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Original Article



# TNS1 and NRXN1 Genes Interacting With Early-Life Smoking Exposure in Asthma-Plus-Eczema Susceptibility

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## ABSTRACT

**Purpose:** Numerous genes have been associated with allergic diseases (asthma, allergic rhinitis, and eczema), but they explain only part of their heritability. This is partly because most previous studies ignored complex mechanisms such as gene-environment (G-E) interactions and complex phenotypes such as co-morbidity. However, it was recently evidenced that the co-morbidity of asthma-plus-eczema appears as a sub-entity depending on specific genetic factors. Besides, evidence also suggest that gene-by-early life environmental tobacco smoke (ETS) exposure interactions play a role in asthma, but were never investigated for asthma-plus-eczema. To identify genetic variants interacting with ETS exposure that influence asthma-plus-eczema susceptibility.
**Methods:** To conduct a genome-wide interaction study (GWIS) of asthma-plus-eczema according to ETS exposure, we applied a 2-stage strategy with a first selection of single nucleotide polymorphisms (SNPs) from genome-wide association meta-analysis to be tested at a second stage by interaction meta-analysis. All meta-analyses were conducted across 4 studies including a total of 5,516 European-ancestry individuals, of whom 1,164 had both asthma and eczema.
**Results:** Two SNPs showed significant interactions with ETS exposure. They were located in 2 genes, *NRXN1* (2p16) and *TNS1* (2q35), never reported associated and/or interacting with ETS exposure for asthma, eczema or more generally for allergic diseases. *TNS1* is a promising candidate gene because of its link to lung and skin diseases with possible interactive effect with tobacco smoke exposure.
**Conclusions:** This first GWIS of asthma-plus-eczema with ETS exposure underlines the importance of studying sub-phenotypes such as co-morbidities as well as G-E interactions to detect new susceptibility genes.

**Keywords:** Genome; genome-wide association study (GWIS); polymorphism, single nucleotide; gene-environment interaction; tobacco smoke; eczema; asthma

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## INTRODUCTION

The 3 most common allergic diseases: asthma, atopic dermatitis (or eczema), and allergic rhinitis (AR) may share genetic determinants as suggested by their strong associations at both the individual and family levels.[1] Most genetic studies have focused specifically on one of the allergic diseases and numerous susceptibility genes for asthma, AR and eczema have been found.[2] These three diseases are also multifactorial diseases depending on multiple genetic and environmental factors that may interact.

All the genes found associated with these diseases explain only part of their heritability.[3] In most genetic studies, complex mechanisms as gene by environment interactions (GxE) or complex phenotypes, such as those accounting simultaneously for the three allergic diseases, were not considered. That may explain part of this missing heritability. A few recent studies have considered the three allergic diseases simultaneously, but rather in the sense of allergic disease defined by the presence of at least one of the diseases: asthma, eczema, or AR. These studies allowed detection of numerous new genes, involved or related to mechanisms affecting function of immune and epithelial cells.[4-6]

Other studies focused on the consideration of these diseases rather in the sense of co-morbidity. Some studies have focused on the co-morbidity of asthma associated with AR and have indeed detected new genes specifically associated to this co-morbidity.[7-9] Other studies have focused on the co-morbidity of asthma associated with eczema. The first meta-analysis of GWAS of asthma-plus-eczema[10] led to detect several genes already found to be associated with asthma and/or eczema and two other loci detected for allergic diseases for the first time. More recently, we conducted a second meta-analysis of GWAS of the co-morbidity of asthma-plus-eczema[11] with the special aim to discover new genes specifically associated to this co-morbidity. Our previous study led to the detection of six new genes for the first time for allergic diseases.

Besides, GxE interactions underlying susceptibility to asthma and asthma-related phenotypes were investigated in several studies. Some of them focused on early life environmental tobacco smoke (ETS) exposure which is a well-known risk factor for asthma[12] and also to a lesser extent to eczema.[13] A meta-analysis of genome-wide interaction studies (GWIS) for childhood asthma suggested interaction between ETS exposure and *PACRG* gene (Parkin coregulated).[14] More recently, a GWIS of time-to-asthma onset with ETS exposure showed a significant interactive effect with *KLHL1* (Kelch-like 1).[15] These genes had never been detected by previous genetic studies ignoring GxETS interaction.

Since it was evidenced that the co-morbidity of asthma-plus-eczema appears as a specific sub-entity depending on specific factors, it seemed also of interest to investigate GxETS interaction underlying the genetic susceptibility of this co-morbidity, which has never been investigated.

Our goal here was to identify genetic variants interacting with ETS exposure on the susceptibility to asthma-plus-eczema co-morbidity. Meta-analyses of GWIS were conducted across four independent populations of European ancestry.

# MATERIALS AND METHODS

## Populations

We studied 5,516 European-ancestry individuals from 4 independent studies, 2 population-based (GABRIELA and ALSPAC) and 2 family (EGEA and SLSJ) studies, which were part of the European GABRIEL consortium on the genetics of asthma.[16] A brief description of these studies with the definition of the phenotypes of asthma and eczema and of ETS exposure, is provided in the online data supplement.

