Interactive effect between ATPase-related genes and early-life tobacco smoke exposure on bronchial hyper-responsiveness detected in asthma-ascertained families

Marie-Hélène Dizier,^{1,2} Patricia Margaritte-Jeannin,^{1,2} Lucile Pain,³ Chloé Sarnowski,^{1,2} Myriam Brossard,^{1,2} Hamida Mohamdi,^{1,2} Nolwenn Lavielle,^{1,2} Marie-Claude C Babron,^{1,2} Jocelyne Just,⁴ Mark Lathrop,⁵ Catherine Laprise,³ Emmanuelle Bouzigon,^{1,2} Florence Demenais,^{1,2} Rachel Nadif^{6,7}

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For numbered affiliations see end of article.

Correspondence to

Marie-Hélène Dizier, UMR946, INSERM/Université Paris-Diderot, Paris F-75010, France; marie-helene.dizier@inserm.fr

CL and EB contributed equally. FD and RN contributed equally.

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ABSTRACT

Background A positional cloning study of bronchial hyper-responsiveness (BHR) at the 17p11 locus in the French Epidemiological study on the Genetics and Environment of Asthma (EGEA) families showed significant interaction between early-life environmental tobacco smoke (ETS) exposure and genetic variants located in *DNAH9*. This gene encodes the heavy chain subunit of axonemal dynein, which is involved with ATP in the motile cilia function.

Our goal was to identify genetic variants at other genes interacting with ETS in BHR by investigating all genes belonging to the 'ATP-binding' and 'ATPase activity' pathways which include *DNAH9*, are targets of cigarette smoke and play a crucial role in the airway inflammation.

Methods Family-based interaction tests between ETS-exposed and unexposed BHR siblings were conducted in 388 EGEA families. Twenty single-nucleotide polymorphisms (SNP) showing interaction signals ($p \leq 5 \cdot 10^{-3}$) were tested in the 253 Saguenay-Lac-Saint-Jean (SLSJ) families.

Results One of these SNPs was significantly replicated for interaction with ETS in SLSJ families ($p=0.003$). Another SNP reached the significance threshold after correction for multiple testing in the combined analysis of the two samples ($p=10^{-5}$). Results were confirmed using both a robust log-linear test and a gene-based interaction test.

Conclusion The SNPs showing interaction with ETS belong to the *ATP8A1* and *ABCA1* genes, which play a role in the maintenance of asymmetry and homeostasis of lung membrane lipids.

INTRODUCTION

Bronchial hyper-responsiveness (BHR) is both a feature and an important risk factor for asthma.¹ The susceptibility genes for asthma and BHR,² identified to date, account for a relatively small proportion of the genetic component of these phenotypes.³ As asthma and BHR are complex diseases which result from both genetic and environmental factors, the effect of genetic factors may be missed if they are tested individually, that is, by ignoring gene by environment (G×E) interactions. Furthermore,

Key messages

What is the key question?

- Could genes belonging to ATP-related pathways interact with exposure to early-life tobacco smoke exposure on bronchial hyper-responsiveness?

What is the bottom line?

- Gene × environment interaction analyses with ATP-related genes allowed to identify promising candidate genes, *ATP8A1* and *ABCA1*, interacting with early-life tobacco smoke exposure in bronchial hyper-responsiveness susceptibility.

Why read on?

- The present study highlights that gene × environment interaction analyses under a pathway-based strategy can greatly contribute to the identification of novel genes involved in complex disease as bronchial hyper-responsiveness.

taking into account the biological function shared by genes or pathways they are involved in may help discovering new genes.⁴

Maternal smoking during pregnancy and early-life environmental tobacco smoke (ETS) exposure are well-known risk factors for asthma and BHR.⁵ Gene×ETS interaction underlying susceptibility to asthma and asthma-related phenotypes have been evidenced by positional cloning studies which detected protocadherin 1 (*PCDH1*) and ADAM metalloproteinase domain 33 (*ADAM33*) genes,^{6–8} by association studies with candidate genes including β 2-adrenergic receptor (*ADRB2*),⁹ and by interaction analysis with genetic variants at the 17q12-21 locus discovered by the first asthma genome-wide association studies (GWAS).^{10 11} More recently, a meta-analysis of genome-wide interaction studies (GEWIS) for childhood asthma¹² suggested interaction between ETS

exposure and Parkin coregulated (*PACRG*)—a gene having a role in motile cilia function.

