

Letters

A case-only genome-wide association study on gene-sex interaction in allergic rhinitis



Allergic rhinitis (AR) is a condition with a significant effect on patient quality of life. Sex differences in the prevalence and clinical presentation of AR have been reported in multiple studies. In addition, AR has comorbidities with asthma, atopic dermatitis, rhinosinusitis, otitis media, anosmia, nasal polyps, and lower airway infection. As a complex phenotype, AR is affected by genetic and environmental factors. Recent literature has focused on difference in the genomic architecture of asthma for the 2 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 with 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 a 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, including 243 siblings and 191 unrelated individuals (184 males and 250 females) (eTable 1). Sample collection and diagnostic criteria have been described in detail elsewhere.¹ Single-nucleotide polymorphism (SNP) genotyping was conducted using the Affymetrix Genome-Wide Human SNP array 5.0, which contains nearly 500,000 SNPs. Before statistical analysis, SNPs with a minor allele frequency less than 5% and a call rate less than 90% were removed, resulting in 363,536 genotyped SNPs. On the basis of the observed SNP genotypes and linkage disequilibrium (LD) information from the 1000 Genomes Project and International HapMap Project, SNP imputation was performed using the IMPUTE2 software package with The Genome Reference Consortium Human build 37 as reference.

After imputation, SNPs with the measured information metric (Info score) less than 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 performed by fitting a logistic regression model of sex on SNP genotype, assuming additive genetic effect using the *geepack* package in R (R Foundation for Statistical Computing, Vienna, Austria), which accounts for sibling correlation. Our analysis showed no genome-wide significant SNP ($P < 5 \times 10^{-8}$) but 12 suggestive SNPs ($P < 1 \times 10^{-5}$). These SNPs are positioned on chromosomes 2, 4, 5, 7, and 16, including 6 imputed and 6 genotyped SNPs (eFig 1). A cluster of suggestive SNPs on chromosome 5 were plotted in eFigure 2 with the top SNP rs566750 ($\beta = 0.67$, $P = 2.8 \times 10^{-6}$) at position 134,513,184 base pair (bp) mapped to the *C5orf66* gene.

Next, we performed a group test of SNPs within a gene to boost the power using VEGAS2 (QIMR Berghofer Medical Research Institute, Brisbane, Australia). 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 a $P < .05$. The list of top 20 genes ordered by P values is presented in Table 1, including the top 4 genes: *TWF1* ($P = 5.0 \times 10^{-5}$), *PUS7L* ($P = 7.2 \times 10^{-5}$), *C5orf66* ($P = 7.2 \times 10^{-5}$), and *IRAK4* ($P = 7.3 \times 10^{-5}$). Note that *C5orf66* located on 5q31.1 was identified by both SNP and gene-based analyses. *C5orf66* is a protein-coding gene that has not yet been reported to be associated with atopy. In Table 1, *IRAK4* on 12q12 hosts SNP rs4251459 ($\beta = 1.06$, $P = 1.28 \times 10^{-5}$). Zhang and colleagues² found 2 SNPs (rs4251431 and rs6582484) in *IRAK4* displaying significant association with AR in males, whereas in our results, rs4251459 appears to increase the risk of AR in females compared with males.

The *GALNT14* gene (Table 1) has been associated with otitis media (OM) in 1 GWAS.³ AR has frequently been reported to be associated with OM in different populations.⁴ Our results provide further evidence that the 2 diseases (AR and OM) could be genetically connected. Chromosome 1p22.2 also showed an important association with AR because it harbors 3 genes: *GBP4*, *GBP7* and *GBP2* (Table 1); each plays a role in the innate immune functions.⁵ Because 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.⁶ In addition, the expression of *TFAP2B* (Table 1) is able to distinguish controlled and therapy-resistant childhood asthma.⁷

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 tests and empirical P values to obtain the significance of each detected pathway. The top 10 pathways are given in eTable 2. The top pathway, trophoblast cell differentiation, comprises 8 genes, among which *CDH1* is known to relate to airway remodeling and lung function in asthma because SNPs within this gene are associated with epithelial E-cadherin expression.⁸ Moreover, dysfunction in epithelial cell morphogenesis, a subpathway of cell morphogenesis, leads to loss of differentiation, reduced junctional integrity, and impaired innate defense, which predate atopy and development of allergic disease.⁹ In addition, children with asthma were likely to have higher triglyceride levels than those without asthma.¹⁰

Finally, we applied HaploReg, version 4 (Broad Institute, Cambridge, Massachusetts) to investigate the significance of noncoding

Ms Mohammadnejad and Dr Brasch-Andersen contributed equally to this work.

