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A novel role for cilia function in atopy: *ADGRV1* and *DNAH5* interactions

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ABSTRACT

Background: Atopy, an endotype underlying allergic diseases, has a substantial genetic component.

Objective: Our goal was to identify novel genes associated with atopy in asthma-ascertained families.

Methods: We implemented a three-step analysis strategy in three datasets: The Epidemiological study on the Genetics and Environment of Asthma (EGEA) dataset: 1,660 subjects; The Saguenay-Lac-Saint-Jean (SLSJ) dataset: 1,138 subjects; and The Medical Research Council (MRC) dataset: 446 subjects). This strategy included a single-SNP genome-wide association study (GWAS), the selection of related gene pairs based on statistical filtering of GWAS results and text-mining filtering using GRAIL and SNP-SNP interaction analysis of selected gene pairs.

Results: We identified the 5q14 locus, harboring the adhesion G protein-coupled receptor V1 (*ADGRV1*) gene, that showed genome-wide significant association with atopy (rs4916831; $P_{\text{meta}}=6.8 \times 10^{-9}$). Statistical filtering of GWAS results followed by text-mining filtering revealed relationships between *ADGRV1* and three genes showing suggestive association with atopy ($P \leq 10^{-4}$). SNP-SNP interaction analysis between *ADGRV1* and these three genes showed significant interaction between *ADGRV1* rs17554723 and two correlated SNPs (rs2134256 and rs1354187) within dynein axonemal heavy chain 5 (*DNAH5*) gene ($P_{\text{meta-int}}=3.6 \times 10^{-5}$ and 6.1×10^{-5} , that met the multiple-testing corrected threshold of 7.3×10^{-5}). Further conditional analysis indicated that rs2134256 alone accounted for the interaction signal with rs17554723.

Conclusion: As both *DNAH5* and *ADGRV1* contribute to function of cilia, this study suggests that cilia dysfunction may represent a novel mechanism underlying atopy. Combining GWAS and epistasis analysis driven by statistical and knowledge-based evidence represents a promising approach for identifying new genes involved in complex traits.

Key Messages:

- *ADGRV1* genetic variants are associated with atopy in asthma families
- Interaction between *ADGRV1* and *DNAH5* variants is associated with atopy; these two genes are involved in ciliary function
- Use of a strategy that combines genome-wide association analysis and epistasis analysis driven by statistical and knowledge-based evidence can successfully identify new genes underlying complex traits.

Capsule summary:

This study in three family-based studies identified association between *ADGRV1* and atopy and interaction between *ADGRV1* and *DNAH5*, two genes that contribute to ciliary functions.

Key words: atopy, asthma, genetics, genome-wide association study, gene-gene interaction, text-mining, *ADGRV1*, *DNAH5*, ciliary function

Abbreviations:

ADGRV1: adhesion G protein-coupled receptor V1

DNAH5: dynein, axonemal, heavy chain 5

SNP: single nucleotide polymorphism

GWAS: genome-wide association study

SPT: skin prick test

EGEA: Epidemiological study on the Genetics and Environment of Asthma

SLSJ: Saguenay-Lac-Saint-Jean study

MRCA: Medical Research Council funded collection of nuclear families with Asthma

MRCE: Medical Research Council funded collection of nuclear families with Eczema

87 GRAIL: Gene Relationships Across Implicated Loci

88 QC: quality control

89 MAF: minor allele frequency

90 PCs: principal components

91 LD: linkage disequilibrium

92 ORs: odds-ratios

93 GTEx: Genotype-Tissue Expression

94 ETS: environmental tobacco smoke

95 CI: confidence interval

96

INTRODUCTION

Allergies and asthma are among the most common diseases in industrialized countries. Although environmental factors play an important role in allergic diseases, estimates of heritability of allergy, which range between 25% and 80%, suggest significant genetic contribution.¹ Genome-wide association studies (GWAS) have identified a number of loci associated with allergic diseases (i.e., asthma, atopic dermatitis, rhinitis),^{2,3} but these loci only explain a small part of the genetic risk. Part of the difficulty encountered in identifying the genetic factors involved in these allergic diseases is due to the heterogeneity of these diseases and the uncertainty of diagnosis. However, this problem can be alleviated by the study of an endotype underlying allergic diseases, such as allergic sensitization or atopy.

Atopy is characterized by the production of allergen-specific immunoglobulin E against environmental allergens. Estimates of heritability of atopy range from 40% to 85%.^{4,5} Many candidate genetic studies of atopy have been conducted but have often led to inconsistent results.⁶ While the first GWAS of allergic sensitization only reported a few loci,⁷⁻¹⁰ two recent large-scale meta-analyses of allergic sensitization¹¹ and self-reported allergy¹² increased the number of associated loci to 10 and 16 loci, respectively. However, other loci may influence atopy as it is well known that GWAS alone cannot reveal the whole genetic landscape underlying complex phenotypes.

Heterogeneity across studies, which may be caused by variability in the genetic background of the populations, environmental exposures, or study design, may be a limitation of meta-analyses of GWAS for identifying new loci associated with a trait. Notably, the importance of data sampling was recently highlighted by a positional cloning study of eczema, where association with *ANO3/MUC15* genetic variants was only found in family samples ascertained through

asthmatic subjects but neither in families ascertained through eczema patients nor in a case/control study of eczema.¹³

Another limitation of GWAS is that they typically focus on the analysis of individual single nucleotide polymorphisms (SNPs) and are underpowered to detect genetic factors which have a small marginal effect but rather interact with each other. Gene-gene interaction analysis (or epistasis analysis) has the ability to reveal novel genes involved in complex traits but raises an enormous multiple-testing problem when performed at the genome-wide level. Statistical and biological filtering pipelines can be used to limit the search for SNP-SNP interactions.¹⁴ Following the “guilt-by-association” assumption which states connected genes are usually participating in the same or related cellular functions,¹⁵ search for interactions can be restricted to genes pointed out by a preliminary GWAS (e.g., interactions of genes harboring significant association signals with genes harboring suggestive associations) and showing relationships based on prior knowledge. One knowledge-based approach that can be particularly useful to prioritize genes for epistasis analysis is text-mining of the literature as it can highlight relationships between genes¹⁶ according to their co-occurrence with the same words in scientific articles.

The objective of this study was to identify novel genetic factors influencing atopy by combining a GWAS and epistasis analysis driven by statistical and knowledge-based evidence in three family samples ascertained through asthmatic subjects: the French Epidemiological study on the Genetics and Environment of Asthma (EGEA; 1,660 subjects), the French-Canadian Saguenay-Lac-Saint-Jean study (SLSJ; 1,138 subjects) and the Medical Research Council UK study (MRC; 446 subjects). Our overall analysis strategy included three main steps: (1) a genome-wide single-SNP association analysis, (2) the selection of related gene pairs based on statistical filtering from

GWAS results and text-mining filtering using the Gene Relationships Across Implicated Loci (GRAIL) approach,¹⁷ and (3) a SNP-SNP interaction analysis for the selected gene pairs.

MATERIALS AND METHODS

Study datasets and definition of atopy

The EGEA study combines a case-control and a family-based study of asthma. The whole study population includes 388 families ascertained through at least one asthmatic proband recruited in chest clinics (1,705 probands and first-degree relatives) plus 415 population-based controls (total of 2,120 subjects). All subjects were born in France and were of European ancestry. The protocol of this study has been described elsewhere.¹⁸⁻²⁰ Atopy was assessed by skin prick tests (SPT) performed in 1,978 subjects. A positive SPT response was defined as a wheal diameter ≥ 3 mm to at least one of 11 aeroallergens belonging to three groups (indoor allergens, outdoor allergens, molds). After quality control (QC) of genotypic data, 925 atopic and 735 non-atopic subjects were included in the analysis.

The Saguenay-Lac-Saint-Jean and Quebec City Familial Asthma Collection (SLSJ) consists of a French-Canadian founder population panel of 253 multigenerational families from Saguenay-Lac-Saint-Jean region, ascertained through two asthmatic probands.²¹ This study has been described elsewhere.²¹ Skin tests were done in 1,195 SLSJ subjects and atopy was defined similarly as in EGEA. After QC of genotypic data, the analysis dataset included 641 atopic and 497 non-atopic subjects.

The Medical Research Council (MRC) UK study includes 207 nuclear families, recruited through at least one proband with childhood-onset asthma (MRCA sample). The study protocol has been described elsewhere.²² Atopy was defined similarly as in EGEA. To increase the number of unaffected subjects (controls), we included subjects from another MRC-UK dataset that were

recruited through probands with eczema (MRCE sample). Only subjects without asthma, without eczema and with low IgE levels were used as controls in this study. We checked that the age and gender distributions were similar in MRCA and MRCE samples. After QC of genotypic data, the analysis sample included 106 atopic and 340 non-atopic subjects. The whole UK sample will be subsequently designated as the MRC sample.

Protocols of EGEA, SLSJ and MRC studies have been approved by the local ethical committees. All adult participants and child's legal guardians provided written informed consent.

Genotyping

Both EGEA and SLSJ datasets were genotyped using the Illumina 610-Quad array (Illumina, San Diego, CA), as part as of the Gabriel asthma consortium GWAS.²³ Stringent quality criteria were applied to select both individuals and SNPs and have been previously detailed.^{23,24} After QC, there was a final set of 501,167 autosomal SNPs for analysis. The offspring in MRCA families and MRCE controls were genotyped using the Illumina Sentrix HumanHap300 BeadChip (307,981 autosomal SNPs), as part of the first asthma GWAS.^{22,23} QC for MRC samples has been detailed elsewhere.^{22,25} In order to get a number of SNPs in MRC sample as large as in EGEA and SLSJ samples, SNP imputation was performed using MACH v1.00 software²⁶ and HapMap2 release 21 CEU haplotypes as reference panel. Imputed SNPs were kept for analysis if their imputation quality score (rsq)²⁷ was ≥ 0.5 and minor allele frequency was $\geq 5\%$.

Descriptive statistics and strategy of analysis

Descriptive statistics of atopy together with sex, age and asthma status were assessed in each dataset using Stata® V14.1 (distributed by Stata Corporation, College Station, Texas, USA). The

workflow of our three-step analysis strategy is summarized in Figure 1 and presented in the following paragraphs.

Genome-wide single-SNP analysis

We performed a two-stage GWAS. In the first stage, association analysis between individual SNPs and atopy was carried out in the EGEA dataset. This analysis was based on a logistic regression model assuming an additive model for SNP effect, using Stata® V14.1. This model was adjusted for significant effects of age and sex and two principal components (PCs) to account for population structure. We took into account familial dependencies using the cluster and robust options of the logit function in Stata®. Test of SNP effect was based on a Wald-test. In a second stage, the SNPs reaching $P \leq 10^{-4}$ in EGEA were followed-up in SLSJ and MRC. The association analysis in SLSJ and in MRC used the same model as in EGEA. The results of stage 2 datasets and, then, of the three datasets were combined using a fixed-effects meta-analysis. SNPs were declared significantly associated with atopy if the three datasets meta-analysis P -value (P_{meta}) reached the genome-wide significance level of 1.5×10^{-7} . This threshold was obtained by dividing the type I error of 5% by the effective number of independent SNPs in the Illumina 610-Quad array.²⁸

Selection of gene pairs using both statistical and text-mining filters

The statistical filtering consisted of selecting two sets of genes using the GWAS results: genes showing significant association with atopy (set-1) and genes showing suggestive association with atopy (set-2). The set-1 included all genes lying at a distance of 50 kb or less from SNPs reaching the genome-wide significance level in the GWAS meta-analysis. The set-2 included all genes that

were at most 50 kb apart from SNPs having $P \leq 10^{-4}$ in the stage 1 EGEA dataset and were not part of set-1. To assign SNPs to genes, we used NCBI dbSNP Build 137 and human Genome Build 37.3. We further filtered gene pairs (formed by crossing set-1 genes with set-2 genes) through GRAIL¹⁷ text-mining of PubMed abstracts (available in October 2014). For each gene, GRAIL builds a vector of words where the elements of this vector are weights that take values between 0 and 1 depending on how often a word is found with a gene in an abstract. Then, GRAIL computes pairwise similarity between genes from gene/word vectors and ranks the similarities between each gene from set-1 and all genes of the genome. The P_{GRAIL} of a gene from set-2 with a gene from set-1 is equal to the proportion of all genes that have similarity with the set-1 gene greater than the similarity between set-2 and set-1 genes (i.e. rank divided by total number of genes across the genome). We used the threshold of $P_{\text{GRAIL}} \leq 0.10$, as recommended,¹⁷ to select related gene pairs for further epistasis analysis.