### *Genetic data*

The EGEA, SLSJ, and GABRIELA individuals were genotyped using the Illumina 610-Quad array (Illumina, Inc., San Diego, CA, USA) as part of the European Gabriel asthma GWAS consortium.[16] The ALSPAC samples were genotyped using the Illumina Human Hap 550-Quad array (Illumina, Inc.) by 23andMe. In all datasets, stringent quality control (QC) was used to select both individuals and single nucleotide polymorphisms (SNPs) as previously described.[16] The following SNPs QCs were applied: genotyping call rates ≥ 97% and departure from the Hardy–Weinberg equilibrium in the controls (*P* value ≥ $10\times^{4}$) and minor allele frequencies (MAFs) ≥ 5%. To control for ethnicity/population stratification in the analysis, ancestry analysis was carried out in each dataset using the EIGENSTRAT2.0 software and HapMap data (CEU, YRI, JPT, and CHB). Based on this analysis, putative non-European samples were flagged as outliers and eliminated from any subsequent genetic analyses.

Genetic association analyses were first conducted for all SNPs included in the 610-Quad chip using genotyped data in EGEA and SLSJ and imputed data for GABRIELA (imputation using HapMap2, release 22) and for ALSPAC (imputation using the panel of the 1,000 Genomes Project, phase 1, version 3, release Dec 2013). Then, to further investigate new loci showing evidence of interaction with ETS exposure in asthma-plus-eczema susceptibility, we used imputed SNPs in a region of 1 Mb, 500 kb on both sides of the top SNP(s) of each locus (imputed SNPs from HapMap2 [release 22] for EGEA, SLSJ, and GABRIELA and 1000 genomes for ALSPAC). Imputed SNPs were kept for analysis if their MAF was equal to or greater than 0.05 and their imputation info score equal to or greater than 0.80.

## Statistical analysis

We conducted a 2-stage strategy to detect GxE interactions. At a first stage, we selected SNP(s) from genome-wide disease-SNP association analysis using as a threshold $P < 0.20$.[17] At a second stage, the selected SNPs were tested for interaction with ETS exposure on asthma-plus-eczema susceptibility. The whole strategy of analyses is described in **Fig. 1**.

### *Meta-analysis of association (stage 1)*

Association analyses in each of the 4 studies were performed by logistic regression using Stata® V17.0 or PLINK 1.9 assuming an additive model for SNP effect. Informative principal components for within-Europe diversity were included as covariates in all analyses. For the 2 family datasets (EGEA and SLSJ), logistic regression considered familial dependencies through the options of the logit function, of clustering within family and of robust variance, *i.e.* by adjusting the standard errors for clustering of observations within family. To take into account the stratified random sampling in GABRIELA, inverse probability weights were introduced in the logistic regression analyses. Then, SNP effect estimates on disease status of the 4 studies were meta-analyzed using a fixed-effects (inverse variance) model in order to


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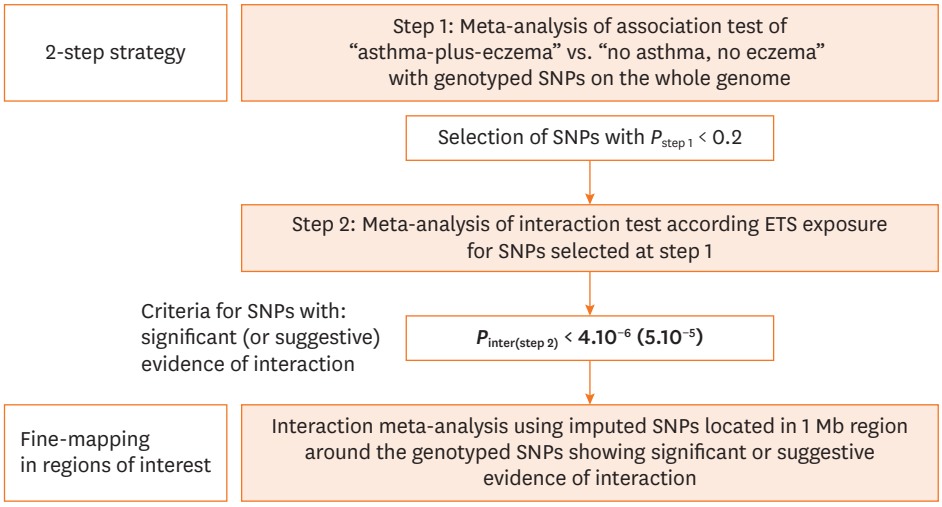


**Fig. 1.** Whole strategy of analyses for the detection of SNPs showing significant (or suggestive) evidence of interactions with ETS exposure on the susceptibility to asthma-plus-eczema co-morbidity.
SNP, single nucleotide polymorphism; ETS, environmental tobacco smoke.

obtain combined SNP effect size. SNPs associated with asthma-plus-eczema phenotype at $P < 0.2$ in the meta-analysis were selected for stage 2.

