Data Article

Data on High Resolution Melting (HRM) and phylogenetic analysis of *P. ovale wallikeri* and *P. ovale curtisi*

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**Abstract**

High Resolution Melting (HRM) analysis is a post-PCR analysis method used for identifying genetic variation in nucleic acid sequences. These data are presenting the identity of the 33 samples used for a qPCR-HRM and a nested snapback methods validation. In addition we are presenting the high resolution melting profiles of *P. ovale curtisi* (Poc) and *P. ovale wallikeri* (Pow) in the following conditions: after a direct qPCR run and after a nested snapback run. The qPCR-HRM of artificial mixture of Poc and Pow plasmids (200 copies/μl, each) at different proportions are showing the melting pattern of co-infections with both species. The sequencing methodology of the clpc gene fragment of 12 randomly selected samples is described and their likeness to published sequences is shown in a maximum likelihood tree. "Novel high resolution melting and snapback assays for simultaneous detection and differentiation of Plasmodium ovale spp." [1].

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**Data on High Resolution Melting (HRM) and phylogenetic analysis of *P. ovale wallikeri* and *P. ovale curtisi***

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**Article Info**

Article history:
Received 2 February 2019
Received in revised form 28 March 2019
Accepted 16 April 2019
Available online 23 April 2019

**Abstract**

High Resolution Melting (HRM) analysis is a post-PCR analysis method used for identifying genetic variation in nucleic acid sequences. These data are presenting the identity of the 33 samples used for a qPCR-HRM and a nested snapback methods validation. In addition we are presenting the high resolution melting profiles of *P. ovale curtisi* (Poc) and *P. ovale wallikeri* (Pow) in the following conditions: after a direct qPCR run and after a nested snapback run. The qPCR-HRM of artificial mixture of Poc and Pow plasmids (200 copies/μl, each) at different proportions are showing the melting pattern of co-infections with both species. The sequencing methodology of the clpc gene fragment of 12 randomly selected samples is described and their likeness to published sequences is shown in a maximum likelihood tree. "Novel high resolution melting and snapback assays for simultaneous detection and differentiation of Plasmodium ovale spp." [1].

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1. Data

This report is presenting in Table 1 the detailed list of all thirty three (33) samples used to validate a qPCR-HRM and a nested snapback methods developed for P. ovale species differentiation [1]. Table 1 is presenting the origin of samples and the results of different genotyping methods (microscopy, nested-PCR,qPCR-HRM and PrimerDesign kit). The P. ovale clpc gene fragments of twelve (12) samples selected randomly were sequenced. The obtained sequences were used for a phylogenetic reconstruction together with previously published P. ovale clpc sequences (Fig. 3). The PCR and HRM melting curves of P. ovale curtisi (Poc) and P. ovale wallikeri (Pow) are shown in Fig. 1 and the differences between the melting temperatures (Tm) are presented in Fig. 2. The nested snapback ΔTm was 3.74°C and that of the direct qPCR-HRM was 0.2°C. Intermediate Tm values of 71.07 ± 0.05°C (for qPCR-HRM reaction) and 60.0 ± 1.5°C (for nested snapback reaction) were observed with artificial mix of Poc/Pow at the proportions of 8/2, 7/3, 5/5, 3/7 and 2/8 (Fig. 4).

2. Experimental design, materials and methods

2.1. qPCR–HRM and nested snapback assays

The melting curves of Poc and Pow in Fig. 1 and the melting temperatures (Tm) shown in Fig. 2 were obtained by PCR and high-resolution melting reactions using a Roche LightCycler 480 qPCR system.
Table 1

P. ovale curtisi (Poc) and P. ovale wallikeri (Pow) samples tested with the qPCR-HRM and snapback assays with their microscopy, nested PCR and PrimerDesign qPCR genotyping.

