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Identification of *OCA2* as a novel locus for the co-morbidity of asthma-plus-eczema

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Abstract

Background: Numerous genes have been associated with the three most common allergic diseases (asthma, allergic rhinitis or eczema) but these genes explain only a part of the heritability. In the vast majority of genetic studies, complex phenotypes such as co-morbidity of two of these diseases, have not been considered. This may partly explain missing heritability.

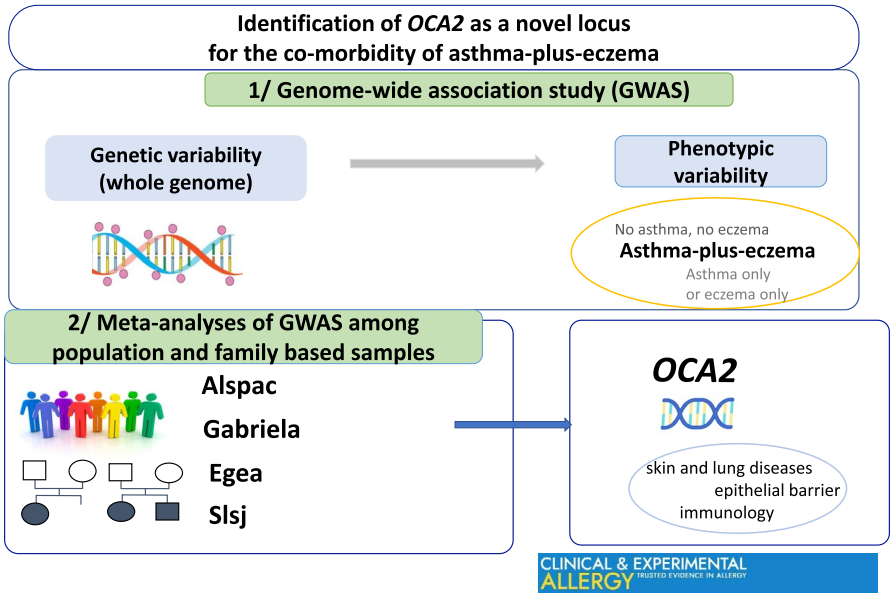
Objective: To identify genetic variants specifically associated with the co-morbidity of asthma-plus-eczema.

Methods: We first conducted a meta-analysis of four GWAS (Genome-Wide Association Study) of the combined asthma-plus-eczema phenotype (total of 8807 European-ancestry subjects of whom 1208 subjects had both asthma and eczema). To assess whether the association with SNP(s) was specific to the co-morbidity, we also conducted a meta-analysis of homogeneity test of association according to disease status ("asthma-plus-eczema" vs. the presence of only one disease "asthma only or eczema only"). We then used a joint test by combining the two test statistics from the co-morbidity-SNP association and the phenotypic heterogeneity of SNP effect meta-analyses.

Results: Seven SNPs were detected for specific association to the asthma-plus-eczema co-morbidity, two with significant and five with suggestive evidence using the joint test after correction for multiple testing. The two significant SNPs are located in the *OCA2* gene (Oculocutaneous Albinism II), a new locus never detected for significant evidence of association with any allergic disease. This gene is a promising candidate gene, because of its link to skin and lung diseases, and to epithelial barrier and immune mechanisms.

Conclusion: Our study underlines the importance of studying sub-phenotypes as co-morbidities to detect new susceptibility genes.

KEYWORDS
ALSPAC, asthma, co-morbidity, eczema, EGEA, GABRIELA, GWAS, phenotypic heterogeneity, SLSJ



GRAPHICAL ABSTRACT
A meta-analysis of four GWAS among population and family based samples to identify new susceptibility genes for the co-morbidity of asthma-plus-eczema. Identification of *OCA2*, a new gene associated specifically with the phenotype of asthma-plus-eczema. The candidate gene OCA2 is linked to skin and lung diseases, epithelial barrier and immunology.

1 | INTRODUCTION

The three 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] However, genes specifically involved in each disease also exist. Most genetic studies have focused specifically on either one of the allergic diseases and numerous susceptibility genes for asthma, AR and eczema have been found.[2] Many of these genes are involved in the immune response, and are not specifically associated to one of the diseases. Other genes related to epithelial barrier dysfunction as *C11orf30* a transcriptional regulator in keratinocyte, are also shared by the three diseases.[3,4] But some other genes were found to be more specifically associated to one disease, such as asthma genes *ORMDL3/GSDML*[5,6] involved in remodelling or eczema genes *SPRR3*,[7] *SPINK5*[8] and *OVOL1*[9] involved in skin or epidermis development.

However, all the genes found associated with these diseases explain only a part of the heritability.[10] In most of the genetic studies, complex mechanisms such as Gene–Gene and Gene–Environment interactions or complex phenotypes, such as those taking into account simultaneously the three allergic diseases, were not considered. That may explain a part of this missing heritability. Few recent studies have considered the three allergic diseases simultaneously, rather in the sense of allergic disease defined by the presence of at least one of the diseases: asthma, eczema or AR. These studies indeed allowed detection of numerous new genes and loci especially involved or related to mechanisms affecting function of immune and epithelial cells.[11–13]

Other studies have focused on the consideration of these diseases rather in the sense of co-morbidity. Studying more precise

Key Messages

- A genome-wide association study to identify new susceptibility genes for the phenotype of asthma-plus-eczema.
- We identified OCA2, a new gene associated specifically with the phenotype of asthma-plus-eczema.
- The candidate gene OCA2 is linked to skin and lung diseases, epithelial barrier and immunology.

sub-phenotypes such as co-morbidities, could allow the detection of new genes. For example, some studies have focused on the co-morbidity of asthma associated with AR and have detected new genes specifically associated to this co-morbidity, such as the *NFIA*,[14] *ZBTB*, *CLEC16A*[15] and *MTNR1A*[16] genes.

