

The *ANO3/MUC15* locus is associated with eczema in families ascertained through asthma

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Background: A previous genome-wide linkage scan in 295 families of the French Epidemiological Study on the Genetics and Environment of Asthma (EGEA) reported strong evidence of linkage of 11p14 to eczema.

Objective: Our purpose was to conduct fine-scale mapping of the 11p14 region to identify the genetic variants associated with eczema.

Methods: Association analyses were first conducted in the family sample from the French EGEA by using 2 methods: the

family-based association method and logistic regression.

Replication of the EGEA findings was sought in French Canadian and United Kingdom family samples, which, similarly to EGEA samples, were ascertained through asthma. We also tested for association in 2 German samples ascertained through eczema.

Results: We found significant association of eczema with 11p14 genetic variants in the vicinity of the linkage peak in EGEA ($P = 10^{-4}$ for rs1050153 by using the family-based association method, which reached the multiple testing-corrected threshold of 10^{-4} ; $P = .003$ with logistic regression). Pooled analysis of the 3 asthma-ascertained samples showed strong improvement in the evidence for association ($P = 6 \times 10^{-6}$ for rs293974, $P = 3 \times 10^{-5}$ for rs1050153, and $P = 8 \times 10^{-5}$ for rs15783). No association was observed in the eczema-ascertained samples.

Conclusion: The significant single nucleotide polymorphisms are located within the overlapping anoctamin 3 (*ANO3*) and mucin 15 (*MUC15*) genes. Several lines of evidence suggest that *MUC15* is a strong candidate for eczema. Further investigation is needed to confirm our findings and to better understand the role of the *ANO3/MUC15* locus in eczema and its relationship with respect to asthma. (J Allergy Clin Immunol 2012;129:1547-53.)

Key words: Positional cloning, association study, family-based association study, logistic regression, eczema, asthma, family sample

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Atopic dermatitis (AD), also called *eczema*, is the most common chronic inflammatory skin disorder. It affects up to 10% to 15% of children and 3% of adults in developed societies. Eczema is also an allergic manifestation strongly associated with asthma and allergic rhinitis. These comorbidities might share genetic determinants, as suggested by their strong associations at both the individual and family levels.¹ Genes with pleiotropic effects underlying these 3 diseases might be involved in allergic processes, barrier mechanisms of the epithelium, or both. Genes specific to eczema might also exist, as supported by the observation that parental history of eczema represents a more potent risk factor for eczema than parental history of asthma or allergic rhinitis.^{1,2}

Numerous genetic studies based on candidate gene and positional cloning approaches and more recently genome-wide association studies (GWASs) have been conducted for asthma and have identified a number of susceptibility genes.³ Fewer genomic studies have been carried out for eczema.⁴ A major breakthrough in the genetics of AD has been the discovery of filaggrin (*FLG*) mutations,^{5,6} which cause ichthyosis vulgaris and were found to

Abbreviations used

AD:	Atopic dermatitis
ANO3:	Anoctamin 3 gene
EGEA:	Epidemiological Study on the Genetics and Environment of Asthma
FBAT:	Family-based association method
GWAS:	Genome-wide association study
LD:	Linkage disequilibrium
MRC:	Medical Research Council Asthma UK Center in Allergic Mechanisms of Asthma
MUC15:	Mucin 15 gene
OR:	Odds ratio
QC:	Quality control
SLSJ:	Saguenay-Lac-Saint-Jean
SNP:	Single nucleotide polymorphism
UK:	United Kingdom

be associated consistently with the risk of AD. Other candidate genes related to epidermal barrier dysfunction have been reported to be associated with eczema, including *SPINK5*,⁷ which was initially characterized in patients with Netherton disease, a rare recessive skin disorder. Another susceptibility gene, *COL29A1*, which is involved in collagen synthesis,⁸ was identified by positional cloning. More recently, the first GWAS for AD, which was conducted in European subjects, showed evidence for association with an intergenic region near the *C11orf30* gene on chromosome 11q13.5 that might play a role in epithelial barrier mechanisms.⁹ A second AD GWAS carried out in Chinese Han subjects revealed 2 new loci. One of these loci, on 5q22, includes the *TMEM232* and *SLC25A46* genes, which have a yet unknown function, whereas the other locus, on 20q13, harbors *TNFRSF6B*, which plays an important role in adaptive immune responses, and *ZGPAT*, which is involved in the epidermal growth factor receptor pathway.¹⁰