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Table 1

Description and Test Statistics of Top 20 Genes From Gene-Based Analysis Using VEGAS2

Chromosome	Gene	Nonsynonymous SNPs	Start	Stop	Test	P Value	Top SNP	Top SNP P Value
12	<i>TWF1</i>	72	44137525	44250178	583.49	5.00×10^{-5}	rs4251459	1.28×10^{-5}
12	<i>PUS7L</i>	80	44072409	44202620	726.80	7.20×10^{-5}	rs4251459	1.28×10^{-5}
5	<i>C5orf66</i>	198	134318969	134730370	653.91	7.20×10^{-5}	rs566750	2.81×10^{-6}
12	<i>IRAK4</i>	83	44102746	44233346	719.80	7.30×10^{-5}	rs4251459	1.28×10^{-5}
2	<i>GALNT14</i>	238	31083330	31411592	1154.93	1.04×10^{-4}	rs2028677	3.87×10^{-4}
5	<i>LOC644936</i>	43	79544916	79646297	302.89	1.12×10^{-4}	rs11744364	1.87×10^{-4}
6	<i>TFAP2B</i>	83	50736438	50865326	566.26	1.39×10^{-4}	rs7772880	5.09×10^{-4}
12	<i>LINC00615</i>	69	91261799	91392446	441.28	1.87×10^{-4}	rs1920765	7.15×10^{-4}
1	<i>GBP4</i>	27	89596830	89714633	271.77	2.08×10^{-4}	rs604593	2.77×10^{-4}
1	<i>GBP7</i>	60	89547433	89691723	566.53	2.20×10^{-4}	rs2026043	2.65×10^{-4}
16	<i>LOC100506172</i>	123	73370703	73505295	512.21	2.24×10^{-4}	rs727014	1.53×10^{-5}
2	<i>CUL3</i>	138	225284866	225500114	1085.94	2.42×10^{-4}	rs11688397	2.23×10^{-4}
5	<i>SPZ1</i>	50	79565789	79667660	303.84	2.64×10^{-4}	rs11744364	1.87×10^{-4}
14	<i>CGRRF1</i>	38	54926586	55055334	233.90	3.10×10^{-4}	rs4143720	3.65×10^{-5}
5	<i>CRSP8P</i>	51	79596423	79697785	268.44	3.68×10^{-4}	rs11744364	1.87×10^{-4}
2	<i>CRXCR1</i>	33	218977567	219081716	237.10	5.13×10^{-4}	rs1008562	6.28×10^{-4}
1	<i>GBP2</i>	59	89521815	89641842	474.76	5.65×10^{-4}	rs2026043	2.65×10^{-4}
11	<i>EI24</i>	71	125389282	125504584	413.15	5.74×10^{-4}	rs515735	7.79×10^{-4}
18	<i>SIGLEC15</i>	63	43355544	43472521	389.03	6.12×10^{-4}	rs8085782	1.60×10^{-4}
6	<i>TFAP2D</i>	65	50631256	50790746	579.81	6.41×10^{-4}	rs7772880	5.09×10^{-4}

Abbreviation: SNP, single-nucleotide polymorphism.

regions related to AR in the regulatory units outside the coding regions. It used the list of query SNPs with $P < 10^{-4}$ from GWAS, 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 $P < .05$. Among the summary results, one enhancer, esophagus, was identified with a binomial $P = .02$. Eosinophilic esophagitis is a chronic allergy, causing inflammation of the esophagus. Interestingly, it has been reported that there are similar histopathologic findings between eosinophilic esophagitis and atopic diseases, and most patients with eosinophilic esophagitis have allergy disorders.¹¹

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

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Supplementary Data

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.janai.2018.07.001>.

Afsaneh Mohammadnejad, MS*,[†]
Charlotte Brasch-Andersen, PhD^{†,§}

Weilong Li, MS*

Annette Haagerup, MD^{||}

Jan Baumbach, PhD^{†,¶}

Qihua Tan, MD, PhD*,[‡]

* Unit of Epidemiology and Biostatistics
Department of Public Health
University of Southern Denmark
Odense, Denmark

[†] Department of Mathematics and Computer Science
University of Southern Denmark
Odense, Denmark

