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Short communication

Term and preterm labour are associated with distinct microbial community structures in placental membranes which are independent of mode of delivery

R.M. Doyle [a, *], D.G. Alber [a], H.E. Jones [a], K. Harris [c], F. Fitzgerald [a], D. Peebles [b], N. Klein [a]

[a] Infection, Inflammation and Rheumatology Section, Institute of Child Health, London, United Kingdom
[b] Department of Maternal and Fetal Medicine, Institute for Womens Health, University College London, London, United Kingdom
[c] Microbiology Department, Camelia Botnar Laboratories, Great Ormond Street Hospital, London, United Kingdom

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ABSTRACT

Infection is considered a possible trigger for preterm labour, supported by evidence showing the presence of bacteria in the placenta and placental membranes from preterm births. In this study, 16S rDNA pyrosequencing was used to identify bacteria in placental membranes. Caesarean sections and vaginal deliveries at term were found to harbour common genera. *Mycoplasma hominis*, *Aerococcus christensenii*, *Gardnerella vaginalis* and *Fusobacterium nucleatum* were either only present in preterm membranes or in greater abundance than at term. These data support previous studies that used either targeted qPCR or broad-range 16S rDNA PCR and cloning but not a recent microbiome analysis of placental tissue using high-throughput sequencing.

1. Introduction

Onset of labour is characterised by placental and amniotic inflammation, which appears to occur as a result of both maternal and fetal triggers. An unscheduled inflammatory response can induce preterm labour, which is associated with substantial morbidity and mortality despite medical attention [1].There are a number of studies that show approximately 90% of very preterm births are associated with the presence of an infectious agent and severe inflammation in the placenta and amniotic fluid [1,2]. However while such evidence is supportive of a role for bacteria in preterm births, questions remain as to the identity of the bacteria involved, and how, if present, these microbes lead to premature labour.

Recent advances in 16S rDNA amplicon high-throughput sequencing have allowed attempts to extensively define bacteria within placenta tissue [3]. The aim of this study is to compare microbiota found on placental membranes of very preterm and term deliveries using the combination of sequencing two separate 16S hypervariable regions (V1–V2 and V5–V7) in the attempt to maximise coverage.

2. Methods

This study received ethical approval from The University College London/University College Hospital Research Ethics Committee (REC number 03/0179). Informed written consent was obtained from all participants.

Placental membranes (chorion and amnion) were collected from vaginal deliveries from spontaneous preterm births with intact membranes at 28–32 weeks gestation (PTL V, $n = 14$), after 37 weeks by caesarean section not in labour with intact membranes (T CS, $n = 4$) and term vaginal deliveries (TL V, $n = 6$). Only preterm placentas were evaluated for histological chorioamnionitis and all were diagnosed positive. Collection and DNA extraction was carried out as previously described [2]. Extracted DNA was amplified twice in separate PCR reactions, pyrosequenced on a 454 FLX Titanium (Roche) and the subsequent sequences analysed as previously described [4]. Reads were filtered above a level to remove spurious Operational Taxonomic Units generated by PCR or sequencing error (had to be present above 1% relative abundance in at least one sample). Six samples collected routinely at Great Ormond Street Hospital for microbiological diagnosis were run in parallel and

* Corresponding author. Infection, Inflammation and Rheumatology Section, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom. Tel.: +44 (0) 20 7905 2892.
E-mail addresses: Ronan.doyle.10@ucl.ac.uk (R.M. Doyle), d.alber@ucl.ac.uk (D.G. Alber), hannah.jones@ucl.ac.uk (H.E. Jones), kathryn.harris@gosh.nhs.uk (K. Harris), felicity.fitzgerald@ucl.ac.uk (F. Fitzgerald), d.peebles@ucl.ac.uk (D. Peebles), n.klein@ucl.ac.uk (N. Klein).

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results compared to those obtained by the clinical microbiology service to provide method validation.