A previous genome-wide linkage scan carried out in 295 families of the French Epidemiological Study on the Genetics and Environment of Asthma (EGEA) showed strong linkage of the 11p14 region to eczema.¹¹ Our objective was to conduct fine-scale mapping of this region to identify the genetic variants associated with eczema. The discovery phase of this association study was based on the EGEA families, which have been genome-wide genotyped as part of the large-scale Gabriel Consortium asthma GWAS,¹² and used 2 statistical methods for internal validation: the family-based association method (FBAT) and logistic regression. The replication phase used 2 family samples of European ancestry, French Canadian families and United Kingdom (UK) families, which, similarly to EGEA families, were ascertained through probands with asthma and were part of the Gabriel Consortium GWAS.¹² Our goal was also to investigate the influence of the mode of ascertainment of the probands on association outcomes by testing for association of the 11p14 region with eczema in German family-based and case-control samples recruited through subjects affected with eczema.

METHODS**Discovery sample**

The EGEA has been described in detail previously.¹³ The EGEA family sample consisted of 388 French nuclear families that included 253 families ascertained through offspring with asthma (1 offspring proband in 90% of

families and 2 offspring probands in the remainder) and 135 families ascertained through 1 parent with asthma. Inclusion criteria for probands¹³ were positive answers to 4 standardized questions ([1] Have you ever had attacks of breathlessness at rest with wheezing? [2] Have you ever had an asthma attack? If yes, [2a] was this diagnosis confirmed by a physician? [2b/] Have you had an asthma attack in the last 12 months?). The definition of eczema was the same as the one used for the previous linkage analysis¹¹ and was based on the answer to the question: Have you had eczema during childhood? Confirmation of eczema disease status was obtained for the majority of subjects who answered a second questionnaire that was used in the 10-year follow-up of the EGEA and included questions on symptoms. After applying stringent quality control (QC) criteria to 1631 family members who had been genome-wide genotyped (see the section on genotyping), 1492 EGEA family members were kept for analysis.

Replication samples ascertained through asthmatic subjects

French Canadian family collection. The Saguenay-Lac-Saint-Jean (SLSJ) asthma study comprised 253 French Canadian multigenerational families ascertained through 2 probands with asthma.¹⁴ Probands were included in the study if they fulfilled at least 2 of the following criteria: (1) a minimum of 3 clinic visits for acute asthma within 1 year; (2) 2 or more asthma-related hospital admissions within 1 year; or (3) steroid dependency, as defined by either 6 months of oral or 1 year of inhaled corticosteroid use. Eczema was defined by a self-reported history of eczema on a questionnaire. Positive answers were validated by physicians or through clinical records. After QC checking of 1712 family members who were genome-wide genotyped, the analysis sample included 1199 subjects.

UK family sample. The MRC UK National family collection included 207 nuclear families recruited through at least 1 proband with asthma.¹⁵ Probands were included if they answered positively to the following question: Has your doctor ever told you that you have asthma? Probands had step 3 asthma or worse according to the British Thoracic Society guidelines (high-dose inhaled steroids or low-dose inhaled steroids and a long-acting β -agonist). Eczema definition was based on answers to questions on the presence of symptoms. After QC checking of a total of 400 siblings (parents were not genotyped) who were genome-wide genotyped, the analysis sample included 254 sibs.