*Meta-analysis of Interaction (stage 2)*

Each dataset was split in ETS-exposed (ETS+) and ETS-unexposed (ETS-) individuals. Association analysis of the co-morbidity of asthma-plus-eczema vs. "no asthma and no eczema" was conducted in each stratified dataset separately. All association analyses were performed similarly to those performed at stage 1. In each ETS+/ETS- stratum, the SNP effect sizes from each study were combined using a fixed-effect meta-analysis with inverse variance weighting. Then, the SNP x ETS interaction effect was estimated as the difference between the 2 combined SNP effect sizes (D) = ($\beta meta_{ETS+}$ - $\beta meta_{ETS-}$).[18] The test statistic for SNP × ETS interaction (square of D divided by its variance) was compared to $\chi^2$ distribution with 1 df. The test of the equality of SNP effect in exposed and unexposed individuals is equivalent to the test of SNP × ETS interaction term in a model including the main effects of SNP, ETS exposure and interaction term with $\beta_{inter}$ = ($\beta meta_{ETS+}$ -$\beta meta_{ETS-}$), se = (var($\beta meta_{ETS+}$) + var($\beta meta_{ETS-}$)$^{0.5}$.

To minimize the false-positive findings and to obtain robust results, we assessed consistency of results by allowing no significant heterogeneity across studies (with *P* value of Cochran's Q statistic > 0.05) separately in each ETS+/ETS- stratum. Because of the independence between the results of the SNP (marginal) association test in stage 1 and the interaction effect in stage 2 that has been established in previous works,[19,20] correction for multiple testing was thus applied to the number of SNPs tested at stage 2. A Bonferroni correction was applied to the Meff, the effective number of independent SNPs calculated after discarding the dependence between tests due to the Linkage Disequilibrium (LD) between SNPs,[21] from the total number of SNPs tested at stage 2. In the EGEA and SLSJ datasets in which genotypic data are analyzed, individuals' stratification according to "asthma-plus-eczema," "neither asthma nor eczema" and to ETS+/ETS- stratum led to small sample size per genotype in each sub-group. We thus selected only SNPs having in each ETS+/ETS- stratum of each of these datasets, sufficient expected sample size per genotypes (≥ 5 in either affected or unaffected individuals). Then, a total of 131,517 SNPs were tested at stage 1, and 23,567 SNPs were selected to be tested at stage 2. The Meff was estimated to $1.38 \times 10^4$. Consequently, a


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threshold for significance level was estimated to $4.0 \times 10^{-6}$ for the interaction test at stage 2. The threshold of $5 \times 10^{-5}$ was used for suggestive evidence of interaction.

Next, to assess the consistency of our results according to the age at onset of asthma, all analyses were also conducted considering for asthma status, childhood-onset asthma before 16 years of age. Finally, to further investigate regions of interest, we repeated all analyses using imputed SNPs located in 1 Mb region around the top SNP(s) of each locus showing significant or suggestive evidence of interaction. We directly applied the interaction test with all imputed SNPs in the regions of interest and used the significance threshold for a genome-wide analysis with a panel of imputed SNPs from Hapmap2 (equal to $5 \times 10^{-8}$).

*Conditional analyses, Bayesian fine-mapping and credible sets of SNPs*

To identify distinct associated SNPs in each region harboring significant signals, we performed approximated conditional analyses using imputed SNPs in each of these regions. We used the Genome-wide Complex Trait Analysis (GCTA) software[22] (see URLs) based on the interaction summary meta-analysis statistics and on the correlations among SNPs (estimated from Hapmap phase2 CEU population). Within each investigated region by conditional analysis, summary meta-analysis data for imputed SNPs belonging to that region were adjusted for the lead SNP, to search for a second distinct signal. If there was a SNP meeting the significance threshold estimated by Bonferroni correction applied to the Meff in the region, after adjustment for the lead SNP, we concluded for a second distinct signal. Furthermore, we defined a credible set of SNPs for each distinct locus in the significant regions using FINEMAP software v1.4.[23] For each locus, we included SNPs in moderate to strong LD with the lead SNP ($r^2 \geq 0.6$) and we ran FINEMAP with default settings while allowing for one causal variant. We used the per SNP posterior probabilities and the Bayes factors (BF) provided by FINEMAP, and defined in each locus the credible sets as the smallest group of SNPs with a total posterior probability for including the causal SNP (PIP) ≥ 95%.[24]

*EQTL, meQTL and functional annotations*

We investigated whether the SNPs belonging to the credible sets for loci showing significant evidence of interaction with ETS exposure on asthma-plus-eczema susceptibility were cis-expression quantitative trait loci (cis-eQTLs) or methylation Quantitative Trait Loci (meQTLs). We queried existing eQTL databases in multiple tissues (GTEx,[25] eQTLGen26, BIOSQTL,[26] Luo Lung,[27] Muther[28] and GHSExpress[29] browsers). For meQTLs, we used the browser Phenoscanner v2 (http://www.phenoscanner.medschl.cam.ac.uk/) that combines several databases. Functional annotations of these SNPs (or proxies) were also done using ROADMAP and ENCODE (Encyclopedia of DNA Elements) data provided by the HaploReg tool.[30] Finally, we also checked if there were deleterious variants using CADD v1.3,[31] which integrates multiple annotations.

