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Investigation into the genetic and functional relevance of the association of rs12477314 with pulmonary function

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Introduction: Recent Genome-Wide Association Study (GWAS) metaanalyses have identified a number of significant association signals for pulmonary function, one of which maps to a locus (rs12477314) in an intergenic region on 2q37.3 flanked by two oppositely transcribed genes – HDAC4 and Twist2, and a lincRNA (FLJ43879). Aim: The aim of this study is to investigate the genetic and functional relevance of the association of single nucleotide polymorphism (SNP) rs12477314 with pulmonary function.

Methods: 3'Rapid amplification of cDNA ends (RACE) was performed on HDAC4 and Twist2 expressed from a number of cell types. The potential involvement of mentioned genes in reduced pulmonary function was assessed by investigating the effect of inflammatory mediators on gene expression in A549 cells, using quantitative polymerase chain reaction (qPCR). To gain further insights into the mechanisms underlying the GWAS signal, linkage disequilibrium, expression and methylation quantitative trait loci, and histone methylation signatures were investigated using publicly available sources.

Results: 3'RACE did not reveal any variants for which the 3'UTR extended to rs12477314 proximity. Treatment of A549 cells with lipopolysaccharide resulted in upregulation of HDAC4 expression. Bioinformatic searches revealed that the intergenic region is enriched for DNA/histone methylation markers suggesting active enhancer regions. We will follow up this work with deletion of selected regions showing enhancer potential using CRISPR/Cas9 system followed by RNAseq, in order to investigate genome regulation in mentioned intergenic region.

Conclusion: This study provides preliminary evidence suggesting that epigenetic regulation at region tagged by rs12477314 may underlie the observed association seen with pulmonary function.