It seemed of interest to study the genetics of the co-morbidity of asthma associated with eczema, which has less been investigated. To our knowledge, only one genetic study has studied this co-morbidity, an asthma-plus-eczema Genome-Wide Association Study (GWAS) meta-analysis.[17] For the phenotype of this co-morbidity, the particular hypothesis of the atopic march was considered. It corresponds to a disease progression from infantile eczema to asthma in childhood. This study led to the detection of several genes already found to be associated with asthma and/or eczema and two other loci detected for allergic disease for the first time: a locus encompassing the *EFHC1* gene and another one located between *TMTC2* and *SLC6A15* genes.

To discover new genes in addition to those already found to be associated with either asthma or eczema, our goal here was to identify genetic variants specifically associated with the co-morbidity of asthma-plus-eczema. GWAS meta-analysis was conducted across four independent populations of European ancestry. Firstly, association with the co-morbidity of asthma-plus-eczema was investigated and then, to assess the specificity of the association with the co-morbidity, the homogeneity of the association was tested according to disease status defined by the presence of the two diseases "asthma-plus-eczema" versus the presence of only one disease "asthma only or eczema only." A joint test was then applied by combining the two test statistics from the co-morbidity-SNP association and the phenotypic heterogeneity of SNP effect meta-analyses. The present study is thus the first GWAS meta-analysis of asthma-plus-eczema, testing the specificity of the associations with this co-morbidity.

2 | MATERIALS AND METHODS

2.1 | Populations

We studied 8807 European-ancestry subjects from four independent studies, two population-based (GABRIELA and ALSPAC) and two family (EGEA and SLSJ) studies, which were part of the European consortium on the genetics of asthma.[18] A brief description of these studies with the definition of asthma and eczema phenotypes is provided in the Supplementary Material.

2.2 | Genetic data

The EGEA, SLSJ and GABRIELA samples were genotyped using the Illumina 610-Quad, as part of the European Gabriel asthma GWAS consortium.[18] The ALSPAC samples were genotyped using the Illumina Human Hap 550-quad array (Illumina, Inc.) by 23andMe. In all datasets, stringent quality criteria were used to select both individuals and SNPs as described previously.[18] 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 using genotyped data in EGEA, SLSJ and GABRIELA and imputed data for ALSPAC (imputation using the panel of the 1000 Genome Project, phase 1, version 3, release Dec 2013) for all SNPs included in both the 610-Quad chip and the 1000 genome panel. Then, to further investigate new loci showing evidence of association with the co-morbidity of asthma-plus-eczema, 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 genome for ALSPAC).

2.3 | Statistical analysis

For all association analyses in each of the four studies, we performed logistic regression using Stata® V14.1 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 two family datasets (EGEA and SLSJ), logistic regression considered familial dependencies through the cluster within family and robust variance options of the logit function. 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 four studies were meta-analysed using a fixed-effects (inverse variance) model in order to increase power and to obtain more robust findings. As described below, different status for cases and/or controls were considered and subsequently meta-analysed. The whole strategy of the analysis is described in Figure 1 and presented in the following paragraphs.

We firstly conducted a genome-wide meta-analysis of association between SNPs and the co-morbidity of asthma-plus-eczema, where cases were defined as having both asthma and eczema, and controls had neither asthma nor eczema; this test will be referred as the "co-morbidity association test" hereafter. However, a SNP associated with asthma (including cases with asthma having or not having eczema) or eczema (including cases with eczema having or not having asthma) can be found associated with the co-morbidity asthma plus eczema. Consequently, an association shown between asthma-plus-eczema versus no asthma no eczema is not sufficient to prove the specificity of the association to the co-morbidity. To verify this specificity of association, we then tested by meta-analysis, the homogeneity of SNP association between the two following phenotypes: "asthma-plus-eczema" versus "asthma alone or eczema alone" comparing by logistic regression SNP-association between these two case groups. The latter test will be referred to hereafter

