Investigating Genome wide DNA methylation in Airway Smooth Muscle cells from Asthmatic and Non-asthmatic Donors

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Rationale: Genetic mechanisms fail to fully explain asthma pathogenesis and environmental factors are considered to play an important role. Environmental factors may lead to permanent changes in epigenetic patterns and contribute to asthma. Epigenetics is the study of heritable changes in gene expression that are not due to changes in DNA sequence. DNA methylation is a reversible modification of DNA structure in which a methyl group is added to cytosine residues. Parental smoking affects the methylation of buccal cell DNA from children and children with early onset wheeze have an altered blood DNA methylation profile to healthy individuals. No studies have compared DNA methylation profiles in the disease relevant cell type of airway smooth muscle (ASM) cells.

Methods: DNA was isolated from ASM cells at passage 5 and bisulphite treated to convert epigenetic information into sequence-based information. Site specific, quantitative genome wide methylation was determined using the Illumina 450K Infinium Methylation BeadChip array. Hits were validated by Pyrosequencing. RNA was extracted simultaneously for mRNA expression analysis by real time PCR.

Results: There were no independent CpG sites associated with asthmatic status of ASM cells following multiple test correction. Without correction over 13000 CpG sites showed a significant difference in methylation (linear modelling, p value>0.05) between asthmatic and non-asthmatic cells, and a biologically relevant difference in methylation of greater that 10% (β value >0.1). 10 of these sites were selected as top hits. 7 sites positively validated by pyrosequencing. They were associated with 7 different genes; LGALS3BP, ATP11A, ZNF696, KLF6, TBX1, RUNX3, and SPINT2. Expression of these genes was measured in ASM cells isolated from asthmatic and non-asthmatic donors. LGALS3BP expression was undetectable while ATP11A and ZNF696 displayed no difference in expression between cells from asthmatic and non-asthmatic donors. KLF6 and SPINT2 showed a trend towards increased expression in cells from asthmatic donors while RUNX3 and TBX1 showed a trend towards decreased expression.

Conclusions: Differences in CpG methylation exist between ASM isolated from asthmatic and non-asthmatic donors. Future work will focus on identifying differentially methylated regions of DNA and further defining the association to gene and protein expression.