Understanding the impact of host polymorphisms on PQ effectiveness: $G6PD$ and $CYP2D6$

APMEN Vivax Working Group
Kuta, Indonesia, 9-11<sup>th</sup> October 2017

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Aim: *P. vivax* elimination

=> maximised access to effective radical cure

- **Maximise access to primaquine to avert morbidity and mortality associated with relapses**
  - Drug availability, prescription, adherence to full courses

- Alongside this, is it necessary to consider the impact of host polymorphisms which may complicate this objective? How these might impact on anticipated effectiveness?

- What do we know about the role of CYP2D6 in primaquine efficacy?

- Quantify the need for complimentary treatment courses for patients not currently eligible or suitable for primaquine therapy
  - Quantify this population
  - Advocate for the need
Preventing relapse in *P. vivax* or *P. ovale* malaria

The G6PD status of patients should be used to guide administration of primaquine for preventing relapse.

*Good practice statement*

To prevent relapse, treat *P. vivax* or *P. ovale* malaria in children and adults (except pregnant women, infants aged < 6 months, women breastfeeding infants aged < 6 months, women breastfeeding older infants unless they are known not to be G6PD deficient, and people with G6PD deficiency) with a 14-day course (0.25-0.5 mg/kg bw daily) of primaquine in all transmission settings.

*Strong recommendation, high-quality evidence*

In people with G6PD deficiency, consider preventing relapse by giving primaquine base at 0.75 mg/kg bw once a week for 8 weeks, with close medical supervision for potential primaquine-induced haemolysis.

*Conditional recommendation, very low-quality evidence*

When G6PD status is unknown and G6PD testing is not available, a decision to prescribe primaquine must be based on an assessment of the risks and benefits of adding primaquine.

*Good practice statement*

**Pregnant and breastfeeding women**

In women who are pregnant or breastfeeding, consider weekly chemoprophylaxis with chloroquine until delivery and breastfeeding are completed, then, on the basis of G6PD status, treat with primaquine to prevent future relapse.

*Conditional recommendation, moderate-quality evidence*
Aim: *P. vivax* elimination

=> maximised access to effective radical cure

What about PQ ineligible patients?
Estimates of the population eligible for PQ radical cure

**Based on population-wide rates, not specific to *Pv*-patients

Limitations to this dataset discussed later

<table>
<thead>
<tr>
<th>WHO region</th>
<th>Total pop (2010)</th>
<th>Sum of PQ_safe</th>
<th>% PQ safe</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFRO</td>
<td>804,871</td>
<td>617,279</td>
<td>77%</td>
</tr>
<tr>
<td>AMRO</td>
<td>525,750</td>
<td>482,826</td>
<td>92%</td>
</tr>
<tr>
<td>EMRO</td>
<td>429,493</td>
<td>338,750</td>
<td>79%</td>
</tr>
<tr>
<td>EURO</td>
<td>129,265</td>
<td>119,619</td>
<td>93%</td>
</tr>
<tr>
<td>SEARO</td>
<td>1,800,932</td>
<td>1,519,632</td>
<td>84%</td>
</tr>
<tr>
<td>WPRO</td>
<td>1,670,054</td>
<td>1,514,653</td>
<td>91%</td>
</tr>
<tr>
<td><strong>Grand Total</strong></td>
<td><strong>5,360,365</strong></td>
<td><strong>4,592,758</strong></td>
<td><strong>86%</strong></td>
</tr>
</tbody>
</table>

**Based on population-wide rates, not specific to *Pv*-patients

Limitations to this dataset discussed later
The clinical and public health problem of relapse due to the periodicity of Plasmodium vivax malaria warrants the use of primaquine to clear liver stage parasites. However, primaquine is a CYP2D6 substrate. We report the first case of primaquine dose dependent toxicity resulting from poor metabolizer status of CYP2D6. Stochastic DNA sequencing revealed a rare homozygous CYP2D6*14/14 (888C>T) mutation in this subject. The CYP2D6 genotyping tests, however, were inconsistent with the observed phenotype, and we showed that a polymorphic AQE (polymorphism at codon 386) was capable of compensating for the CYP2D6*14/14 deficient genotype.
Role of CYP2D6 in primaquine metabolism

- Recent reports have shown that PQ requires metabolic activation by CYP2D6 isoenzymes for liver-stage antimalarial activity in both mouse and human studies.