| No. | Sample ID | Origin      | Microscopy | Parasite density/μl | Nested PCR | qPCR-HRM genotyping | Snapback genotyping | PrimerDesign Kitb |
|-----|-----------|-------------|------------|---------------------|------------|---------------------|---------------------|-------------------|
| 1   | F3        | Ethiopia    | Pv         | –                   | Pow        | Pow                 | Pow                 | Pow               |
| 2   | T52       | Ethiopia    | n.d.       | –                   | Pow        | Pow                 | Pow                 | Pow               |
| 3   | K21       | Ethiopia    | Pv         | –                   | Pow        | Pow                 | Pow                 | Pow               |
| 4   | K28       | Ethiopia    | Pf         | –                   | Poc        | Poc                 | Poc                 | Poc               |
| 5   | K41       | Ethiopia    | Pv         | –                   | Poc/Pf     | Poc                 | Poc                 | Poc               |
| 6   | K46       | Ethiopia    | Pv         | –                   | Pow        | Pow                 | Pow                 | Pow               |
| 7   | Pro2      | Ethiopia    | Pv         | 4920                | Poa        | n.d.                | Pow                 | n.d.              |
| 8   | Pro4      | Ethiopia    | Pv         | 5600                | Poa        | n.d.                | Pow                 | n.d.              |
| 9   | Pro6      | Ethiopia    | Pv         | 15200               | Poa        | Pow                 | Pow                 | Pow               |
| 10  | Pro9      | Ethiopia    | Pv         | 4920                | Pf/Po      | Pow                 | Pow                 | Pow               |
| 11  | Pro12     | Ethiopia    | Pv         | 6000                | Poa        | Poc                 | Poc                 | Poc               |
| 12  | Pro21     | Ethiopia    | Pv         | 7200                | Pf/Iova    | Pow                 | Pow                 | Pow               |
| 13  | 5         | Ethiopia    | n.d.       | –                   | Poc        | Poc                 | Poc                 | n.d.              |
| 14  | SG9255    | Ethiopia    | n.d.       | –                   | Pow        | Pow                 | Pow                 | Pow               |
| 15  | Po1       | Bangladesh  | Pv         | 2240                | Pow        | Pow                 | Pow                 | Pow               |
| 16  | Po2       | Bangladesh  | Pm         | 6680                | Pow        | Pow                 | Pow                 | Pow               |
| 17  | Po3       | Bangladesh  | Pm         | 2600                | Pow        | Pow                 | Pow                 | Pow               |
| 18  | Po4       | Bangladesh  | Pv         | 280                 | Poc        | Poc                 | Poc                 | Poc               |
| 19  | Po5       | Bangladesh  | Pv         | 120                 | Pow        | Pow                 | Pow                 | Pow               |
| 20  | Po7       | Bangladesh  | Pv         | 440                 | Pow/pf     | n.d.                | Pow                 | n.d.              |
| 21  | Po8       | Bangladesh  | Pv         | 320                 | Pow/pm/pf  | Pow                 | Pow                 | Pow               |
| 22  | Po9       | Bangladesh  | Pf         | 14520               | Pow/Pf     | n.d.                | Pow                 | n.d.              |
| 23  | Po10      | Bangladesh  | Pf+Pv      | 480                 | Poc/Pf/Pv  | Poc                 | Poc                 | Poc               |
| 24  | Po11      | Bangladesh  | neg        | –                   | Pow        | Pow                 | Pow                 | Pow               |
| 25  | Po12      | Bangladesh  | neg        | –                   | Poc        | Poc                 | Poc                 | Poc               |
| 26  | Po14      | Bangladesh  | neg        | –                   | Poc        | Poc                 | Poc                 | Poc               |
| 27  | Po15      | Bangladesh  | neg        | –                   | Poc        | Poc                 | Poc                 | Poc               |
| 28  | Po16      | Bangladesh  | neg        | –                   | Poc/Pf     | Poc                 | Poc                 | Poc               |
| 29  | Po17      | Bangladesh  | Pf         | 3080                | Poc/Pf/Pm  | Poc                 | Poc                 | Poc               |
| 30  | Po18      | Bangladesh  | neg        | –                   | Poc/Pf     | Poc                 | Poc                 | Poc               |
| 31  | Po20      | Bangladesh  | neg        | –                   | Poc/Pf/Pv/Pm| Poc                 | Poc                 | Poc               |
| 32  | Po21      | Bangladesh  | neg        | –                   | Poc/pf/pv/pm| Pow                 | Pow                 | Pow               |
| 33  | Po22      | Bangladesh  | neg        | –                   | Pow/Pf/Pv/Pm| Pow                 | Pow                 | Pow               |

n.d. (not detected); Po (Plasmodium ovale); Pf (Plasmodium falciparum); Pv (Plasmodium vivax); Pm (Plasmodium malariae).

