Sequence Analysis of *Plasmodium falciparum* histidine-rich protein 2 and 3 genes from returning travelers to Africa

Rachel Lau¹, Ayomide Olubanwo², Filip Ralevski¹, Aisha Khatib², Andrea K Boggild¹,²,³

¹ Public Health Ontario Laboratories, Public Health Ontario, Toronto, Canada,
² Tropical Disease Unit, UHN-Toronto General Hospital, Toronto, Canada,
³ Department of Medicine, University of Toronto, Toronto, Canada

**Background:**
- WHO reported 219 million new cases of malaria with half million deaths in 2017
- Sub-Saharan Africa and Children under 5 accounted for majority of cases and deaths.
- 5 human species – *Plasmodium falciparum, P. vivax, P. ovale, P. malariae, P. knowlesi*
- *Plasmodium falciparum* accounts for 99% of Malaria deaths
- Malaria is not endemic in Canada but we have competent vectors for transmission
- Travellers from endemic areas account for 500 cases/year
- Current diagnosis of Malaria in Canada include microscopy, rapid diagnostic test by antigen detection of histidine-rich proteins 2 and 3, and PCR
- We aimed to study the sequence heterogeneity of histidine-rich protein 2 and 3 genes in *Plasmodium falciparum* cases which failed detection by HRP2 specific rapid diagnostic test

**Materials and Methods:**
- *Plasmodium falciparum* whole blood samples from cases which failed BinaxNow rapid diagnostic test were identified
- DNA extraction was performed and species were confirmed by real time PCR
- Plasmodium histidine-rich protein 2 (*pfhrp2*) and 3 (*pfhrp3*) genes were PCR amplified and Sanger Sequenced
- Sequence alignment and analysis were performed by MEGA 6.06 software

**Results:**
- Four *Plasmodium falciparum* cases that failed BinaxNow rapid diagnostic test were identified
- Real time PCR confirmed three were mono-*Plasmodium falciparum* infections whereas one was a mixed infection

**Table 1. Deletion of pfhrp2 and pfhrp3 genes described in the literature**

<table>
<thead>
<tr>
<th>Gene</th>
<th>basepair</th>
<th>Gene Structure</th>
<th>Expression</th>
<th>Mutation/Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfhrp2</td>
<td>1064</td>
<td>2 exons, 1 intron</td>
<td>HRP2</td>
<td>Deletion (Brazil, Peru) Partial Deletion (Bangladesh) Partial and full deletion (Kenya)</td>
</tr>
<tr>
<td>Pfhrp3</td>
<td>983</td>
<td>2 exons, 1 intron</td>
<td>Epitopes homologous to HRP2</td>
<td>Deletion (Brazil, Peru) Mutation (Bangladesh)</td>
</tr>
</tbody>
</table>

**Table 2. *Plasmodium falciparum* cases that failed BinaxNow rapid diagnostic test**

<table>
<thead>
<tr>
<th>Case</th>
<th>Travel (Country of Acquisition)</th>
<th>Year</th>
<th>Age</th>
<th>Sex</th>
<th>Microscopy</th>
<th>Parasitaemia</th>
<th>BinaxNow T1 (PFHRP2)</th>
<th>BinaxNow T2 (Pfhrp3)</th>
<th>Pfhrp2</th>
<th>Pfhrp3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nigeria</td>
<td>2016</td>
<td>39</td>
<td>M</td>
<td><em>Pf</em>, Rings</td>
<td>&lt;0.1%</td>
<td>-</td>
<td>-</td>
<td>No mutation compared to control (400bp of exon2)</td>
<td>No mutation compared to control (400bp of exon 2)</td>
</tr>
<tr>
<td>2</td>
<td>Nigeria</td>
<td>2017</td>
<td>42</td>
<td>M</td>
<td><em>Pf</em>, Rings</td>
<td>&lt;0.1%</td>
<td>-</td>
<td>+</td>
<td>No mutation compared to control (700bp of exon 2)</td>
<td>TBD</td>
</tr>
<tr>
<td>3</td>
<td>Tanzania</td>
<td>2017</td>
<td>13</td>
<td>M</td>
<td>Neg but <em>Pf</em> PCR Pos, low level infection</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>TBD</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ivory Coast</td>
<td>2018</td>
<td>50</td>
<td>M</td>
<td><em>Pf</em> and <em>Pm</em> mixed infection. Rings, Trophozoites, Schizonts</td>
<td>0.2%</td>
<td>-</td>
<td>-</td>
<td>TBD</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:**
- No mutation was found in exon 2 region of *pfhrp2* and *pfhrp3* DNA sequence analysis for the cases where primary material was available for sequencing
- Detection failure may be due to low expression of PFHRP2 -> detection failure by BinaxNow RDT
- In non-endemic lab setting with waning microscopy expertise
  - Ambiguous microscopy smear + PFHRP2 RDT Neg may lead to:
    - -> Misdiagnosis as non-*falciparum* Malaria or Neg for Malaria
    - -> Inappropriate treatment with Chloroquine for presumed *Plasmodium vivax* or other non *falciparum* malaria
    - -> Outcome can be detrimental (Death)!

**Future Analysis:**
- Further DNA analysis of exon 1 and full exon 2 of both genes
  - -> Insight on any sequence heterogeneity that may affect binding to the RDT
- Analysis of remaining PFHRP2 RDT neg Plasmodium falciparum cases
  - -> Etiology of detection failure
- Continual Surveillance
  - -> Inform lab diagnostics and physicians of potential pitfalls
  - -> Possible modification in diagnostic algorithm

**Contact:** Dr. Andrea Boggild
Lab website: www.boggildlab.ca
Email: andrea.boggild@utoronto.ca @boggildlab