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Interaction between the *DNAH9* gene and early smoke exposure in bronchial hyperresponsiveness

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ABSTRACT   A previous genome-wide linkage scan of bronchial hyperresponsiveness (BHR) in the French Epidemiological study on the Genetics and Environment of Asthma (EGEA) families, performed in the presence of a gene×early-life environmental tobacco smoke (ETS) exposure interaction, showed the strongest interaction in the 17p11 region where linkage was detected only among unexposed siblings. Our goal was to conduct fine-scale mapping of 17p11 to identify single nucleotide polymorphisms (SNPs) interacting with ETS that influence BHR.

Analyses were performed in 388 French EGEA asthmatic families, using a two-step strategy: 1) selection of SNPs displaying family-based association test (FBAT) association signals ($p \leqslant 0.01$) with BHR in unexposed siblings, and 2) a FBAT homogeneity test between exposed and unexposed siblings plus a robust log-linear interaction test.

A single SNP reached the threshold ($p \leqslant 3 \times 10^{-3}$) for significant interaction with ETS using both interaction tests, after accounting for multiple testing. Results were replicated in 253 French-Canadian families, but not in 341 UK families, probably due in part to differences in phenotypic features between datasets.

The SNP showing significant interaction with ETS belongs to *DNAH9* (dynein, axonemal, heavy chain 9), a promising candidate gene involved in respiratory cilia mobility and associated with primary ciliary dyskinesia, a disease associated with abnormalities of pulmonary function.

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Introduction

Asthma is a common respiratory disease characterised by airway inflammation, reversible airflow obstruction and bronchial hyperresponsiveness (BHR). BHR is a feature of asthma and it also constitutes an important risk factor for asthma [1]. BHR is due to increased sensitivity of the airways to physical and chemical stimulants, such as cold air and cigarette smoke, and to pharmacological agents, such as methacholine and histamine. Both asthma and BHR result from multiple genetic and environmental factors that may interact. Many genetic studies based on positional cloning approaches and genome-wide association studies (GWAS) have been conducted for asthma and BHR [2]. These studies, which have largely focused on association between disease and individual genetic polymorphisms, have been successful in identifying susceptibility genes [2]. However, all these genes account for only a small proportion of heritability [3]. To further our understanding of the genetic basis of complex diseases, current studies are now extending to more complex analyses, taking into account phenotypic heterogeneity and gene×gene or gene×environment interactions. For asthma and BHR, for which the involvement of environment factors has been shown to be substantial, it appears of major interest to include gene×environment interactions in genetic studies. Among the environmental factors associated with asthma and BHR, maternal smoking during pregnancy and early-life environmental tobacco smoke (ETS) exposure are important risk factors [4]. Gene×ETS interactions underlying susceptibility to asthma and asthma-related phenotypes were first evidenced in the context of genome-wide linkage scans [5–7] and by association studies with candidate genes, including *ADRB2* (β_2-adrenergic receptor) [8], *GSTM1* (glutathione *S*-transferase M1) [9], *GSTT1* (glutathione *S*-transferase T1) [9] and *GSTP1* (glutathione *S*-transferase P1) [10]. Genetic variants at the 17q21 locus that were detected by a GWAS of childhood asthma [11] were shown to confer an increased risk of early-onset asthma in subjects exposed to early-life ETS first in the French Epidemiological study on the Genetics and Environment of Asthma (EGEA) study [12] and, subsequently, in other studies [13–15]. *PCDH1* (protocadherin 1) and *ADAM33* (ADAM metallopeptidase domain 33) genes that were identified as susceptibility genes for BHR through positional cloning [16, 17] were also found to interact with ETS exposure *in utero* and/or in early life [16, 18, 19]. More recently, a meta-analysis of genome-wide interaction studies (GWIS) of childhood asthma suggested the potential interaction of a number of genes with ETS exposure [20].

A previous genome-wide linkage scan of BHR in EGEA families, performed in the presence of gene×ETS exposure interaction, showed the strongest interaction in the 17p11 region [7], where linkage was detected only among unexposed siblings. Our goal was to conduct fine-scale mapping of this region to identify genetic variants interacting with ETS exposure in BHR susceptibility. A two-step strategy using the family-based association test (FBAT) [21] was conducted in the discovery sample of 388 French EGEA families ascertained through asthmatic probands. We validated our results by another interaction test based on a log-linear model in case–parent triads [22], and by replication analysis in two other asthma-ascertained family samples that consisted of 253 French-Canadian families and 341 UK families.

