13, 17}.

The G6PD literature search is summarized in figure 3.

\textit{Figure 3: Flowchart on G6PD literature search.}

\textbf{PQ failure}

We found very few studies that have examined connection of PQ failure with CYP2D6 variants in \textit{P. vivax} malaria. In their 2012 research article, Pybus \textit{et al.} first showed the importance of genetic variability of CYP2D6 and efficacy of PQ as a treatment against relapsing malaria, especially with PMs and IMs\textsuperscript{21}. Hemolytic toxicity could also be increased in poor, intermediate and to some extent in extensive metabolizers\textsuperscript{22}. In 2013, Bennett \textit{et al.} reported PQ failures in two clinical cases of \textit{P. vivax} linked to poor and intermediate activity of CYP2D6\textsuperscript{23}. In 2016, St Jean \textit{et al.} reported that TQ treatment efficiency in \textit{P. vivax} malaria individuals is not
reduced to the same extent as PQ. However, their conclusions were determined by only looking at IM phenotypes as no PM phenotypes were in the study population\textsuperscript{24}.

Thus when we examine studies done on CYP2D6, PMs and IMs, we can conclude that host genetics plays a major role towards PQ failure in people with relapsing \textit{P. vivax} malaria, but its role in TQ efficacy is less clear.

The results of the literature survey are summarized in a conceptual diagram connecting the \textit{P. vivax} malarial population and showing the subsets of G6PD deficient group and CYP2D6 PMs and IMs (figure 4). G6PD deficient population along with PQ treatment failure in CYP2D6 PMs and IMs among the \textit{P. vivax} malarial population can be predicted in the overlapping subgroups.

\textit{Figure 4: Conceptual Diagram}
**Database analyses**

Data were extracted for each of the twenty-one malaria endemic countries. The malaria cases and the percentage of *P. vivax* in every country were determined from the malaria atlas database and the percentages of G6PD deficiencies and CYP2D6 data were collected from various literature surveys\textsuperscript{18}. These data are summarized in table 1.

**Table 1: Data showing malaria endemic countries, *P. vivax* cases, G6PD deficiency, PM and IM**

**CYP2D6 percentages**

<table>
<thead>
<tr>
<th>Countries</th>
<th>Population</th>
<th>Malaria Cases</th>
<th>% of <em>P. vivax</em> malaria</th>
<th>% of G6PD Deficiency</th>
<th>% of CYP2D6 PM &amp; IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>1252100000</td>
<td>881730</td>
<td>47</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Indonesia</td>
<td>249800000</td>
<td>343527</td>
<td>45</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>China</td>
<td>1385597000</td>
<td>4086</td>
<td>23</td>
<td>7</td>
<td>55</td>
</tr>
<tr>
<td>Brazil</td>
<td>200410000</td>
<td>178546</td>
<td>82</td>
<td>7</td>
<td>18</td>
</tr>
<tr>
<td>Pakistan</td>
<td>182180000</td>
<td>281755</td>
<td>83</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>94141000</td>
<td>2645454</td>
<td>36</td>
<td>1</td>
<td>8.6</td>
</tr>
<tr>
<td>Viet_Nam</td>
<td>91700000</td>
<td>17128</td>
<td>40</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Thailand</td>
<td>66960000</td>
<td>33302</td>
<td>47</td>
<td>17</td>
<td>40</td>
</tr>
<tr>
<td>Malaysia</td>
<td>29750000</td>
<td>3859</td>
<td>13</td>
<td>10</td>
<td>55</td>
</tr>
<tr>
<td>Colombia</td>
<td>48270000</td>
<td>51722</td>
<td>66</td>
<td>7</td>
<td>1.2</td>
</tr>
<tr>
<td>Myanmar</td>
<td>53200000</td>
<td>333871</td>
<td>26</td>
<td>17</td>
<td>27</td>
</tr>
<tr>
<td>Venezuela</td>
<td>30421000</td>
<td>78643</td>
<td>65</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Philippines</td>
<td>98360000</td>
<td>6514</td>
<td>20</td>
<td>7</td>
<td>54</td>
</tr>
<tr>
<td>Peru</td>
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<td>43139</td>
<td>84</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>Ecuador</td>
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<td>378</td>
<td>57</td>
<td>7</td>
<td>0.4</td>
</tr>
<tr>
<td>Bolivia</td>
<td>10662000</td>
<td>7342</td>
<td>84</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>Cambodia</td>
<td>15130000</td>
<td>21309</td>
<td>45</td>
<td>17</td>
<td>62</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>6079000</td>
<td>1194</td>
<td>82</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Guyana</td>
<td>8000000</td>
<td>31479</td>
<td>44</td>
<td>10</td>
<td>27</td>
</tr>
<tr>
<td>Solomon Islands</td>
<td>561610</td>
<td>25609</td>
<td>47</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Afghanistan</td>
<td>30580000</td>
<td>39263</td>
<td>95</td>
<td>10</td>
<td>27</td>
</tr>
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</table>