SNP-SNP interaction analysis for selected gene pairs

As for the single-SNP association analysis, we performed a two-stage SNP-SNP interaction analysis. At stage 1, we analyzed all SNP-SNP interactions for the GRAIL-selected gene pairs in the EGEA dataset. For each gene, we considered all SNPs lying within gene boundaries. Pairwise SNP-SNP interactions were evaluated by logistic regression assuming an additive model for SNP main effects and interaction and adjusting for the same covariates (age, sex, PCs) as in the GWAS, using Stata® V14.1. We used the same coding scheme as usually proposed for SNP-SNP interaction modelling.²⁹ We modeled the additive effect of a SNP by coding the genotypes of homozygotes for the minor allele, heterozygotes and homozygotes for the major allele as 1, 0, and -1; the interaction term between two SNPs was obtained by multiplication of these genotypic values for the two SNPs. Test of interaction was based on a likelihood-ratio test which follows a

Chi-square distribution with one degree of freedom. We discarded all SNP pairs for which one or more of the nine genotypic combinations appeared in fewer than five subjects (cases or controls). In a second stage, all SNP pairs showing suggestive evidence for interaction in EGEA ($P_{\text{int}} \leq 5 \times 10^{-3}$) were followed-up in SLSJ and MRC. The results of the stage 2 datasets and, then, of the three datasets were meta-analyzed using a fixed-effects model.

To correct for multiple testing, we computed, for each gene pair investigated, the effective number of independent interaction tests from the eigenvalues of the correlation matrix of products of SNP variables, using an extension of Li and Ji's method.³⁰ The corrected threshold to declare an interaction statistically significant was equal to the 5% type I error divided by the sum of effective number of independent interaction tests over all gene pairs tested.

Stratified analyses according to asthma status

Because family samples were ascertained through asthmatic probands, we investigated whether SNP associations and SNP-SNP interactions detected with atopy might be related to the presence of asthma. Single-SNP and SNP-SNP interaction analyses were repeated in the two groups of asthmatic and non-asthmatic subjects separately. These analyses were performed for the SNPs that showed significant results in the meta-analyses of the three datasets. Homogeneity of the odds-ratios (ORs) between the two groups was tested using the Cochran's Q statistic.³¹

RESULTS

Descriptive statistics

A total of 1,660 EGEA, 1,138 SLSJ and 446 MRC subjects were included in this study. The proportion of atopic subjects was similar in EGEA and SLSJ (55.7% and 56.3% respectively) but

was lower in MRC (23.8%; $P \leq 10^{-3}$). In each study, there was a higher proportion of males in atopic than in non-atopic subjects and atopic subjects were younger than non-atopic subjects (see Table E1 in the Online Repository). As expected, the proportion of asthmatic subjects was higher in atopic than in non-atopic subjects in all datasets (Table E1). In EGEA (respectively in SLSJ and MRC), 78.0% (75.0% and 78.3%) of atopic subjects had positive SPT to indoor allergens, 55.5% (77.5% and 52.8%) to outdoor allergens, and 34.8% (14.8% and 12.3%) to molds.

Genome-wide single-SNP analysis

In the stage 1 EGEA dataset, no SNP reached the genome-wide significance level of 1.5×10^{-7} (see quantile-quantile (QQ) plot and Manhattan plot in Figures E1 and E2). However, 73 SNPs lying in 47 loci showed associations with atopy exceeding the screening threshold of $P \leq 10^{-4}$. These SNPs were followed-up in the stage 2 SLSJ and MRC datasets and meta-analyzed (Table E2). The SNP rs4916831 within *ADGRV1* gene at 5q14 reached the genome-wide significance level ($P_{\text{meta}} = 6.8 \times 10^{-9}$) in the overall meta-analysis of the three datasets (Table I). Four other SNPs at that locus, in moderate linkage disequilibrium (LD) with rs4916831 (r^2 ranging between 0.51 and 0.79), showed suggestive association ($4.3 \times 10^{-7} \leq P_{\text{meta}} \leq 3.8 \times 10^{-6}$; Table I).

Selection of gene pairs using both statistical and text-mining filters

The gene set-1 included *ADGRV1*, the only gene significantly associated with atopy. There were 30 genes that lied fewer than 50 kb apart from the 65 SNPs at 46 loci having $P \leq 10^{-4}$ in EGEA (after exclusion of *ADGRV1* SNPs) and formed gene set-2 (Table E3). When GRAIL was applied to 30 gene pairs (date accessed: 04/24/2015), formed by each of these 30 genes with *ADGRV1*, three genes were related with *ADGRV1* at $P_{\text{GRAIL}} < 0.10$: *DNAH5* on 5p15 ($P_{\text{GRAIL}} = 0.084$), *CHD7* on 8q12 ($P_{\text{GRAIL}} = 3.2 \times 10^{-3}$) and *ATP8B1* on 18q21 ($P_{\text{GRAIL}} = 0.016$).

SNP-SNP interaction analysis for selected gene pairs

In the stage 1 EGEA dataset, the three GRail-selected gene-pairs (*ADGRV1/DNAH5*, *ADGRV1/CHD7*, *ADGRV1/ATP8B1*) were each examined for SNP-SNP interactions, making a total of 5,324 SNP pairs. There were 37 SNP pairs that reached $P_{\text{int}} \leq 5 \times 10^{-3}$ in EGEA and were followed-up in SLSJ and MRC at stage 2. Two of these SNPs pairs, which are related to the *ADGRV1* and *DNAH5* gene pair, met the multiple-testing corrected threshold, estimated to be 7.3×10^{-5} (see Table E4), in the meta-analysis of the three datasets (Table II). The two significant interactions involved the same SNP rs17554723 within *ADGRV1* and two SNPs within *DNAH5*, rs2134256 ($P_{\text{meta-int}} = 3.6 \times 10^{-5}$) and rs1354187 ($P_{\text{meta-int}} = 6.1 \times 10^{-5}$), that are in moderate LD ($r^2 = 0.50$; $D' = 0.95$). However, further conditional regression analysis in each of the strata defined by genotypes at *ADGRV1* rs17554723 showed that *DNAH5* rs1354187 was no longer significantly associated with atopy ($P \geq 0.15$) when conditioning on *DNAH5* rs2134256. The most significant SNP pair shows a pattern of interaction in which the ORs for atopy associated with TT (or CC) genotype at *DNAH5* rs2134256 are in opposite direction according to the genotype, AA (or GG), at *ADGRV1* rs17554723 (Figure 2). This pattern was consistent in all three datasets (Figure 2).

Stratified analyses according to asthma status

Association analyses of atopy with the genome-wide significant *ADGRV1* rs4916831 SNP in asthmatic and non-asthmatic subjects did not show any relationship with presence of asthma in the stage 1 and stage 2 datasets and meta-analysis of the three datasets (P_{Cochran} for test of homogeneity between the two groups ≥ 0.82 ; Table E5A). In the meta-analysis, the evidence for association was even stronger although not significantly in non-asthmatics ($P = 7.8 \times 10^{-6}$) than in

asthmatics ($P=1.4 \times 10^{-4}$). Similarly, interaction analyses for *ADGRV1* and *DNAH5* SNPs did not show any relationship with asthma ($P_{\text{Cochran}} \geq 0.30$; Table E5B). The evidence for interaction was only significant in non-asthmatic subjects (Table E6B); this can be at least partly explained by the larger sample size of non-asthmatic ($N=1,849$) than asthmatic subjects ($N=1,354$).

Functional annotations of SNPs showing significant results

All SNPs that show significant association (or interaction) with atopy are intronic. The two *ADGRV1* SNPs, rs4916831 and rs17554723 on 5q14, detected through GWAS and interaction analysis, lie 120 kb apart in introns 83 (rs4916831) and 70 (rs17554723) and are in low LD ($r^2=0.20$, $D'=0.75$). The two *DNAH5* SNPs (rs2134256 and rs1354187) at 5p15.2 are located in introns 58 and 60 (8 kb apart) but only rs2134256 accounts for the interaction signal (see above). By interrogating the Genotype-Tissue Expression (GTEx) database,³² rs4916831 was found associated with *ADGRV1* expression in esophagus mucosa ($P=7.5 \times 10^{-7}$).³² We also investigated whether the *ADGRV1* and *DNAH5* SNPs (as well as their proxies, $r^2 \geq 0.80$) map to functionally important regulatory regions using HaploRegV4.³³ As shown in Table E6, these SNPs and/or proxies map to binding sites of various transcription factors (TFs). In addition, four proxies of *ADGRV1* rs4916831 map to enhancer histone marks in lung and skin while a proxy of *DNAH5* rs2134256 maps to promoter and enhancer marks in hematopoietic stem cells.

DISCUSSION

By combining genome-wide single-SNP analysis and epistasis analysis driven by statistical and knowledge-based evidence in three asthma-ascertained family datasets, we identified significant association of atopy at a novel 5q14 locus harboring *ADGRV1* gene and significant interaction between *ADGRV1* and *DNAH5* genetic variants.

The interaction between *ADGRV1* and *DNAH5* variants has biological relevance as these two genes are both involved in ciliopathies and ciliary function. Ciliopathies comprise a group of disorders associated with genetic mutations encoding defective proteins, which result in either abnormal formation or function of cilia.³⁴ Mutations in the adhesion G protein-coupled receptor V1 (*ADGRV1*) gene cause Usher syndrome type IIC, a ciliopathy characterized by hearing loss and visual impairment,^{35,36} while mutations of dynein axonemal heavy chain 5 (*DNAH5*) gene cause primary ciliary dyskinesia type 3, a ciliopathy which combines upper and lower tract respiratory manifestations, male infertility, and situs inversus.³⁷ The *ADGRV1* protein (also called GPR98) is a component of the Usher protein network that functions in stereocilia of inner ear hair cells and photoreceptor cilia. The heavy chain of axonemal dynein, encoded by *DNAH5*, is part of a microtubule-associated motor protein complex that is responsible for cilia mobility, especially in respiratory epithelial cells where cilia motility is essential for mucus transport and airway clearance.³⁸ Although the respective function of *ADGRV1* and *DNAH5* proteins was initially described in different organs, these proteins may also have related functions. Indeed, the cilium in photoreceptors is ultrastructurally very similar to the nasal ciliated epithelium and the nasal ciliated epithelium of Usher syndrome II patients was found to have a lower ciliary beat frequency than healthy controls.³⁹ Moreover, Usher syndrome has been reported to be associated with bronchiectasis, sinusitis and reduced nasal mucociliary clearance.⁴⁰

Besides the involvement of both *ADGRV1* and *DNAH5* proteins in cilia functions, which supports the statistical interaction found between these two genes, both *DNAH5* and *ADGRV1* genes have been previously associated with respiratory diseases and related phenotypes. Recent GWAS reported significant association of *DNAH5* variants with total lung capacity in chronic obstructive pulmonary disease⁴¹ and suggestive association with Immunoglobulin E grass sensitization.⁹ However, the SNP reported by that latter study was not in LD with the *DNAH5*

SNPs interacting with *ADGRV1* SNP in this study ($r^2 < 0.13$). Based on an approach similar to ours, which combined genome-wide expression data in nasal epithelial cells, allele frequency variation between populations and literature search to select candidate genes, nominal association of asthma with *DNAH5* was reported and stronger association was found with *KIF3*, a gene involved in transport of protein complexes within cilia and potentially in allergen clearance as *DNAH5*.⁴² In addition, *DNAH5* belongs to the same gene family as *DNAH9* which showed interaction with environmental tobacco smoke (ETS) exposure for bronchial hyperresponsiveness in EGEA and SLSJ families.⁴³ Moreover, suggestive association of *ADGRV1* with asthma has been recently reported by a meta-analysis of GWAS.⁴⁴ Though most previously reported associations concern asthma or respiratory phenotypes, the interaction between *ADGRV1* and *DNAH5* SNPs associated with atopy in the present study appears independent of asthma, as shown by the stratified analysis on asthma. Although the mechanism by which these two genes influence atopy is still unknown, we can hypothesize that they are involved in dysfunction of cilia that move secreted mucus containing trapped foreign particles up and out of the airways, which favors allergic sensitization. This is supported by recent observations of a differential mRNA expression of both *ADGRV1* and *DNAH5* genes in sputum from House Dust Mite (HDM)-sensitized wheezing subjects as compared to non-atopic controls.⁴⁵ Furthermore, *DNAH5* as well as other genes of the same family including *DNAH9* were among the highest-ranking co-expression hubs in one of the HDM-wheezing associated gene modules, which was strongly enriched with genes involved in the function of ciliated epithelial cells.⁴⁵ All these observations suggest cilia-related genes may constitute an important emerging pathway for atopy.