Replication samples ascertained through subjects with eczema

The German family and case-control samples were included in a recent GWAS for eczema.⁹ In both samples the physician's diagnosis of eczema was made according to standard criteria in the presence of a chronic or chronically relapsing pruritic dermatitis with the typical morphology and distribution.¹⁶ The family sample included 270 nuclear families with a total of 1097 subjects, of whom 529 offspring were affected with eczema. These families were recruited through 2 siblings with moderate-to-severe eczema. The case-control study included 939 eczema cases. All 975 German control subjects were derived from the previously described population-based Popgen Biobank.¹⁷

Genotyping and imputations

The EGEA sample was genotyped with the Illumina 610 Quad array (Illumina, San Diego, Calif) at the Centre National de Génomique (CNG, Evry, France) as part of the European Gabriel Consortium (<http://www.gabriel-fp6.org/>) asthma GWAS.¹² Stringent quality criteria, as detailed by Moffatt et al,¹² were used to select both subjects and single nucleotide polymorphisms (SNPs) for analysis. For this study, we used 394 SNPs which spanned 2.5 Mb on each side of the linkage peak located at 27.5 Mb from pter using fine linkage mapping of 11p14.¹¹

The SLSJ and Medical Research Council Asthma UK Center in Allergic Mechanisms of Asthma (MRC) replication samples were also genotyped at

TABLE I. Phenotypic characteristics of genotyped siblings in the 3 asthma-ascertained samples

Phenotypes	EGEA			SLSJ			MRCA		
	All	ECZ+	ECZ−	All	ECZ+	ECZ−	All	ECZ+	ECZ−
No. (%)	789 (100)	255 (32)	534 (68)	639 (100)	193 (30)	446 (70)	254 (100)	80 (31)	174 (69)
Male sex (%)	53	54	53	46	38	49	57	60	55
Mean age (SD)	16.4 (8.1)	14.6 (7.3)	17.3 (8.3)	24.5 (15.7)	22.8 (15.5)	25.2 (15.8)	11.2 (4.7)	11.6 (4.3)	11 (4.9)
Asthma (%)	52	70	43	65	75	61	67	86	59
Atopy (%)	65	75	60	70	81	65	41	61	32
Mean log(IgE) (SD)	2.17 (0.73)	2.37 (0.65)	2.07 (0.76)	2.23 (0.68)	2.35 (0.75)	2.17 (0.65)	2.15 (0.81)	2.48 (0.72)	2.00 (0.82)

ECZ+, With eczema; ECZ−, without eczema.

CNG by using the Illumina 610 Quad array for the SLSJ sample¹² and the Illumina 317K array for the MRCA sample, which was part of a previous GWAS.^{12,15} The same QC criteria for subjects and markers as used for EGEA applied to these datasets. Imputations of ungenotyped SNPs were performed with MACH version 1.00 software (<http://www.sph.umich.edu/csg/abecasis/MACH/>) and HapMap2 release 21 CEU haplotypes as the reference panel to have the same number of SNPs across the EGEA, SLSJ, and MRCA samples and to increase SNP density. These imputations were carried out in each of the 3 datasets (EGEA, SLSJ, and MRCA) separately. Imputed SNPs were kept for analysis if their imputation quality score (rsq)¹⁸ was greater than or equal to 0.9 and if their minor allele frequency was 5% or greater.

The German family and case-control samples were genotyped with the Illumina 500K array and Affymetrix 6.0 array, respectively. Imputations were conducted with Mach version 1.0.16 software and HapMap2 release 24 CEU haplotypes as the reference sample.

Statistical methods

Because the 11p14 region was detected through linkage analysis, association of eczema with the 394 genotyped SNPs spanning the linkage region was first investigated in the EGEA families by using the FBAT approach,¹⁹ which tests for association in the presence of linkage. We assumed an additive genetic model and took into account the presence of linkage by using the $-e$ option.²⁰ The sibship distribution of EGEA families contributing to FBAT analysis is shown in Table E1 in this article's Online Repository at www.jacionline.org. A threshold corresponding to a type 1 error of 5% was calculated by using the method of Gao et al²¹ and estimated to be 10^{-4} to correct for multiple testing. We also investigated to what extent the most significantly associated SNP accounted for the linkage signal.¹¹ For that purpose, we tested for linkage of eczema to the microsatellite markers used in our previous linkage scan¹¹ by using the maximum likelihood binomial^{11,22} method in EGEA sibships stratified according to the genotypes at the most significant SNP.