[‡] Unit of Human Genetics
Department of Clinical Research

University of Southern Denmark
Odense, Denmark

[§] Department of Clinical Genetics
Odense University Hospital
Odense, Denmark

^{||} NIDO | Danmark

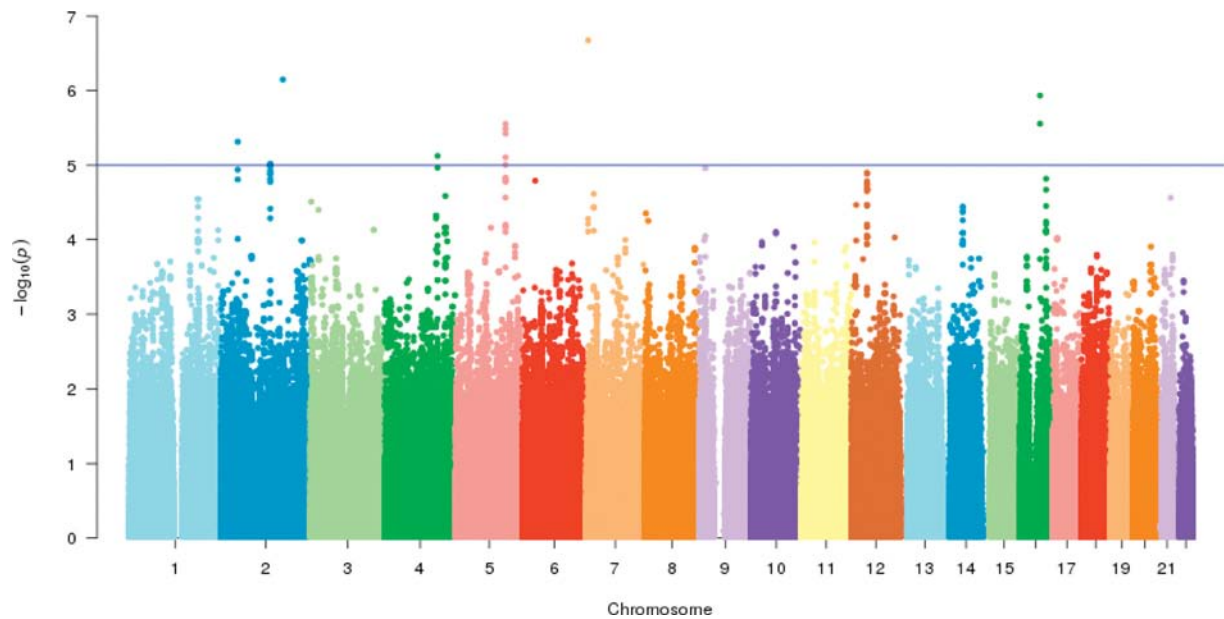
Gødstrup Hospital, and Human Genetics
Institute of Clinical Biomedicine
Aarhus University
Aarhus, Denmark

[¶] Department of Experimental Bioinformatics
School of Life Sciences

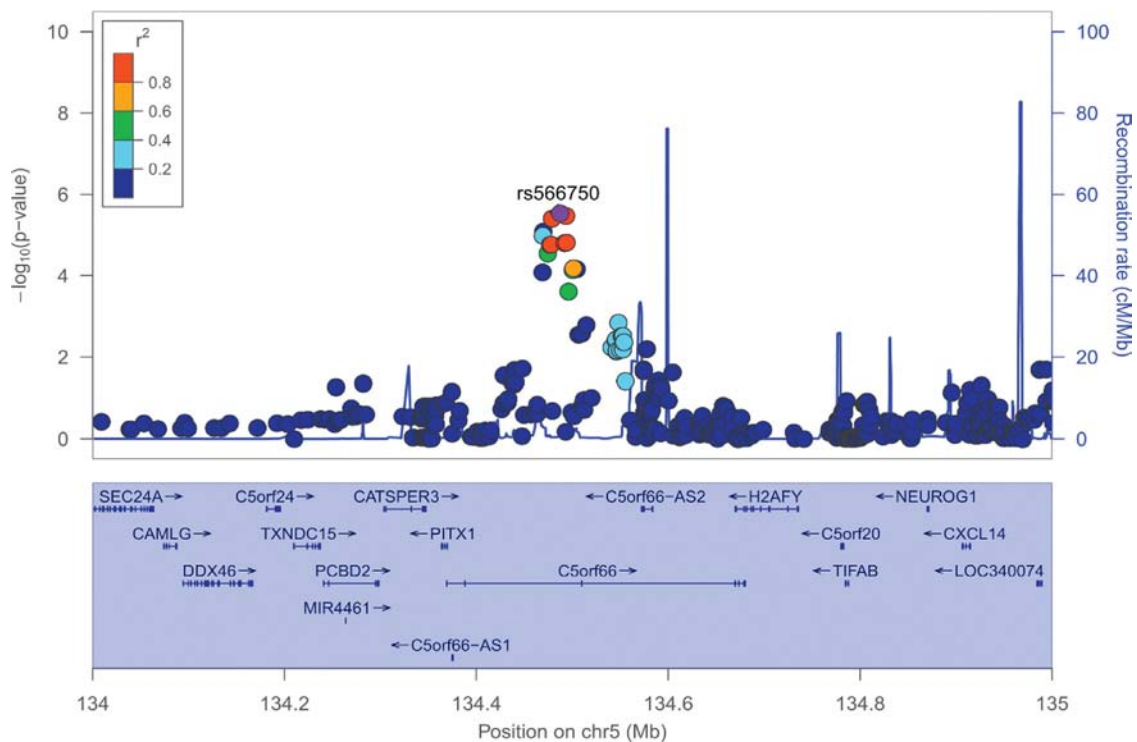
Technical University of Munich
Munich, Germany
qtan@health.sdu.dk