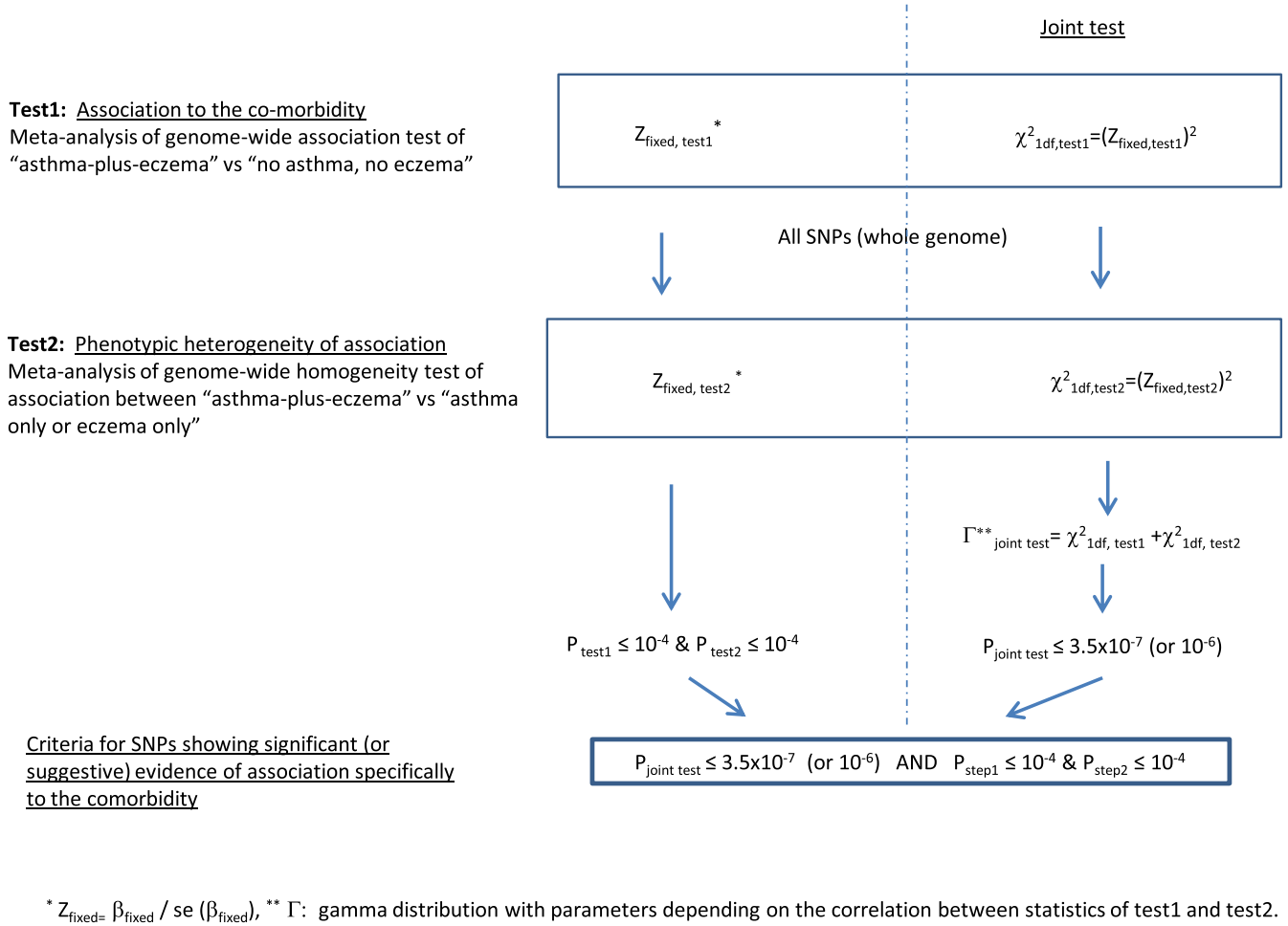


FIGURE 1 Whole analysis strategy for the detection of SNPs showing significant (and suggestive) evidence of association specifically with the co-morbidity asthma-plus-eczema. Note that $^{*}Z_{fixed} = \beta_{fixed}/SE\ (\beta_{fixed})$, $^{**}\Gamma$: gamma distribution with parameters depending on the correlation between statistics of test1 and test2

as "the phenotypic homogeneity test." Such homogeneity tests of SNP effect between sub-phenotypes have been shown to have equivalent power to that of a multinomial regression-based test of heterogeneity.[19]

We then conducted on the whole genome, a joint test by combining the two test statistics obtained from the co-morbidity-SNP association and the phenotypic heterogeneity of SNP effect meta-analyses. These two tests follow a χ^2 distribution with 1 df, we summed their χ^2_{1df} results obtained at each SNP. However, we could not assume that this sum of χ^2_{1df} results follows a χ^2_{2df} distribution because the two tests were not independent due to sample overlapping, the two tests shared the individuals having asthma plus eczema. We used, as proposed by Ferrari,[20] the approximation of the sum of correlated χ^2 variables by a parametrized gamma distribution with parameters depending on the Pearson correlation estimated between statistics obtained by the two tests.

For the joint test, the Bonferroni correction was applied to the Meff (effective number of independent SNPs calculated after discarding the dependence between tests due to Linkage Disequilibrium (LD) between SNPs from the total number of SNPs). In each of the four datasets, subjects' stratification according to "asthma plus eczema," "neither asthma nor eczema" or "asthma only or eczema only" status, led in each sub-phenotype group to small sample size per genotype. We thus selected only SNPs with MAF ≥ 0.10 and/or SNPs having in each dataset sufficient expected sample size per genotypes (≥5 in either affected or unaffected subjects) depending on whether imputed or genotyped data were available. From the total number of tested SNPs (286,679), the Meff was estimated to be 143,414 with the method of Li and Ji[21] and the threshold for significance to be $p = 3.5 \times 10^{-7}$ (0.05/143414).