- CYP2D6 implicated in metabolism of ~25% of all drugs in clinical use, well documented in context of cancer therapy:
  - Genetically highly polymorphic, with over 74 described alleles.
  - Metabolic activity of key alleles phenotypes characterised.
Pharmacogenetic variation at CYP2C9, CYP2C19, and CYP2D6 at global and microgeographic scales

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Objectives CYP2C9, CYP2C19, and CYP2D6 belong to a subfamily of cytochrome P450 (CYP) enzymes, associated mainly with the metabolism of exogenous compounds in the human body. The genes coding for these enzymes are highly polymorphic and thus of major pharmacogenetic importance. By systematically retrieving data from the literature and genotyping new population samples, we aimed at describing the worldwide distribution of genetic variation at these loci. We created a comprehensive resource of frequency data for the most important CYP2C9, CYP2C19, and CYP2D6 genetic variants in 129, 146, and 138 different population samples, respectively. Furthermore, we showed how demographic history can affect pharmacogenetic variation at a microgeographic scale by analyzing regional samples from Finland, which represents a well-known genetic isolate.

globally in all geographic regions, reaching extremely high frequencies in some populations; (ii) each of the CYP genes studied shows a distinct geographic pattern of variation; (iii) population substructure can strongly affect the variation seen in pharmacogenetic loci; and (iv) several geographic regions of pharmacogenetic interest are still poorly characterized. 


Pharmacogenetics and Genomics 2009, 19:170–179

Keywords: CYP2C9, CYP2C19, CYP2D6, genetic variation, human populations, polymorphism

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Supplementary Table 3. *CYP2D6* genetic variant frequencies in different populations<sup>a,b</sup>