SNPs reaching the critical threshold in the FBAT analysis and all SNPs spanning 15 kb on each side of each significant SNP were tested in EGEA siblings by using logistic regression to validate the FBAT results. A robust sandwich estimation of the variance,²³ as implemented in the STATA logit function, was used to model clustering of family genotypes. An additive genetic model was assumed, and principal components for within-France diversity were included as covariates in the model.¹²

Replication of EGEA results was sought in SLSJ and MRCA asthma-ascertained families by using imputed SNPs in the region selected from the EGEA FBAT analysis. We applied logistic regression to allele dosage to take into account the uncertainty of imputing missing genotypes¹⁸ and used the same modeling approach as in EGEA. All sibships from MRCA nuclear families and extended SLSJ pedigrees were included in that analysis. All data from EGEA, SLSJ, and MRCA samples were then pooled for single-marker logistic regression analysis followed by multiple regression while adjusting for study and within-study principal components. Multiple regression was applied to SNPs with a single-marker analysis P value 10^{-3} or less and a pairwise linkage disequilibrium (LD) coefficient (r^2) of 0.7 or less. A stepwise procedure was used to select the best-fitting model. Association analyses were repeated in

TABLE II. Phenotypic characteristics of genotyped siblings in the German eczema-ascertained samples: case-control and family samples

Phenotypes	German cases/control subjects		German families
	Cases	Control subjects	Genotyped offspring with eczema
No. (%)	915 (100)	975 (100)	529 (100)
Male sex (%)	51	52	49
Mean age (SD)	17.4 (17.6)	50.9 (14.6)	8.45 (6.0)
Asthma (%)	24	—	25
Atopy (%)	69	—	71
Mean log(IgE) (SD)	2.27 (0.91)	—	2.11 (0.79)

—, No information.

siblings with and without asthma separately to investigate whether evidence for association with eczema might be related to the presence of asthma in these asthma-ascertained samples.

Finally, we tested whether the SNPs found to be associated with eczema in the asthma-ascertained families were associated with eczema in the 2 German samples recruited through eczema. We used FBAT with the best-imputed genotype in the family set and logistic regression analysis with the imputed allele dosage in the case-control set.

RESULTS

Table I shows the phenotypic distribution of genotyped sibs with and without eczema in the EGEA, SLSJ, and MRCA families. The proportion of sibs with eczema (about 30%) was similar in these 3 samples. In contrast, the proportion of sibs with asthma varied from 50% in EGEA families to 65% to 67% in SLSJ and MRCA families, which might at least in part be due to the mode of ascertainment of the families that differed according to the number of asthmatic probands and the parental or offspring generation to which they belonged. As expected, the proportions of sibs with asthma and atopy were higher among siblings with eczema than among those without eczema in all 3 samples (Table I). Similarly, the mean log(IgE) value was higher among sibs with eczema than among those without eczema. The proportion of male subjects and the sibs' mean ages did not differ according to eczema affection status within each sample but varied across samples. The older age of SLSJ subjects is partly due to the existence of 3-generation pedigrees in that dataset.