We reported SNPs as showing significant (or suggestive) evidence of association specifically to the co-morbidity, those showing (1) a significant (or suggestive $p \leq 10^{-6}$) result with the joint test and (2) strong signals ($p \leq 10^{-4}$) for both the "co-morbidity association test" and the "phenotypic homogeneity test."

Consistency of results across the four studies was assessed by use of the I^2 statistic, which describes the percentage of variation across studies that is due to heterogeneity rather than chance.[22,23] Thus to select only consistent results across studies, we applied $I^2 \leq 24\%$ as criteria to retain SNPs for both "co-morbidity association

test" and "phenotypic homogeneity test," indicating no or little heterogeneity[24] of results across studies.

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, we repeated all analyses using imputed SNPs located in 1 Mb region around the top genotyped SNPs of each locus showing significant or suggestive evidence of association.

2.4 | Expression quantitative trait loci analysis and functional annotation

We investigated whether the SNPs (or their proxies, $r^2 \geq .8$) showing significant or suggestive evidence of association specifically to asthma-plus-eczema were cis-eQTL. We queried existing expression quantitative trait loci (eQTL) databases in multiple tissues (GTEx,[25] eQTLGen,[26] BIOSQTL,[27] Muther[28] and GHSExpress[29] browsers). 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]

3 | RESULTS

3.1 | Phenotypic description of the samples

The sample size of each of the four studies by affection status stratum is indicated in Table 1. Due to the mode of ascertainment, the proportion of subjects having both asthma and eczema was the strongest in EGEA (26%) and in SLSJ (23%), and the smallest in GABRIELA (16%) and in ALSPAC (10%). The proportion of men was similar in the four datasets, ranging from 46% to 57%. In contrast, the subjects were the oldest in SLSJ with a mean age equal to 23.7, then in EGEA and ALSPAC with respective mean ages equal to 16.5 and 13.9 and they were the youngest in GABRIELA with a mean age of 9.

3.2 | Results of meta-analyses

The SNPs detected as specifically associated with the co-morbidity of asthma-plus-eczema are presented in Table 2 and in more details in Table S1. In addition, for SNPs presented in Table 2, the results obtained in each dataset are shown in Table S2. There was no inflation in the statistical tests with genomic inflation factor estimated to 1.02, 0.99 and 1.01 for the "co-morbidity association test," 'phenotypic homogeneity test' and joint test respectively (see QQ plots in Figure S1). Results of the meta-analysis of the joint test are shown in the Manhattan plot in Figure 2.

When testing the association between SNPs and the co-morbidity of asthma-plus-eczema, 32 SNPs were found associated with "asthma-plus-eczema" at $p \leq 10^{-4}$, among which two were excluded because of $I^2 \geq 24\%$. When testing the specificity of this association, 25 SNPs reached the threshold of 10^{-4}, with none of them excluded because of $I^2 \geq 24\%$. Finally, two SNPs and five SNPs showed respectively significant ($p \leq 3.5 \times 10^{-7}$) and suggestive ($p \leq 10^{-6}$) results using the joint test, and had $p \leq 10^{-4}$ for both the "co-morbidity association test" and the "phenotypic homogeneity test." None of these seven SNPs were excluded because of $I^2 \geq 24\%$. The two SNPs (rs4778192 and rs2703978) showing significant evidence of association with the joint test were located in *OCA2* gene. The five SNPs with suggestive evidence, were located in *OCA2* (rs2311469, rs4778189 and rs2594897), *TBC1D14* (rs10937762) and *LRP1B* (rs1402470) genes. Forest plots are shown in Figure S2 for association and phenotypic homogeneity tests for each of the two significant SNPs (rs4778192 and rs2703978).

When the analyses were restricted to childhood-onset asthma, the five SNPs located in *OCA2* gene were detected in the same manner (two of them with significant evidence of association specifically to asthma-plus-eczema and the three others with suggestive evidence), while no SNP, including those in *LRP1* and *TBC1D14* was detected elsewhere.

In the regions of the three detected loci, we repeated association analyses using imputed SNPs located in a 1 Mb region around the genotyped lead SNPs. These analyses supported all our initial findings, with similar or slightly improved significance of the results compared to those observed with genotyped SNPs. Moreover, additional signals with similar significance level were detected at imputed SNPs in *OCA2* gene (see Figure 3). The imputed SNP with highest significance level, rs924318, was in LD with the genotyped significant SNPs, strong LD with rs2703978 ($r^2 = .87$) but not with rs4778192 ($r^2 = .30$). However, further conditional regression analyses of both co-morbidity association and phenotypic homogeneity tests showed that rs4778192 and rs2703978 were each with

TABLE 1 Phenotypic description of the EGEA, SLSJ, GABRIELA and ALSPAC samples

	EGEA	SLSJ	GABRIELA	ALSPAC
N	783	614	1649	5761
Age, years, mean (SD)	16.46 (0.29)	23.75 (0.62)	9.00 (0.04)	15.46 (0.33)
Gender-men (%)	410 (52.4)	282 (45.9)	943(57.2)	2869 (49.8)
Asthma and eczema (%)	207 (26)	144 (23)	269 (16)	588 (10)
Neither asthma nor eczema (%)	260 (33)	163 (27)	713 (43)	3297 (57)
Asthma only or eczema only (%)	316 (40)	307 (50)	667 (40)	1876 (33)