References

- [1] Kruse LV, Nyegaard M, Christensen U, et al. A genome-wide search for linkage to allergic rhinitis in Danish sib-pair families. *Eur J Hum Genet*. 2012;20:965–972.
- [2] Zhang Y, Lin X, Desrosiers M, et al. Association pattern of interleukin-1 receptor-associated kinase-4 gene polymorphisms with allergic rhinitis in a Han Chinese population. *PLoS One*. 2011;6:e21769.
- [3] Rye MS, Warrington NM, Scaman ESH, et al. Genome-wide association study to identify the genetic determinants of otitis media susceptibility in childhood. *PLoS One*. 2012;7:e48215.
- [4] Kreiner-Møller E, Chawes BLK, Caye-Thomasen P, Bünnelykke K, Bisgaard H. Allergic rhinitis is associated with otitis media with effusion: a birth cohort study. *Clin Exp Allergy*. 2012;42:1615–1620.
- [5] Praefcke GJK. Regulation of innate immune functions by guanylate-binding proteins. *Int J Med Microbiol*. 2018;308:237–245.
- [6] Ortiz RA, Barnes KC. Genetics of allergic diseases. *Immunol Allergy Clin North Am*. 2015;35:144.
- [7] Persson H, Kwon AT, Ramilowski JA, Silberberg G, et al. Transcriptome analysis of controlled and therapy-resistant childhood asthma reveals distinct gene expression profiles. *J Allergy Clin Immunol*. 2015;136:638–648.
- [8] Ierodiakonou D, Postma DS, Koppelman GH, et al. E-cadherin gene polymorphisms in asthma patients using inhaled corticosteroids. *Eur Respir J*. 2012;38:1044–1052.
- [9] Schleimer RP, Berdnikovs S. Etiology of epithelial barrier dysfunction in patients with type 2 inflammatory diseases. *J Allergy Clin Immunol*. 2017;139:1752–1761.
- [10] Cottrell L, Neal WA, Ice C, Perez MK, Piedimonte G. Metabolic abnormalities in children with asthma. *Am J Respir Crit Care Med*. 2011;183:441–448.
- [11] Ram G, Lee J, Ott M, et al. Seasonal exacerbation of esophageal eosinophilia in children with eosinophilic esophagitis and allergic rhinitis. *Ann Allergy Asthma Immunol*. 2015;115:224–228.e1.



eFigure 1. Manhattan plot of genotype-sex interaction from case-only genome-wide association study (GWAS). The figure plots $-\log_{10}(P \text{ value})$ for each single-nucleotide polymorphism (SNP) against chromosomal location. Each dot represents an SNP, and the blue horizontal line is the suggestive GWAS threshold (1×10^{-5}).



eFigure 2. Regional LocusZoom plot (<https://genome.sph.umich.edu/wiki/LocusZoom>) of chromosome 5 within the coordinate 134-135 megabase (Mb). The Y-axis to the left represents $-\log_{10}(P \text{ value})$ of single-nucleotide polymorphisms (SNPs) and the Y-axis to the right represents the recombination rate in centiMorgan per megabase (cM/Mb). Each dot represents an SNP with color indicating the degree of linkage disequilibrium. Genes in the region are annotated according to their physical location.

eTable 1

Detailed Information of Sample

	Siblings	Unrelated individuals	Male	Female	Total
Sample	243	191	184	250	434

eTable 2

Description and Test Statistics of Top 10 Pathways From Pathway Analysis Using VEGAS2

Pathway ID	Pathway length	Pathway <i>P</i> value	Empirical <i>P</i> value
GO:0001829 trophoblast differentiation	8	4.70×10^{-5}	1.50×10^{-4}
GO:0009982 pseudouridine synthase activity	6	5.80×10^{-5}	1.90×10^{-4}
GO:0001522 pseudouridine synthesis	6	5.80×10^{-5}	2.20×10^{-4}
GO:0001825 blastocyst formation	10	1.60×10^{-4}	5.10×10^{-4}
GO:0031625 ubiquitin protein ligase binding	27	1.90×10^{-4}	5.80×10^{-4}
GO:0000904 cell morphogenesis involved in differentiation	148	3.20×10^{-4}	1.40×10^{-3}
GO:0009309 amine biosynthetic process	65	5.10×10^{-4}	1.70×10^{-3}
Path Toll Endogenous pathway	18	1.10×10^{-3}	2.30×10^{-3}
GO:0008652 amino acid biosynthetic process	37	8.00×10^{-4}	2.80×10^{-3}
GO:0006641 triglyceride metabolic process	30	9.20×10^{-4}	3.00×10^{-3}