Materials and methods

Discovery sample

The EGEA study and the inclusion criteria have been described in detail previously [23]. 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.

As in the previous linkage analysis [7], the BHR phenotype was defined according to the results of the methacholine bronchial challenge test. Participants with a provocative dose of methacholine causing a ⩾20% fall in their baseline forced expiratory volume in 1 s (FEV1) (PD20) of ⩽4 mg·mL^{-1} had BHR, while participants that did not show a fall in FEV$_1$ did not have BHR. This binary BHR phenotype (yes/no for BHR) will be subsequently designated as BHR. The protocol of the methacholine challenge test has been described in detail elsewhere [23].

The definition of ETS exposure in early childhood in the current study was the same as that used for the previous linkage analysis [7], and in the identification of interaction of ETS with 17q21 variants in EGEA

families [12] and subsequent replication studies [13–15]. ETS exposure was defined as follows: 1) for an adult, by a positive answer to the question: "Did your mother or your father smoke during your early childhood?"; 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 studies

The Saguenay–Lac-Saint-Jean (SLSJ) asthma study comprised 253 French-Canadian multigenerational families ascertained through two probands with asthma [24]. Inclusion criteria of probands have been described previously [24].

The UK Southampton asthma family cohort (SOTON) has been described in detail previously [25]. A total of 341 Caucasian families were recruited from the Southampton area with at least two siblings (age 5–21 years) with a current physician diagnosis of asthma and taking asthma medication on a regular basis [25]. The BHR (PD20) phenotype and ETS exposure were defined in the SLSJ and SOTON studies in an identical manner as in the EGEA study.

Genotyping

The EGEA subjects were genotyped using an Illumina 610 Quad array (Illumina, San Diego, CA, USA) at the Centre National de Génotypage (CNG, Evry, France), as part of the European GABRIEL asthma consortium [26]. Stringent quality criteria, as detailed previously [26], were used to select both individuals and SNPs for analysis. For this study, we used 1732 SNPs belonging to the 17p11 region within an interval of 5 Mb on each side of the linkage peak, located at 16 Mb from pter. The SLSJ sample was also genotyped at CNG using an Illumina 610 Quad array. The same quality control criteria for individuals and markers as used for EGEA applied to this dataset. The SOTON family sample was genotyped using custom (not genome-wide) genotyping which was performed by LGC Genomics (Hoddesdon, Hertfordshire, UK; www. lgcgenomics.com), using a proprietary competitive allele-specific PCR genotyping system (KASPar).

Statistical analysis
Analysis of the EGEA discovery sample
Two-step analysis using FBAT

As the 17p11 region was detected through linkage analysis, analyses were conducted using the FBAT approach [21], which tests for association in presence of linkage. We assumed an additive genetic model and used the "-e" option [27] for an empirical estimator of the variance, which makes the association test robust to the dependency between siblings and allows use of all siblings in a family.

To limit the problem of multiple testing, we applied a two-step strategy consisting in a first step of SNP selection followed by a second step of interaction tests. Such a strategy is currently used for interaction studies based on a large panel of markers to reduce the loss of power due to the correction for multiple testing [28, 29]. As linkage to 17p11 had been previously detected in unexposed siblings only [7], in the first step we selected SNPs indicating FBAT association signals with BHR in the same group of unexposed siblings using a nonstringent threshold (as usually recommended in two-step strategies) of 1%. In the second step, we searched for SNP×ETS interactions by testing the homogeneity of the SNP–BHR association between exposed and unexposed siblings using FBAT [30]. This test was applied to SNPs selected at the first step. As presence of association signals in unexposed siblings does not rely on the presence of a SNP×ETS interaction (*i.e.* different effect of SNPs between exposed and unexposed siblings), these two steps are independent. Consequently, correction for multiple testing at the second step used the Bonferroni correction for the number of SNPs selected at the first step.

Validation analysis using the Umbach–Weinberg method

In order to validate 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 [22]. 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 matings between exposed *versus* 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 thus less robust than the Umbach–Weinberg method as FBAT only adjusts for the genotypic parental distribution within the exposed and unexposed groups of siblings. However, the Umbach–Weinberg method is less powerful than FBAT since it can be applied to only one sibling per family. The log-linear analysis was first 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 least influenced by potential recall bias.