### Ethical statement

Information may be found online in **Supplementary Data S1**.

## RESULTS

### Phenotypic description of the samples

The sample size of each of the 4 studies by affection status stratum is indicated in **Table 1**. Due to the mode of ascertainment, the proportion of individuals having both asthma and eczema


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**Table 1.** Phenotypic description of the EGEA, SLSJ, GABRIELA, and ALSPAC datasets

| Phenotypes | EGEA | SLSJ | GABRIELA | ALSPAC |
|---|---|---|---|---|
| Total* | 754 | 427 | 1,637 | 5,761 |
| Age (yr) (mean ± SD) | 16.23 ± 0.39 | 20.15 ± 0.91 | 8.96 ± 0.05 | 15.46 ± 0.33 |
| Gender, men (%) | 227 (50.4) | 96 (45.7) | 528 (54.4) | 1,938 (49.9) |
| Asthma AND Eczema (%) | 201 (26.7) | 109 (25.5) | 266 (16.2) | 588 (10.2) |
| Exposed to early life ETS† (%) | 102 (50.7) | 73 (67.0) | 78 (29.3) | 245 (41.7) |
| NEITHER Asthma NOR eczema (%) | 249 (33.0) | 101 (23.7) | 705 (43.1) | 3,297 (57.2) |
| Exposed to early life ETS† (%) | 154 (61.8) | 59 (58.4) | 200 (28.4) | 1,268 (38.5) |
| Asthma alone OR Eczema alone (%)‡ | 304 (40.3) | 217 (50.8) | 666 (40.7) | 1,876 (32.6) |
| Exposed to early life ETS† (%) | 170 (55.9) | 131 (60.4) | 220 (33.0) | 732 (39.0) |

SD, standard deviation; ETS, environmental tobacco smoke.
*In the total number of individuals N presented here, individuals with no information available for ETS exposure are not taken into account, because they are not included in the analyses.
†Exposure to early life ETS.
‡Number of individuals having "asthma only OR eczema only" are presented here but are not included in the analyses.

was the strongest in EGEA (44.7%) and in SLSJ (51.9%), and the smallest in GABRIELA (27.4%) and in ALSPAC (15.1%). The proportion of men was similar in the 4 datasets, ranging from 46% to 54%. In contrast, the individuals were the oldest in SLSJ with a mean age equal to 20.15 years, then in EGEA and ALSPAC with respective mean ages equal to 16.23 and 15.46, and they were the youngest in GABRIELA with a mean age of 8.96. Proportion of individuals exposed to ETS was the strongest in SLSJ and EGEA whatever the phenotypic status (ranging from 51% to 67%) then in ALSPAC (around 40%) and the smallest in GABRIELA (28%–29%).

## Results of 2-stage interaction meta-analyses

The SNPs detected as interacting (at a significant or suggestive level) with ETS exposure on asthma-plus-eczema susceptibility are presented in **Table 2**. In addition, the results obtained in each dataset for these SNPs are shown in **Supplementary Table S1**. There was no inflation in the statistical tests with genomic inflation factor estimated to 1.02 and 1.02 for the association and interaction meta-analysis respectively (see QQ plots in **Supplementary Fig. S1**).

Among the $2.35 \times 10^4$ SNPs selected at stage 1, significant interactions ($P \leq 4.0 \times 10^{-6}$) with ETS exposure were found for 2 SNPs, the SNP rs10194978 located in *NRXN1* gene (2p16.3) and the SNP rs918949 located in *TNS1* gene (2q35). The odds ratios (95% confidence interval) in exposed and unexposed individuals were estimated for rs10194978_G allele to be equal to 0.80 (0.68–0.95) and 1.34 (1.17–1.54), respectively, and for rs918949_T allele equal to 0.82 (0.69–0.96) and 1.37 (1.18–1.59), respectively. Except the lead SNP at these loci, there were three additional variants that showed interaction signals with $P \leq 10^{-5}$ in these loci. The other top SNPs (reaching the suggestive level of $5.0 \times 10^{-5}$) were located in *ABCA4* and in *GRID2* genes. The top SNPs located in *NRXN1 and TNS1* genes interacting with ETS exposure were also detected by the statistical analyses restricted to "childhood-onset asthma-plus-eczema" at significant or suggestive levels (data not shown).

Note that for all SNPS detected in *NRXN1, TNS1, ABCA4* and *GRID2* genes, specificity of the interaction with ETS exposure on the co-morbidity asthma-plus-eczema was also verified by smaller evidence (at least *P* multiplied by a factor 10) of interaction with each one of the diseases, asthma and eczema when tested separately (**Supplementary Table S2**).

