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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

*These authors shared second authorship.

‡These authors shared senior authorship.

[a]Genetic Variation and Human Diseases Unit, INSERM, Université Paris Diderot, Université Sorbonne Paris Cité, Paris, France

[b]Université Pierre et Marie Curie, Paris, France

[c]McGill University and Génome Québec Innovation Centre, Montréal, Canada

[d]Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, QC, Canada

[e]Section of Genomic Medicine, National Heart and Lung Institute, London, UK

Correspondance:

Florence Demenais, MD

UMR-946, INSERM, Université Paris-Diderot

27 rue Juliette Dodu

75010 Paris

Tel: 33 (0) 1 53 72 50 02

Fax: 33 (0) 1 53 72 50 49

Email: florence.demenais@inserm.fr

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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; $P_{meta}=6.8x10^{-9}$). Statistical filtering of GWAS results followed by text-mining filtering revealed relationships between *ADGRV1* and three genes showing suggestive association with atopy ($P\leq10^{-4}$). 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 ($P_{meta\text{-}int}=3.6x10^{-5}$ and $6.1x10^{-5}$, that met the multiple-testing corrected threshold of $7.3x10^{-5}$). 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*, *DNAH*5, 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

GRAIL: Gene Relationships Across Implicated Loci

QC: quality control

MAF: minor allele frequency

PCs: principal components

LD: linkage disequilibrium

ORs: odds-ratios

GTEx: Genotype-Tissue Expression

ETS: environmental tobacco smoke

CI: confidence interval

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 sensitization[11] and self-reported allergy[12] 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 genes[16] 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 ≥ 3mm 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

166 recruited through probands with eczema (MRCE sample). Only subjects without asthma, without
167 eczema and with low IgE levels were used as controls in this study. We checked that the age and
168 gender distributions were similar in MRCA and MRCE samples. After QC of genotypic data, the
169 analysis sample included 106 atopic and 340 non-atopic subjects. The whole UK sample will be
170 subsequently designated as the MRC sample.
171 Protocols of EGEA, SLSJ and MRC studies have been approved by the local ethical committees.
172 All adult participants and child's legal guardians provided written informed consent.
173
174 ***Genotyping***
175 Both EGEA and SLSJ datasets were genotyped using the Illumina 610-Quad array (Illumina, San
176 Diego, CA), as part as of the Gabriel asthma consortium GWAS.[23] Stringent quality criteria were
177 applied to select both individuals and SNPs and have been previously detailed.[23,24] After QC, there
178 was a final set of 501,167 autosomal SNPs for analysis. The offspring in MRCA families and
179 MRCE controls were genotyped using the Illumina Sentrix HumanHap300 BeadChip (307,981
180 autosomal SNPs), as part of the first asthma GWAS.[22,23] QC for MRC samples has been detailed
181 elsewhere.[22,25] In order to get a number of SNPs in MRC sample as large as in EGEA and SLSJ
182 samples, SNP imputation was performed using MACH v1.00 software[26] and HapMap2 release 21
183 CEU haplotypes as reference panel. Imputed SNPs were kept for analysis if their imputation quality
184 score (rsq)[27] was ≥ 0.5 and minor allele frequency was $\geq 5\%$.
185
186 ***Descriptive statistics and strategy of analysis***
187 Descriptive statistics of atopy together with sex, age and asthma status were assessed in each
188 dataset using Stata® V14.1 (distributed by Stata Corporation, College Station, Texas, USA). The

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

191

192 ***Genome-wide single-SNP analysis***

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

205

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

207 The statistical filtering consisted of selecting two sets of genes using the GWAS results: genes
208 showing significant association with atopy (set-1) and genes showing suggestive association with
209 atopy (set-2). The set-1 included all genes lying at a distance of 50 kb or less from SNPs reaching
210 the genome-wide significance level in the GWAS meta-analysis. The set-2 included all genes that
211 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 GRAIL[17] 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 $P_{GRAIL} \leq 0.10$, 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 ($P_{int} \leq 5x10^{-3}$) 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 $1.5x10^{-7}$ (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 ($P_{meta}=6.8x10^{-9}$) 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 ($4.3x10^{-7} \leq P_{meta} \leq 3.8x10^{-6}$; 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 $P_{GRAIL}<0.10$: *DNAH5* on 5p15 ($P_{GRAIL}=0.084$), *CHD7* on 8q12 ($P_{GRAIL}=3.2x10^{-3}$) and *ATP8B1* on 18q21 ($P_{GRAIL}=0.016$).