Further information of Bangladeshi ovale samples can be found in Ref.[5].

a P. ovale spp. was not known to be endemic in the sampling areas.

b P. ovale species was not characterized.

c Parasite density/μl was not evaluated for all samples.

**Fig. 1.** Separated melting curves of Pow (red) and Poc (green). The lines at the middle of the curves are presenting the accuracy of the Tm with equal Tm value of the sample.
2.2. Clpc gene fragments amplification and sequencing

A fragment of the clpc gene (640 bp) of 12 randomly selected samples was amplified by PCR from total genomic DNA using primers previously designed by Perkins et al. [2]: Perkins_clpcF (5′-GGTAAAACTGAATTAGCAAAAATATTA-3′) and Perkins_clpcR (5′-GGACGAGCTCCATATAAAGGATT-3′). The PCR reaction was performed with initial denaturation at 95°C (4 min) and 40-cycles of denaturation (95°C, 20 sec), annealing (50°C, 30 sec) and extension (72°C, 50 sec). The PCR products were separated by electrophoresis in a 2% agarose gel. The PCR positive products were sequenced commercially by LGC Genomics.

The sequences were edited using Vector NTI version 11.5 and BioEdit software package version 7.2.6. Multiple sequence alignments were performed using the clustal W algorithm, as implemented in MEGA 7, to compare the obtained sequences to a set of published P. ovale clpc sequences from other studies [2–4] retrieved from GenBank (Accession numbers KP050438 – KP050448, AB649417, AY634623, HQ842632, KX611805, LT594596, LT5994519).

2.3. Phylogenetic reconstructions

For phylogenetic reconstructions, the most appropriate model of molecular evolution was determined by the Akaike Information Criterion (AIC) using MEGA7. Maximum likelihood (ML) analyses with 1000 bootstrap replicates were performed using the program MEGA7 with the predetermined model of molecular evolution (GTR+I+G for both datasets) using all sites. All the Plasmodium species clustered separately with strong bootstrap support. Additionally, the P. ovale wallikeri and P. ovale...
Fig. 3. Maximum likelihood tree of 61 Plasmodium sp. clpc gene. The clpc gene fragment of 12 samples was amplified, sequenced, aligned and compared to those from other studies [2–4] in order to confirm their identity. The green diamonds are indicating the selected 12 samples. The scale bar shows the number of nucleotide substitutions per site.
Fig. 4. qPCR-HRM and Nested snapback melting curves obtained from artificial mixes from Poc and Pow. The following five (5) artificial mixes with various ratios were done: 8/2, 7/3, 5/5, 3/7, 2/8, for respectively Poc/Pow. All mix samples produced an intermediate Tm (71.07 ± 0.05 °C) with the qPCR-HRM assay. For the snapback reaction, the intermediate Tm values were 60.0 ± 1.5 °C.
curtisi formed two distinct sub-clusters with strong bootstrap support. Among the 12 samples that were sequenced, 7 (Po1, Po2, Po4; K46, Pro2, Pro6, Pro21) were clustered with *P. ovale wallikeri* and 5 (Po3, Pro9, Pro12, K28, K41) were clustered with *P. ovale curtisi*.

**Acknowledgements**

We wish to thank all collaborators involved in collecting the samples used in the validation of the assay as well as all malaria patients participating in these studies. We also wish to thank our colleagues from the Epidemiology & Diagnostic of Zoonoses Research Group, Institute of Specific Prophylaxis and Tropical Medicine, Medical University of Vienna, Austria for providing the Non-plasmodium DNA.

**Transparency document**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2019.103937.

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