3. Results

Term deliveries shared many of the same genera, across both primer sets (Fig. 1) such as *Streptococcus*, *Microbacterium*, *Rhodococcus* and *Corynebacterium*. There was a marked difference between the relative levels of *Lactobacillus* in the vaginal deliveries compared to caesarean sections; however this was expected and is easily identifiable. 16S rDNA amplicon pyrosequencing identified 6 genera (*Fusobacterium*, *Streptococcus*, *Mycoplasma*, *Aerococcus*, *Gardnerella* and *Ureaplasma*) and 1 family (*Enterobacteriaceae*) which were either present in greater relative abundances in preterm samples or absent in term deliveries. Both hypervariable regions were consistent with reduced abundances of the genus *Lactobacillus* recovered from the PTL V group, as well as increased abundances of the genera *Streptococcus*, *Aerococcus* and *Ureaplasma*. Unique or abundant sequences found in the PTL V group were compared with the sequences found on the Genbank database to see if any could be identified to species level. The criteria used for a species level assignment was 99%–100% similarity to only a single species using BLAST, with the following organisms meeting that criteria: *Fusobacterium nucleatum*, *Mycoplasma hominis*, *Aerococcus christensenii*, *Streptococcus anginosus*, *Streptococcus agalactiae* and *Gardnerella vaginalis*. *Streptococcus mitis* group was also identified, but could not be speciated further.

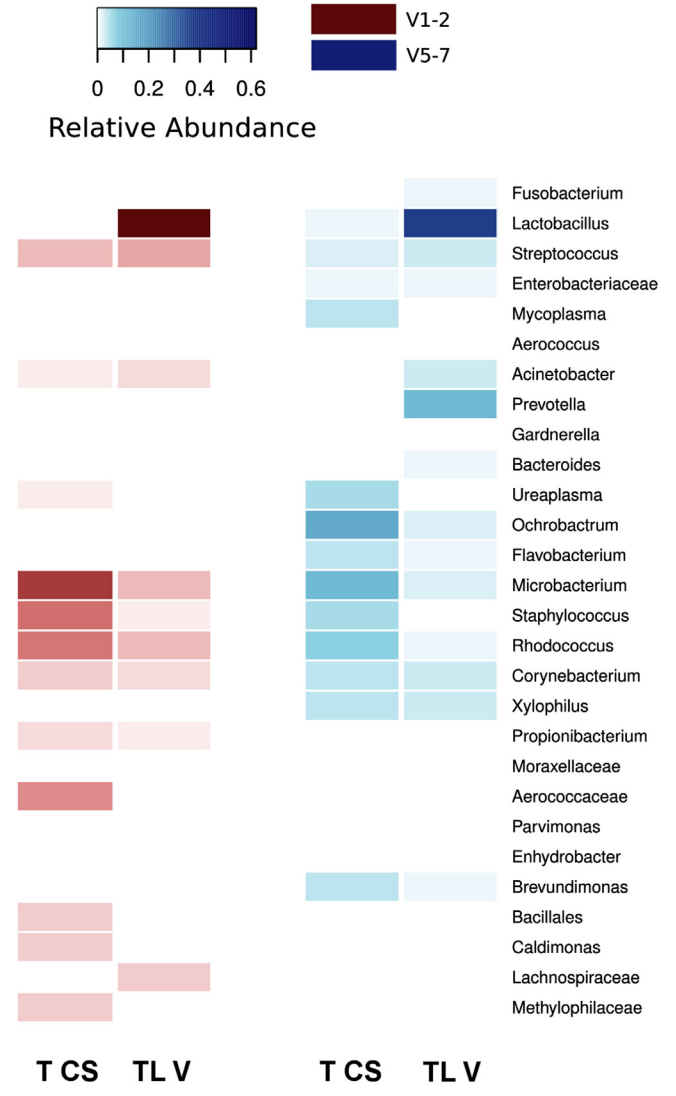

Fig. 1. Bacteria present in placental membranes of both elective caesarean sections (T CS) and vaginal deliveries (TL V) at term. Pyrosequencing of 16S rDNA isolated from placental membranes are grouped by primer pair used to amplify library. Numbers of reads per individual were normalised before being grouped by mode of delivery. Each block of colour represents the varying relative abundance of each genera to that specific group.

4. Discussion

We have previously shown an increased frequency of bacterial detection as well as a wider spectrum of bacteria in preterm placental membranes as compared to term [2]. Bacteria can either invade during labour [5], or colonise at some point prior to labour, eventually triggering an inflammatory response and the onset of labour. This latter scenario is consistent with previous work suggesting intra-amniotic infection and inflammation as a trigger of preterm birth [6]. What was unclear was whether the bacteria detected were an accurate representation of the placental bacterial microbiome associated with preterm birth.