In the French Epidemiological study on the Genetics and Environment of Asthma (EGEA) families, we previously performed a positional cloning study in the 17p11 region that showed interactive effect between ETS and Dynein, Axonemal, Heavy Chain 9 (*DNAH9*)^{13,14} genetic variant on BHR. This gene encodes the heavy chain subunit of axonemal dynein, a component responsible for cilia mobility. Interestingly, a recent study of atopy, another asthma-related phenotype, showed significant interaction between *DNAH5*, a gene of the same family as *DNAH9*, and *ADGRV1* (adhesion G protein-coupled receptor V1), both genes being involved in ciliary function.¹⁵ Overall, these findings suggest that ciliary dysfunction may represent a novel mechanism underlying asthma-related phenotypes.

The heavy chain subunit of axonemal dynein contains all of the elements that are needed to convert the energy into movement through a process in which dynein, ATP binding and ATP hydrolysis are involved.^{16–18} Interestingly, in patients with asthma, ATP was shown to be accumulated in the airways and to trigger BHR, suggesting an important role played by ATP in the airway inflammation.¹⁹ Moreover, ATP-binding cassette (ABC) transporters were implicated in pulmonary lipid homeostasis and inflammation, indicating a crucial and protective role in lung.²⁰

Smoking and exposure to cigarette smoke have been shown to affect the number and function of human bronchial cilia,^{18,21} while a significant loss of Na, K-ATPase activity was observed in human lung cell lines exposed to cigarette smoke²² and in platelet membrane in cigarette smokers compared with controls.²³

In the present study, we hypothesised that genes belonging to ‘*ATPase activity*’ and ‘*ATP binding*’ pathways, the two ATP-related pathways that include *DNAH9*, may also interact with exposure to ETS in BHR. We tested for interaction all genes of these two pathways using the discovery sample of 388 French EGEA families, ascertained through asthmatic probands. We first applied Family-Based Association Test (FBAT)²⁴ homogeneity test between exposed and unexposed BHR siblings to detect interactions for single-nucleotide polymorphisms (SNP) located in the genes belonging to the two pathways. Replication of results was sought in an independent sample of 253 French-Canadian asthma-ascertained families. We also validated our results by using another SNP×E interaction test based on log-linear modelling case-parent triads,²⁵ and finally by gene-based interaction analysis.

MATERIALS AND METHODS

Discovery sample

The EGEA study and inclusion criteria have been described in detail previously.²⁶ The EGEA family sample consisted of 388 French nuclear families that included 253 families ascertained through offspring with asthma (one offspring proband in 90% of families and two offspring probands in the remainder) and 135 families ascertained through one parent with asthma.

The BHR phenotype was defined according to the results of the methacholine bronchial challenge test, as done previously.^{13,14} Participants who had a fall in their baseline FEV₁ ≥20% at ≤4 mg/mL of methacholine (PD20) had BHR while participants who did not show a fall in FEV₁ did not have BHR. The protocol of the methacholine challenge test has been described in detail elsewhere.²⁶

The ETS exposure in early childhood was defined, as previously^{10,13,14} through questionnaires: (1) for an adult, by a positive answer to the question: ‘*Did your mother or your father smoke*

during your early-childhood?’ and (2) for a child, by a positive answer to the question asked to the child’s mother (or father): ‘*Did you or the father (or the mother) of your child smoke when your child was less than 2 years old?*’ We did not use information on in utero exposure to tobacco smoke since all mothers who smoked during pregnancy continued to smoke during the early childhood of their offspring.

Replication study

The Saguenay-Lac-Saint-Jean (SLSJ) asthma study comprised 253 French-Canadian multigenerational families ascertained through two probands with asthma.²⁷ Inclusion criteria of probands have been described previously.²⁷

The BHR (PD20) phenotype and ETS exposure were defined in SLSJ in an identical manner as in the EGEA study.