In the 4 regions showing significant or suggestive interactions with ETS exposure, we repeated interaction analyses using imputed SNPs located around the lead SNPs. These analyses supported all our initial findings, with similar or slightly improved significance


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Allergy, Asthma & Immunology Research AAIR

**Table 2.** Meta-analysis results of genotyped SNPs selected at stage 1 and showing at stage 2 significant (or suggestive) evidence of interaction with ETS exposure on the susceptibility to the "asthma-plus-eczema" co-morbidity

| Chr | SNP | Effect/ Baseline allele | Effect Allele Freq* | Position (kb)† | Gene | Previous gene | Next gene | βmeta$_{ETS+}$ | Se | *P* | βmeta$_{ETS-}$ | Se | *P* | βmeta$_{ALL}$ | Se | *P* | $\beta_{inter}$ | Se | $\chi^2_{1df}$ | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | rs3789395 | C/A | 0.54 | 94 036 | *ABCA4* | *GCLM* | *ARHGAP29* | 0.377 | 0.085 | $8.67 \times 10^{-6}$ | −0.094 | 0.074 | $2.05 \times 10^{-1}$ | 0.108 | 0.053 | $4.27 \times 10^{-2}$ | 0.470 | 0.112 | 17.530 | $2.83 \times 10^{-5}$ |
| 1 | rs1320502 | T/C | 0.54 | 94 036 | *ABCA4* | *GCLM* | *ARHGAP29* | 0.377 | 0.085 | $8.41 \times 10^{-6}$ | −0.096 | 0.074 | $1.97 \times 10^{-1}$ | 0.107 | 0.053 | $4.44 \times 10^{-2}$ | 0.472 | 0.112 | 17.670 | $2.63 \times 10^{-5}$ |
| 2 | rs601010 | T/C | 0.54 | 50 222 | *NRXN1* | *FSHR* | *ASB3* | 0.190 | 0.083 | $2.22 \times 10^{-2}$ | −0.295 | 0.072 | $4.18 \times 10^{-5}$ | −0.073 | 0.051 | $1.54 \times 10^{-1}$ | 0.484 | 0.110 | 19.470 | $1.02 \times 10^{-5}$ |
| 2 | rs1715970 | T/C | 0.54 | 50 237 | *NRXN1* | *FSHR* | *ASB3* | 0.187 | 0.083 | $2.39 \times 10^{-2}$ | −0.298 | 0.072 | $3.60 \times 10^{-5}$ | −0.077 | 0.051 | $1.35 \times 10^{-1}$ | 0.485 | 0.110 | 19.500 | $1.00 \times 10^{-5}$ |
| **2** | **rs10194978** | **G/A** | **0.39** | **50 298** | ***NRXN1*** | ***FSHR*** | ***ASB3*** | **−0.221** | **0.085** | $\mathbf{9.16 \times 10^{-3}}$ | **0.296** | **0.071** | $\mathbf{3.10 \times 10^{-5}}$ | **0.069** | **0.051** | $\mathbf{1.78 \times 10^{-1}}$ | **−0.517** | **0.111** | **21.840** | $\mathbf{2.97 \times 10^{-6}}$ |
| **2** | **rs918949** | **T/C** | **0.62** | **217 810** | ***TNS1*** | ***TNP1*** | ***RUFY4*** | **−0.203** | **0.082** | $\mathbf{1.30 \times 10^{-2}}$ | **0.313** | **0.076** | $\mathbf{3.58 \times 10^{-5}}$ | **0.086** | **0.053** | $\mathbf{1.02 \times 10^{-1}}$ | **−0.516** | **0.111** | **21.410** | $\mathbf{3.70 \times 10^{-6}}$ |
| 2 | rs1035672 | G/A | 0.38 | 217 810 | *TNS1* | *TNP1* | *RUFY4* | 0.200 | 0.082 | $1.49 \times 10^{-2}$ | −0.312 | 0.076 | $3.75 \times 10^{-5}$ | −0.087 | 0.053 | $1.00 \times 10^{-1}$ | 0.511 | 0.111 | 21.020 | $4.54 \times 10^{-6}$ |
| 4 | rs989927 | G/A | 0.56 | 93 195 | *GRID2* | *CCSER1* | *ATOH1* | 0.342 | 0.083 | $3.99 \times 10^{-5}$ | −0.109 | 0.073 | $1.35 \times 10^{-1}$ | 0.113 | 0.052 | $2.91 \times 10^{-2}$ | 0.451 | 0.111 | 16.600 | $4.61 \times 10^{-5}$ |

In bold: SNPs detected with significant evidence of interaction with ETS, i.e., detected at $P \leq 4 \times 10^{-6}$ by SNP × ETS interaction meta-analysis. The other SNPs were detected with suggestive evidence of interaction with ETS, i.e., detected at $P \leq 5 \times 10^{-5}$ by SNP × ETS interaction meta-analysis.
SNP, single nucleotide polymorphism; ETS, environmental tobacco smoke.
*Estimated in CEU population from Phase 3 of the 1000 Genomes Project.
†SNP position in kilo base pairs (GRCh38.p13: Genome Reference Consortium Human Build 38 patch release 13).
‡Exposure to early life ETS.



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of the results compared to those observed with initial result obtained for the lead SNPs. Moreover, additional signals of interaction with ETS showed a strong improvement in significance level for imputed SNPs in *NRXN1*, with *P* values reaching $5 \times 10^{-9}$ (vs. $P = 3 \times 10^{-6}$) and thus quite strongly exceeding the significance threshold for a genome-wide analysis with imputed SNPs from Hapmap2 ($P = 5.0 \times 10^{-8}$), and for imputed SNPs in *TNS1*, $5.4 \times 10^{-7}$ (vs. $P = 3.7 \times 10^{-6}$) (Regional interaction plots are shown in **Fig. 2**).