In the German family and case-control samples (Table II), subjects with eczema had a frequency of atopy and mean log(IgE) values of the same order of magnitude as those observed in siblings with eczema from EGEA, SLSJ, and MRCA samples. In contrast, the proportion of subjects with asthma among German

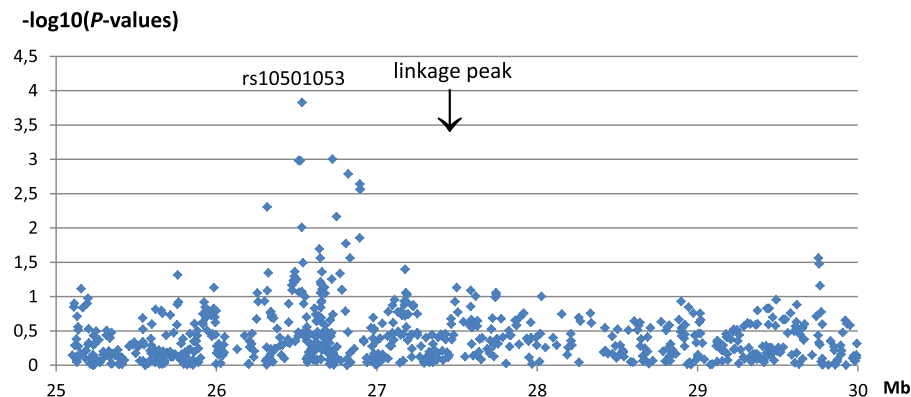


FIG 1. Plot of FBAT results (expressed as $-\log_{10}[P \text{ values}]$) in EGEA families using 394 genotyped SNPs in the 11p14 linkage region (25-30 Mb from pter). The linkage peak is located at 27.5 Mb. The name of the most significant SNP (rs10501053) is indicated.

TABLE III. Association tests for eczema in the EGEA sample by using genotyped SNPs

SNP*	Position†	Minor/ major allele	Frequency of minor allele	FBAT		Logistic regression analysis				
				Z	P value	Z	P value	OR‡	ORI	ORu
rs2703422	26 517 496	T/C	0.28	-3.28	.0010	-2.19	.029	0.73	0.55	0.97
rs2703404	26 524 212	C/T	0.28	-3.28	.0010	-2.19	.029	0.73	0.55	0.97
rs293969	26 532 067	T/C	0.23	1.71	.0900	0.54	.591	1.07	0.83	1.38
rs11029614	26 532 469	T/G	0.35	-2.58	.0090	-2.80	.005	0.69	0.53	0.89
rs12418975	26 532 767	G/A	0.49	-1.73	.0800	-2.09	.037	0.78	0.62	0.99
rs10501053	26 533 301	C/T	0.25	-3.79	.0001	-2.96	.003	0.64	0.47	0.86
rs17309195	26 536 696	A/G	0.14	0.95	.3400	0.89	.375	0.87	0.64	1.18
rs423207	26 539 637	G/A	0.25	-2.15	.0300	-2.92	.004	0.66	0.50	0.87
rs15783	26 543 377	G/A	0.34	-1.53	.1300	-2.84	.005	0.68	0.52	0.89
rs2063278	26 548 553	G/A	0.31	0.88	>.10	0.18	.861	1.02	0.79	1.32

ORI, Lower bound of the 95% CI of the OR; ORu, upper bound of the 95% CI of the OR; Z, FBAT test statistic.

*The SNPs span 15 kb on each side of rs10501053, the SNP significantly associated with eczema by using FBAT in the EGEA sample.

†The position is expressed in base pairs (dbSNP, build 36.3).

‡ORs were computed by using the minor allele as the risk allele.

patients with eczema (25%) was at least 2.8-fold lower than that observed in the asthma-ascertained samples (70% to 86%).

Association results of FBAT analysis in EGEA families showed association of eczema with SNPs in the vicinity of the linkage peak (Fig 1 and Table III), with 3 SNPs having a P value of .001 or less and one of them (rs10501053) reaching the multiple testing-corrected threshold ($P = .0001$). This SNP is located at 26.53 Mb from pter (1 Mb from the linkage peak). We further verified that this SNP accounted for most of the linkage signal because the lod score for linkage was equal to 2.42 in sibships with the risk allele of rs10501053, whereas it decreased to 0.25 in the remaining sibships.