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 $P_{int} \leq 5x10^{-3}$ 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 $7.3x10^{-5}$ (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 ($P_{meta\text{-}int}=3.6x10^{-5}$) and rs1354187 ($P_{meta\text{-}int}=6.1x10^{-5}$), 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 ($P=7.8x10^{-6}$) than in asthmatics ($P=1.4x10^{-4}$). Similarly, interaction analyses for *ADGRV1* and *DNAH5* SNPs did not show any relationship with asthma ($P_{Cochran} \geq 0.30$; 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 *DNAH*5 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=7.5x10^{-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 disease[41] and suggestive association with Immunoglobuline 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 *DNAH*5.[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 *ADGRV1,* two genes involved in cilia functions, is of biological relevance and provides a novel mechanism

403 underlying atopy. Further studies, including functional and experimental studies, are needed to
404 confirm the current findings and to identify the functional variants.
405

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

				Stage 1		Stage 2						Overall Meta-Analysis		
				EGEA (N=1,660)		SLSJ (N=1,138)		MRC (N=446)		Meta-Analysis				
SNP	**Position (kb)***	**Alleles†**	**MAF‡**	**beta (se) §**	***P*‖**	**beta (se) §**	***P*‖**	**beta (se) §**	***P*‖**	**beta (se) §**	P_{stage2}**	**beta (se) §**	P_{meta}††	$P_{Cochran}$‡‡
rs4244205	90,188	A/G	0.41	-0.35 (0.08)	$1.1x10^{-5}$	-0.19 (0.10)	$6.0x10^{-2}$	-0.14 (0.18)	0.45	-0.18 (0.09)	$4.4x10^{-2}$	-0.27 (0.06)	$3.8x10^{-6}$	0.35
rs4916831	90,212	A/G	0.44	-0.40 (0.08)	$1.0x10^{-6}$	-0.32 (0.11)	$2.3x10^{-3}$	-0.21 (0.17)	0.23	-0.29 (0.09)	$1.2x10^{-3}$	-0.35 (0.06)	**$6.8x10^{-9}$**	0.59
rs10060641	90,213	T/C	0.38	-0.39 (0.08)	$7.2x10^{-7}$	-0.17 (0.11)	0.11	-0.24 (0.19)	0.22	-0.18 (0.09)	$4.8x10^{-2}$	-0.30 (0.06)	$4.3x10^{-7}$	0.23
rs12054681	90,217	C/A	0.37	-0.39 (0.08)	$9.7x10^{-7}$	-0.14 (0.11)	0.18	-0.29 (0.21)	0.16	-0.17 (0.09)	$6.5x10^{-2}$	-0.30 (0.06)	$7.8x10^{-7}$	0.18
rs949787	90,251	G/T	0.28	-0.33 (0.08)	$5.5x10^{-5}$	-0.23 (0.10)	$2.0x10^{-2}$	-0.19 (0.20)	0.36	-0.22 (0.09)	$1.2x10^{-2}$	-0.28 (0.06)	$3.2x10^{-6}$	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 $1.5x10^{-7}$.

‡‡$P_{Cochran}$ 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 EGEA (N=1,660)			Stage 2 SLSJ (N=1,138)			Stage 2 MRC (N=446)			Stage 2 Meta-Analysis			Overall Meta-analysis			
					Main effect	*Interaction*		*Main effect*	*Interaction*		*Main effect*	*Interaction*		*Main effect*	*Interaction*		*Main effect*	*Interaction*		
					beta (se)‖	beta (se)‖	P_{int}**	beta (se)‖	beta (se)‖	P_{int}**	beta (se)‖	beta (se)‖	P_{int}**	beta (se)‖	beta (se)‖	$P_{stage2\text{-}int}$††	beta (se)‖	beta (se)‖	$P_{meta\text{-}int}$‡‡	$P_{Cochran}$§§
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).

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

‡‡$P_{meta\text{-}int}$ is the *P*-values associated with the Wald test of meta-analyzed interaction effect in the three datasets (EGEA, SLSJ, MRC); $P_{meta\text{-}int}$ 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.

Figure 1.

