BMFMS Bursary, recipient Dr Gareth J Waring

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Interim progress report:

Title: The Microbiome of chorioamnionitis

Introduction:
Though preterm birth (PTB) is the leading cause of neonatal morbidity and mortality its aetiology remains elusive. Infection is the principle pathologic process with an established causal link to PTB and a defined molecular pathophysiology. Ascending infection via the vagina and cervix is the most commonly recognised pathway and histologic chorioamnionitis (HCA) is the most specific and sensitive marker for infection. Our group previously has described the inflammatory signalling associated with PTB and chorioamnionitis in the fetal membranes suggestive of polymicrobial involvement (*Waring et al, PLoS ONE 2015*).
Culturing organisms in this setting has always been challenging with an estimated 90% of microbes making up the human microbiome thought to be uncultivable. However, as shown by the human microbiome project, 16s based metagenomics is a powerful technique that allows the research community to identify the composition of the microbial community even down to the species level. The microbiome in various reproductive tissues have been assessed. Of note, the placental parenchymal microbiome appears to most closely resemble the microbiome of the non pregnant oral tract and not closely resemble either the urogenital or GI tract. This suggests the placental parenchymal microbiome is seeded via haematogenous spread as opposed to ascending colonisation. Given the membranes are the interface between the maternal and fetal compartments the composition of the microbiome at this point should be informative as to the mechanism of inflammation related PTB.

Work carried out:
Fetal membrane explants from 29 patients were collected and phenotype histologically; 9 were from term patients following spontaneous labour without evidence of HCA, 20 were preterm samples (<34 weeks), 12 with HCA and 8 without HCA.
DNA has been extracted and isolated using a protocol that has been optimised for fetal membranes (based on work with term membranes). Quantity and quality of isolated DNA has been assessed by nano-drop and gel electrophoresis.

Work to complete:

The DNA will be analysed by 16S rRNA PCR using barcoded primers to be ran on the MiSeq. Sequences that pass QC will be checked for chimeras generated during PCR and any matches to the chimera database will be excluded from any further analysis. The remaining sequences will be compared against the SILVA 16S rRNA database for identification of bacteria and called at the genus level. The current aim of the MiSeq analysis is to generate 10,000 sequence reads per sample.

Interpretation of results will be aided by comparison alongside the previously generated gene expression profiles for each of these samples for key TLRs and IL8. Specifically, we will use principle component analysis to analyse the microbial communities to see if community membership is different between disease samples and controls. We will then scrutinise the data further using partial least squares discriminant analysis to examine the effect of the microbiome on TLR and IL-8 expression.

Expected completion date is October 2017 and this work will be submitted for the BMFMS conference 2018 before the deadline of 4/12/17.