TABLE 2 Meta-analysis results of SNPs showing significant (or suggestive) evidence of association specifically to the co-morbidity "asthma-plus-eczema"

									Co-morbidity association test[c]			Phenotypic homogeneity test[d]			Joint test[e]
SNP	chr	Effect/ baseline allele	Effect allele freq[a]	Position (kb)[b]	SNP location	Gene	Previous gene	Next gene	β_{fixed}	SE (β_{fixed})	p	β_{fixed}	SE (β_{fixed})	p	p
rs1402470	2	G/A	0.556	141 362	intron	*LRP1B*	*NXPH2*	*KYNU*	−0.250	0.055	4.90×10^{-6}	−0.230	0.056	4.41×10^{-5}	4.52×10^{-7}
rs10937762	4	G/A	0.495	6 917	intron	*TBC1D14*	*KIAA0232*	*CCDC96*	−0.244	0.054	6.10×10^{-6}	−0.222	0.053	3.29×10^{-5}	4.32×10^{-7}
rs2311469	15	T/G	0.444	27 824	intron	*OCA2*	*GABRG3*	*HERC2*	0.231	0.052	7.41×10^{-6}	0.221	0.054	3.84×10^{-5}	5.41×10^{-7}
rs4778189	15	G/A	0.556	27 827	intron	*OCA2*	*GABRG3*	*HERC2*	−0.232	0.052	6.63×10^{-6}	−0.224	0.054	3.24×10^{-5}	4.51×10^{-7}
rs4778192	**15**	**T/C**	**0.556**	**27 835**	**intron**	***OCA2***	***GABRG3***	***HERC2***	**−0.236**	**0.052**	$\mathbf{4.63 \times 10^{-6}}$	**−0.229**	**0.054**	$\mathbf{2.26 \times 10^{-5}}$	$\mathbf{2.84 \times 10^{-7}}$
rs2594897	15	T/C	0.278	27 900	intron	*OCA2*	*GABRG3*	*HERC2*	0.269	0.056	1.70×10^{-6}	0.225	0.058	9.58×10^{-5}	3.75×10^{-7}
rs2703978	**15**	**T/C**	**0.278**	**27 902**	**intron**	***OCA2***	***GABRG3***	***HERC2***	**0.270**	**0.056**	$\mathbf{1.60 \times 10^{-6}}$	**0.229**	**0.058**	$\mathbf{7.14 \times 10^{-5}}$	$\mathbf{2.98 \times 10^{-7}}$

Note: In bold: SNPs detected with significant evidence of association specifically to "asthma plus eczema", i.e., detected at $p \leq 10^{-4}$ for "co-morbidity association test" AND for "phenotypic homogeneity test" AND $p \leq 3.5 \times 10^{-7}$ for the joint test. The other SNPs were detected with suggestive evidence of association specifically to "asthma plus eczema", i.e. detected at $p \leq 10^{-4}$ for "co-morbidity association test" AND for "phenotypic homogeneity test" AND $p \leq 1 \times 10^{-6}$ for the joint test.

[a]Estimated in CEU population from Phase 3 of the 1000 Genomes Project.

[b]SNP position in kilo base pairs (GRCh38.p12:Genome Reference Consortium Human Build 38 patch release 12).

[c]Meta-analysis of genome-wide association test of "asthma-plus-eczema" versus "no asthma, no eczema".

[d]Meta-analysis of homogeneity test of association between "asthma-plus-eczema" vs. "asthma only or eczema only".

[e]Test combining statistics of the association and phenotypic homogeneity tests and following a gamma distribution with parameters depending on the correlation between statistics of the two tests.