The analysis was also performed by considering the oldest siblings, but similar results were obtained and thus are not presented. Note that in EGEA, more than two-thirds of families have only one sibling having BHR, making the choice of siblings irrelevant, and explaining at least in part the similarity of results with the youngest and oldest siblings. Moreover, this indicates that the overall familial dependency was not large.

Finally, to avoid any bias caused by the selection of one sibling per family, we applied a Umbach–Weinberg-like method to all siblings while taking into account the dependency between siblings. For that purpose, we used the log-linear (Poisson regression) model with the robust estimator of variance [31], as implemented in Stata version 12 (Stata Corporation, College Station, TX, USA). Note that such analysis was performed in EGEA only because of problems encountered in estimating robust standard errors in the other datasets.

As for FBAT analysis, all these analyses assumed an additive genetic model and the same correction for multiple testing was applied, *i.e.* Bonferroni correction according to the number of SNPs selected at the first step.

Replication analysis in SLSJ and SOTON families
SNPs showing significant interaction with ETS from both FBAT homogeneity and Umbach–Weinberg tests in the discovery sample were analysed in the replication samples using the same two interaction tests. To declare an interaction to be validated in replication samples, we again applied a Bonferroni correction to the number of SNPs tested in these samples.

Results
Data description
The characteristics of genotyped siblings having BHR in EGEA, SLSJ and SOTON families are shown in table 1.

A total of 304 siblings (from 189 families) had BHR in EGEA, 145 (from 120 families) in SLSJ and 382 (from 258 families) in SOTON. Among these siblings, the proportion of atopic subjects was similar in the three datasets (ranging between 76% and 86%), while the proportion of asthmatics was higher in SLSJ and SOTON (95%) than in EGEA (60%). The proportions of females in siblings with BHR were similar in the three datasets, ranging between 43% and 52%. In contrast, these siblings were younger in SOTON with a mean age of 10.6 years (95% CI 8.0–13.8) than those in EGEA and SLSJ with respective mean ages of 16.1 years (95% CI 15.2–17.0) and 20.3 years (95% CI 18.6–22.0). Similarly, the mean of age at onset of asthma was lower in SOTON (4.4 years, 95% CI 4.0–4.7) than in EGEA (7.4 years, 95% CI 6.3–8.6) and SLSJ (9.4 years, 95% CI 7.7–11.0). Finally, only 29.8% of siblings with BHR were exposed to ETS in SOTON, while that proportion was 58% in EGEA and 64% in SLSJ.

Analysis of gene×ETS interactions
The results of the EGEA discovery sample are shown in table 2.

FBAT analysis of the 17p11 region, conducted in EGEA unexposed siblings, showed association of 16 SNPs (after removing SNPs in complete linkage disequilibrium) with BHR ($p \leqslant 0.01$). When using the FBAT homogeneity test between exposed *versus* unexposed siblings, one of these SNPs showed a significant interaction after accounting for multiple testing ($p=0.05/16=3\times10^{-3}$), *i.e.* $p=8\times10^{-4}$ for rs7225157, and one

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TABLE 1 Phenotypic features of genotyped siblings having bronchial hyperresponsiveness in the Epidemiological study on the Genetics and Environment of Asthma (EGEA), Saguenay–Lac-Saint-Jean (SLSJ) asthma study and Southampton asthma family cohort (SOTON) asthma-ascertained families

	EGEA	SLSJ	SOTON
Subjects	304	145	382
Age years	16.1 (15.2–17.0)	20.3 (18.6–22.0)	10.6 (8.0–13.8)
Women	43.4 (132)	52.4 (76)	44.5 (170)
Asthma	59.5 (181)	95.2 (138)	94.8 (362)
Age at asthma onset years	7.4 (6.3–8.6)	9.4 (7.7–11.0)	4.4 (4.0–4.7)
Atopy	76.0 (228)	85.8 (121)	77.5 (296)
ETS exposure	57.9 (176)	64.1 (93)	29.8 (114)

Data are presented as n, mean (95% CI) or % (n). ETS: environmental tobacco smoke.