| Population         | Code | Geographic Origin                  | Chr<sup>c</sup> | *2   | *3   | *4   | *5   | *6   | *9   | *10  | *17  | *29  | *41  | *1xN | *2xN | *4xN | *10xN | *41xN | Others | Ref<sup>a</sup> | Additional Information                                                                 |
|--------------------|------|------------------------------------|----------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|--------|------------------|---------------------------------------------------------------------------------------|
| **AFRICA**         |      |                                    |                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |        |                                |
| Southern Africa    |      |                                    |                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |        |                                |
| South African Bantu| AFs1 | South Africa                       |                | 182  | 0.315| 0    | 0.011| 0.125| 0    | 0    | 0    | 0.187| 0.031| 0    | 0.071| 0    | 0    | 0    | 0    | 0.292 | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Venda              | AFs2 | Tsikikundamakela, Venda, South Africa|                | 152  | 0.178| 0    | 0.033| 0.046| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.240| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.500 | [2] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| San                | AFs3 | Namibia                            |                | 14   | 0.643| 0    | 0.143| 0    | 0    | 0    | 0    | 0.071| 0    | 0.143| 0    | 0    | 0    | 0    | 0    |       | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| **Middle Africa**  |      |                                    |                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |        |                                |
| Mbuti Pygmies      | AFm1 | Democratic Republic of Congo       |                | 30   | 0.600| 0    | 0    | 0.100| 0    | 0    | 0    | 0.042| 0.079| 0.031| 0.007| 0.067| 0    | 0.007| 0    | 0.165 | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Biaka Pygmies      | AFm2 | Central African Republic           |                | 72   | 0.500| 0    | 0.014| 0.014| 0    | 0    | 0    | 0.083| 0.125| 0.028| 0.014| 0    | 0    | 0    | 0    | 0.209 | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| **Western Africa** |      |                                    |                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |        |                                |
| Yoruba             | AFw1 | Nigeria                            |                | 50   | 0.120| 0    | 0    | 0.040| 0    | 0    | 0    | 0.040| 0.060| 0.120| 0.020| 0    | 0.040| 0.140| 0    |       | 0.420 | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Ghanaian           | AFw2 | Ghana                              |                | 386  | 0.109| 0    | 0.070| 0.060| 0    | 0    | 0    | 0.031| 0.277| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.016| ND<sup>d</sup> | ND<sup>d</sup> | 0.437 | [3] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Mandenka           | AFw3 | Senegal                            |                | 48   | 0.125| 0    | 0.125| 0.063| 0    | 0    | 0    | 0.063| 0.188| 0.063| 0.104| 0    | 0    | 0    | 0    | 0.271 | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| **Eastern Africa** |      |                                    |                |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |       |        |                                |
| Zimbabwesian       | AFe1 | Zimbabwe                           |                | 228  | 0.130| 0    | 0.020| 0.040| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.340| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.470 | [2] CYP2D6*4xN includes CYP2D6*2xN; Same samples as in Wesnerholm et al. Pharmacogenetics 1999;9:707-714 |
| Tanzanian Bantu     | AFe2 | Tanzania                           |                | 212  | 0.203| 0    | 0.009| 0.061| ND<sup>d</sup> | 0.038| 0.170| 0.198| ND<sup>d</sup> | 0.033| ND<sup>d</sup> | 0.009| ND<sup>d</sup> | 0.278 | [2] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Tanzanian           | AFe3 | Tanzania                           |                | 212  | 0.184| 0.005| 0.014| 0.033| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.203| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.561 | [2] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Tanzanian           | AFe4 | North-Eastern Tanzania            |                | 392  | ND<sup>d</sup> | 0    | 0.040| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.203| ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | ND<sup>d</sup> | 0.96  | [5] Samples from the National Laboratory for the Genetics of Israeli Populations, Tel-Aviv |
| Kenyan Bantu        | AFe5 | Kenya                              |                | 24   | 0.250| 0    | 0    | 0.042| 0    | 0    | 0    | 0.167| 0.167| 0    | 0    | 0    | 0.042| 0    |       | 0.334 | [1] Samples from the Human Genome Diversity Cell Line Panel, CEPH                   |
| Ethiopian           | AFe6 | Addis Ababa, Ethiopia              |                | 244  | 0.352<sup>a</sup> | 0    | 0.041| 0.033| ND<sup>d</sup> | ND<sup>d</sup> | 0.086| 0.113| ND<sup>d</sup> | ND<sup>d</sup> | 0.190| ND<sup>d</sup> | ND<sup>d</sup> | 0.215 | [6] Same samples as in Aklilu et al. J Pharmacol Exp Ther 1996;278:441-446          |
| Ethiopian           | AFe7 | Ethiopia                           |                | 134  | 0.395<sup>a</sup> | 0    | 0.059| 0.031| 0    | ND<sup>d</sup> | 0.080| 0.149| ND<sup>d</sup> | ND<sup>d</sup> | 0.101| ND<sup>d</sup> | ND<sup>d</sup> | 0.185 | [6] Samples from the National Laboratory for the Genetics of Israeli Populations, Tel-Aviv |
| Ethiopian Jews      | AFe8 | Ethiopia                           |                | 56   | 0.392<sup>a</sup> | 0    | 0.054| 0.036| ND<sup>d</sup> | ND<sup>d</sup> | 0.054| 0.143| ND<sup>d</sup> | ND<sup>d</sup> | 0.089| ND<sup>d</sup> | ND<sup>d</sup> | 0.232 | [7] Samples from the National Laboratory for the Genetics of Israeli Populations, Tel-Aviv |
The CYP2D6 Activity Score: Translating Genotype Information into a Qualitative Measure of Phenotype

A Gaedigk¹, SD Simon², RE Pearce¹, LD Bradford³, MJ Kennedy⁴ and JS Leeder¹

Inferring CYP2D6 phenotype from genotype is increasingly challenging, considering the growing number of alleles and their range of activity. This complexity poses a challenge in translational research where genotyping is being considered as a tool to personalize drug therapy. To simplify genotype interpretation and improve phenotype prediction, we evaluated the utility of an “activity score” (AS) system. Over 25 CYP2D6 allelic variants were genotyped in 672 subjects of primarily Caucasian and African-American heritage. The ability of genotype and AS to accurately predict phenotype using the CYP2D6 probe substrate dextromethorphan was evaluated using linear regression and clustering methods. Phenotype prediction, given as a probability, was compared to Caucasians. The AS tool warranted further exploration in ethnic populations.