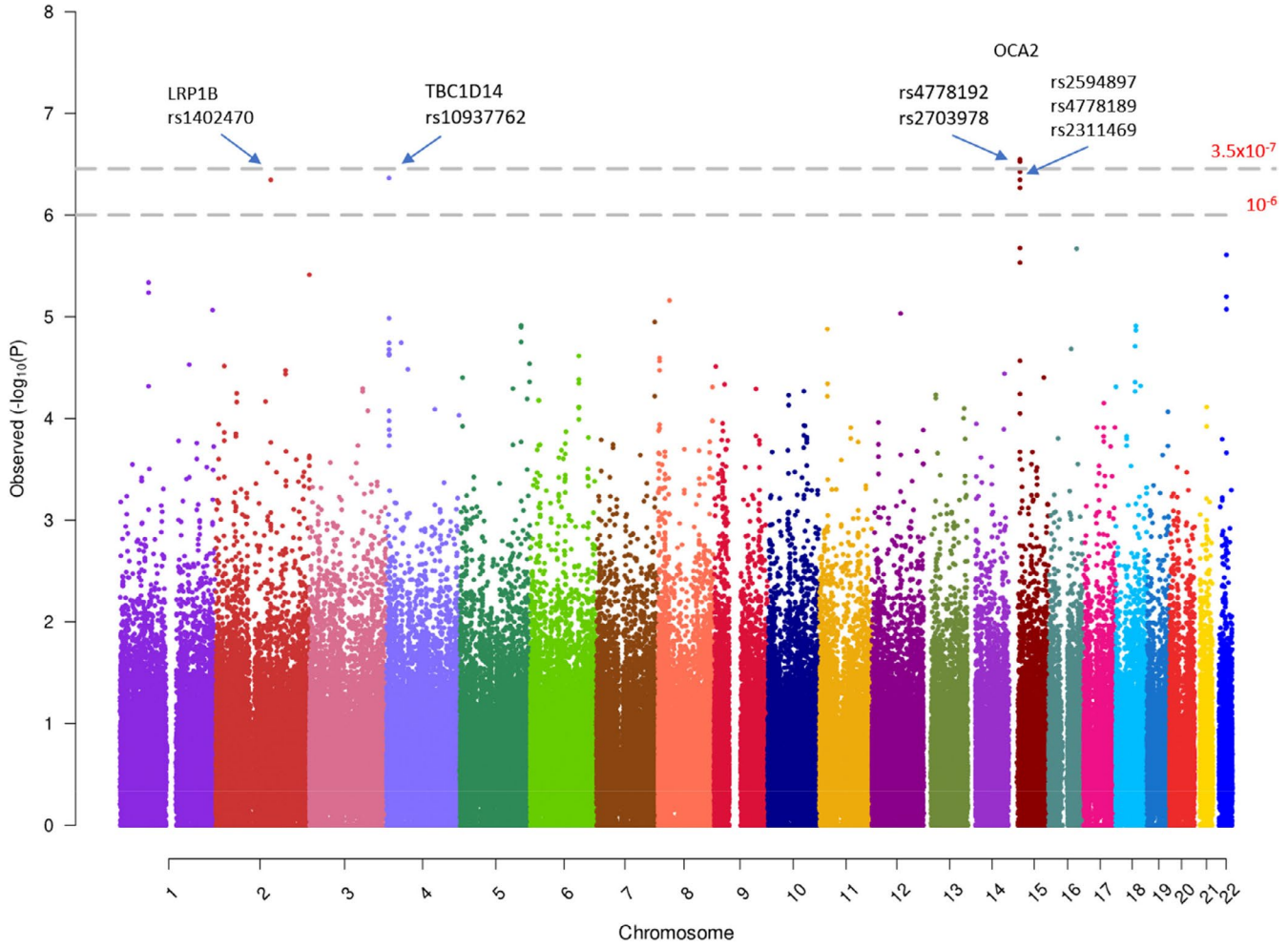


FIGURE 2 Manhattan plot of meta-analysis results for the joint test. The *x* axis represents chromosomal location and the *y* axis represents $-\log_{10} p_{joint}$; the first horizontal line denotes $p = 3.5 \times 10^{-7}$, corresponding to the significance threshold, the second horizontal line denotes $p = 10^{-6}$, corresponding to the suggestive threshold used for the joint test

the joint test no longer significantly associated with asthma-plus-eczema ($p \geq .05$) when conditioning on rs924318. This indicates that the three SNPs represent the same association signal.

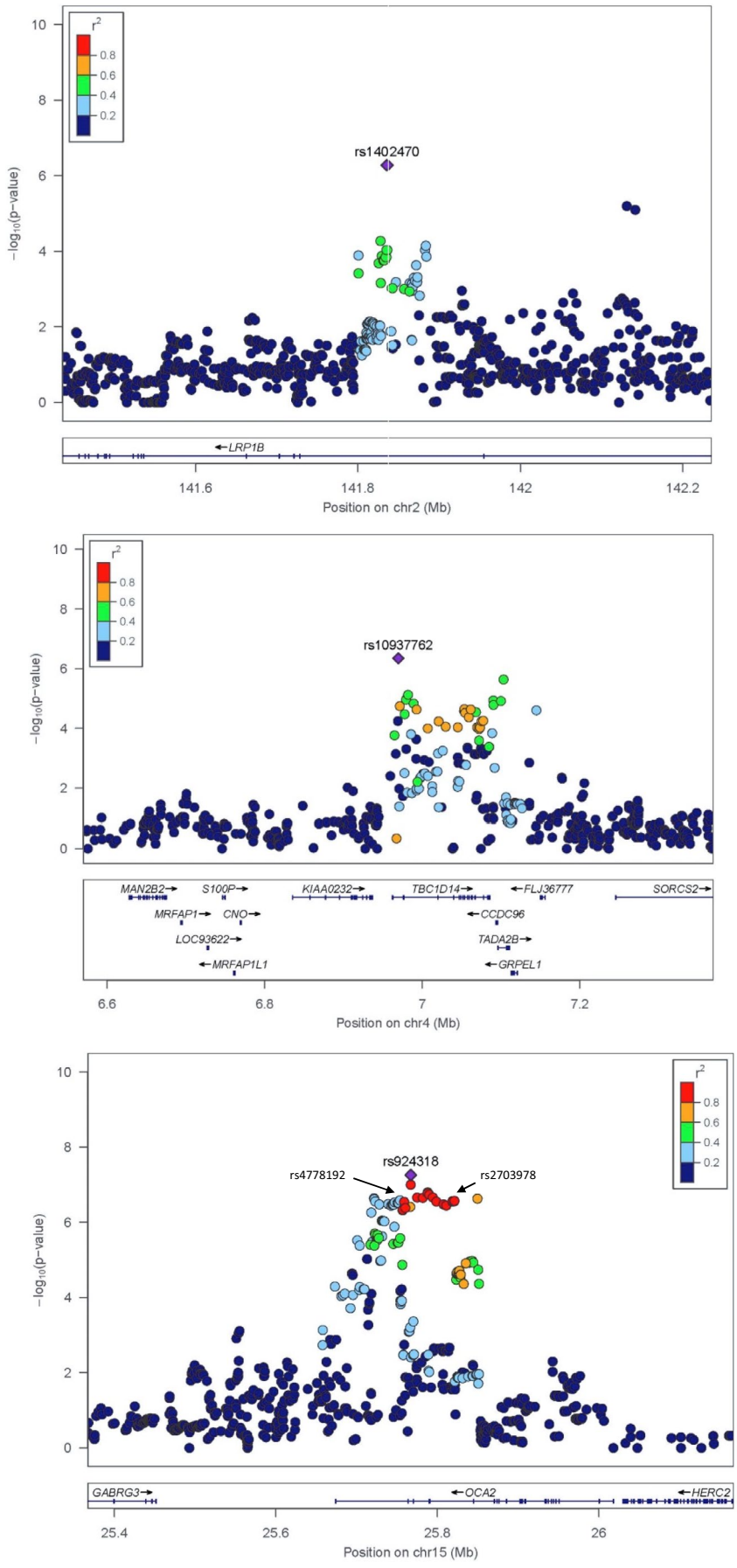
3.3 | eQTL analysis and functional annotation