TABLE 2 Results of the two-step strategy investigating gene×environmental tobacco smoke (ETS) exposure in the 17p11 region in the Epidemiological study on the Genetics and Environment of Asthma (EGEA) discovery sample

SNP[#]	Position bp[¶]	Gene	Location	MAF	ETS unexposed (1)			ETS exposed (2)			FBAT homogeneity test between (1) and (2)		Umbach–Weinberg interaction test[f]		
					n[+]	Z	p	n[+]	Z	p	$\chi^{2\S}$	p	n[##]	Z	p
rs7225157	11680785	DNAH9	Intron	0.17	39	−2.66	0.008	52	2.09	0.037	11.22	0.0008	188	3.08	0.0016
rs1519255	12583672	MYOCD	Intron	0.13	28	3.12	0.002	35	0.91	0.362	8.31	0.0040	189	−2.07	0.0356
rs1233855	12613063	MYOCD	Intron	0.25	37	2.95	0.003	60	0.62	0.537	6.84	0.0089			
rs9905908	12655081	MYOCD	Intron	0.40	45	3.03	0.002	61	−0.32	0.748	6.30	0.0120			
rs2323052	13780923	COX10	Flanking 5′ UTR	0.07	29	−2.72	0.007	23	0.25	0.803	2.79	0.0947			
rs6502310	13784815	COX10	Flanking 5′ UTR	0.09	34	−2.68	0.007	32	0.52	0.600	4.01	0.0452			
rs4792411	13797101	COX10	Flanking 5′ UTR	0.10	28	−2.89	0.004	34	1.19	0.236	7.21	0.0072			
rs9891555	14239843	HS3ST3B1	Intron	0.32	46	3.06	0.002	69	−2.11	0.035	1.09	0.2954			
rs3785655	14249167	HS3ST3B1	3′ UTR	0.39	50	−2.96	0.003	64	−0.75	0.455	2.44	0.1185			
rs11652573	14542140	FLJ45831	Flanking 3′ UTR	0.08	22	2.76	0.006	29	−1.07	0.285	7.66	0.0057			
rs11649913	14547787	FLJ45831	Flanking 3′ UTR	0.26	40	3.16	0.002	58	1.61	0.108	1.57	0.2102			
rs6502405	14834158	FLJ45831	Flanking 5′ UTR	0.39	44	2.57	0.010	66	−0.33	0.742	4.50	0.0339			
rs12942402	15371906	FLJ36674	Flanking 5′ UTR	0.26	48	2.69	0.007	60	−0.75	0.451	5.48	0.0192			
rs1971043	20043515	SPECC1	Flanking 5′ UTR	0.16	33	−2.97	0.003	51	−2.31	0.021	0.04	0.8358			
rs7209889	20217122	SPECC1	Flanking 3′ UTR	0.15	32	−2.75	0.006	49	−1.72	0.086	−0.15				
rs4473257	20823268	MGC87631	Flanking 5′ UTR	0.26	44	2.60	0.009	58	0.43	0.665	2.58	0.1085			

SNP: single nucleotide polymorphism; MAF: minor allele frequency; FBAT: family-based association test; UTR: untranslated region. [#]: all SNPs showing p⩽0.01 with FBAT in unexposed siblings; [¶]: dbSNP (build 37.1); [+]: number of informative families for FBAT; [§]: Chi-squared test (1 degree of freedom); [f]: applied only to SNPs reaching or being close to the significance level of interaction (p⩽3×10^{-3}) with the FBAT homogeneity test; [##]: number of siblings (only one per family using the Umbach–Weinberg method).

approached significance, i.e. p=4×10^{-3} for rs1519255 (figure 1). The interaction with rs7225157 was also found significant with the Umbach–Weinberg method (p=1.6×10^{-3}) and was thus validated, while this was not the case for rs1519255 (p=0.04). Note that when using the Umbach–Weinberg-like method applied to all siblings in EGEA, the interaction with rs7225157 reached p=10^{-5}, thus largely exceeding the significance threshold and strengthening our finding.

The minor allele of rs7225157 was negatively associated with BHR in ETS-unexposed siblings and positively in ETS-exposed siblings.

Thus, only rs7225157 was taken forward in the replication samples (table 3).

In SLSJ, interaction with ETS exposure was significantly detected with the same direction effect as in EGEA using both the FBAT homogeneity test (p=0.04) and Umbach–Weinberg method (p=0.03).

However, there was no replication of interaction of ETS with rs7225157 in SOTON families, using either the FBAT homogeneity test (p=0.2) or Umbach–Weinberg method (p=0.43).

