

Wandering out of the GWAS wilderness: a new pathway paradigm for complex disease genetics

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A genetic contribution to asthma susceptibility has long been recognised.¹ Despite multiple approaches, from candidate genes to genome-wide association studies (GWAS), a large proportion of this heritability remains unexplained.² The reasons for this are myriad and are largely generalisable to similar approaches in other complex diseases and phenotypes. Candidate gene studies are well based in known pathophysiology and can be powered to identify small effect sizes, but often provide limited insight into new biology or therapeutic opportunities. GWAS are largely unbiased (tagging single-nucleotide polymorphism (SNP) selection not withstanding) and provide opportunities for discovery, but identifying the biologically functional variant can prove daunting, and other important variants likely do not survive genome-wide significance corrections where sample sizes, and hence statistical power, are limited. Both of these approaches in isolation are also likely to miss the influence of genetics on the complex phenotypic response to environmental exposures. In this issue of *Thorax*, Dizier *et al* report an interesting pathway-based approach to a gene by environment (G×E) analysis of a well-established epidemiological association between environmental tobacco smoke (ETS) exposure and bronchial hyper-responsiveness (BHR).³ This hybrid method has some of the benefits of multiple modalities and may provide a new avenue for further clarifying the genetic underpinnings of other complex diseases.

The basis for the current study comes from a preceding positional cloning study of the 17p11 region in the French Epidemiological study on the Genetics and Environment of Asthma (EGEA) families. This showed an interactive effect between ETS exposure and a variant in Dynein, Axonemal, Heavy Chain 9 (DNAH9) on BHR.^{4,5} The DNAH9 gene encodes a

subunit of axonemal dynein, a cytoskeletal motor protein critical for ciliary motility. Importantly, this subunit contains the components necessary for ATP binding and hydrolysis needed for conversion of stored chemical energy into mechanical work.⁶ Dizier *et al* reasoned that variants in other genes encoding for proteins with either ATP binding or ATPase functions might have similar interactions with ETS in BHR. This rationale was further supported by studies showing (1) a linkage between airway extracellular ATP and asthmatic inflammation⁷ and (2) reduced ATPase activity with cigarette smoke exposure.⁸

To test the hypothesis that genes belonging to 'ATPase activity' and 'ATP-binding' pathways interact with exposure to ETS in BHR, the authors first tested for this interaction with 4252 SNPs within all 296 genes in these pathways. Selected SNPs were restricted to within gene boundaries to limit the number of tests and to maintain power for interaction testing. This analysis was done in a discovery cohort of 388 French EGEA families using the Family-Based Association Test (FBAT) approach and yielded a significant SNP×ETS interaction for 20 SNPs from 11 different genes.⁹ These 20 SNPs were then tested for replication in 253 French-Canadian multigenerational families from the Saguenay-Lac-Saint-Jean (SLSJ) asthma study¹⁰ and again in a combined analysis of the two cohorts. Importantly, the definitions of the clinical measures (BHR and ETS) were highly consistent between the discovery and replication cohorts. Two SNP×ETS interactions were replicated between these analyses: rs2253304, an intronic SNP in ABCA1, and rs17448506, an intronic SNP in ATP8A1. The authors also validated the findings from the FBAT approach with a second method—the Umbach and Weinberg method. Interestingly, these two genes, ABCA1 and ATP8A1, are both involved in membrane lipid regulation. Additionally, both SNPs showed variable association with BHR based on exposure, a so-called 'flip-flop' interaction where the same allele was positively associated with BHR in ETS-exposed siblings and negatively associated with BHR in

ETS-unexposed siblings. Functionally, both SNPs map to enhancer and promoter histone marks and transcription factor binding sites, providing opportunities for altered regulation of expression. Finally, a review of curated data from the Comparative Toxicogenomics Database revealed that tobacco smoke pollution and soot have been shown to alter ATP8A1 and ABCA1 mRNA expression levels.

There are data to support a cellular mechanism for interaction between variants in membrane lipid regulatory genes and ETS in BHR. The ATP8A1 gene encodes for a transmembrane protein which is thought to maintain phospholipid asymmetry in membranes. Little is known about its functional role in the lung, though its expression does appear to be dynamically regulated, for example, by cigarette smoke. Knowledge of the role of ABCA1, however (along with ABCG1 and ABCA3) in lung biology is growing.¹¹ ABCA1 is a transmembrane protein which mediates the efflux of cholesterol and phospholipids to the extracellular acceptor apolipoprotein A-I (apoA-I) in the process of reverse cholesterol transport. Mice deficient in ABCA1 develop significant pulmonary lipid accumulation.¹² A number of studies suggest that this process may be important in pulmonary inflammation and asthma. In a study of patients with atopic and non-atopic asthma, serum high-density lipoprotein and apoA-I levels were positively correlated with FEV₁ in subjects with atopy and asthma.¹³ ApoA-I levels have also been found to be lower in bronchoalveolar lavage samples from asthmatic patients as compared with healthy controls, and intranasal delivery of apoA-I decreased inflammation in house dust mite challenged mice.¹⁴ Likewise, overexpression of ABCA1 reduced inflammation in ovalbumin-challenged mice.¹⁵

So how might the identified variants in ABCA1 and ATP8A1 differentially modulate the effect of ETS exposure on BHR? And what might account for the 'flip-flop' interaction observed? One explanation might be that ABCA1 and ATP8A1 play different roles in airway homeostasis in healthy versus disease states. Alternatively, ETS exposure may differentially modulate ABCA1 and ATP8A1 expression through effects on transcriptional regulatory machinery active at the sites of the interacting SNPs. Future mechanistic studies may focus on the relative expression and function of ABCA1 and ATP8A1 in patients of differing genotypes, with and without ETS exposure. When able, the focus should be on disease-relevant tissues, such as alveolar macrophages or airway smooth muscle.

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The pathway-based G×E interaction testing employed by Dizier *et al* combines the pathophysiological foundation of a hypothesis-driven approach with the breadth of a hypothesis-generating one to allow for discovery of novel genetic bases for complex diseases. In the end, this revealed a novel pair of genes involved in membrane lipid regulation, providing support for further studies of pulmonary lipid homeostasis in asthma. Following this pathway-based G×E interaction approach in other complex diseases may yield similar promising avenues for future investigation.

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