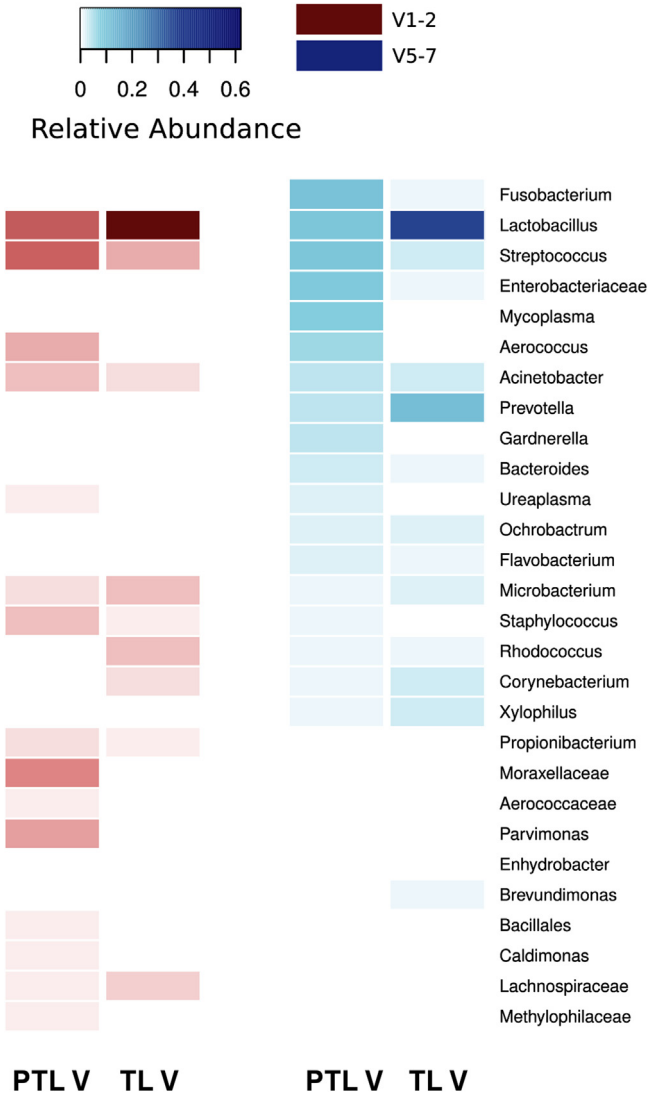

Fig. 2. Relative abundance of bacteria in placental membranes of term (TL V) and preterm (PTL V) vaginal deliveries. Heat map was generated using the same method as previously mentioned (Fig. 1). Bacteria are in descending order from highest relative abundance in PTL V group by primers targeting V5-7 region of 16S rRNA gene.

Bacterial species detected in this study were broadly similar to those previously associated with the placenta or preterm birth such as *Steptococcus agalactiae*, *Streptococcus mitis group* and *Mycoplasma hominis* (Fig. 2), [2,7] and to species recovered from the amniotic fluid of preterm deliveries such as *Fusobacterium nucleatum* and *Gardnerella vaginalis* [8]. However, for the first time *Aerococcus christensenii*, an organism associated with the vaginal microbiota [9] was detected in preterm membranes. Interestingly there were few similarities to the most common genera recovered in a recent study of the placenta microbiome [3] that also used high-throughput sequencing to analyse the same region (V1–V3) of the 16S rRNA gene. We have shown here, that this region on its own does not capture the full diversity present, and that in combination with another hypervariable region, organisms that might otherwise be missed can be recognised. Interestingly, shot-gun metagenomic analyses [3] also identified different organisms from our data, and from other published studies.

In conclusion, this study provides further evidence for the role of bacteria in preterm birth. Bacterial DNA is present in the majority of placental membranes from both term and preterm deliveries, irrespective of mode of delivery and there are consistently identifiable bacterial species in preterm labour. The relative roles of bacterial diversity, bacterial load and host in the induction of preterm birth remain to be clarified. A greater understanding of when and how such bacteria are related to preterm delivery should lead to novel ways of limiting preterm births.

Conflict of interest

All authors wish to confirm there is no conflict of interest associated with this publication.

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