Transcriptomic and next generation sequencing approaches to understanding infection with *Treponema pallidum*

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I am an infectious diseases clinician and epidemiologist and NIHR Clinical Lecturer based at the London School of Hygiene & Tropical Medicine. This was a proof of concept study which aimed to utilise next generation sequencing and transcriptomic approaches to understand infection with both Syphilis and Yaws.

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**Study Report**  
The human treponematoses comprise syphilis and the endemic treponematoses. Infections with these organisms cause a variety of complex diseases, characterised by multisystem involvement. Although the genome sequences of *T. pallidum pallidum* and *T. p. pertenue* are extremely similar with greater than 99% sequence homology the clinical spectrum and severity of yaws and syphilis differs significantly. How host responses to infection with *T.p.pallidum* and *T.p.pertenue* differs and the extent to which this influences clinical phenotype is unknown. This pilot study aimed to address this question.

**Patient Recruitment**  
Patients with clinically suspected yaws were recruited in Papua New Guinea as part of a large-scale yaws treatment study. Additional follow-up funding was obtained from the British Infection Association which also allowed patients with yaws to be recruited in Ghana. Patients with syphilis were recruited at the Mortimer Market Centre in London. From both suspected yaws and syphilis patients we obtained lesion samples into RNA-Later.

**Sample Processing**  
We extracted DNA and RNA from lesion samples. RNA libraries were prepared and samples multiplexed and sent for RNA transcriptional profiling at Lexogen. DNA samples were sent to the Wellcome Trust Sanger Institute. qPCR was performed and samples with a CT value of <31 were selected for whole genome sequencing using a SureSelect DNA enrichment protocol on an Illumina platform.

**Transcriptional Analysis**  
Transcriptional analysis of RNA data was performed in collaboration with colleagues at the UCL Division of Infection and Immunity. Data were mapped to the latest reference transcriptome (release 98), using Kallisto for single-end reads and the dataset annotated with biomart and filtered for protein-coding genes. Expression profiles across different disease subsets are being assessed to identify transcriptional pathways that might be implicate in difference between study groups.

A total of 106 samples were collected from patients with syphilis in the United Kingdom and yaws in both Papua New Guinea and Ghana. Transcriptional analysis is still in process and we are exploring differential expression of immune and other pathways which might be important in the pathogenesis of both diseases.
Use of Funds:
Funding from the Association of Physicians was used to cover travel and field work costs for sample collection in Papua New Guinea, supporting sample collection in the UK and performing the RNA Transcriptional profiling.

Next Steps:
Analysis of the RNA Transcriptional profiles is ongoing. We will use our rich dataset to try and identify difference in gene expression between different stages of syphilis (Primary and Secondary syphilis) and between Syphilis and Yaws. We also plan to consider unsupervised assessment of variability in gene expression across all samples and explore clinical variables that associate with different gene expression profiles.

In the longer term it is hoped that this pilot work can support an application for larger scale transcriptomic studies exploring host immune responses and the pathogenesis of these important human diseases.