Genotyping

The EGEA subjects were genotyped using Illumina 610 Quad array (Illumina, San Diego, CA) at the Centre National de Génotypage (CNG, Evry, France), as part of the European Gabriel consortium asthma GWAS.²⁸ Stringent quality control criteria, as detailed previously,²⁸ were used to select both individuals and genotyped SNPs for analysis. For this study, we selected from the Gene Ontology (GO) database (<http://amigo.geneontology.org/>) 296 genes belonging to both molecular functions ‘*ATPase binding*’ (GO: 0005524) and ‘*ATPase activity*’ (GO: 0016887) and the corresponding 4252 SNPs located within these genes (see online supplementary table S1).

The SLSJ sample was also genotyped at CNG using the Illumina 610 Quad array. The same quality control criteria for individuals and SNPs as those used for EGEA, were applied to this data set.

Statistical analysis

G×E interaction analysis using FBAT homogeneity test.

Analysis of the EGEA discovery sample

Analyses were conducted using the FBAT approach,²⁴ which tests for association in presence of linkage. We assumed an additive genetic model and used the option for an empirical estimator of the variance,²⁹ which makes the association test robust to the dependency between siblings and allows use of all siblings in a family. FBAT was applied separately to ETS-exposed and unexposed siblings. We searched for SNP×ETS interaction by testing homogeneity of FBAT association results between ETS exposed and unexposed.³⁰ Note that after exclusion of SNPs leading to insufficient sample size (<30) of informative families for FBAT analyses in the EGEA sample, only 4112 SNPs belonging to only 266 genes were analysed.

Replication analysis with the SLSJ sample

SNPs showing interaction signals ($p \leq 5 \times 10^{-3}$) in the EGEA sample were followed up in the SLSJ sample. The arbitrary threshold of $5 \cdot 10^{-3}$ was chosen to obtain both a strong indication of association and a reasonable number of SNPs (here 20, see the Results section) tested in the replication data set. For replication study of these SNPs detected in EGEA, both analysis in SLSJ and combined analysis in EGEA and SLSJ were conducted.

First, analyses were conducted in SLSJ and results were declared as significant if they meet the Bonferroni corrected significance p threshold applied to the Meff (effective number of independent tests after discarding dependence due to linkage disequilibrium (LD) between the SNPs)³¹ calculated from the 20

SNPs tested in SLSJ. The Meff was estimated to 15 and thus the significance p threshold equal to 3.10^{-3} .

Second, combined FBAT analysis of the EGEA and SLSJ samples was conducted separately in ETS-exposed and unexposed siblings using the Stouffer's Z-score method, and homogeneity test was applied between exposed and unexposed siblings to the combined results. The results of this combined analysis are not independent from the results obtained in EGEA. We thus declared results as significant if they meet the significance p threshold ($p=2.10^{-5}$), calculated after correction for multiple tests by the Bonferroni correction applied to the Meff (2500) estimated from the total number of SNPs tested in EGEA.

Validation analysis using Umbach and Weinberg method

In order to validate the significant interactions found with the FBAT homogeneity test, we applied the log-linear modelling approach for testing interaction in triads (case and parents), as proposed by Umbach and Weinberg (UW),²⁵ to the pooled sample of EGEA and SLSJ. This approach allows adjusting on the genotypic parental mating of each sibling and thus avoids bias due to population stratification. A different distribution of parental genotypic mating between exposed and unexposed siblings, due to population stratification, may lead to different transmission probabilities in the two groups and consequently to false detection of interaction. FBAT is less robust because it only adjusts for the genotypic parental distribution within the exposed and unexposed groups of siblings. However, the UW method is less powerful than FBAT because it can be applied to only one sib per family. The log-linear analysis was conducted using the youngest siblings for whom the information on ETS exposure was the closest in time with respect to BHR occurrence and thus the less influenced by a potential recall bias. The analysis was also performed by considering the oldest siblings, but similar results were obtained and are thus not presented.

As for FBAT analysis, all UW analyses assumed an additive genetic model and the same correction for multiple testing was applied, that is, Bonferroni correction applied to the Meff of 2500.

Gene-based analysis

For each of the 266 genes, interactions with ETS exposure in BHR were also investigated at the gene level by using the versatile gene-based test (Versatile Gene-based Association Study).³² The gene-based statistic was defined as the best SNP test statistic (or min p value) using the results of FBAT homogeneity tests in the combined analysis of EGEA and SLSJ samples. Empirical p value of the gene-based statistic was computed through Monte Carlo simulations using the LD pattern of HapMap CEU reference sample. The empirical p values were adjusted for multiple testing using the Bonferroni correction (significance p threshold = $0.05/266 = 2.10^{-4}$).