A mathematical model was developed at PATH (by Michael Kalnoky, Senior Data Analyst, Diagnostics) based on the above data. This model aims at developing an integrated global *P. vivax* elimination strategy. The model would work in determining the impact potential of *P. vivax* diagnosis and radical cure in different malaria endemic countries\textsuperscript{10, 11}. It would also be used as a tool to understand the target group for treatment and responsiveness to PQ.
In this model the key parameters are: **estimated *P. vivax* cases:** (accessed from the WHO malaria report), **tested cases:** cases that received a diagnostic test, **confirmed *P. vivax* cases:** diagnostic test positive (WHO malaria report), **treatable cases:** G6PD normal (G6PD calculator from the model and malaria atlas database) and **responsive cases:** accounts for resistance (patients resistant to PQ treatment regimen), poor adherence (non-compliant patients) and CYP2D6 haplotype\textsuperscript{10,11}. The following table (table 2) illustrates these data worldwide.

*Table 2: World *P. vivax* Statistics*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated Cases*</td>
<td>16,386,162</td>
<td>100%</td>
</tr>
<tr>
<td>Tested Cases</td>
<td>3,164,714</td>
<td>19%</td>
</tr>
<tr>
<td>Confirmed Cases*</td>
<td>2,215,300</td>
<td>14%</td>
</tr>
<tr>
<td>Treatable Cases</td>
<td>2,074,101</td>
<td>13%</td>
</tr>
<tr>
<td>Responsive Cases</td>
<td>1,729,952</td>
<td>11%</td>
</tr>
</tbody>
</table>

* Obtained from WHO world malaria report 2014\textsuperscript{11}.

Using this model, predictions can be made about the various aforementioned criteria in individual *P. vivax* endemic countries as well. An example where the model can be used to study different factors associated with malaria is illustrated in the following graph (figure 5). [Image courtesy: Dr. Gonzalo Domingo, PATH].
Figure 5: *P. vivax* diagnosis and radical cure: assessing impact potential (An example illustrating an application of the model)

**False negatives** = Tested cases – Confirmed cases (*assumption 70% sensitivity for *P. vivax*)

**Treatable cases** = Confirmed cases – G6PDd*  *G6PD model for this figure, threshold set at 30%

**Responsive cases** accounts for operational factors: adherence and CYP2D6

* WHO world malaria report 2014\textsuperscript{11}.
DISCUSSION

From various literature search, it is evident that PMs and, to a lesser extent, IMs are prone to exaggerated side effects from drugs metabolized by CYP2D6, whereas normal dose of the same drugs tend not to be detrimental in UM5, 8. From this study we also found that PM and IM variants exist in malaria endemic regions in small but significant populations and that they might play a role in PQ failure and in relapsing episodes of malaria. In the future, more extensive research is needed with larger populations determining prevalence of PM and IM related SNPs in CYP2D6 variants and PQ effect in vitro and in vivo15. Furthermore, G6PD deficiency leading to AHA when exposed to PQ treatment along with CYP2D6 IM and PM mutations shown to cause relapses in malarial episodes might be a major problem in P. vivax endemic regions of the world14, 22. This research was a step towards understanding these sub-populations in P. vivax malaria endemic countries and will eventually help to develop effective methods for radical cure and elimination of the disease.

While we have extensive data on G6PD deficiency in P. vivax malaria endemic regions and why PQ treatment is lethal for those deficient patients, more research is needed with larger study populations to show the relationships among CYP2D6 activity, geographic regional variance and clinical failure of PQ treatment21. A comprehensive understanding of every factor affecting P. vivax malaria is needed for developing effective treatment, radical cure and eradication of the disease21, 22, 23.

Cost effective and more sensitive rapid point-of care diagnostic tests for detecting malaria and these mutations need to be developed in the future for preventing fatalities from PQ treatment in overlapping populations with CYP2D6 PM and IM mutations and/or G6PD
deficiency. At present there are low testing rates and moreover, the tests available for *P. vivax*
are not very sensitive, which poses a major barrier into the impact of radical cure and elimination
of the disease\textsuperscript{1,11}. The impact of radical cure on elimination will vary from country to country.
This will mainly depend on access to proper and accurate diagnostics and drugs, G6PD deficient
population, CYP2D6 PM and IM population and other causes for non-responsiveness\textsuperscript{1}. Among
them, access to diagnostics and drugs and sensitivity of the *P. vivax* test are the most consistent
barriers to impact of radical cure\textsuperscript{1}.

The malaria model developed at PATH will work in predicting the impact potential of *P.
vivax* diagnosis and radical cure in different malaria endemic countries\textsuperscript{25}. It would also be used
as a tool to understand the target group for treatment and responsiveness to PQ. The interactive
information in the mathematical model is a step towards developing an integrated *P. vivax*
elimination strategy.
2. Wiselogle FY, A survey of antimalarial drugs, 1941-1945, Ann Arbor, Michigan; 1946.