Discussion

This study identified a genetic variant in the 17p11 region involved in BHR and interacting with ETS exposure. Significant interaction with ETS was detected in the linkage region previously reported in the French EGEA families [7]. Evidence for this interaction rests on the significant results obtained in the large EGEA discovery family sample using FBAT and validated by the log-linear modelling approach, which is robust to population stratification. Replication of these results was further obtained in
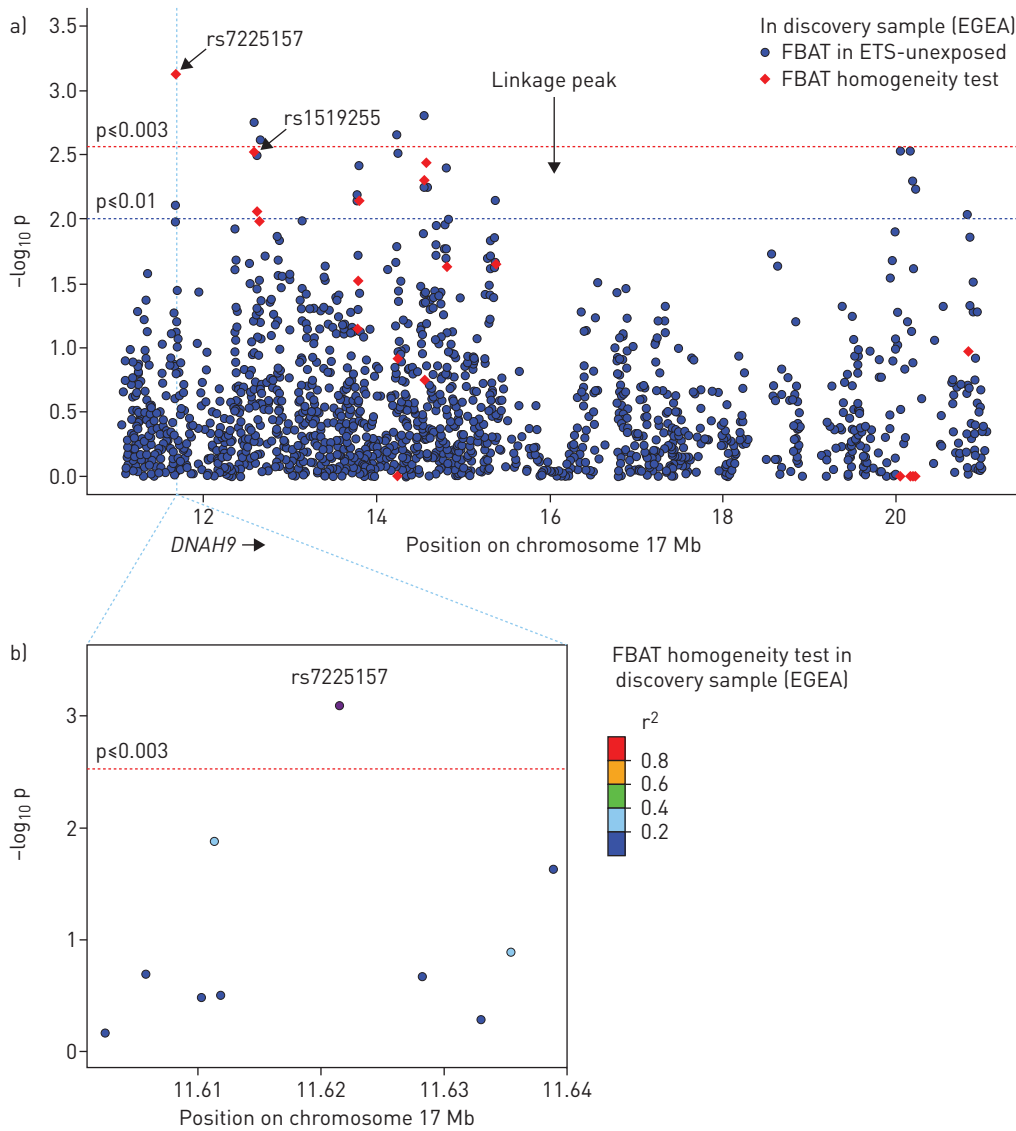
FIGURE 1 a) Plots using the 1732 single nucleotide polymorphisms (SNPs) in the 17p11 linkage region (11–21 Mb from pter) of family-based association test (FBAT) results (expressed as $-\log_{10} p$) in French Epidemiological study on the Genetics and Environment of Asthma (EGEA) early-life environmental tobacco smoke (ETS)-unexposed siblings and results of the FBAT homogeneity test (expressed as $-\log_{10} p$) applied to EGEA siblings for SNPs having FBAT $-\log_{10} p \geqslant 2$ in unexposed siblings. b) Visualisation of the results in the interval of associated SNPs (spanning 20 kb on each side of rs7225157) using LocusZoom software (http://locuszoom.sph. umich.edu/locuszoom/). The y-axis shows the $-\log_{10} p$ values of FBAT homogeneity tests in the EGEA discovery sample. The position of the SNPs in megabases (dbSNP, build 37.1) is shown on the x-axis. The SNP rs7225157 is coloured purple and the degree of linkage disequilibrium (from the 1000 Genomes reference panel; www.1000genomes.org) between that SNP and the other SNPs is indicated by colour according to the key (red being the highest degree of linkage disequilibrium).