Expression quantitative trait loci analysis, functional annotation and chemical–gene/protein interactions

We investigated whether the SNPs (or their proxies, $r^2 \geq 0.8$) found to interact with ETS were cis-expression quantitative trait loci (cis-eQTLs). We used the GTEx browser (<http://www.gtexportal.org/home/>)³³ that includes e-QTL data from many tissues. Functional annotations of these SNPs (or proxies) were done using the HaploReg tool (<http://www.broadinstitute.org/mammals/haploreg/haploreg.php>). HaploReg annotates SNPs in terms of colocalisation with regulatory elements, such as promoter and enhancer marks, DNase I hypersensitivity sites,

Table 1 Phenotypic features of genotyped siblings having BHR in EGEA and SLSJ asthma-ascertained families

	EGEA	SLSJ
Siblings (n)	304	145
Age (years), mean (SD)	16.1 (8.1)	20.3 (10.3)
Sex, n (%) women	132 (43.4)	76 (52.4)
Asthma, n (%)	181 (59.5)	138 (95.2)
Age at asthma onset (years), mean (SD)	7.4 (7.7)	9.4 (9.3)
Allergic sensitisation*, n (%)	228 (76.0)	121 (85.8)
ETS exposure, n (%)	176 (57.9)	93 (64.1)

*Allergic sensitisation: a positive response of skin prick test to at least one allergen. BHR, bronchial hyper-responsiveness; EGEA, Epidemiological study on the Genetics and Environment of Asthma; ETS, environmental tobacco smoke; SLSJ, Saguenay-Lac-Saint-Jean.

and transcription factor (TF) and protein-binding sites, based on Roadmap Epigenomics data and Encyclopedia of DNA Elements data.

Furthermore, curated (chemical–gene interactions|chemical–disease|gene–disease) data were retrieved from the Comparative Toxicogenomics Database (CTD),³⁴ MDI Biological Laboratory, Salisbury Cove, Maine, and NC State University, Raleigh, North Carolina. World Wide Web (URL: <http://ctdbase.org/>) (May 2018). CTD is a robust, publicly available database that aims to advance understanding about how environmental exposures affect human health. It provides manually curated information about chemical–gene/protein interactions, chemical–disease and gene–disease relationships.

RESULTS

Data description

The characteristics of genotyped siblings having BHR in EGEA and SLSJ families are shown in table 1.

Three hundred and four siblings (from 189 families) had BHR in EGEA and 145 (from 120 families) in SLSJ. They were younger in EGEA than in SLSJ with respective mean ages equal to 16.1 and 20.3 years ($p < 10^{-4}$). The proportion of siblings with allergic sensitisation was similar in the two data sets (76% in EGEA and 86% in SLSJ), while the proportion of asthmatics was higher in SLSJ (95%) than in EGEA (60%) ($p < 10^{-4}$). The proportion of female was similar in the two data sets (43% in EGEA and 52% in SLSJ). The mean age at onset of asthma was slightly lower in EGEA (7.4) than in SLSJ (9.4) ($p = 0.05$). Finally, the proportion of exposed siblings was similar in EGEA and in SLSJ (58% vs 64% respectively).

Analysis of gene×ETS interactions using FBAT homogeneity test

The results in the EGEA, SLSJ and combined samples are shown in table 2 (for more details see online supplementary table S2).

FBAT homogeneity test in EGEA detected 20 SNPs (at the level of $p \leq 5.10^{-3}$), for which replication study was then conducted. The analysis in SLSJ detected a significant association ($p = 3.10^{-3}$) with one of these SNPs, rs2253304, which is located in *ABCA1* intron. The combined analysis of the two EGEA and SLSJ samples detected another SNP, rs17448506, which is located in *ATP8A1* intron, and reached the significance threshold for interaction with ETS ($p = 10^{-5}$). The SNP rs2253304 was also the second top signal in the combined analysis ($p = 6.10^{-5}$).