The strategy used in this study, that enabled identifying novel relevant candidates for atopy, combined genome-wide single-SNP analysis and gene-gene interaction analysis based on both statistical filtering of GWAS results and text-mining filtering. It is of note that our three-step

strategy was designed *a priori* and SNP-SNP interaction tests were only performed for gene pairs selected through our two filtering processes. The genome-wide single-SNP analysis pointed towards one gene (*ADGRV1*) which harbored the lead SNP rs4916831 reaching genome-wide significance and four other SNPs showing suggestive association. By increasing the density of SNPs through Hapmap2-based imputation at that locus, an additional SNP ($r^2=0.80$ with rs4916831) reached genome-wide significance and six other SNPs had P-values within one order of magnitude of the genome-wide threshold (results not shown), which strengthens our finding. Further conditional analysis in that region showed that association with atopy was only accounted for by the lead genotyped SNP. The subsequent statistical and text-mining filters, used prior to epistasis analysis, made it possible to detect gene-gene interaction by lowering the multiple testing burden. Indeed, use of both filters reduced the number of interaction tests by 9-fold as compared to using the statistical filter only. The text-mining filter was based on GRAIL that was shown to be successful in pointing out true disease regions that were validated.¹⁷ Although many sources of biological information can be used to connect genes, such as co-expression gene networks or protein-protein interaction networks, the advantage of GRAIL is to provide a broader framework for revealing gene-gene relationships of any origin through literature search. However, GWAS and candidate gene studies, which are driven by researchers' expectations, can create a bias towards genes that are frequently reported in the literature. An appropriate approach would be to utilize the existing knowledge and to correct for potential bias but, to our knowledge, such method does not exist yet.

In conclusion, this study shows that the proposed strategy that combines GWAS and epistasis analysis driven by statistical and knowledge-based evidence can successfully identify strong candidate genes for complex phenotypes as atopy. The interaction between *DNAH5* and

406 *ADGRV1*, two genes involved in cilia functions, is of biological relevance and provides a novel
407 mechanism underlying atopy. Further studies, including functional and experimental studies, are
408 needed to confirm the current findings and to identify the functional variants.

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REFERENCES

1. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev* 2011; 242:10-30.
2. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, *et al.* The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 2014; 42:D1001-6.
3. Bonnelykke K, Sparks R, Waage J, Milner JD. Genetics of allergy and allergic sensitization: common variants, rare mutations. *Curr Opin Immunol* 2015; 36:115-26.
4. Los H, Postmus PE, Boomsma DI. Asthma genetics and intermediate phenotypes: a review from twin studies. *Twin Res* 2001; 4:81-93.
5. Thomsen SF, Ulrik CS, Kyvik KO, Ferreira MA, Backer V. Multivariate genetic analysis of atopy phenotypes in a selected sample of twins. *Clin Exp Allergy* 2006; 36:1382-90.
6. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008; 8:169-82.
7. Andiappan AK, Wang de Y, Anantharaman R, Parate PN, Suri BK, Low HQ, *et al.* Genome-wide association study for atopy and allergic rhinitis in a Singapore Chinese population. *PLoS One* 2011; 6:e19719.
8. Castro-Giner F, Bustamante M, Ramon Gonzalez J, Kogevinas M, Jarvis D, Heinrich J, *et al.* A pooling-based genome-wide analysis identifies new potential candidate genes for atopy in the European Community Respiratory Health Survey (ECRHS). *BMC Med Genet* 2009; 10:128.
9. Ramasamy A, Curjuric I, Coin LJ, Kumar A, McArdle WL, Imboden M, *et al.* A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol* 2011; 128:996-1005.
10. Wan YI, Strachan DP, Evans DM, Henderson J, McKeever T, Holloway JW, *et al.* A genome-wide association study to identify genetic determinants of atopy in subjects from the United Kingdom. *J Allergy Clin Immunol* 2011; 127:223-31, 31 e1-3.

11. Bonnelykke K, Matheson MC, Pers TH, Granell R, Strachan DP, Alves AC, *et al.* Meta-analysis of genome-wide association studies identifies ten loci influencing allergic sensitization. *Nat Genet* 2013; 45:902-6.
12. Hinds DA, McMahon G, Kiefer AK, Do CB, Eriksson N, Evans DM, *et al.* A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. *Nat Genet* 2013; 45:907-11.
13. Dizier MH, Margaritte-Jeannin P, Madore AM, Esparza-Gordillo J, Moffatt M, Corda E, *et al.* The ANO3/MUC15 locus is associated with eczema in families ascertained through asthma. *J Allergy Clin Immunol* 2012; 129:1547-53 e3.
14. Sun X, Lu Q, Mukherjee S, Crane PK, Elston R, Ritchie MD. Analysis pipeline for the epistasis search - statistical versus biological filtering. *Front Genet* 2014; 5:106.
15. Li ZC, Huang MH, Zhong WQ, Liu ZQ, Xie Y, Dai Z, *et al.* Identification of drug-target interaction from interactome network with 'guilt-by-association' principle and topology features. *Bioinformatics* 2016; 32:1057-64.
16. Luo Y, Riedlinger G, Szolovits P. Text mining in cancer gene and pathway prioritization. *Cancer Inform* 2014; 13:69-79.
17. Raychaudhuri S, Plenge RM, Rossin EJ, Ng AC, Purcell SM, Sklar P, *et al.* Identifying relationships among genomic disease regions: predicting genes at pathogenic SNP associations and rare deletions. *PLoS Genet* 2009; 5:e1000534.
18. Kauffmann F, Dizier MH, Annesi-Maesano I, Bousquet J, Charpin D, Demenais F, *et al.* EGEA (Epidemiological study on the Genetics and Environment of Asthma, bronchial hyperresponsiveness and atopy)-- descriptive characteristics. *Clin Exp Allergy* 1999; 29 Suppl 4:17-21.
19. Kaufmann F, Dizier MH, Pin I, Paty E, Gormand F, Vervloet D, *et al.* Epidemiological Study of the Genetics and Environment of Asthma, Bronchial Hyperresponsiveness, and Atopy. *Am J Respir Crit Care Med* 1997; 156:123-9.

20. Bouzigon E, Nadif R, Le Moual N, Dizier MH, Aschard H, Boudier A, *et al.* [Genetic and environmental factors of asthma and allergy: Results of the EGEA study]. *Rev Mal Respir* 2015; 32:822-40.
21. Laprise C. The Saguenay-Lac-Saint-Jean asthma familial collection: the genetics of asthma in a young founder population. *Genes Immun* 2014; 15:247-55.
22. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, *et al.* Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007; 448:470-3.
23. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, *et al.* A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010; 363:1211-21.
24. Sarnowski C, Sugier PE, Granell R, Jarvis D, Dizier MH, Ege M, *et al.* Identification of a new locus at 16q12 associated with time to asthma onset. *J Allergy Clin Immunol* 2016.
25. Liang L, Morar N, Dixon AL, Lathrop GM, Abecasis GR, Moffatt MF, *et al.* A cross-platform analysis of 14,177 expression quantitative trait loci derived from lymphoblastoid cell lines. *Genome Res* 2013; 23:716-26.
26. Li Y, Abecasis GR. Mach 1.0: Rapid haplotype reconstruction and missing genotype inference. *Am. J. Hum. Genet.* 2006; S79:2290.
27. Li Y, Willer C, Sanna S, Abecasis G. Genotype imputation. *Annu Rev Genomics Hum Genet* 2009; 10:387-406.
28. Li MX, Yeung JM, Cherny SS, Sham PC. Evaluating the effective numbers of independent tests and significant p-value thresholds in commercial genotyping arrays and public imputation reference datasets. *Hum Genet* 2012; 131:747-56.
29. Herold C, Steffens M, Brockschmidt FF, Baur MP, Becker T. INTERSNP: genome-wide interaction analysis guided by a priori information. *Bioinformatics* 2009; 25:3275-81.
30. Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. *Heredity* 2005; 95:221-7.
31. Cochran WG. The comparison of percentages in matched samples. *Biometrika* 1950; 37:256-66.

32. The GTEx Consortium. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* 2015; 348:648-60.
33. Ward LD, Kellis M. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 2012; 40:D930-4.
34. Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol* 2007; 8:880-93.
35. Weston MD, Luijendijk MW, Humphrey KD, Moller C, Kimberling WJ. Mutations in the VLGR1 gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am J Hum Genet* 2004; 74:357-66.
36. Besnard T, Vache C, Baux D, Larrieu L, Abadie C, Blanchet C, *et al.* Non-USH2A mutations in USH2 patients. *Hum Mutat* 2012; 33:504-10.
37. Leigh MW, Zariwala MA, Knowles MR. Primary ciliary dyskinesia: improving the diagnostic approach. *Curr Opin Pediatr* 2009; 21:320-5.
38. Olbrich H, Horvath J, Fekete A, Loges NT, Storm van's Gravesande K, Blum A, *et al.* Axonemal localization of the dynein component DNAH5 is not altered in secondary ciliary dyskinesia. *Pediatr Res* 2006; 59:418-22.
39. Armengot M, Salom D, Diaz-Llopis M, Millan JM, Milara J, Mata M, *et al.* Nasal ciliary beat frequency and beat pattern in retinal ciliopathies. *Invest Ophthalmol Vis Sci* 2012; 53:2076-9.
40. Bonneau D, Raymond F, Kremer C, Klossek JM, Kaplan J, Patte F. Usher syndrome type I associated with bronchiectasis and immotile nasal cilia in two brothers. *J Med Genet* 1993; 30:253-4.
41. Lee JH, McDonald ML, Cho MH, Wan ES, Castaldi PJ, Hunninghake GM, *et al.* DNAH5 is associated with total lung capacity in chronic obstructive pulmonary disease. *Respir Res* 2014; 15:97.
42. Kovacic MB, Myers JM, Wang N, Martin LJ, Lindsey M, Ericksen MB, *et al.* Identification of KIF3A as a novel candidate gene for childhood asthma using RNA expression and population allelic frequencies differences. *PLoS One* 2011; 6:e23714.

