ABSTRACT

THE IDENTIFICATION OF ATPAF1

AS A NOVEL ASTHMA SUSCEPTIBILITY GENE AND

THE CHARACTERIZATION OF FUNCTIONAL REGULATORY VARIANTS.

By

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Asthma, the most common chronic disease of childhood, is driven by genetic and environmental determinants. To identify genes that increase the risk of asthma in children, a multiple stage genome-wide association study was conducted in a nested case-control study of a wholepopulation birth cohort from the Isle of Wight, UK. This study resulted in the identification of a cluster of associated SNPs and SNP haplotypes in the ATPAF1 gene (ATP synthase mitochondrial F1 complex assembly factor 1) on human chromosome 1p33, with two SNPs achieving significance at a genome-wide level (P=2.26E-5 to 2.2E-8). SNP, haplotype, and/or gene-level associations were confirmed in three of five replication populations.

The ATPAF1 gene contains 303 reported variants, which were assessed using in silico techniques and prioritized through annotated function in public databases, and/or inferred function based on their location in experimentally reported or predicted functional DNA sequences. Twenty-seven variants were prioritized based on the in silico results, of which several variants had predicted function as coding, splicing, and/or gene expression regulation. These prioritized variants were targeted in addition to exons, conserved, and regulatory regions for selective resequencing in 40 cohort individuals using Sanger sequencing.

Selective resequencing of 14.6kb resulted in the identification of 35 total variants. This included validation of 9 (of the 27) prioritized variants from the in silico screen and 9 new rare variants, including 1 nonsynonymous mutation. Three variants with gene expression regulatory potential were found to be clustered within 600 bp of each other in the promoter/exon 1 of ATPAF1 in four haplotypes. This region was targeted for analysis using luciferase reporter gene assays in BEAS-2B and COS-7 cell lines. These cell culture assays confirmed promoter functionality and indicated a statistically significant difference in luciferase expression (means ranging between 2-3 fold differences) among the promoter haplotypes.

In conclusion, ATPAF1 was identified as a childhood asthma susceptibility gene. In silico studies

coupled with selective resequencing of the ATPAF1 region provided an efficient method to identify functional variants. DNA variant haplotypes within the ATPAF1 promoter demonstrated the ability to differentially regulate gene expression. However, the roles of these and other functional variants in the ATPAF1 promoter and their ability to modulate asthma susceptibility need further study.