At both SNPs, there was a 'Flip-Flop' interaction effect (ie, an inverse effect depending on exposure): the C allele (vs T allele)

Table 2 Results of FBAT homogeneity test in the EGEA and SLSJ samples for SNPs detected in EGEA with $p \leq 5.10^{-3}$

Chromosome	SNP	Gene	Location	Position* (kb)	MAF†	EGEA	SLSJ	EGEA+SLSJ‡
						P values	P values	P values
1	rs10924249	KIF26B	flanking_3UTR	243837	0.18	0.004	0.302	0.059
2	rs6736802	KIF5C	flanking_5UTR	149566	0.43	0.002	0.320	0.056
2	rs6435220	KIF5C	flanking_5UTR	149569	0.31	0.003	0.659	0.032
4	rs1460354	ATP8A1	Intron	42266	0.22	0.001	0.115	6.0E-04
4	rs13124088	ATP8A1	Intron	42280	0.20	0.005	0.227	0.004
4	rs17448506	ATP8A1	Intron	42343	0.27	1.7E-05	0.133	1.2E-05
6	rs160666	WRNIP1	flanking_3UTR	2719	0.32	0.003	0.398	0.041
8	rs2279444	KIF13B	Intron	29053	0.15	0.005	0.400	0.006
9	rs2253304	ABCA1	Intron	106658	0.27	0.004	0.003	6.1E-05
9	rs2253182	ABCA1	Intron	106659	0.27	0.004	0.005	9.2E-05
9	rs2253175	ABCA1	Intron	106660	0.27	0.004	0.004	7.2E-05
9	rs2253174	ABCA1	Intron	106660	0.27	0.004	0.004	7.2E-05
9	rs2230805	ABCA1	Coding	106663	0.25	0.005	0.017	2.0E-04
11	rs762667	MYO7A	Coding	76546	0.38	0.002	0.446	0.003
16	rs2914819	ATP2C2	Intron	83026	0.19	0.003	0.353	0.0018
17	rs7225157	DNAH9§	Intron	11621	0.17	8.1E-04	0.038	6.8E-05
18	rs12458154	ATP9B	Intron	75187	0.28	0.002	0.551	0.0047
20	rs6067867	ATP9A	Intron	49698	0.46	0.005	0.376	0.0056
20	rs6067892	ATP9A	Intron	49731	0.47	0.002	0.410	0.0036
20	rs1475670	ATP9A	Intron	49777	0.50	0.004	0.058	7.0E-04

Significant results are in bold.

*SNP position in kilobase (dbSNP, build 37.1).

†Minor Allele Frequency estimated in EGEA.

‡Combined analysis of the EGEA and SLSJ samples using the Stouffer's Z-score method.

§Previously detected by our positional cloning.¹⁴

EGEA, Epidemiological study on the Genetics and Environment of Asthma; FBAT, Family-Based Association Test; SLSJ, Saguenay-Lac-Saint-Jean; SNP, single-nucleotide polymorphism.

of rs17448506 was positively associated with BHR in ETS-exposed siblings and negatively in ETS-unexposed siblings, while G allele (vs A allele) of rs2253304 was positively associated with BHR in ETS-exposed siblings and negatively in ETS-unexposed siblings.

Validation analysis using UW method

For the two SNPs retained by the analyses based on FBAT homogeneity test (rs17448506 and rs2253304), results of UW analysis in the pooled EGEA and SLSJ samples are presented in table 3.

Both SNPs showed quite strong signals of interaction ($p \leq 10^{-3}$) with the UW method, although they did not reach the significance threshold of 2×10^{-5} .

Gene-based analysis

Results by the gene-based test using the combined results of EGEA and SLSJ were given for all 266 genes in online supplementary table S3. *ATP8A1* and *ABCA1* were the first and third

top genes, respectively, interacting with ETS exposure in BHR that were detected ($p = 3.10^{-4}$ and $p = 3.10^{-3}$, respectively). Only the p value for *ATP8A1* was close to the significance threshold of 2.10^{-4} adjusted for multiple testing. The second top gene was *ATP9B* and the fourth top gene was *DNAH9*.

eQTL, functional annotations and chemical-gene/protein interactions

No eQTL was found among the SNPs (or proxies) interacting with ETS at *ATP8A1* and *ABCA1* loci. Using the functional annotation tool HaploReg-v4.1, we found that both SNPs map to enhancer and promoter histone marks and that rs2253304 and its proxies map to DNase hypersensitivity sites, notably in fetal lung fibroblast cell line, lung carcinoma cell line and lung fibroblast primary cells. They also map to binding sites of many TFs including the redox-sensitive nuclear factor (NF)-kappaB (NF-κB) for rs17448506 and the histone deacetylase 2, activator protein 1, Smad and STAT for rs2253304 and proxies.

