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SCIENTIFIC REP⚙RTS

New insights into the *Plasmodium vivax* transcriptome using RNA-Seq

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Historically seen as a benign disease, it is now becoming clear that *Plasmodium vivax* can cause significant morbidity. Effective control strategies targeting *P. vivax* malaria is hindered by our limited understanding of *vivax* biology. Here we established the *P. vivax* transcriptome of the Intraerythrocytic Developmental Cycle (IDC) of two clinical isolates in high resolution by Illumina HiSeq platform. The detailed map of transcriptome generates new insights into regulatory mechanisms of individual genes and reveals their intimate relationship with specific biological functions. A transcriptional hotspot of *vir* genes observed on chromosome 2 suggests a potential active site modulating immune evasion of the *Plasmodium* parasite across patients. Compared to other eukaryotes, *P. vivax* genes tend to have unusually long 5′ untranslated regions and also present multiple transcription start sites. In contrast, alternative splicing is rare in *P. vivax* but its association with the late schizont stage suggests some of its significance for gene function. The newly identified transcripts, including up to 179 *vir* like genes and 3018 noncoding RNAs suggest an important role of these gene/transcript classes in strain specific transcriptional regulation.

Malaria remains a global problem impacting hundreds of million lives around the world. Over recent years significant progress has been made in reducing the global burden of *Plasmodium falciparum* and major steps towards an operational vaccine have been recently made[1,2]. The successful control of *P. falciparum* has though highlighted how little progress has been made to control the second species of the malaria pathogen; the geographically most widely spread parasite, *P. vivax*. It is of particular concern that in areas where *P. falciparum* control is successful, *P. vivax* becomes dominant[3]. This suggests that the overall morbidity caused by *P. vivax* has been significantly underestimated in the past[4-6]. The distinct pathophysiology of *P. vivax* that is now being fully appreciated, calls for specific disease control programs that need to be different from those that until now are being used in most endemic regions to target *P. falciparum*[3,5,7].

The specificities of *vivax* malaria are underlined by unique biology of the pathogen itself and its interaction with the host[7-9]. The difficulty in studying this parasite in the laboratory due to a lack of a continuous *in vitro* culture system, limits the understanding of molecular mechanisms that characterize this parasite. Nonetheless publication of the genome[10] and the transcriptome of the blood stage life cycle[11,12] in 2008 provided invaluable insights into *P. vivax* biology. The subsequent high throughput sequencing[13] and the assembly of a genetic map helped us to understand the genetic diversity of this pathogen[9,14-18] showing specific differences from other parasites[10,19-21]. Altogether these genomic studies provided a broader platform for identifying new molecular targets for malaria intervention strategies[22-25]. More insights were also obtained into the genetic nature of *P. vivax* relapses resulting from the activation of the dormant hypnozoite stage[26].

Genome-wide transcriptional analyses throughout the life cycle stages of *Plasmodium* using microarray approaches revealed many features of gene expression and utilization in respect to both the parasite growth and adaptation to its host[10-12]. Nonetheless, some properties of gene expression remained uncharacterized including absolute expression levels, the structures of the untranslated regions (UTRs) or the full extent of alternative splicing (AltSpl). RNA-Seq studies of *P. falciparum* parasites failed in deriving these information due to the high abundance of AT-rich low complexity sequences in the genome[27-29]. Although the *P. vivax* genome is highly

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