Project Number: CC002

Title: DNA methylation in blood and tissue of children with cleft lip/palate

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Scientific Outline:

Ongoing research aims to provide improved strategies to predict, prevent and treat CL/P. A greater understanding of the biological mechanisms causing clefts and cleft-related outcomes will be crucial to inform such strategies.

Non-syndromic CL/P has a complex, multifactorial aetiology involving interactions between genetics and the environment. The biological mechanism by which the environment influences risk of cleft is unknown, but epigenetics may play a role. DNA methylation is the most commonly studied epigenetic mechanism and Illumina 450k array technology can be used to measure DNA methylation levels at over 450,000 sites throughout the human genome.

The aim of this project is to generate Illumina 450k data for 150 individuals from the Cleft Collective postnatal cohort. The Cleft Collective is a richly phenotyped cohort with tissue and blood samples from a large number of children with cleft lip/palate. This will provide the opportunity to investigate associations between DNA methylation and cleft lip/palate subtypes, as well as other phenotypes. The availability of tissue and blood samples provides further opportunities for cross-tissue comparison, which is an area of interest in epigenetics.

DNA methylation data will allow us to explore several research questions under four main themes:

1) Causes of CL/P – identifying differentially methylated regions associated with different subtypes of CL/P (i.e. CL, CP, CL+P, unilateral, bilateral) and maternal risk factors for CL/P will help us understand the aetiology of CL/P and the extent to which DNA methylation might play a role.
2) Outcomes of CL/P – clinical subtypes of CL/P as well as cleft-associated outcomes such as anxiety, difficulties feeding or recurrent ear infections may affect DNA methylation, which may mediate associations with other longer-term outcomes such as poor educational attainment or a higher risk of cancer.
3) Tissue-specificity of DNA methylation – unlike genetic material, which is the same in all cells of the body, epigenetic marks can vary between cells and tissues. Having DNA methylation data on blood and lip/palate tissue for 150 individuals will allow us to investigate the extent to which DNA methylation is tissue-specific. For example, we can explore whether or not associations between methylation and CL/P subtype are stronger in the relevant tissue than

in peripheral blood. Few existing cohorts have the necessary biological samples to allow them to address such questions, so this will be of interest to the wider scientific community.

4) Associations between DNA methylation and other traits/disease – DNA methylation data will allow us to explore associations between DNA methylation and other traits/diseases, not necessarily related to CL/P. By contributing results to analyses by consortia such as the Pregnancy and Childhood Epigenetics (PACE) consortium, we will aid efforts to understand the aetiology of other diseases, while widening awareness of the Cleft Collective in the molecular epidemiology community and strengthening opportunities for further collaboration.

A sufficient number of biological samples have already been collected and are stored at the Bristol Bioresource Laboratory. DNA extraction and running of the Illumina 450k arrays will be carried out by Karen Ho. RNA will also be extracted from tissue and stored at -80^{o}C for future analysis (not part of the current project). Data QC, normalisation and statistical analysis will be carried out by Gemma Sharp using the R package “meffil”, developed at the MRC Integrative Epidemiology Unit.

Initially, the essential required variables are: CL/P subtype (CL, CP, CLP), unilateral/bilateral, age of child at biological sample collection, sex of child.

When the data become available, other requested variables are: maternal smoking status, folic acid supplements during pregnancy, maternal alcohol intake, maternal age, paternal age, maternal BMI, paternal BMI, birth weight, parity, socioeconomic status (mother, father, household), ethnicity. Amendments to request additional variables will be submitted as and when required.