By interrogating databases of gene expression in target tissues, we identified a proxy rs10013696 of rs10937762 ($r^2 = .82$) located in *TBC1D14* that was associated with *TBC1D14* expression in skin ($p = 5 \times 10^{-9}$).[25] We also identified two SNPs in *OCA2*, rs2594897 and rs2703978, associated in the whole blood with *HERC2* expression ($p = 5 \times 10^{-12}$).[26] Functional annotation of the three loci is presented in detail in Table S3.

The associated SNPs located in *OCA2* and in *TBC1D14*, map to promotor and enhancer histone marks and DNase I hypersensitivity sites in numerous lung and skin cells, and included transcription factor (TF) binding sites that will be described in more detail in the discussion.

4 | DISCUSSION

The aim of this study was to discover new genes, different from those already found associated with asthma or eczema, by identifying genetic variants specifically associated with the co-morbidity of asthma-plus-eczema. Our study identified a new locus significantly and specifically associated with the co-morbidity and located on chromosome 15q13 in the *OCA2* gene. Two other loci located on chromosomes 2q22 in the *LRP1B* gene and 4p16 in the *TBC1D14* gene were detected with a suggestive evidence of specific association to the co-morbidity. We did not conduct analysis in discovery sample(s) followed by replication analyses in independent sample(s). We directly conducted meta-analyses of four independent datasets and to ensure validity of our findings, we required strong consistency of results across studies assessed by I^2 (percentage of variation across studies that is due to heterogeneity rather than chance[22,23]) ≤24% indicating no or little heterogeneity.[24] We found strong consistency of results ($I^2 < 10\%$) for the two *OCA2* and *TBC1D14* loci, but somehow less consistent results for the *LRP1B* locus ($20\% < I^2 < 24\%$).



Region around the genotyped SNP rs1402470 located in *LRP1B*

Region around the genotyped SNP rs10937762 located in *TBC1D14*

Region around the genotyped SNPs rs4778192 and rs2703978 located in *OCA2*

FIGURE 3 Regional plots for the three regions of interest (imputed SNPs around 400 kb of best genotyped SNPs). In regional plots (A–F), the *x* axis presents physical distance in megabases (build 37.3 coordinates) and the *y* axis presents $-\log_{10} p_{\text{joint}}$ values for the joint test statistic

For the three loci detected (*OCA2*, *TBC1D14 and LRP1B*), specificity of the association to the co-morbidity was also verified by an absence of association with the phenotype of "asthma only or eczema only" versus "no asthma and no eczema" and by a smaller evidence of association with each one of the diseases, asthma and eczema when tested separately.

All our findings were also well supported, by repeated association analyses using imputed SNPs to obtain a denser map of the regions of the three detected loci. These analyses strengthened the original findings, particularly for those in the *OCA2* gene.

To our knowledge, the single genetic study of asthma-plus-eczema reported to date in the literature is a GWAS meta-analysis of the co-morbidity defined as the atopic march.[17] This study was conducted to search for genetic associations with this co-morbidity but without testing the specificity of the associations. The study led to the detection of five loci already found associated with one of the allergic diseases. For three of these loci (IKZF3,[15,31,32] *AP5B1/OVOL1*[9] and *IL4/KIF3A*[9]), we found suggestive evidence of association with asthma-plus-eczema ($10^{-5} < p < 10^{-2}$) in our present study. These results are not surprising since the "atopic march" meta-analysis enclosed the four datasets included in the current study. But for these three loci, there was no indication of the specificity of this association with the asthma-plus-eczema co-morbidity. Furthermore, the two new loci identified in the "atopic march" meta-analysis (*EFHC1* and between *SLC6A15* and *TMTC2*) did not show indication of association in the current study ($p > 0.01$). Regarding *CRNN/LCE5A* (*FLG*),[33] a known eczema locus that was also identified in the atopic march meta-analysis, we found evidence of association with asthma-plus-eczema ($p = 2 \times 10^{-6}$). However, we did not retain this locus due to the large measure of heterogeneity detected across studies ($I^2 > 0.50$).