Conditional analyses performed in each of the 2 significant loci, evidenced no secondary distinct signal in these regions. We further applied FINEMAP to identify a credible set of causal SNPs at each of the two top significant regions. The first top signal located in *NRXN1* was fine-mapped to a small credible set of putative causal variants of two SNPs with respective PIP of 0.51 and 0.47 and respective Log10 BF of 3.1 and 3.0 (with Log10 BF > 2 indicating considerable evidence that the SNP is causal). The second signal located in *TNS1* was fine-mapped to a credible set of five SNPs with PIP ranging from 0.64 to 0.02 and with respective Log10 BF ranging from 3.1 to 1.2. The list of the credible set of SNPs for each locus is provided in **Supplementary Table S3**.

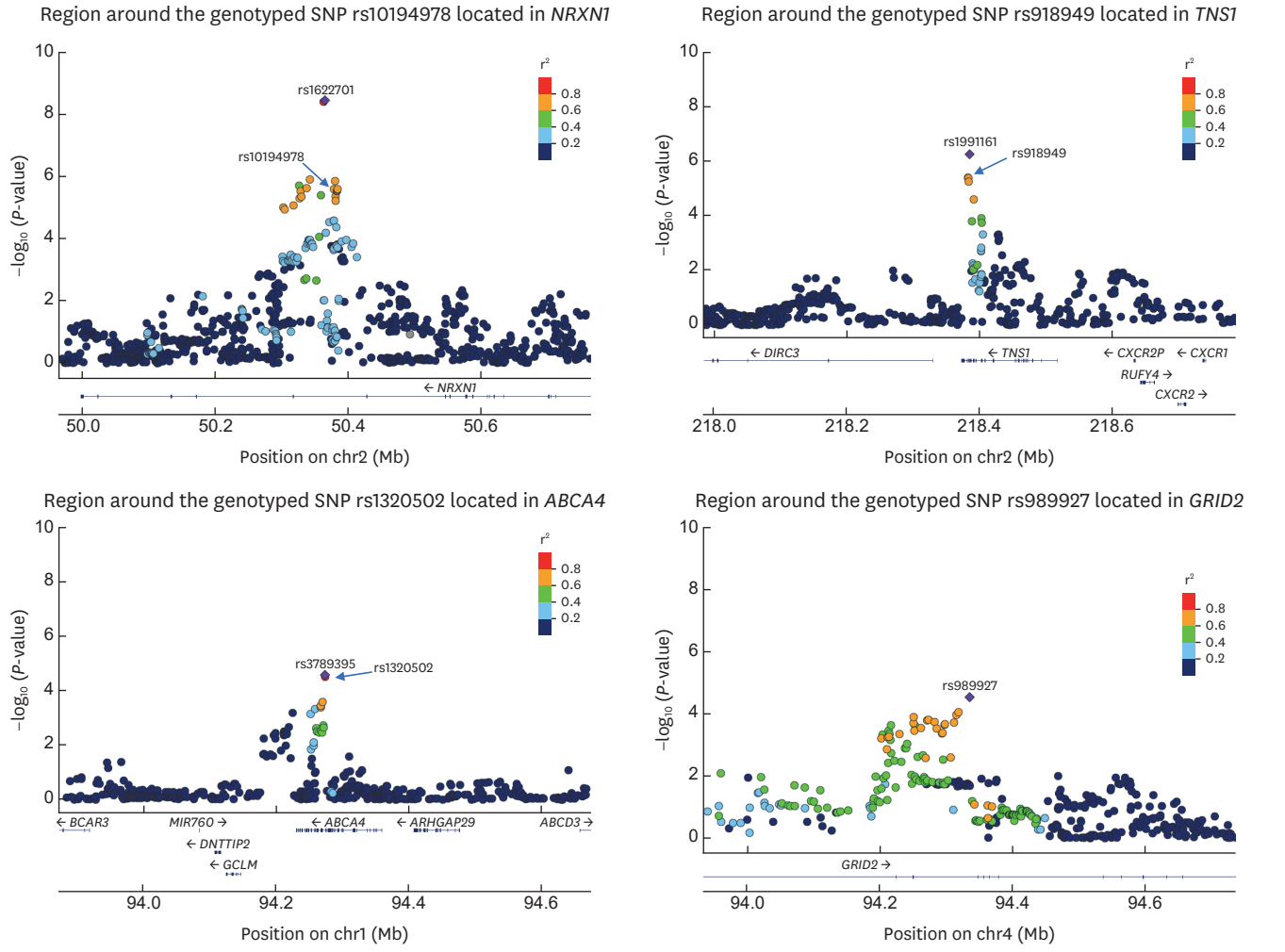


**Fig. 2.** Regional plots for the four regions of interest (imputed SNPs in a +/-400 kb window around the best genotyped SNP). In regional plots, the x axis presents physical distance in megabase (build 37.3 coordinates) and the y axis presents $-\log_{10}$ *P*-value for the interaction meta-analysis statistic.
SNP, single nucleotide polymorphism.


