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A Case-only Genome-Wide Association Study on Gene-Sex Interaction in Allergic Rhinitis

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Abbreviations: none

Allergic rhinitis (AR) is a condition with a significant impact on patient’s quality of life. Sex differences in the prevalence and clinical presentation of rhinitis have been reported in multiple studies. Additionally, it has comorbidities with asthma, atopic dermatitis, rhinosinusitis, otitis media, anosmia, nasal polyps and lower airway infection. As a complex phenotype, AR is affected by both genetic and environmental factors. Recent literature has focused on difference in the genomic architecture of asthma for the two sexes by considering gene-sex interaction, whereas AR has been neglected. The cost-effective case-only design is an efficient approach for detecting interactions, compared to the case-control design. In addition, the hypothesis-free genome-wide association study (GWAS) is a promising approach to discovering novel phenotype-associated variants. Application of the case-only design to GWAS should help with detecting interaction effects efficiently.

We conducted a case-only GWAS to detect gene-sex interaction in AR in 434 Danish patients, among which 243 siblings and 191 unrelated individuals, 184 males and 250 females (eTable 1). Sample collection and diagnostic criterions have been described in detail elsewhere. SNP genotyping was conducted using the Affymetrix Genome-Wide Human SNP array 5.0 containing nearly 500,000 SNPs. Before statistical analysis, SNPs with a minor allele frequency < 5% and call rate < 90% were removed resulting in 363,536 genotyped SNPs. Based on the observed SNP genotypes and linkage disequilibrium (LD) information from the 1,000 Genomes
Project and International HapMap Project, SNP imputation was performed using IMPUTE2 software package with The Genome Reference Consortium Human build 37 as reference. After imputation, SNPs with the measured information metric (Info score) < 0.5 were removed. The final number of genotyped and imputed SNPs included in the analysis was 1,348,667. The case-only GWAS was carried out by fitting a logistic regression model of sex on SNP genotype, assuming additive genetic effect using the geepack package in R which accounts for sibling correlation. Our analysis showed no genome-wide significant SNP with p-value < 5 × 10^{-8} but 12 suggestive SNPs with p-value < 1× 10^{-5}. These SNPs are positioned on chromosomes 2, 4, 5, 7 and 16 including six imputed and six genotyped SNPs (eFigure 1). A cluster of suggestive SNPs on chromosome 5 were plotted in eFigure 2 with top SNP rs566750 (β = 0.67, p-value 2.8× 10^{-6}) at position 134,513,184 base pair (bp) mapped to C5orf66 gene.

Next, we performed a group test of SNPs within a gene to boost the power using VEGAS2. The gene-based test considers LD between SNPs estimated from the 1000 Genome Project and assigns SNPs to genes based on hg19 genomic location, and then uses a simulation approach for gene-based testing. The analysis found 1194 genes with p-value < 0.05. The list of top 20 genes ordered by p-values are presented in Table 1, with the top four genes TWFL (p-value = 5.0 × 10^{-5}), PUS7L (p-value = 7.2 × 10^{-5}), C5orf66 (p-value = 7.2 × 10^{-5}) and IRAK4 (p-value = 7.3 × 10^{-5}). Note that C5orf66 located on 5q31.1 was identified by both SNP and gene-based analyses. C5orf66 is a protein coding gene and has not yet been reported to associate with atopy. In Table 1, IRAK4 on 12q12 hosts SNP rs4251459 (β = 1.06, p-value = 1.28 × 10^{-5}). Zhang and colleagues^2 found two SNPs (rs4251431 and rs6582484) in IRAK4 displaying significant association with AR in males, while in our result, rs4251459 appears to increase the risk of AR in females compared to males. The GALNT14 gene (Table 1) has been associated
with otitis media (OM) in one GWAS. AR has been frequently reported to associate with OM in different populations. Our result provides further evidence that the two diseases (AR and OM) could be genetically connected. Chromosome 1p22.2 also showed an important association to AR because it harbors three genes GBP4, GBP7 and GBP2 (Table 1), each plays a role in the innate immune functions. Since the innate immune system is indispensable for autoimmunity and AR is an autoimmune disease, the detected genes are sensible although they are subject to further evaluations. From chromosome 2, CUL3 hosting top SNP rs11688397 is a protein-coding gene related to the innate immune system. This gene is near DOCK10 on 2q36.2 which was identified as being associated with asthma. In addition, the expression of TFAP2B (Table 1) has been shown to distinguish controlled and therapy-resistant childhood asthma.

The summary statistics from the gene-based test were then used for pathway-based analysis to cluster genes into pathways using VEGAS2 which calculated pathway-based test and empirical p-values to obtain the significance of each detected pathway. The top 10 pathways are shown in eTable 2. The top pathway “Trophectodermal cell differentiation” comprises eight genes, among which CDH1 is known to relate with airway remodeling and lung function in asthma, as SNPs within this gene are associated with epithelial E-cadherin expression. Moreover, dysfunction in epithelial cell morphogenesis, a sub-pathway of cell morphogenesis, leads to loss of differentiation, reduced junctional integrity, and impaired innate defense which predates atopy and development of allergic disease. Children with asthma were likely to have higher triglyceride level than those without asthma.
Finally, we applied HaploReg v4 to investigate the significance of non-coding regions related to AR in the regulatory units outside the coding regions. It used the list of query SNPs with p-values $< 10^{-4}$ from GWAS and calculated the coverage of strong enhancers for each cell type and then compared with background set using a binomial test statistic. At the end, enrichment was reported based on p-values $< 0.05$. Among the summary results, one enhancer, “Esophagus” was identified with a binomial p-value of 0.02. Eosinophilic esophagitis (EE) is a chronic allergy, causing inflammation of the esophagus. Interestingly, it has been reported that there is a similar histopathology between EE and atopic diseases and most of the patients with EE have allergy disorders.\textsuperscript{10}

Overall, our case-only GWAS revealed evidence of gene-sex interaction in AR either in support of previously published results or presenting novel findings that could be considered as a reference for future verifications.

Acknowledgements

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REFERENCE


Table 1: Description and test statistics of top 20 genes from gene-based analysis using VEGAS2.
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