Table 3 Results with UW in the pooled sample of EGEA and SLSJ for SNPs detected by FBAT homogeneity test

Chromosome	SNP	Gene	Location	Position* (kb)	MAF	P values
4	rs17448506	ATP8A1	Intron	42343	0.27	9.0E-04
9	rs2253304	ABCA1	Intron	106658	0.27	5.0E-04

*SNP position in kilobase (dbSNP, build 37.1).

EGEA, Epidemiological study on the Genetics and Environment of Asthma; FBAT, Family-Based Association Test; MAF, Minor Allele Frequency; SLSJ, Saguenay-Lac-Saint-Jean; SNP, single-nucleotide polymorphism; UW, Umbach and Weinberg.

Further, from the CTD, we found that tobacco smoke pollution and soot have been reported to modify the expression of *ATP8A1* and *ABCA1* mRNA (see online supplementary table S4). We also found that air pollutant exposures known to contain compounds with irritant properties such as in tobacco smoke modified the expression of *ABCA1* (see online supplementary table S4).

DISCUSSION

This study identified genetic variants at two novel loci, in chromosomes 4 and 9, interacting with ETS exposure in BHR. After a selection of SNPs showing signal of interaction with ETS in EGEA, interaction was significantly replicated in SLSJ for a first SNP intronic to *ABCA1* gene. Significant interaction with ETS was detected for a second SNP intronic to *ATP8A1* gene in the combined analysis of EGEA and SLSJ samples. Furthermore, in the gene-based analysis, *ATP8A1* and *ABCA1* were among the three top genes interacting with ETS exposure, this interaction being very close to the significance level for *ATP8A1*.

Most of previous interaction studies of genetic variants with ETS on BHR or asthma risk had difficulties to show significant interaction and/or replication in independent samples.^{6 12 35} Indeed, replication of G×E interaction is much more difficult to achieve than replication of single SNP association. It is well recognised that interaction tests have low power. Moreover, replication of interaction is affected by heterogeneity in the outcome and in exposure definition of the participating studies. The distribution of exposure may also differ across populations and therefore change the potential to identify the interaction. It is well known that ETS is influenced by socioeconomic position and generation effect. Tobacco use seems to be almost universally more prevalent in low socioeconomic groups than in high socioeconomic groups.³⁶ Over the last 10 years, the prevalence of ETS exposure at home among children and the percentage of children whose parents smoke have declined in several industrialised countries.³⁷ Difference in the distribution of ETS may thus result in different relationships among the gene, environment and disease. It was difficult to find replication samples that showed consistent definitions of BHR and ETS exposure and similar distribution of ETS exposure with those of the EGEA discovery sample. Only one replication sample from the SLSJ study showed similarities with EGEA in terms of ascertainment through asthmatic subjects, definition of both BHR and ETS, distribution of ETS and genotype information available in parents and siblings. To keep reasonable the number of tests and then limit the problem of low power of interaction test, we examined only SNPs located within gene boundaries. Even if SNPs lying at some distance upstream and downstream from the gene are often considered, the choice of that distance is not obvious.

Nevertheless, we were able to identify two SNPs, with significant evidence for interaction with ETS. These results were confirmed by the UW approach, which is more robust although less powerful than FBAT as described in the Methods section. Our study relies on an original strategy to select and enlarge a list of candidate genes. Supported by biological knowledge, we think our pathway selection approach allows a good trade-off between GEWIS and candidate gene approaches, and offers the possibility to identify new genes as we previously showed.⁴ We cannot exclude that our selection may lose a number of relevant genes that are not targeted by our analysis. In particular, we choose the stringent option to investigate genes, which similar to *DNAH9*, belong simultaneously to the two ATP-related

pathways. We thus used the intersection and not the union of these two pathways. The advantage of this strategy was to limit the number of SNPs to be tested, leading to a reduction of the multiple testing burden and to a gain of power. Furthermore, after the analysis in the discovery sample (EGEA), we used two different replication analyses of top EGEA results: first analysis in the SLSJ sample, and then a combined analysis in the two samples (EGEA and SLSJ). Although these two types of analyses are not independent (indeed combined analysis of EGEA and SLSJ included SLSJ results), they led to detect two different genes that appear both interesting. Note that the *DNAH9* gene evidenced by our previous positional cloning study¹⁴ was also detected in the present study, ranked as the third top SNP and as the fourth top gene, although results did not reach significance.