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### eQTL, meQTL analysis and functional annotation

By interrogating databases of eQTL and meQTL in target tissues, we identified that in the region of *NRXN1*, the 2 SNPs of the credible set were associated with methylation level at CpG sites located in *NRXN1*. In the region of *TNS1*, the 5 SNPs of the credible set were associated with TNS1 expression in blood. These SNPs were also associated with methylation level at CpG sites located in *TNS1*. Note that 2 SNPs of the credible set of SNPs located in *TNS1* (rs918949 and rs2571445) had CADD score equal to 18 and 17, respectively (with CADD ≥ 15 indicating deleterious effect of these SNPs) and were missense SNPs. EQTL and meQTL with SNPs of the credible set for each locus are described in **Supplementary Tables S4** and **S5**.

Moreover, rs2571445 maps to enhancer histone marks in lung cells. Finally, SNPs in the credible set of SNPs located in the 2 loci include TF binding sites that are described in more detail in the discussion. Functional annotations of all loci are presented in details in **Supplementary Table S6**.

## DISCUSSION

The aim of our study was to detect new loci interacting with ETS exposure on the susceptibility of the co-morbidity of asthma-plus-eczema. This study led to detect significant interactions between ETS exposure and genetic variants located in *NRXN1* (Neurexin I-Alpha)/*TNS1* (tensin 1) gene. SNP by ETS interactions were also suggested for variants located in *ABCA4* (ATP Binding Cassette Subfamily A Member 4) and in *GRID2* (Glutamate Ionotropic Receptor Delta Type Subunit 2) genes.

Genome-wide analyses are underpowered for detecting GxG and GxE interactions, due to the joint impact of multiple testing on the whole genome and of the poor power of interaction test itself. To increase the power to identify such interactions, 2-stage strategies have been proposed to select SNP(s) that will be tested for interaction and thus to decrease the number of multiple tests.[32] The 2-step strategy applied here was to select SNP(s) from genome-wide disease-SNP association analysis at the first stage, before testing SNP-by-ETS exposure interaction at the second stage.[17] This approach has the advantage of being robust against gene-environment (G-E) correlation at the population level, but its power depends, like all the other strategies, on the threshold used for filtering SNP at the first stage. We here used, at the first stage, a modest SNP marginal effect of $P < 0.20$ as a threshold. Indeed, the problem, when using a too stringent threshold for selecting SNPs using their marginal effects on the phenotype of interest, is to discard strong interaction such as flip flop effect, *i.e.*, inversion of the effect of a given allele according to the exposure (E), this model leading to small marginal effect of the variant on the disease.[33] Opposite effects as a function of ETS exposure, were indeed observed here for variants in *NRXN1* and *TNS1* genes, suggesting a flip-flop model. However, as already discussed above, all results obtained here with the 2-step strategy, depend on this threshold chosen at step 1.

We here conducted meta-analyses of 4 independent datasets instead of conducting analysis in some of these datasets as discovery sample(s) followed by replication analyses in the remaining independent sample(s). To ensure validity of our findings, we required strong consistency of results across studies assessed by no significant heterogeneity separately in each ETS+/ETS- stratum across studies. Replication study in independent samples of results obtained in discovery samples is somewhat equivalent to show a homogeneity of results across all samples (discovery and replication samples), as it is the case in the present study.


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All our findings were also well supported, by repeated analyses using imputed SNPs to obtain a denser map at the 4 suggestive or significant loci. That was done by interaction analyses using the significant level of $5 \times 10^{-8}$ for genome-wide analysis with imputed data and thus without dependency on a threshold used at a first step. These analyses strengthened the original findings, in an extensive manner for *NRXN1*, in which a variant greatly exceeded the significant threshold with $P = 5 \times 10^{-9}$ and also with an improvement of association signal in *TNS1* ($P = 5 \times 10^{-7}$ instead of $3 \times 10^{-6}$ with the top genotyped SNP).