Table 4 Values assigned to alleles in AS-Model A

<table>
<thead>
<tr>
<th>Value assigned to allele</th>
<th>Alleles¹</th>
<th>AS-Model A</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>*1xN, *2xN, *35xN</td>
<td></td>
</tr>
</tbody>
</table>

AS, activity score. The AS of a genotype is the sum of the values assigned to each allele (e.g., CYP2D6*1/*1 and CYP2D6*2/*5 genotypes have AS of 2 and 1, respectively). ¹Only observed alleles are listed.
CYP2D6 activity scores (AS)

Allele frequencies

Hardy-Weinberg inheritance assumptions

Genotype frequencies

Phenotype AS

<table>
<thead>
<tr>
<th>ALLELE 2</th>
<th>Activity Score (AS)</th>
<th>Increased</th>
<th>Normal</th>
<th>Intermediate</th>
<th>Non-functional</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>2.0</td>
<td>4.0</td>
<td>3.0</td>
<td>2.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>3.0</td>
<td>2.0</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.5</td>
<td>2.5</td>
<td>1.5</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Non-functional</td>
<td>0</td>
<td>2.0</td>
<td>1.0</td>
<td>0.5</td>
<td>0.0</td>
</tr>
</tbody>
</table>
Recent meta-analysis of CYP2D6 polymorphic SNPs

How does this vary sub-regionally?
How representative are these population groups of the overall populations?
Limitations to these preliminary estimates of ineffective CYP2D6 primaquine metabolisers

- CYP2D6 allelic activity scores are based on metabolism of a range of drug compounds, not necessarily all transferrable to primaquine
- Activity score threshold for inadequate PQ metabolism (≤1) based on a single study from single population group
- CYP2D6 allele frequency data from selected ethnic groups not necessarily widely representative
- Variable sample sizes across surveys
- Masked sub-regional variation
- Sistonen dataset assembled in 2007: published articles for “CYP2D6” and “population” have doubled in number since then = needs updating

⇒ Preliminary indication, but strongly warrants further investigation and refinement
⇒ Supports case for continuing to expand innovative approaches to radical cure for the subset of patients unable to safely metabolise effective PQ metabolites
Limitations to current G6PDd maps & population estimates

- Denominator currently used is the background population prevalence of G6PDd, not specifically *P. vivax* cases
  - Estimates of G6PDd rates among *Pv* patients is a priority
  - Suggested differences based on infection-driven erythropoiesis
  - Protective effect of G6PDd which led to selection of the numerous *G6PD* mutations

- Analysis shown here excludes all G6PD hemi/homo/hetero patients: should be adjusted to local G6PD variants and MoH policies

- Current prevalence maps do not differentiate between mutations; aim to integrate genetic variants into the smooth frequency maps to give added resolution to PQ-haemolytic risk calculations

- Lots of recent high-quality data been generated which is not yet incorporated
315 new publications, equivalent to 21% of all published articles
Work nested within APMEN VxWG objectives and in context of APMEN surveys

APMEN surveys:

Standardized “gold standard” protocols across the region
MAP’s new Online Data Portal: a centralized repository for malaria resources coming end 2017

www.map.ox.ac.uk
Layer catalogue
Spatial analysis tools
Country profiles

Data Sources

- B. Annis (1990) *Comparison of the effectiveness of two formulations of deet against Anopheles fluviatilis.*
- Department of Health, National Epidemiology Centre, Public Health Surveillance and...
Compiling the evidence-base

• Goal is to facilitate access to comprehensive data compilations and epidemiological data

• Providing population-specific epidemiological parameters for planning strategies to increase radical cure effectiveness suitable to the local context

• Identify areas where the evidence-base would justify further surveys
Thank you

• Kevin Baird & Katherine Battle

• VxWG team, and the Gates Foundation grant to APMEN-VxWG to update G6PD datasets and maps

• Mike Thorn & ROAD-MAP team, also funded by Bill & Melinda Gates Foundation