- 550 43. Dizier MH, Nadif R, Margaritte-Jeannin P, Barton SJ, Sarnowski C, Gagne-Ouellet V, *et al.*
551 Interaction between the DNAH9 gene and early smoke exposure in bronchial hyperresponsiveness.
552 *Eur Respir J* 2016; 47:1072-81.
- 553 44. Almoguera B, Vazquez L, Mentch F, Connolly J, Pacheco JA, Sundaresan AS, *et al.* Identification of
554 Four Novel Loci in Asthma in European and African American Populations. *Am J Respir Crit Care*
555 *Med* 2016 Sep 9; [Epub ahead of print].
- 556 45. Jones AC, Troy NG, White E, Hollams EM, Alexander MG, Ling KM, *et al.* Persistent activation of
557 interlinked Th2-airway epithelial gene networks in sputum-derived cells from aeroallergen-sensitized
558 symptomatic atopic asthmatics. *bioRxiv* 2016; <https://doi.org/10.1101/063602>
- 559

Table I. *ADGRV1* locus on 5q14 showing significant association with atopy

SNP	Position (kb)*	Alleles†	MAF‡	Stage 1		Stage 2						Overall Meta-Analysis		
				EGEA (N=1,660)		SLSJ (N=1,138)		MRC (N=446)		Meta-Analysis		beta (se) §	$P_{meta}^{††}$	$P_{Cochran}^{‡‡}$
				beta (se) §	$P^{\parallel}$	beta (se) §	$P^{\parallel}$	beta (se) §	$P^{\parallel}$	beta (se) §	P_{stage2}^{**}			
rs4244205	90,188	A/G	0.41	-0.35 (0.08)	1.1×10^{-5}	-0.19 (0.10)	6.0×10^{-2}	-0.14 (0.18)	0.45	-0.18 (0.09)	4.4×10^{-2}	-0.27 (0.06)	3.8×10^{-6}	0.35
rs4916831	90,212	A/G	0.44	-0.40 (0.08)	1.0×10^{-6}	-0.32 (0.11)	2.3×10^{-3}	-0.21 (0.17)	0.23	-0.29 (0.09)	1.2×10^{-3}	-0.35 (0.06)	6.8×10^{-9}	0.59
rs10060641	90,213	T/C	0.38	-0.39 (0.08)	7.2×10^{-7}	-0.17 (0.11)	0.11	-0.24 (0.19)	0.22	-0.18 (0.09)	4.8×10^{-2}	-0.30 (0.06)	4.3×10^{-7}	0.23
rs12054681	90,217	C/A	0.37	-0.39 (0.08)	9.7×10^{-7}	-0.14 (0.11)	0.18	-0.29 (0.21)	0.16	-0.17 (0.09)	6.5×10^{-2}	-0.30 (0.06)	7.8×10^{-7}	0.18
rs949787	90,251	G/T	0.28	-0.33 (0.08)	5.5×10^{-5}	-0.23 (0.10)	2.0×10^{-2}	-0.19 (0.20)	0.36	-0.22 (0.09)	1.2×10^{-2}	-0.28 (0.06)	3.2×10^{-6}	0.65

*Position in kilobases (kb) according to NCBI dbSNP Build 137

†Major allele/Minor allele

‡Minor allele frequency

§beta is the regression coefficient for a one-unit increase of the effect allele in logistic regression assuming an additive model; se is the standard error associated with the regression coefficient.

||P is the P-value associated with the Wald test of SNP effect.

** P_{stage2} is the P-values associated with the Wald test of meta-analyzed SNP effect in the stage 2 datasets (SLSJ and MRC).

†† P_{meta} is the P-value associated with the Wald test of meta-analyzed SNP effect in the three datasets (EGEA, SLSJ, MRC); the P-value is shown in bold when it reached the multiple-testing corrected threshold of 1.5×10^{-7} .

‡‡ $P_{Cochran}$ is the P-value associated with Cochran's Q test of homogeneity across the three datasets.

Table II. SNP pairs showing significant interaction for atopy

SNPs	Chr*	Genes [†]	Alleles [‡]	MAF [§]	Stage 1			Stage 2									Overall Meta-analysis			
					EGEA (N=1,660)			SLSJ (N=1,138)			MRC (N=446)			Meta-Analysis						
					<i>Main effect</i>			<i>Main effect</i>			<i>Main effect</i>			<i>Main effect</i>			<i>Main effect</i>			
					<i>beta</i> (se)	<i>beta</i> (se)	<i>P</i> _{int} ^{**}	<i>beta</i> (se)	<i>beta</i> (se)	<i>P</i> _{int} ^{**}	<i>beta</i> (se)	<i>beta</i> (se)	<i>P</i> _{int} ^{**}	<i>beta</i> (se)	<i>beta</i> (se)	<i>P</i> _{stage2-int} ^{††}	<i>beta</i> (se)	<i>beta</i> (se)	<i>P</i> _{meta-int} ^{‡‡}	<i>P</i> _{Cochran} ^{§§}
rs17554723	5	ADGRV1	A/G	0.33	-0.06 (0.10)	-0.38 (0.12)	3.0x10 ⁻³	0.02 (0.13)	-0.42 (0.16)	1.1x10 ⁻²	0.13 (0.23)	-0.28 (0.33)	0.40	0.04 (0.11)	-0.39 (0.14)	6.1x10 ⁻³	-0.02 (0.08)	-0.38 (0.09)	3.6x10⁻⁵	0.84
rs2134256	5	DNAH5	T/C	0.25	0.13 (0.09)			-0.06 (0.13)			0.03 (0.26)			-0.04 (0.12)			0.06 (0.07)			
rs17554723	5	ADGRV1	A/G	0.33	0.03 (0.09)	-0.34 (0.11)	3.3x10 ⁻³	0.13 (0.11)	-0.35 (0.14)	1.4x10 ⁻²	0.19 (0.21)	-0.16 (0.29)	0.59	0.14 (0.10)	-0.32 (0.12)	9.3x10 ⁻³	0.08 (0.06)	-0.33 (0.08)	6.1x10⁻⁵	0.92
rs1354187	5	DNAH5	T/C	0.36	0.08 (0.08)			-0.04 (0.11)			0.05 (0.22)			-0.02 (0.10)			0.04 (0.06)			

*Chr is the chromosome number where the SNP is located

[†]Gene symbol of gene where SNP lies

[‡]Major allele/Minor allele

[§]Minor allele frequency

^{||}beta for the main effect is the regression coefficient for a one-unit increase of the effect allele in logistic regression assuming an additive model; beta for interaction is the regression coefficient for homozygotes for the minor allele at the two loci or homozygotes for the major allele at the two loci with respect to heterozygotes at either one or the two loci using the coding scheme under an additive genetic model described in the methods section; se is the standard error associated with the regression coefficient.

^{**}*P*_{int} is the *P*-value of the likelihood-ratio test for interaction between SNPs (which follows a chi-square distribution with one degree of freedom assuming an additive model).

^{††}*P*_{stage2-int} is the *P*-values associated with the Wald test of meta-analyzed interaction effect in the stage 2 datasets (SLSJ and MRC).

^{‡‡}*P*_{meta-int} is the *P*-values associated with the Wald test of meta-analyzed interaction effect in the three datasets (EGEA, SLSJ, MRC); *P*_{meta-int} is shown in bold when it reached the multiple-testing corrected threshold of 7.3x10⁻⁵.

^{§§}*P*_{Cochran} is the *P*-value associated with Cochran's Q test of homogeneity across the three datasets.

|||The two *DNAH5* SNPs, rs1354187 and rs2134256, showing significant interaction with *ADGRV1* SNP are in moderate linkage disequilibrium ($r^2=0.50$; $D'=0.95$).

FIGURE LEGENDS

Figure 1. Three-step analysis strategy

Figure 2. Odds-ratio (ORs) and 95% confidence intervals for atopy associated with each genotype at *DNAH5* rs2134256 (TT, CT, CC) in each of the strata defined by genotypes at *ADGRV1* rs17554723 (AA, AG, or GG). These ORs were calculated using the genotype coding scheme defined in the text and are shown for each of the three datasets (EGEA, SLSJ, MRC) and for the combined dataset.

Step 1**Two-stage Genome-wide Association Study of atopy (GWAS)**

Stage 1: EGEA (n=1,660)

Stage 2: SLSJ (n=1,138) & MRC (n=460)

↳ *Meta-analysis of stage 1 & stage 2***Step 2****Selection of gene pairs using two filters**➤ **Statistical filter to select 2 gene sets**

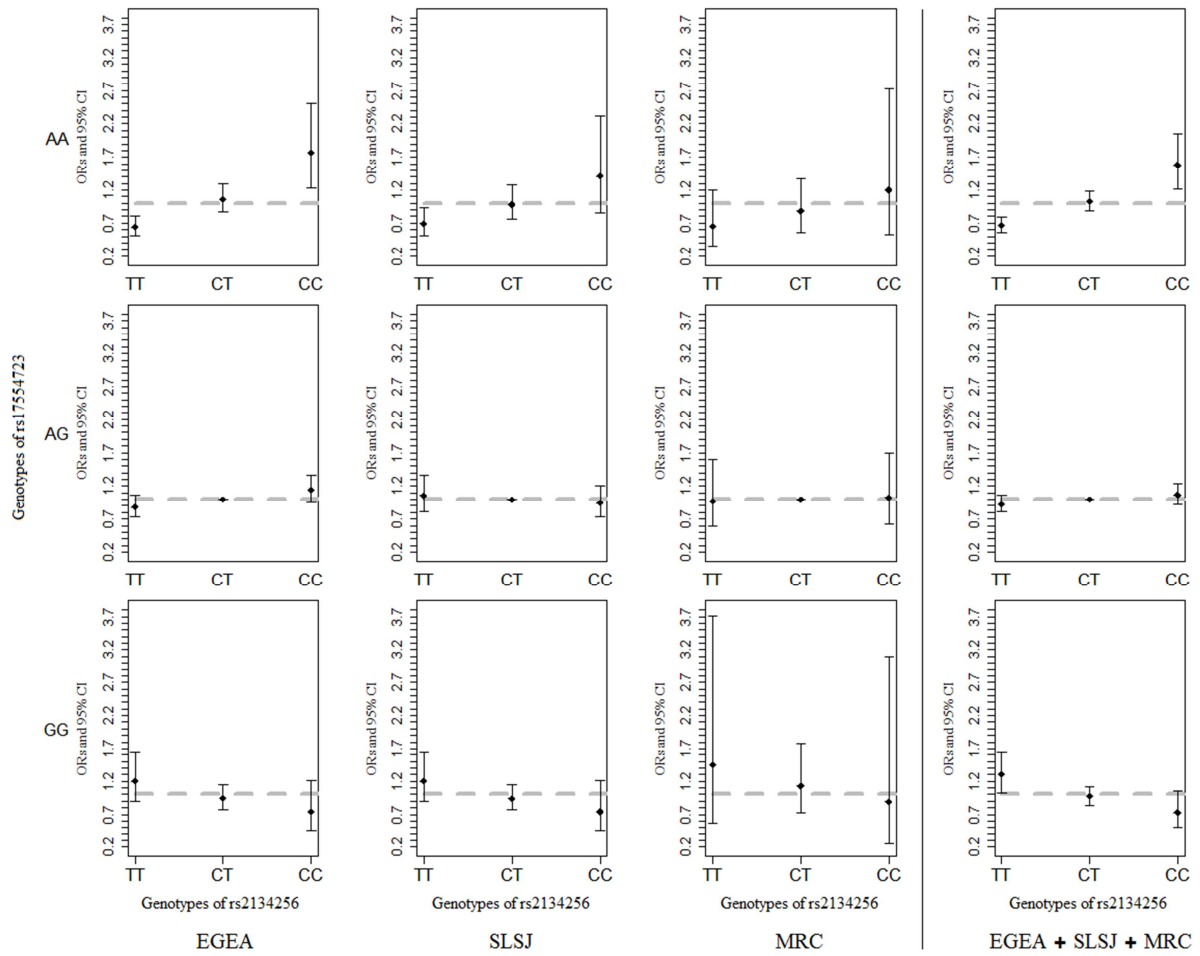
- Gene set-1: genes significantly associated with atopy ($P \leq 5 \times 10^{-8}$)
- Gene set-2: genes showing suggestive association with atopy ($P \leq 10^{-4}$)