None of our novel findings have been previously reported by published GWAS (GWAS-Catalog of Published Genome-Wide Association Studies, http://genome.gov/gwastudies) with significant evidence of association for asthma, eczema or more generally for allergic diseases. However, previous GWAS showed significant evidence of association between genetic variants belonging to *OCA2* loci/gene and diseases or phenotypes related to skin such as melanoma,[34,35] cutaneous squamous cell carcinoma[36] and skin pigmentation.[37] Note that, underlying mechanisms shared by melanoma and asthma have been already suggested, due to genes found associated with both melanoma and asthma, as the *TYRP1* gene.[38,39] Suggestive evidence of associations of *OCA2* genetic variants with allergic sensitization, eczema and lung function phenotypes (FEV1/FVC ratio) have been also reported.[40,41] Moreover, a polymorphism, located within an intron of the *HERC2* gene an adjacent neighbour of *OCA2* which is involved in the regulation of OCA2 expression, was significantly associated with asthma response to diisiocyanate.[42]

The *OCA2* gene (Oculocutaneous Albinism II) codes for a melanosomal transmembrane protein which is involved in many biological processes as tyrosine transport. The tyrosine is a precursor to melanin synthesis. Interestingly, tyrosine is related to various pathological mechanisms of eczema.[43] *OCA2* is also involved in melanocyte differentiation, melanin biosynthesis and pigmentation. Melanocytes, along with keratinocytes and Langerhans cells, being positioned within the epidermis, form a physical skin barrier. A link between pigmentation, which depends on melanin synthesis, and the skin barrier function was shown, both in humans[44] and in mice[45] indicating that melanocytes producing melanin influence epidermal barrier function. In addition, accumulating evidence has shown that melanocytes are also active factors in the skin immune system and participate in immune responses.[46,47]

The five SNPs detected in *OCA2* map to a large number of enhancer and promoter histone marks and to DNase I hypersensitivity sites in numerous lung and skin cells. Moreover, rs4778192 belongs to the binding sites of the TF CEBPB, an important TF involved in the regulation and expression of genes involved in immune and inflammatory responses.[48] The SNP rs2703978 belongs to the binding site of hoxa5 which probably activates antagonist genes against the release of keratinocyte growth factors and epidermal formation.[49] Lastly, rs8035720 a proxy of rs2594897, belongs to the binding site of the TF 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.[50] The two SNPs rs2594897 and rs2703978 were found associated in the whole blood with expression of *HERC2*, this gene being like *OCA2*, also found associated to melanoma.[51]

Among the loci showing suggestive evidence of association to asthma-plus-eczema, the SNP rs10937762 is an intronic variant located in the *TBC1D14* gene (TBC1 Domain Family. Member 14). A variant of *TBC1D14* was found to be associated with eosinophil count, an important biomarker of allergy. The SNP rs10937762 in *TBC1D14* maps to a large number of enhancer and promoter histone marks and to DNase I hypersensitivity sites in numerous lung and skin cells. This SNP belongs to the binding site of the redox-sensitive nuclear factor (NF)-kappaB which is an important participant in a broad spectrum of inflammatory networks that regulate cytokine activity in airway pathology.[52] In addition, rs10013696 a proxy of rs10937762 was strongly associated with *TBC1D14* expression in skin.[25]

Lastly, the SNP rs1402470 which showed suggestive evidence of association to asthma-plus-eczema, is an intronic variant located in the *LRP1B* gene (Low Density Lipoprotein Receptor-Related Protein 1B). Suggestive evidence of association was shown between *LRP1B* genetic variants and post-bronchodilator FEV1,[39] Diisocyanate-induced asthma[40] and childhood-onset asthma.[53] Besides that, a *LRP1B* mutation was shown to be a predictive marker for the presence of chronic obstruction pulmonary disease in patients with lung adenocarcinoma.[54]

In conclusion, the present study highlights that studying well-defined sub-phenotypic entities as the co-morbidity is a critical feature for the identification of new genes. Among the new three loci detected here for the co-morbidity of "asthma-plus-eczema," *OCA2* is emerging as the most relevant candidate gene given the reached significance level of association, the high consistency across studies and its links to skin and lung diseases, and to epithelial barrier

mechanisms and/or immune response, which have crucial roles in both asthma and eczema. Further confirmations of these findings as well as functional studies are needed to bring greater insight into the role of these loci on the co-morbidity of asthma-plus-eczema. Deciphering molecular determinants of this co-morbidity could point to novel therapeutic approaches.

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CONFLICT OF INTEREST

All authors have no conflicts of interest.

AUTHOR CONTRIBUTIONS

MHD conducted the design. PMJ performed the largest part of data analysis, ABA, CLinhard and HM a remaining part. MHD interpreted the findings and drafted the initial version of the manuscript. EB and PMJ contributed to drafting the manuscript. EvM, CLaprise, AMM, RG, ME, EB and MHD contributed to the data acquisition. All authors revised the manuscript and provided final approval of the version to be published.

ETHICAL APPROVAL

Information may be found online in Supplementary Material.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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