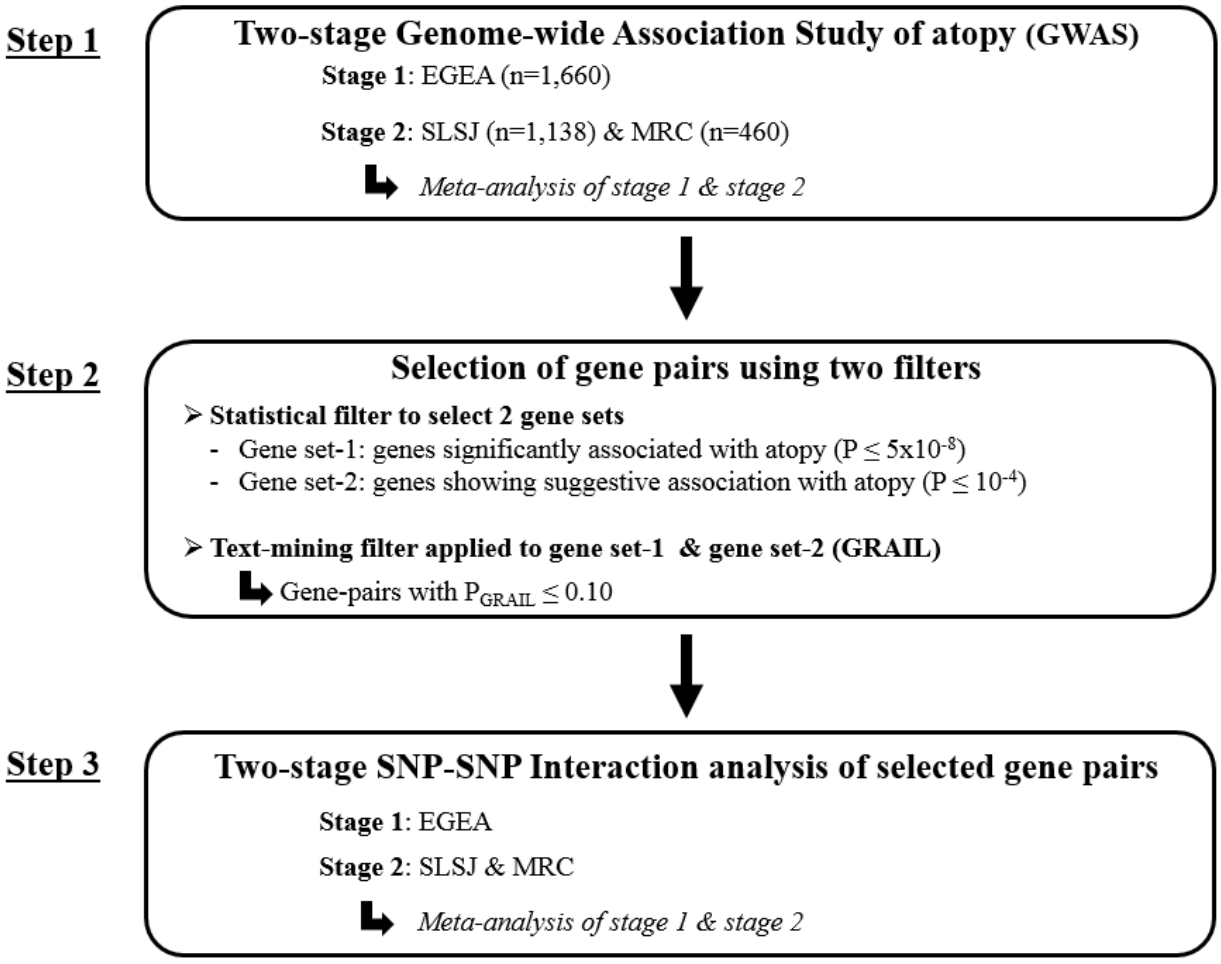
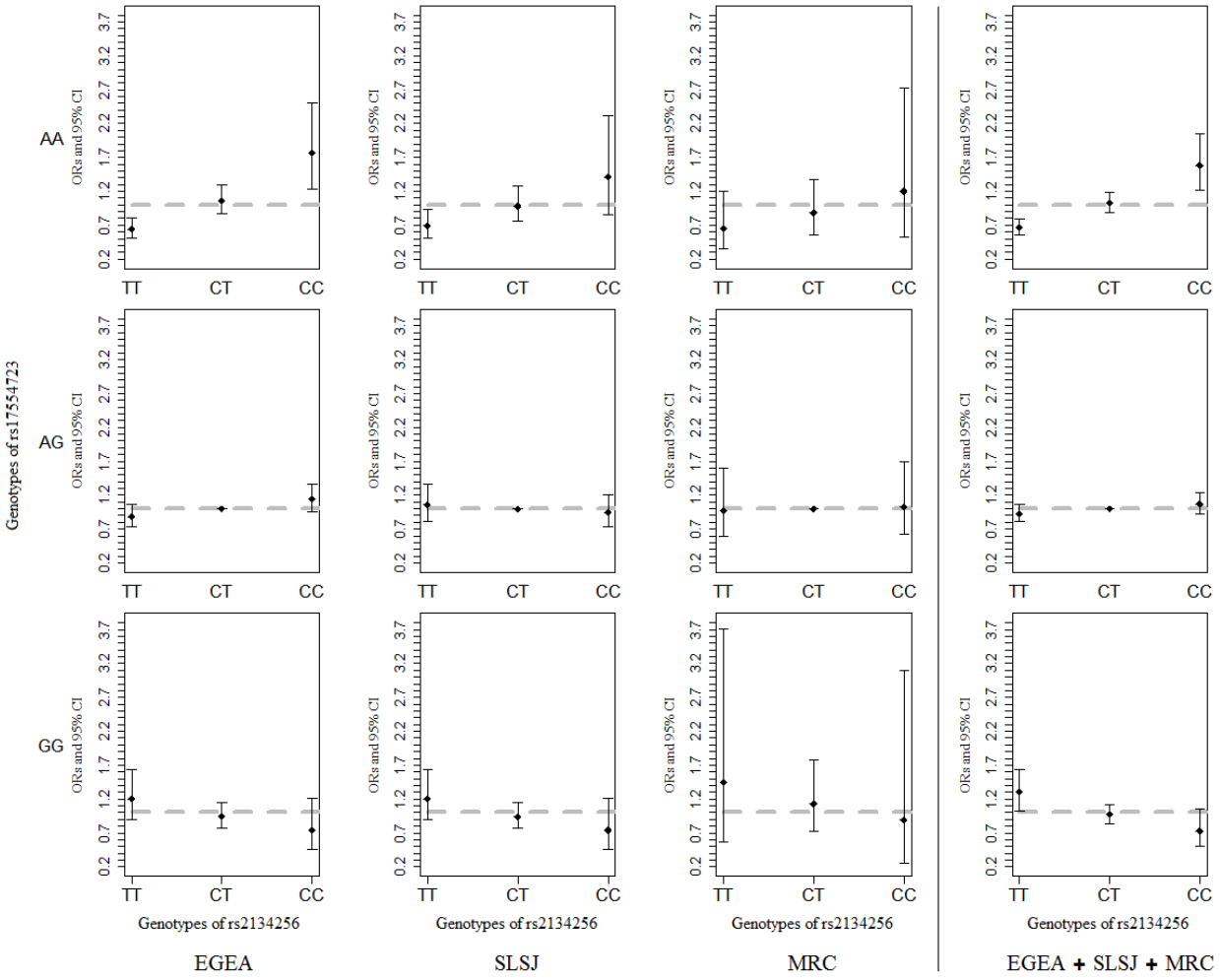

Step 1
Two-stage Genome-wide Association Study of atopy (GWAS)
Stage 1: EGEA (n=1,660)
Stage 2: SLSJ (n=1,138) & MRC (n=460)
Meta-analysis of stage 1 & stage 2
Step 2
Selection of gene pairs using two filters
Statistical filter to select 2 gene sets
- Gene set-1: genes significantly associated with atopy (P ≤ 5x10-8)
- Gene set-2: genes showing suggestive association with atopy (P ≤ 10-4)
Text-mining filter applied to gene set-1 & gene set-2 (GRAIL)
Gene-pairs with PGRAIL ≤ 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

Figure 2.


Genotypes of rs17554723
AA
AG
GG
ORs and 95% CI
0.2
0.7
1.2
1.7
2.2
2.7
3.2
3.7
TT
CT
CC
Genotypes of rs2134256
EGEA
SLSJ
MRC
EGEA + SLSJ + MRC