➤ **Text-mining filter applied to gene set-1 & gene set-2 (GRAIL)**↳ Gene-pairs with $P_{\text{GRAIL}} \leq 0.10$ **Step 3****Two-stage SNP-SNP Interaction analysis of selected gene pairs**

Stage 1: EGEA

Stage 2: SLSJ & MRC

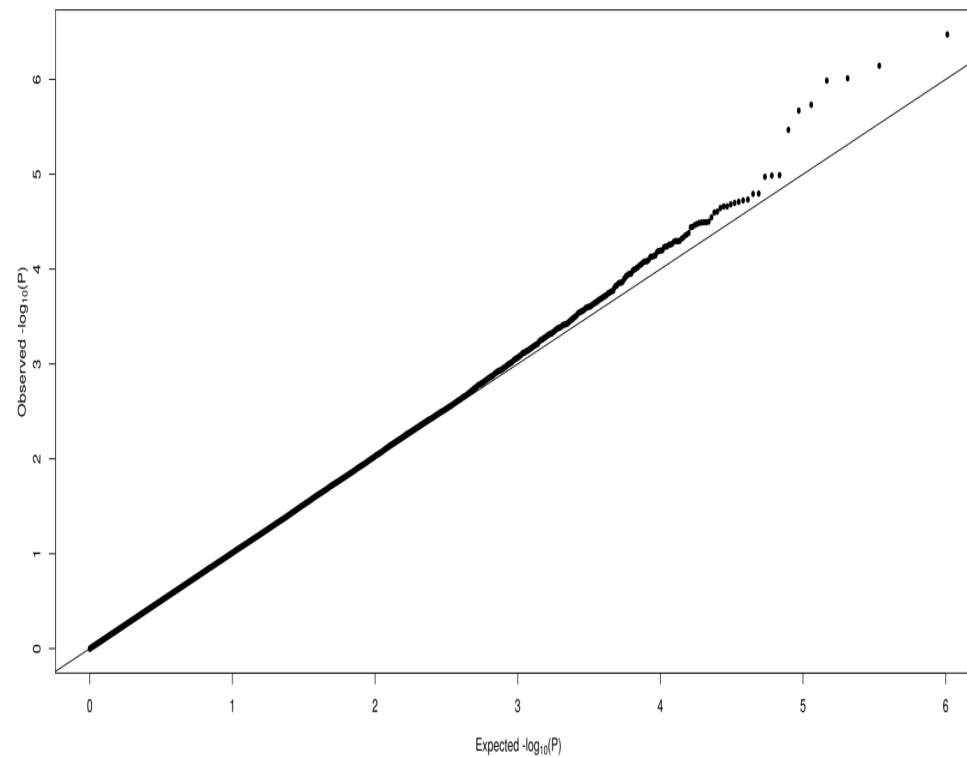
↳ *Meta-analysis of stage 1 & stage 2*



Online Repository**A novel role for cilia function in atopy: *ADGRV1* and *DNAH5* interactions**

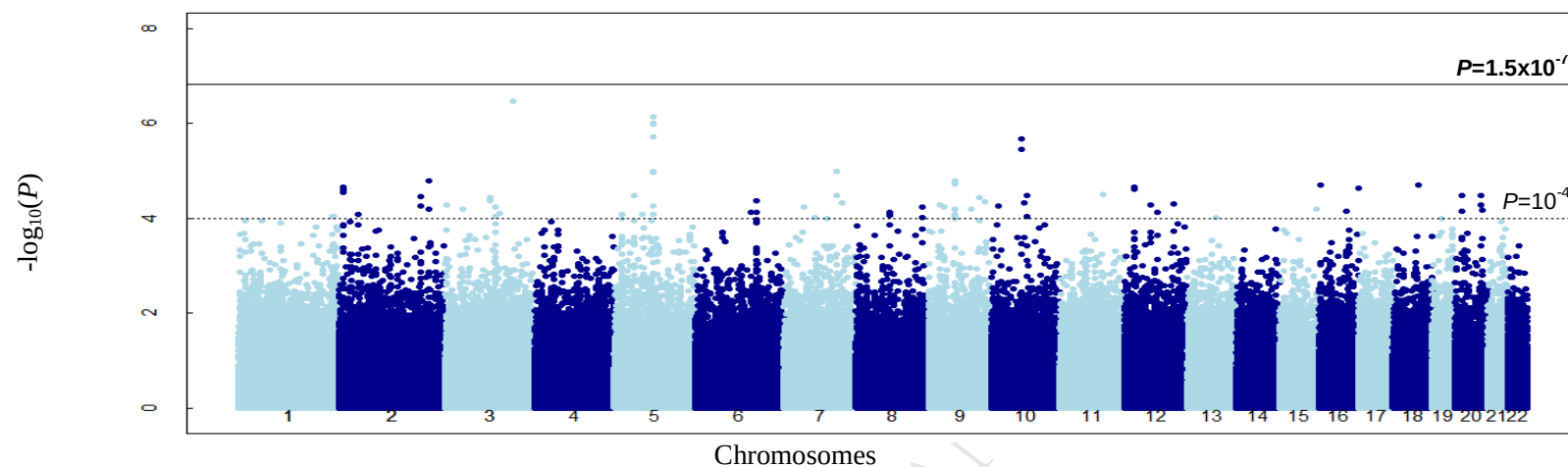
Pierre-Emmanuel Sugier^{a,b}, MSc, Myriam Brossard^{a*}, PhD, Chloé Sarnowski^{a*}, PhD, Amaury Vaysse^a, PhD, Andréanne Morin^{c,d}, MSc, Lucile Pain^d, MSc, Patricia Margaritte-Jeannin^a, PhD, Marie-Hélène Dizier^a, PhD, William O.C.M. Cookson, MD, D.Phil.^e, Mark Lathrop^c, PhD, Miriam F. Moffatt, D.Phil.^e, Catherine Laprise^{d‡}, PhD, Florence Demenais^{a‡}, MD, Emmanuelle Bouzigon^{a‡}, MD, PhD

Figure E1: Quantile-quantile plot of GWAS results of atopy in the stage 1 EGEA dataset



Quantile-quantile (QQ) plot for the association of atopy with 501,167 genotyped SNPs that passed quality control (QC) in the stage 1 EGEA dataset. The dots represent the distribution of observed $-\log_{10}(P)$ values against the expected $-\log_{10}(P)$ values from a theoretical chi-square distribution. The straight line represents the theoretical distribution of expected $-\log_{10}(P)$ values under the null hypothesis of no association. There was no evidence of any systematic bias: the genomic inflation factor (λ) was equal to 1.017.

Figure E2: Manhattan plot of GWAS results for atopy in the stage 1 EGEA dataset



The y axis shows the $-\log_{10} P$ -values of the association test of individuals SNPs with atopy in the stage 1 EGEA dataset and the x axis shows the SNP chromosomal positions. The solid horizontal line indicates the genome-wide significance level ($P=1.5 \times 10^{-7}$); the dotted horizontal line indicates the suggestive association level ($P=10^{-4}$) to select SNPs for follow-up in stage 2 SLSJ and MRC datasets.

Table E1. Descriptive statistics of EGEA, SLSJ and MRC datasets

	EGEA			SLSJ			MRC		
	Non-atopics N=735	Atopics N=925	P^*	Non-atopics N=497	Atopics N=641	P^*	Non-atopics N=340	Atopics N=106	P^*
Sex (male), n (%)	329 (44.8)	539 (58.3)	$<10^{-4}$	205 (41.3)	317 (49.5)	5.9×10^{-3}	117 (34.4)	68 (64.2)	$<10^{-4}$
Age (yrs), mean (SD)	35.9 (16.7)	27.5 (15.7)	$<10^{-4}$	45.1 (23.4)	32.5 (18.4)	$<10^{-4}$	19.6 (14.7)	12.7 (4.3)	$<10^{-4}$
Asthma, n (%)	114 (16.0)	514 (56.3)	$<10^{-4}$	144 (29.0)	405 (63.2)	$<10^{-4}$	84 (24.7)	93 (87.7)	$<10^{-4}$
Indoor allergens [†] , n (%)	-	720 (78.0)		-	481 (75.0)		-	83 (78.3)	
Outdoor allergens [‡] , n (%)	-	512 (55.5)		-	497 (77.5)		-	56 (52.8)	
Molds [§] , n (%)	-	320 (34.8)		-	95 (14.8)		-	13 (12.3)	

* P -values for tests of distribution of each feature (sex, age, asthma) between atopic and non-atopic subjects in each dataset (chi-square test for sex and asthma; Wilcoxon-Mann-Whitney test for age).

[†]Indoor allergens included house dust mite, cockroach and cat in EGEA; house dust mite, dust and cat in SLSJ; dust mite and cat in MRC.

[‡]Outdoor allergens included timothy grass, olive, birch, ragweed and pellitory in EGEA; herbs, trees and animals in SLSJ; timothy grass in MRC.

[§]Molds included *Alternaria*, *Aspergillus* and *Cladosporium* in EGEA; *Alternaria*, *Aspergillus*, *Cladosporium*, *Hormodendrum* and *Penicillium* in SLSJ; *Aspergillus* and *Cladosporium* in MRC.

Table E2. Loci showing suggestive association with atopy at $P \leq 10^{-4}$ in the stage 1 EGEA dataset and followed-up in stage 2 SLSJ and MRC datasets