To our knowledge, this study is the first GWIS of asthma-plus-eczema according to ETS exposure reported to date in the literature. None of our findings have previously been reported by published Catalog of Published Genome-Wide Association Studies, https://www.ebi.ac.uk/gwas/) with significant evidence of association and/or interaction with ETS exposure for asthma, eczema, or more generally for allergic diseases. However, previous GWAS showed strong evidence of association between genetic variants belonging to *TNS1* among which rs2571445 and phenotypes related to the lungs, such as forced expiratory volume in 1 second (FEV1), FEV1/forced vital capacity (FVC) ratio,[34-36] and chronic obstructive pulmonary disease (COPD).[37] Moreover, interactive effects were shown between variants in *TNS1* with active smoking on FEV1[38,39] and on COPD.[40] Such interaction was also shown with ETS exposure on FEV1 in children.[41] Interestingly, the SNP rs2571445 being one of the genetic variants showing all these associations and interactions was part of the credible set of putative causal variants detected by the present study in *TNS1*. Additionally, rs2571445 and rs918949, another SNP in this credible set, are missense variants with high score of deleteriousness. Rs2571445 maps to enhancer histone marks in lung cells and to binding site of the transcription factor CTCF, which controls MHC class II gene expression. It was recently shown that CTCF is a major driver of gene co-expression in the airways of asthmatic patients.[42] It also includes binding site of the AP-1 transcription factor, involved in asthmatic inflammation.[43] In addition, TNS1 expression is increased in COPD airways[44] and also in fibroblastic foci from the lungs with idiopathic pulmonary fibrosis.[45] Additionally TNS1 expression is profoundly up-regulated by transforming growth factors-β,[45] a major mediator involved in inflammatory responses and fibrotic tissue remodeling within the asthmatic lungs. Previous studies showed also a link between *TNS1* and skin-related phenotypes. This gene was associated to skin lipoma and is involved in the migration of fibroblasts that are found in the dermis where they produce collagen fibers and elastin. It was also speculated that in human dermal fibroblasts, reduced expression of *TNS1* might contribute to the dermal alterations observed during skin ageing.[46] Furthermore, skin diseases like atopic dermatitis and lung-related phenotypes and/or diseases like asthma, lung function, and COPD as well as lung injury by several chemicals are in part inferred via interactions between *TNS1* and chemicals such as Tobacco Smoke Pollution (Comparative Toxicogenomics Database, http://ctd.mdibl.org/).

The other SNPs showing significant interactions with ETS exposure were located in *NRXN1* (Neurexin I-Alpha) gene. Previous GWAS showed strong evidence of associations between genetic variants belonging to *NRXN1* and phenotypes related to smoking (smoking status measurement,[47] nicotine dependence,[48]) or phenotypes and/or diseases related to the lungs (response to bronchodilator, FEV1/FVC ratio[49]).

Apart from *TNS1* and *NRXN1* genes, the next 2 SNPs showing suggestive evidence of interaction with ETS exposure were located in *ABCA4* (ATP Binding Cassette Subfamily A Member 4). *ABCA4* enables ATP binding and ATP hydrolysis activity. Interestingly,


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ATP was shown to be accumulated in the airways of asthmatic individuals and to trigger bronchial hyper-responsiveness, suggesting an important role played by ATP in the airway inflammation.[50]

The last SNP showing suggestive evidence of interaction with ETS exposure was located in *GRID2* (Glutamate Ionotropic Receptor Delta Type Subunit 2). Previous GWAS showed associations between variants in this gene and smoking status measurement,[47] and others suggested associations with response to bronchodilator and FEV1/FVC ratio.[50]

In conclusion, our study underlines the importance of studying sub-phenotypes as co-morbidities, including G-E interaction, to detect new susceptibility genes. Among the 4 new loci detected here interacting with ETS exposure on the co-morbidity of "asthma-plus-eczema," *TNS1* is emerging as the most relevant candidate gene given it is one of the loci showing significant interactions with ETS and given its links to skin and lung phenotypes, its possible interactive effect with tobacco smoke pollution in skin and lung diseases and strong evidence of causality and of deleteriousness of SNP(s) detected in this gene. At the same time, *NRXN1* is a relevant gene too, given its highest level of significance for its interaction with ETS exposure ($P = 5.0 \times 10^{-9}$ vs. $5.0 \times 10^{-7}$ for *TNS1*).

To confirm the findings of this first GWIS of asthma-plus-eczema with ETS exposure, further replication analyses in other studies with independent samples would be necessary. Further functional studies will also be necessary to prove the effect of the 2 genes and ETS exposure and then to bring better insights into the role of these loci interacting with ETS exposure on the co-morbidity of asthma-plus-eczema. Deciphering underlying molecular mechanisms of this co-morbidity in interaction with ETS exposure could point to novel therapeutic approaches.

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## SUPPLEMENTARY MATERIALS

### Supplementary Data 1

Online data supplement.

**Click here to view**

**Supplementary Table S1**

results per study of genotyped SNPs showing significant (or suggestive) evidence at stage 2 of interaction with ETS exposure in susceptibility of the "asthma-plus-eczema" co-morbidity by meta-analysis of the four datasets

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**Supplementary Table S2**

Meta-analysis results for eczema and for asthma when analyzed separately of genotyped SNPs showing significant (or suggestive) evidence of interaction with ETS exposure in susceptibility of the "asthma-plus-eczema" co-morbidity

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**Supplementary Table S3**

95% credible sets of SNPs using FINEMAP for the two distinct signals showing significant interaction with ETS exposure

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**Supplementary Table S4**

eQTLs for SNPs belonging to 95% credible sets of the two distinct signals showing significant interaction with ETS exposure

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**Supplementary Table S5**

mQTLs for SNPs belonging to 95% credible sets of the two distinct signals showing significant or suggestive interaction with ETS exposure

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**Supplementary Table S6**

Annotation using Haploreg of SNPs belonging to 95% credible sets of the two distinct signals showing significant interaction with ETS exposure

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**Supplementary Fig. S1**

QQ plots of -log10 *P* value of the test statistics of SNP "asthma-plus-eczema" association and of SNP×ETS exposure interaction meta-analyses.

Click here to view

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