French-Canadian families using both methods. Our results were thus strengthened both by validation at the statistical methodology level and by replication at the population level.

No replication of 17p11 SNP×ETS exposure interaction was observed in the SOTON families. This lack of replication may reflect heterogeneity between datasets in terms of genetic background, ETS exposure, phenotypic characteristics and/or ascertainment mode. Indeed, the SOTON siblings differ from EGEA and SLSJ siblings with respect to both frequency of ETS exposure and phenotypic features. The proportion of ETS-exposed siblings is smaller in SOTON than in EGEA and SLSJ siblings (one-third *versus* two-thirds). Moreover, the frequency of asthma in SOTON siblings with BHR (95%) is much larger than in EGEA siblings (60%). Furthermore, SOTON siblings are younger and have a younger mean age at asthma onset than EGEA and SLSJ siblings, as described in the Results section. Asthma with a younger age at onset may indeed depend on a stronger gene effect and a smaller ETS effect, making gene×ETS interaction more

TABLE 3 Analysis of gene×environmental tobacco smoke (ETS) interaction in the discovery (Epidemiological study on the Genetics and Environment of Asthma (EGEA)) and replication (Saguenay–Lac-Saint-Jean (SLSJ) asthma study and Southampton asthma family cohort (SOTON)) samples for rs7225157[#]

SNP	Position bp	Minor/ major allele	MAF	FBAT homogeneity test								
				EGEA			SLSJ			SOTON		
				$n^{\P}$	χ^{2+}	p	$n^{\P}$	χ^2	p	$n^{\P}$	χ^{2+}	p
rs7225157	11680785	G/A	0.172	89	11.22	0.0008	56	4.32	0.038	97	1.60	0.2

	Umbach–Weinberg test								
	EGEA			SLSJ			SOTON		
	n^f	Z	p	n^f	Z	p	n^f	Z	p
	188	3.08	0.0016	120	2.06	0.032	240	−0.78	0.43

SNP: single nucleotide polymorphism; MAF: minor allele frequency; FBAT: family-based association test. [#]: the SNP showing a significant interaction with ETS using both FBAT homogeneity and Umbach–Weinberg tests in the EGEA discovery sample; [¶]: number of informative families; [+]: Chi-squared test (1 degree of freedom); [f]: number of siblings (only one per family using the Umbach–Weinberg method).

difficult to detect. Genetic heterogeneity according to age is well documented. For instance, the effect of 17q21 variants was found to differ according to age of onset of asthma [12, 26], and variants associated with exhaled fraction nitric oxide fraction (FeNO) levels, an asthma-related phenotype, also differ in children and adults [32].

Another difficulty in replicating results across studies is that the SNP showing the most significant result (here interaction of rs7225157 with ETS) may not be the causal SNP, but a SNP in linkage disequilibrium with it. However, when we repeated our analysis with HapMap2-imputed SNPs in EGEA, a few interaction signals were observed which supported our initial result, but were less significant than this result. Thus, there was no indication that an imputed SNP might be the causal variant or in strong linkage disequilibrium with it and might have a better chance to be replicated in other samples. In addition, the linkage disequilibrium patterns between SNPs are not expected to differ between French and UK populations.

It is, however, noteworthy that interpretation of the imputation results should be conditioned by the possibility that some proportion of genetic variation has been missed.