SNP	Chr*	Position (kb)	Nearest genes (kb distance) [†]	Alleles [‡]	EAF [§]	Stage 1		Stage 2						Overall Meta-Analysis	
						EGEA (N=1,660)		SLSJ (N=1,138)		MRC (N=446)		Meta-Analysis		OR (95% CI)	P_{meta}^{++}
						OR (95% CI)	$P^{††}$	OR (95% CI)	$P^{††}$	OR	$P^{††}$	OR (95% CI)	P_{stage2}^{**}		
rs1252146	1	237 154	<i>MTR (87);RYR2 (51)</i>	C/T	0.31	1.41 (1.19;1.67)	8.9×10^{-5}	0.75 (0.60;0.93)	8.9×10^{-3}	1.03 (0.68;1.58)	0.88	0.80 (0.66;0.97)	0.02	1.10 (0.97;1.25)	0.15
rs9428818	1	239 172	<i>ZP4 (1 118);CHRM3 (620)</i>	C/T	0.08	1.74 (1.32;2.29)	8.9×10^{-5}	1.60 (1.07;2.41)	2.3×10^{-2}	1.07 (0.52;2.21)	0.86	1.46 (1.02;2.08)	0.04	1.63 (1.31;2.02)	1.3×10^{-5}
rs13409750	2	7 610	<i>RNF144A (425);LINC00299 (538)</i>	C/T	0.80	0.64 (0.53;0.79)	2.5×10^{-5}	1.05 (0.84;1.32)	0.66	0.95 (0.63;1.45)	0.83	1.03 (0.84;1.26)	0.78	0.82 (0.71;0.94)	5.9×10^{-3}
rs7567384	2	7 611	<i>RNF144A (426);LINC00299 (537)</i>	C/T	0.80	0.65 (0.53;0.79)	2.8×10^{-5}	1.05 (0.84;1.32)	0.66	0.96 (0.63;1.45)	0.84	1.03 (0.85;1.25)	0.77	0.82 (0.71;0.95)	6.8×10^{-3}
rs1561574	2	7 647	<i>RNF144A (463);LINC00299 (501)</i>	C/T	0.15	1.60 (1.29;2.00)	2.2×10^{-5}	1.07 (0.83;1.37)	0.6	1.13 (0.73;1.75)	0.57	1.08 (0.87;1.35)	0.46	1.32 (1.13;1.54)	4.3×10^{-4}
rs3819892	2	37 510	<i>PRKD</i>	G/T	0.86	0.63 (0.50;0.79)	8.1×10^{-5}	1.09 (0.81;1.48)	0.56	0.89 (0.46;1.7)	0.72	1.05 (0.80;1.39)	0.71	0.78 (0.65;0.93)	5.7×10^{-3}
rs10497664	2	186 260	<i>ZNF804A (456);FSIP2(343)</i>	G/T	0.41	1.38 (1.18;1.61)	5.5×10^{-5}	0.89 (0.74;1.08)	0.24	0.79 (0.54;1.14)	0.21	0.87 (0.74;1.03)	0.11	1.11 (0.99;1.25)	0.06
rs13006333	2	186 268	<i>ZNF804A (464);FSIP2 (335)</i>	G/T	0.43	1.38 (1.18;1.61)	3.4×10^{-5}	0.89 (0.74;1.08)	0.23	0.76 (0.52;1.11)	0.16	0.86 (0.73;1.02)	0.09	1.12 (1.00;1.25)	0.05
rs12328639	2	211 676	<i>CPS1 (132);ERBB4 (564)</i>	A/G	0.19	1.50 (1.23;1.84)	6.4×10^{-5}	1.11 (0.89;1.39)	0.35	1.04 (0.64;1.69)	0.88	1.10 (0.90;1.34)	0.36	1.29 (1.12;1.48)	4.8×10^{-4}
rs1016403	2	211 704	<i>CPS1 (161);ERBB4 (536)</i>	A/G	0.16	1.60 (1.29;1.99)	1.6×10^{-5}	1.17 (0.92;1.48)	0.19	1.01 (0.63;1.63)	0.95	1.14 (0.92;1.40)	0.23	1.35 (1.16;1.56)	9.9×10^{-5}
rs2689860	3	801	<i>CHL1 (349);CNTN6 (334)</i>	A/C	0.63	1.37 (1.18;1.59)	5.1×10^{-5}	0.83 (0.68;1.02)	7.9×10^{-2}	1.10 (0.77;1.57)	0.60	0.89 (0.75;1.06)	0.20	1.14 (1.02;1.28)	0.02
rs445518	3	28 004	<i>EOMES (240);CMC1 (279)</i>	C/T	0.32	0.71 (0.61;0.84)	6.5×10^{-5}	1.21 (0.99;1.47)	6.6×10^{-2}	1.08 (0.75;1.55)	0.69	1.17 (0.99;1.40)	0.07	0.90 (0.80;1.02)	0.09
rs1007368	3	93 961	<i>NSUN3 (115);EPHA6 (2 573)</i>	A/G	0.87	1.59 (1.28;1.99)	4.2×10^{-5}	0.99 (0.71;1.40)	0.98	1.37 (0.75;2.48)	0.30	1.07 (0.80;1.44)	0.63	1.38 (1.16;1.65)	3.8×10^{-4}
rs1584930	3	93 962	<i>NSUN3 (116);EPHA6 (2 572)</i>	A/G	0.13	0.62 (0.50;0.78)	3.6×10^{-5}	1.00 (0.71;1.41)	1	0.73 (0.4;1.33)	0.31	0.92 (0.69;1.25)	0.61	0.72 (0.60;0.86)	3.1×10^{-4}
rs3900940	3	108 148	<i>MYH15</i>	C/T	0.68	1.39 (1.18;1.64)	9.3×10^{-5}	0.93 (0.74;1.16)	0.51	1.20 (0.84;1.73)	0.32	1.00 (0.82;1.21)	0.98	1.21 (1.07;1.37)	3.1×10^{-3}
rs11917965	3	108 189	<i>MYH15</i>	A/C	0.41	0.72 (0.62;0.85)	5.9×10^{-5}	1.05 (0.85;1.29)	0.66	0.81 (0.57;1.15)	0.24	0.98 (0.82;1.17)	0.83	0.83 (0.74;0.93)	1.6×10^{-3}
rs7633227	3	116 831	<i>LSAMP-AS4 (180);IGSF11 (1 789)</i>	C/T	0.62	0.73 (0.62;0.85)	7.9×10^{-5}	0.97 (0.79;1.18)	0.73	1.13 (0.77;1.65)	0.54	1.00 (0.84;1.19)	0.98	0.84 (0.75;0.94)	3.2×10^{-3}
rs4681369	3	147 419	<i>FLJ30375 (279);AGTR1 (997)</i>	G/T	0.20	0.62 (0.51;0.74)	3.4×10^{-7}	1.01 (0.80;1.29)	0.91	1.03 (0.69;1.54)	0.89	1.02 (0.83;1.25)	0.87	0.77 (0.67;0.89)	2.3×10^{-4}
rs795540	5	13 818	<i>DNAH5</i>	C/T	0.56	0.73 (0.62;0.85)	8.3×10^{-5}	1.02 (0.84;1.23)	0.86	0.95 (0.67;1.35)	0.76	1.00 (0.85;1.18)	0.99	0.85 (0.75;0.95)	4.4×10^{-3}
rs17194068	5	39 821	<i>DAB2 (396);PTGER4 (859)</i>	A/G	0.28	1.44 (1.21;1.70)	3.3×10^{-5}	0.81 (0.66;1.00)	5.1×10^{-2}	0.87 (0.6;1.26)	0.45	0.82 (0.69;0.99)	0.04	1.11 (0.98;1.26)	0.11
rs7711329	5	65 126	<i>NLN (1);ERBB2IP (96)</i>	C/T	0.19	0.68 (0.56;0.83)	8.3×10^{-5}	1.07 (0.83;1.37)	0.61	1.07 (0.65;1.77)	0.80	1.07 (0.85;1.33)	0.57	0.82 (0.71;0.95)	8.7×10^{-3}
rs10942608	5	90 083	<i>ADGRV1</i>	C/T	0.23	0.71 (0.60;0.84)	8.2×10^{-5}	0.99 (0.81;1.21)	0.92	0.75 (0.49;1.16)	0.19	0.94 (0.78;1.13)	0.52	0.81 (0.71;0.92)	8.4×10^{-4}
rs4244205	5	90 189	<i>ADGRV1</i>	A/G	0.41	0.71 (0.61;0.83)	1.1×10^{-5}	0.83 (0.68;1.01)	6.0×10^{-2}	0.87 (0.62;1.24)	0.45	0.84 (0.70;1.00)	0.04	0.76 (0.68;0.86)	3.8×10^{-6}
rs4916829	5	90 195	<i>ADGRV1</i>	T/G	0.35	0.70 (0.61;0.83)	1.0×10^{-5}	0.94 (0.78;1.16)	0.59	0.88 (0.59;1.31)	0.52	0.93 (0.75;1.18)	0.44	0.79 (0.70;0.89)	1.2×10^{-4}
rs4916831	5	90 212	<i>ADGRV1</i>	A/G	0.44	0.67 (0.58;0.79)	1.0×10^{-6}	0.72 (0.59;0.89)	2.3×10^{-3}	0.81 (0.58;1.14)	0.23	0.75 (0.63;0.89)	1.2×10^{-3}	0.71 (0.63;0.79)	6.8×10^{-9}
rs10060641	5	90 213	<i>ADGRV1</i>	T/C	0.38	0.67 (0.58;0.79)	7.2×10^{-7}	0.85 (0.68;1.04)	0.11	0.79 (0.54;1.16)	0.22	0.83 (0.69;1.00)	0.05	0.74 (0.66;0.83)	4.3×10^{-7}
rs12054681	5	90 218	<i>ADGRV1</i>	C/A	0.37	0.68 (0.58;0.79)	9.7×10^{-7}	0.87 (0.70;1.06)	0.18	0.75 (0.5;1.12)	0.16	0.84 (0.70;1.01)	0.06	0.74 (0.66;0.84)	7.8×10^{-7}
rs949787	5	90 251	<i>ADGRV1</i>	G/T	0.28	0.72 (0.61;0.84)	5.5×10^{-5}	0.79 (0.66;0.96)	1.9×10^{-2}	0.83 (0.56;1.23)	0.36	0.80 (0.67;0.95)	0.01	0.76 (0.67;0.85)	3.2×10^{-6}
rs12522571	5	90 259	<i>ADGRV1</i>	A/G	0.22	0.66 (0.56;0.78)	1.9×10^{-6}	0.90 (0.73;1.12)	0.36	0.75 (0.46;1.23)	0.25	0.88 (0.72;1.07)	0.19	0.74 (0.65;0.85)	7.4×10^{-6}
rs7769042	6	106 156	<i>PREP (305);PRDM1 (378)</i>	A/G	0.57	1.38 (1.18;1.62)	7.4×10^{-5}	1.14 (0.93;1.39)	0.21	0.61 (0.43;0.87)	0.01	0.98 (0.82;1.16)	0.79	1.18 (1.05;1.33)	5.9×10^{-3}
rs12660166	6	120 894	<i>MIR3144 (557);C6orf170 (507)</i>	C/T	0.25	0.70 (0.59;0.84)	7.4×10^{-5}	1.11 (0.87;1.42)	0.41	0.95 (0.62;1.45)	0.80	1.07 (0.86;1.32)	0.55	0.83 (0.73;0.95)	7.2×10^{-3}
rs12194792	6	121 085	<i>MIR3144 (348);C6orf170 (316)</i>	C/T	0.58	1.37 (1.18;1.60)	4.3×10^{-5}	0.90 (0.74;1.09)	0.27	1.04 (0.69;1.58)	0.83	0.92 (0.78;1.10)	0.36	1.16 (1.03;1.30)	0.01
rs1880617	7	53 138	<i>POM121L12 (33);FLJ45974 (585)</i>	G/T	0.39	1.36 (1.17;1.59)	9.5×10^{-5}	1.02 (0.81;1.27)	0.89	0.85 (0.57;1.25)	0.40	0.97 (0.80;1.18)	0.77	1.19 (1.06;1.35)	4.3×10^{-3}
rs12530936	7	91 187	<i>FZD1 (289);MTERF (315)</i>	C/T	0.13	0.64 (0.51;0.80)	9.9×10^{-5}	0.86 (0.63;1.17)	0.34	0.83 (0.43;1.6)	0.57	0.85 (0.65;1.13)	0.27	0.72 (0.60;0.85)	1.8×10^{-4}
rs10279056	7	115 847	<i>TFEC (176);TES (4)</i>	A/G	0.57	1.40 (1.20;1.62)	1.0×10^{-5}	0.90 (0.74;1.10)	0.30	1.05 (0.75;1.48)	0.77	0.94 (0.79;1.11)	0.45	1.17 (1.05;1.31)	4.9×10^{-3}
rs17138756	7	116 135	<i>TES (236);CAV2 (4)</i>	A/G	0.10	0.58 (0.45;0.75)	3.4×10^{-5}	0.99 (0.77;1.28)	0.95	0.93 (0.51;1.7)	0.83	0.98 (0.78;1.24)	0.89	0.77 (0.65;0.92)	3.9×10^{-3}
rs10253511	7	130 797	<i>MKLN1</i>	C/T	0.91	0.59 (0.46;0.76)	4.8×10^{-5}	0.88 (0.61;1.25)	0.46	1.97 (0.82;4.74)	0.13	0.98 (0.71;1.37)	0.92	0.71 (0.58;0.87)	1.6×10^{-3}
rs4237038	8	61 731	<i>CHD7</i>	A/G	0.25	1.44 (1.20;1.73)	7.3×10^{-5}	0.97 (0.80;1.18)	0.77	1.08 (0.7;1.67)	0.74	0.99 (0.83;1.18)	0.89	1.19 (1.05;1.35)	7.6×10^{-3}
rs10957162	8	61 758	<i>CHD7</i>	A/G	0.27	1.44 (1.20;1.72)	8.5×10^{-5}	0.99 (0.82;1.21)	0.95	0.98 (0.63;1.51)	0.92	0.99 (0.83;1.18)	0.92	1.19 (1.05;1.35)	7.3×10^{-3}
rs7357565	8	134 817	<i>ST3GAL1 (233);ZFAT (673)</i>	A/G	0.51	0.73 (0.62;0.86)	9.7×10^{-5}	1.00 (0.82;1.22)	1	0.63 (0.41;0.96)	0.03	0.92 (0.77;1.10)	0.36	0.81 (0.72;0.91)	3.9×10^{-4}