To our knowledge, none of our findings have been previously reported by published GWAS (GWAS-Catalogue of Published Genome-Wide Association Studies; <http://genome.gov/gwastudies>), or by GEWIS for asthma, BHR or lung function. Although a few G×ETS interaction studies^{16 18} have been published for BHR to date, neither the *ATP8A1* nor the *ABCA1* gene has been mentioned.

The *ATP8A1* gene codes for a putative aminophospholipid transporting enzyme which helps maintain phospholipid asymmetry in cell membranes.³⁸ Recently, in non-small cell lung cancer, *ATP8A1* was found to be a novel direct target of miR-140-3p,³⁹ a small non-coding RNA molecule known to regulate gene expression, and that may contribute substantially to airway epithelium abnormalities.⁴⁰ Moreover, exposure of rats to cigarette smoke causes extensive alterations in miRNA expression of the miR-140 family in the lung.⁴¹ The *ABCA1* gene encodes a transport protein known to participate to the maintenance of lung lipid homeostasis by interacting with the apolipoprotein A-I (apoA-I), and which expression and function are affected by smoking.⁴² The critical role of *ABCA1* in lung inflammation was evidenced from murine knockout models²⁰ and a recent review reported that the apoA-I/*ABCA1* pathway was involved in the modulation of the function of airway structural cells, and associated with neutrophilic airway inflammation and airway hyper-responsiveness.⁴³ Furthermore, rs17448506 located in *ATP8A1* and rs2253304 in *ABCA1* are both intronic variants, and map near regulatory elements, and TF-binding sites include that of NF-κB, an important participant in a broad spectrum of inflammatory networks that regulate cytokine activity in airway pathology.⁴⁴ Lastly, tobacco smoke pollution and soot have been reported to modify the expression of *ATP8A1* and *ABCA1* mRNA.

Interestingly, some of the previous genes detected to interact with ETS in BHR belong to or interact experimentally with genes belonging to the 'ATPase activity' or 'ATP binding' pathways. Among them, the *ADRB2* gene⁹ belongs to the 'ATPase activity' pathway. The *PCDH1* gene⁶ was shown to interact with *ABCA2* and *ATF7IP* genes, which both belong to 'ATP binding' and/or 'ATPase activity' pathways. Similarly the *PACRG* and the *EPB41L3* genes¹² were also shown to interact with numerous genes belonging to 'ATP binding' and/or 'ATPase activity' pathways. Overall, all these data suggest that *ATP8A1* and *ABCA1* may play a role in BHR in relationship with early tobacco smoke exposure.

In conclusion, the present study highlights that G×E interaction analyses under a pathway-based strategy allowed to identify promising candidate genes interacting with ETS exposure in BHR susceptibility. Further confirmation of the interaction of *ATP8A1* and *ABCA1* with ETS exposure as well as functional studies is needed to bring greater insight into the role of these genes in BHR. Our study suggests that the two pathways are promising to be further explored in the search of more effective

therapies for inflammatory lung diseases, especially for BHR which is a feature and an important risk factor for asthma.

Author affiliations

- ¹INSERM, UMR-946, Genetic Variation and Human Diseases Unit, Paris, France
- ²Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Université Paris Diderot, Paris, France
- ³Département des Sciences Fondamentales, Université du Québec, Chicoutimi, Québec, Canada
- ⁴Service d'Allergologie Pédiatrique, Centre de l'Asthme et des Allergies, Hôpital d'Enfants Armand-Trousseau (APHP), UPMC Paris 06, Paris, France
- ⁵Department of Human Genetics, McGill University and Genome Quebec's Innovation Centre, Montréal, Québec, Canada
- ⁶Aging and Chronic Diseases—Epidemiological and Public Health Approaches (VIMA), Inserm, U1168, Villejuif, France
- ⁷UMR-S 1168, Université de Versailles Saint-Quentin-en-Yvelines, Paris, France

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