Most previous interaction studies of genetic variants with ETS on asthma risk had difficulties in showing a significant interaction and/or replication in independent samples [14, 16, 20]. Indeed, replication of gene×ETS interaction is much more difficult to achieve than replication of single SNP association. The interaction tests are known to have low power and, moreover, as outlined earlier, there are many sources of heterogeneity between studies that are more crucial for detection of gene×ETS interaction than single SNP association. It should also be noticed that we applied here a particularly stringent strategy, which retained only SNPs showing significant interaction by two robust interaction tests. This strategy avoids false detection of interaction due to population stratification, but has consequently low power.

We verified that no association between the 17p11 locus and BHR, asthma or other lung function-related phenotypes has been reported by any of the published GWAS (http://www.genome.gov/gwastudies). Although a few gene×ETS interaction studies [16, 18] have been published for BHR to date, the 17p11 locus has never been mentioned. The SNP showing the significant interaction, *i.e.* rs7225157, is located in an intronic region of the *DNAH9* gene (dynein, axonemal, heavy chain 9). The minor allele of the SNP is protective in ETS-unexposed siblings, but increases risk in exposed siblings. Examination of functional annotations using the HaploReg tool (http://compbio.mit.edu/HaploReg) and the UCSC Genome Browser (http://genome.ucsc.edu) shows that the rs7225157 SNP and its proxies map to binding sites of various transcription factors. These transcription factors include *FAC1* (fetal Alz-50-reactive clone 1), which was found to be associated with lung cancer and lung adenocarcinoma, *CTCF* (CCCTC-binding factor (zinc finger protein)), which plays a critical role in epigenetic regulation, *NR4A1* (nuclear receptor subfamily 4 group A member 1), which was found associated with lung cancer in ETS-exposed subjects [33], and *GATA2* (GATA binding protein 2), which was reported to be associated with an immunodeficiency disease (dendritic cell, monocyte lymphocyte, B and natural killer lymphoid deficiency) [34].

DNAH9 encodes the heavy chain subunit of axonemal dynein, a component of intra-flagellar transport responsible for cilia mobility. Interestingly, a loss of airway cilia mobility was associated with an increase in airway reactivity in mice [35]. In the airways of individuals who have asthma, some areas of the epithelial basement membrane seem to be denuded of ciliated cells [36]. *DNAH9* is the most abundant isoform of the axonemal dynein heavy chain genes in ciliated cells of airways of the human body [37] and its expression has been recently reported to be downregulated in bronchial biopsies of patients with primary ciliary dyskinesia (PCD) [38], a disease associated with bronchiectasis and abnormalities of pulmonary function. BHR may play a part in the pathogenesis of bronchiectasis, the major pulmonary complication of PCD, by reducing the efficiency of respiratory clearance mechanisms, thereby promoting microbial colonisation and inflammation [39]. Finally, acute and chronic exposures to cigarette smoke were associated with an increase in dynein protein concentration in bronchoalveolar lavage, altered cilia motility in bovine and mouse lungs [40, 41], and epithelial epigenetic changes in the small airway epithelium [42].

All these observations showing the involvement of *DNAH9* and ETS exposure in respiratory cilia mobility, which is linked to BHR, support our finding of a potential interactive effect of *DNAH9* with ETS exposure on BHR. Interestingly, the recent GWIS for childhood asthma and ETS exposure [20] indicated interaction of ETS exposure with *PACRG* (Parkin coregulated gene), which, similar to *DNAH9*, has an important role in motile cilia function. Additionally, the pathway of cilia-related genes has been shown recently to be highly relevant to a biomarker of asthma, *i.e. F*NO, by a bronchial epithelial cell gene expression study [43]. An immediate practical utility at the clinical level cannot be inferred from the results obtained in the framework of this genetic epidemiological study. However, taken together with the recent literature, our results add to the interest of studying cilia-related genes [44]. This emerging topic has already led to the identification of new genes correlated to clinical subphenotypes of asthma, suggesting that changes in ciliary function may be particularly relevant for the development of asthma in children.

In conclusion, the present study identified a promising candidate gene interacting with ETS exposure in BHR susceptibility. It highlights that gene×environment interaction analyses, beyond single SNP association analysis, can greatly contribute to gene identification. Further confirmation of the interaction of *DNAH9* with ETS exposure as well as functional studies are needed to bring better insight into the role of this gene in BHR.

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