rs4399592	8	134 824	ST3GAL1 (240);ZFAT (666)	C/T	0.65	1.41 (1.19;1.66)	5.8x10 ⁻⁵	1.00 (0.81;1.22)	0.99	1.67 (1.11;2.52)	0.01	1.11 (0.92;1.33)	0.27	1.26 (1.12;1.43)	2.1x10 ⁻⁴
rs1408793	9	12 628	PTPRD (2 000);TYRP1 (65)	A/C	0.21	0.68 (0.57;0.82)	5.1x10 ⁻⁵	0.94 (0.73;1.21)	0.62	1.67 (1.02;2.72)	0.04	1.06 (0.85;1.32)	0.62	0.81 (0.71;0.94)	5.00x10 ⁻³
rs1433831	9	18 869	ADAMTSL1	A/G	0.34	0.72 (0.61;0.84)	5.7x10 ⁻⁵	1.13 (0.91;1.41)	0.27	0.79 (0.54;1.14)	0.20	1.03 (0.85;1.24)	0.78	0.83 (0.74;0.94)	4.0x10 ⁻³
rs2309394	9	71 671	FXN	A/G	0.56	0.72 (0.61;0.85)	8.4x10 ⁻⁵	1.02 (0.84;1.24)	0.83	1.49 (1.01;2.18)	0.04	1.1 (0.93;1.30)	0.27	0.88 (0.78;0.99)	0.04
rs9314854	9	71 686	FXN	C/T	0.52	1.37 (1.18;1.61)	6.3x10 ⁻⁵	1.18 (0.97;1.44)	9.9x10 ⁻²	0.85 (0.56;1.28)	0.43	1.11 (0.93;1.33)	0.24	1.25 (1.11;1.41)	1.6x10 ⁻⁴
rs7870295	9	71 686	FXN	A/G	0.55	1.42 (1.21;1.66)	1.6x10 ⁻⁵	1.15 (0.94;1.40)	0.17	0.79 (0.52;1.19)	0.25	1.07 (0.89;1.28)	0.46	1.25 (1.11;1.41)	2.0x10 ⁻⁴
rs4745580	9	71 690	FXN	C/T	0.55	1.41 (1.21;1.65)	1.9x10 ⁻⁵	1.16 (0.95;1.41)	0.14	1.19 (0.78;1.79)	0.42	0.89 (0.75;1.07)	0.21	0.8 (0.71;0.90)	1.5x10 ⁻⁴
rs7859021	9	71 694	FXN	A/G	0.48	0.73 (0.63;0.86)	8.6x10 ⁻⁵	0.84 (0.69;1.02)	7.5x10 ⁻²	1.14 (0.65;1.99)	0.64	1.02 (0.73;1.44)	0.89	1.41 (1.15;1.72)	1.1x10 ⁻³
rs2771064	9	103 921	PLPPR1	A/G	0.89	1.68 (1.30;2.16)	6.4x10 ⁻⁵	0.96 (0.63;1.48)	0.86	1.20 (0.82;1.75)	0.35	1.19 (1.00;1.42)	0.05	0.90 (0.80;1.02)	0.09
rs13286744	9	117 444	TMEM268 (35);TNFSF15 (103)	C/T	0.42	0.71 (0.60;0.84)	3.6x10 ⁻⁵	1.19 (0.98;1.45)	8.7x10 ⁻²	0.88 (0.49;1.57)	0.66	1.17 (0.87;1.57)	0.31	1.46 (1.19;1.79)	2.4x10 ⁻⁴
rs12000625	9	126 686	DENND1A	A/C	0.93	1.78 (1.35;2.34)	4.4x10 ⁻⁵	1.29 (0.91;1.82)	0.15	0.95 (0.58;1.57)	0.85	0.86 (0.67;1.10)	0.22	1.20 (1.02;1.41)	0.03
rs17515236	10	9 540	GATA3 (1 423);CEL2 (1 507)	A/G	0.87	1.57 (1.26;1.95)	5.4x10 ⁻⁵	0.83 (0.63;1.10)	0.2	0.86 (0.51;1.45)	0.57	0.73 (0.54;0.97)	0.03	1.24 (1.05;1.47)	0.01
rs11005971	10	59 649	MIR3924 (584);IPMK (302)	C/T	0.15	1.64 (1.33;2.01)	3.4x10 ⁻⁶	0.68 (0.48;0.96)	2.7x10 ⁻²	1.13 (0.67;1.9)	0.65	1.38 (1.03;1.84)	0.03	0.80 (0.68;0.95)	0.01
rs7101032	10	59 672	MIR3924 (607);IPMK (279)	C/T	0.85	0.61 (0.49;0.74)	2.1x10 ⁻⁶	1.50 (1.07;2.12)	2.0x10 ⁻²	0.69 (0.38;1.26)	0.23	0.89 (0.67;1.17)	0.41	1.24 (1.01;1.54)	0.04
rs12771265	10	71 411	C10orf35 (18);COL13A1 (150)	A/G	0.52	1.38 (1.19;1.61)	3.2x10 ⁻⁵	0.87 (0.72;1.05)	0.14	1.04 (0.71;1.52)	0.85	0.90 (0.76;1.07)	0.22	1.14 (1.02;1.28)	0.02
rs4746906	10	71 516	C10orf35 (122);COL13A1 (45)	C/T	0.16	1.53 (1.24;1.90)	9.1x10 ⁻⁵	0.93 (0.74;1.17)	0.52	0.73 (0.44;1.21)	0.22	0.89 (0.72;1.10)	0.28	1.16 (1.00;1.35)	0.05
rs618929	11	96 229	JRKL (102);CNTN5 (2 662)	A/G	0.54	1.39 (1.19;1.63)	3.2x10 ⁻⁵	0.81 (0.66;1.00)	4.7x10 ⁻²	1.23 (0.87;1.76)	0.24	0.90 (0.75;1.08)	0.27	1.16 (1.03;1.30)	0.02
rs4475974	12	18 262	RERGL (19);PIK3C2G (152)	G/T	0.60	0.71 (0.61;0.83)	2.5x10 ⁻⁵	1.05 (0.86;1.29)	0.62	0.73 (0.5;1.06)	0.10	0.97 (0.81;1.16)	0.73	0.81 (0.72;0.92)	7.1x10 ⁻⁴
rs4457807	12	18 306	RERGL (63);PIK3C2G (109)	A/G	0.39	0.70 (0.60;0.83)	2.2x10 ⁻⁵	1.09 (0.92;1.29)	0.32	1.16 (0.78;1.72)	0.45	1.10 (0.94;1.29)	0.23	0.89 (0.79;0.99)	0.04
rs10783599	12	54 186	CALCOCO1 (65);HOXC13 (147)	A/G	0.32	1.39 (1.18;1.62)	5.1x10 ⁻⁵	0.80 (0.66;0.97)	2.0x10 ⁻²	1.24 (0.81;1.89)	0.33	0.86 (0.72;1.02)	0.09	1.11 (0.99;1.25)	0.07
rs7315435	12	69 284	CPM	A/G	0.80	1.45 (1.21;1.75)	7.4x10 ⁻⁵	1.03 (0.80;1.34)	0.81	1.04 (0.64;1.69)	0.87	1.03 (0.82;1.30)	0.77	1.27 (1.10;1.47)	1.1x10 ⁻³
rs2434080	12	106 454	C12orf75 (689);NUAK1 (3)	A/G	0.59	0.72 (0.62;0.85)	5.0x10 ⁻⁵	1.04 (0.84;1.28)	0.73	0.90 (0.58;1.41)	0.66	1.01 (0.84;1.22)	0.90	0.83 (0.74;0.94)	2.4x10 ⁻³
rs1327751	13	75 282	LINC00347 (150);TBC1D4 (577)	C/T	0.11	1.64 (1.28;2.11)	9.7x10 ⁻⁵	1.05 (0.74;1.47)	0.79	0.89 (0.52;1.52)	0.67	1.00 (0.75;1.33)	0.99	1.33 (1.10;1.60)	3.2x10 ⁻⁴
rs8030108	15	98 056	SPATA8 (726);ARRDC4 (448)	C/T	0.44	0.74 (0.64;0.86)	6.4x10 ⁻⁵	1.02 (0.84;1.23)	0.84	1.37 (0.94;1.99)	0.10	1.08 (0.91;1.28)	0.36	0.87 (0.78;0.97)	0.02
rs13338087	16	4 105	ADCY9	G/T	0.39	1.41 (1.20;1.65)	2.0x10 ⁻⁵	0.84 (0.69;1.02)	8.4x10 ⁻²	0.84 (0.56;1.25)	0.39	0.84 (0.70;1.00)	0.05	1.12 (1.00;1.26)	0.05
rs12929999	16	76 915	MIR4719 (13);MON1B (309)	C/T	0.19	1.49 (1.22;1.81)	7.2x10 ⁻⁵	1.09 (0.85;1.40)	0.5	0.94 (0.62;1.43)	0.78	1.05 (0.85;1.30)	0.66	1.27 (1.10;1.47)	1.2x10 ⁻³
rs11647758	16	89 094	CBFA2T3 (51);ACSF3 (66)	C/T	0.82	1.53 (1.26;1.86)	2.3x10 ⁻⁵	1.10 (0.88;1.38)	0.38	1.04 (0.66;1.64)	0.86	1.09 (0.89;1.33)	0.39	1.3 (1.13;1.49)	2.7x10 ⁻⁴
rs2571225	18	55 462	ATP8B1	C/T	0.72	0.68 (0.57;0.81)	2.0x10 ⁻⁵	0.96 (0.78;1.18)	0.67	0.69 (0.47;1.03)	0.07	0.89 (0.74;1.07)	0.22	0.77 (0.68;0.88)	8.3x10 ⁻⁵
rs6056732	20	9 577	PAK5	A/C	0.91	0.56 (0.42;0.73)	3.2x10 ⁻⁵	0.73 (0.52;1.03)	7.0x10 ⁻²	0.55 (0.28;1.06)	0.07	0.68 (0.51;0.93)	0.01	0.61 (0.50;0.75)	2.5x10 ⁻⁶
rs6056733	20	9 577	PAK5	A/G	0.12	1.62 (1.28;2.06)	7.2x10 ⁻⁵	1.28 (0.98;1.67)	7.2x10 ⁻²	1.80 (1.06;3.07)	0.03	1.37 (1.08;1.74)	0.01	1.49 (1.26;1.77)	3.7x10 ⁻⁶
rs8117366	20	52 365	ZNF217 (65);BCAS1 (439)	G/T	0.81	1.51 (1.24;1.84)	3.2x10 ⁻⁵	0.94 (0.73;1.21)	0.64	1.47 (0.88;2.46)	0.14	1.03 (0.82;1.29)	0.81	1.28 (1.11;1.49)	9.5x10 ⁻⁴
rs6013784	20	52 365	ZNF217 (65);BCAS1 (439)	C/T	0.21	0.68 (0.57;0.82)	5.2x10 ⁻⁵	1.08 (0.84;1.40)	0.54	0.68 (0.41;1.13)	0.14	0.99 (0.79;1.24)	0.91	0.79 (0.68;0.91)	1.3x10 ⁻³
rs4389378	20	54 592	CBLN4 (12);MC3R (232)	A/G	0.24	0.72 (0.62;0.85)	6.8x10 ⁻⁵	0.93 (0.75;1.15)	0.51	1.12 (0.76;1.63)	0.57	0.97 (0.80;1.17)	0.77	0.82 (0.72;0.92)	1.1x10 ⁻³

*Chr is the chromosome number where the SNP is located

†The gene where the SNP is located is indicated otherwise the genes on either side of the SNP are indicated together with the distance between the SNP and gene boundary (using Build 37.3)

‡Baseline/Effect allele

§Effect allele frequency

||OR is the Odds Ratio associated with the effect allele under an additive model. 95% CI is the 95% confidence interval associated with the Odds Ratio.

**P is the P-value associated with the Wald test statistic of SNP effect

††P_{stage2} is the P-value associated with the Wald test of meta-analyzed SNP effect in the stage 2 datasets (SLSJ and MRC)

‡‡P_{meta} is the P-values associated with the Wald test of meta-analyzed SNP effect in the three datasets (EGEA, SLSJ & MRC)

Table E3. List of genes (gene set-2) tested for their relationship with *ADGRV1* (gene set-1) using GRAIL

Gene symbol*	Nearest SNP (kb distance) [†]	Cytogenetic Band	Position (kb) [‡]	GWAS <i>P</i> -values in EGEA	<i>P</i> _{GRAIL}
<i>CHD7</i>	rs4237038	8q12	61,731	7.3x10 ⁻⁵	3.2x10⁻³
<i>ATP8B1</i>	rs2571225	18q21	55,462	2.0x10 ⁻⁵	0.016
<i>DNAH5</i>	rs795540	5p15	13,818	8.3x10 ⁻⁵	0.084
<i>NDUFAF7</i>	rs3819892 (34)	2p22	37,510	8.1x10 ⁻⁵	1
<i>PRKD3</i>	rs3819892	2p22	37,510	8.1x10 ⁻⁵	1
<i>MYH15</i>	rs3900940	3q13	108,148	9.3x10 ⁻⁵	1
<i>NLN</i>	rs7711329 (1)	5q12	65,126	8.3x10 ⁻⁵	1
<i>PDE1C</i>	rs11763324	7p14	32,171	2.3x10 ⁻⁵	1
<i>POM121L12</i>	rs1880617 (33)	7p12	53,138	9.5x10 ⁻⁵	1
<i>TES</i>	rs10279056 (4)	7q31	115,847	1.0x10 ⁻⁵	1
<i>CAV2</i>	rs17138756 (4)	7q31	116,135	3.4x10 ⁻⁵	1
<i>LINC-PINT</i>	rs10253511 (3)	7q32	130,797	4.8x10 ⁻⁵	1
<i>MKLN1</i>	rs10253511	7q32	130,797	4.8x10 ⁻⁵	1
<i>ADAMTSL1</i>	rs1433831	9p22	18,869	5.7x10 ⁻⁵	1
<i>FXN</i>	rs2309394	9q21	71,671	8.4x10 ⁻⁵	1
<i>PRKACG</i>	rs2309394 (42)	9q21	71,671	8.4x10 ⁻⁵	1
<i>TJP2</i>	rs7859021 (42)	9q21	71,694	8.6x10 ⁻⁵	1
<i>PLPPR1</i>	rs2771064	9q31	103,921	6.4x10 ⁻⁵	1
<i>TMEM268</i>	rs13286744 (36)	9q32	117,444	3.6x10 ⁻⁵	1
<i>DENND1A</i>	rs12000625	9q33	126,686	4.4x10 ⁻⁵	1
<i>JMJD1C</i>	rs10995495	10q21	65,062	4.7x10 ⁻⁵	1
<i>C10orf35</i>	rs12771265 (18)	10q22	71,411	3.2x10 ⁻⁵	1
<i>COL13A1</i>	rs4746906 (46)	10q22	71,516	9.1x10 ⁻⁵	1
<i>RERGL</i>	rs4475974 (19)	12p12	18,262	2.5x10 ⁻⁵	1
<i>CPM</i>	rs7315435	12q15	69,284	7.4x10 ⁻⁵	1
<i>MDM2</i>	rs7315435 (45)	12q15	69,284	7.4x10 ⁻⁵	1
<i>NUAK1</i>	rs2434080 (3)	12q23	106,454	5.0x10 ⁻⁵	1
<i>ADCY9</i>	rs13338087	16p13	4,105	2.0x10 ⁻⁵	1
<i>PAK5</i>	rs6056732	20p12	9,577	3.2x10 ⁻⁵	1
<i>CBLN4</i>	rs4389378 (12)	20q13	54,592	6.7x10 ⁻⁵	1

*Genes are located at most 50 kb apart from SNPs having $P \leq 10^{-4}$ in the stage 1 EGEA dataset[†]The distance (in kilobases; build 37.3) of nearest SNP to each gene is indicated in parentheses when the SNP lies outside of a gene.[‡]SNP position in kilobases (Build 37.3)

Table E4. Number of SNP pairs tested for the three selected gene pairs and multiple-testing corrected threshold

Gene set 1	Gene set 2	Number of SNP pairs tested	Effective number of independent SNP pairs tested	Total effective number of independent SNP pairs tested*
<i>ADGRV1</i>	<i>CHD7</i>	808	110	688
<i>ADGRV1</i>	<i>ATP8B1</i>	1450	223	
<i>ADGRV1</i>	<i>DNAH5</i>	3066	355	

*The total effective number of independent SNP pairs tested is estimated by the sum of the effective number of independent SNP pairs tested by gene pair over all gene pairs examined.

The multiple-testing corrected threshold is thus equal to the 5% type I error divided by the total effective number of independent SNP pairs tested ($T=7.3 \times 10^{-5}$).

Table E5A. Stratified analysis on asthma for the *ADGRV1* SNP significantly associated with atopy at the genome-wide level

SNPs	Gene*	Alleles [†]	MAF [‡]	Group	Stage 1		Stage 2		Overall Meta-Analysis	
					OR (95% CI) [§]	<i>P</i>	OR (95% CI) [§]	<i>P</i> _{stage2} ^{**}	OR (95% CI) [§]	<i>P</i> _{meta} ^{††}
rs4916831	<i>ADGRV1</i>	A/G	0.44	Asthmatics	0.67 (0.48-0.92)	0.01	0.69 (0.53-0.89)	3.9x10 ⁻³	0.68 (0.56-0.83)	1.4x10 ⁻⁴
				Non-asthmatics	0.69 (0.57-0.84)	1.8x10 ⁻⁴	0.72 (0.55-0.94)	1.4x10 ⁻²	0.70 (0.60-0.82)	7.8x10 ⁻⁶
				<i>P</i> _{Cochran} ^{‡‡}		0.86		0.82		0.82

Table E5B. Stratified analysis on asthma for the interacting *ADGRV1/DNAH5* SNP pair significantly associated with atopy

SNPs	Genes*	Alleles [†]	MAF [‡]	Group	Stage 1		Stage 2		Overall Meta-Analysis	
					Interaction Effect		Interaction Effect		Interaction Effect	
					OR (95% CI) [§]	<i>P</i> _{int}	OR (95% CI) [§]	<i>P</i> _{stage2-int} ^{**}	OR (95% CI) [§]	<i>P</i> _{meta-int} ^{††}
rs17554723	<i>ADGRV1</i>	A/G	0.33	Asthmatics	0.77 (0.47-1.24)	0.28	0.73 (0.48-1.12)	0.15	0.74 (0.54-1.02)	0.07
rs2134256	<i>DNAH5</i>	T/C	0.25	Non-asthmatics	0.64 (0.46-0.89)	7.3x10 ⁻³	0.53 (0.34-0.82)	4.2x10 ⁻³	0.60 (0.46-0.78)	1.1x10 ⁻⁴
				<i>P</i> _{Cochran} ^{‡‡}		0.55		0.29		0.30

The two groups contained 1,354 asthmatics (628, 549, 177 for EGEA, SLSJ and MRC respectively) and 1,849 non-asthmatics (997, 583, 269 for EGEA, SLSJ and MRC)

*Gene symbol

[†]Major allele/Minor allele

[‡]Minor allele frequency

[§]OR is the Odds-Ratio for single SNP effect (Table E5A) or interaction between SNPs (Table E5B) associated with atopy assuming an additive model. 95% CI is the 95% Confidence Interval of the OR.

^{||}*P* and *P*_{int} are the *P*-values associated with the Wald test of SNP effect (Table E5A) and interaction effect (Table E5B).

^{**}*P*_{stage2} and *P*_{stage2-int} are the *P*-values associated with the Wald test of meta-analyzed single SNP effect (Table E5A) and interaction between SNPs (Table E5B) in the stage 2 datasets (SLSJ and MRC).

^{††}*P*_{meta} and *P*_{meta-int} are the *P*-values associated with the Wald test of meta-analyzed single SNP effect (Table E5A) and interaction between SNPs (Table E5B) in the three datasets (EGEA, SLSJ and MRC).

^{‡‡}*P*_{Cochran} is the *P*-value associated with the Cochran's Q test for homogeneity between the two groups of asthmatic and non-asthmatic subjects.

Table E6. Functional annotations of *ADGRV1* and *DNAH5* SNPs

Gene	SNP	Position on chromosome 5	Variant Location	r^2 with the significant SNP(s) in our analysis	Regulatory elements			
					Promoter	Enhancer	DNaseI hyper	Transcription factor binding sites
					histones marks	histones marks	sensitivity site	
<i>ADGRV1</i>	rs4916831	90,916,459	intronic	-	No	No	No	SOX, TCF12
	rs4637585	90,889,700	intronic	0.8	No	Yes (lung)	No	DBX, FOXO, GATA, MEF2, NCX, PAX, PDX, SOX, ZFP
	rs10045202	90,890,830	intronic	0.8	No	No	No	NERF1A
	rs4244205	90,893,069	intronic	0.8	No	No	No	DOBOX4
	rs4580808	90,894,483	intronic	0.82	No	Yes (lung)	No	PAX4, SREBP, SIN3AK20
	rs4496735	90,894,600	intronic	0.82	No	No	No	EVI1, OSF2, SIX5
	rs10035307	90,906,182	intronic	0.83	No	No	No	FOX, SP2
	rs6858917	90,909,376	intronic	0.96	No	Yes (skin, lung)	No	AHR, CTCF, RAD21, SMAD
	rs6889986	90,911,582	intronic	0.99	No	Yes (lung)	No	ARID3A, CDX, DBX1, FOXL1, HOXB13, HOXB9, HOXD10, MEF2, NCX, PBX1, POU2F2, POU3F2, SOX, TATA, TEF, ZFP105
	rs10078568	90,915,831	intronic	0.8	No	No	No	CDX
	rs17554723	90,793,497	intronic	-	No	No	No	-
	rs11745546	90,794,895	intronic	1	No	No	No	CCNT2, E2F, MZF1
	rs13186025	90,827,511	intronic	0.8	No	No	No	DMRT2, HDAC2
<i>DNAH5</i>	rs2134256	13,768,544	intronic	-	No	No	No	SMC3, TCF12
	rs6862904	13,750,128	intronic	0.83	No	No		MRG, NRSF, PBX3, TGIF1, ZNF143
	rs11745096	13,750,128	intronic	0.84	Yes (blood)	Yes (blood)	No	-
	rs34789506	13,757,027	intronic	0.93	No	No	No	GR
	rs11742383	13,762,604	intronic	0.95	No	No	No	GR
	rs13156606	13,765,320	intronic	0.93	No	No	No	BATF, HDAC2, IRF
	rs60499013	13,772,775	intronic	0.94	No	No	No	CDX, DMRT2, DMRT3, DBX1, FOXA, HDAC2, HOXA10, HOXB13, HOXD10, NF, PAX, STAT

The *ADGRV1* rs4916831 SNP is significantly associated with atopy; the *ADGRV1* rs17554723 and *DNAH5* rs2134256 SNPs show significant interaction in atopy. These three SNPs and their proxies ($r^2 \geq 0.8$, using the 1,000 Genomes project database) were mapped to regulatory elements using the HaploReg v4.1 tool (<http://archive.broadinstitute.org/mammals/haploreg/haploreg.php>)