Logistic regression analysis of genotyped SNPs spanning 15 kb on each side of rs10501053 supported the FBAT results (Table III). All SNPs showed the same direction of effect, and 4 SNPs had P values of .005 or less, including rs10501053 ($P = .003$) and the most significant SNP, rs15783 ($P = 1.6 \times 10^{-3}$, Table III). EGEA regression analysis based on both genotyped and imputed SNPs showed good agreement between results obtained from imputed allele dosage and original genotypes (Tables III and IV) plus additional association signals (4 imputed SNPs had $P \leq .004$, Table IV). EGEA findings were replicated in SLSJ families, in which 3 SNPs had P values of less than or equal to .005, including rs10501053 ($P = .003$) and rs15783 ($P = .004$).

In MRCA families an association signal ($P = .07$) was observed at SNP rs293974 near rs15783. Logistic regression of the pooled EGEA, SLSJ, and MRCA samples showed strong improvement in the evidence for association, with a P value of 6×10^{-6} for rs293974, a P value of 3×10^{-5} for rs10501053, and a P value of 8×10^{-5} for rs15783 (Table IV and Fig 2).²⁴ These 3 SNPs had minor allele frequencies ranging between 0.25 and 0.37, and their pairwise LD coefficient (r^2) was between 0.6 and 0.8 (see Fig E1 in this article's Online Repository at www.jacionline.org). The pooled odds ratios (ORs) were 0.66 (95% CI, 0.55-0.79) for rs293974, 0.68 (95% CI, 0.57-0.82) for rs10501053, and 0.70 (95% CI, 0.59-0.84) for rs15783. All 3 SNPs are located within the anoctamin 3 gene (*ANO3*), but rs15783 and rs293974 belong to the part of *ANO3* that overlaps with the mucin 15 gene (*MUC15*; Fig 2). Stepwise regression in the region of interest showed significant association with a single SNP (rs293974, $P = 6 \times 10^{-6}$).

In the asthma-ascertained family samples association analyses were repeated in siblings with and without asthma separately, but these analyses did not show a relationship between the association of 11p14 SNPs with eczema, and the presence of asthma. FBAT analysis showed a decrease in evidence for association with eczema in siblings with asthma ($P = .01$ instead of .0001 in the whole sample for rs10501053), which is in agreement with a

TABLE IV. Logistic regression analysis of eczema in each of the 3 asthma-ascertained samples and in the pooled sample by using genotyped and imputed SNPs

SNP*	Position†	Minor/ major allele	Frequency of minor allele	EGEA		SLSJ		MRCA		Pooled sample				
				Z	P value	Z	P value	Z	P value	OR‡	ORI	ORu	Z	P value
rs2703422	26 517 496	T/C	0.33	-2.19	.028	-2.52	.012	0.07	.945	0.76	0.63	0.91	-3.03	.002
rs398314	26 517 526	A/C	0.26	-2.19	.028	-2.52	.012	0.07	.945	0.76	0.63	0.91	-3.03	.002
rs11823219	26 518 069	A/T	0.25	-2.46	.014	-2.56	.010	0.14	.892	0.74	0.61	0.89	-3.20	.001
rs420143	26 520 228	T/G	0.26	-2.19	.028	-2.52	.012	0.07	.942	0.76	0.63	0.91	-3.03	.002
rs2703404	26 524 212	C/T	0.33	-2.19	.028	-2.52	.012	0.08	.940	0.76	0.63	0.91	-3.03	.002
rs2663168	26 525 542	A/G	0.30	-2.94	.003	-2.73	.006	0.28	.780	0.72	0.61	0.87	-3.52	4.10 ⁻⁴
rs440645	26 527 384	T/C	0.26	-3.02	.002	-2.29	.022	0.12	.901	0.70	0.57	0.86	-3.34	.001
rs293969	26 532 067	T/C	0.22	0.53	.596	-0.47	.635	0.99	.323	0.96	0.81	1.15	-0.44	.661
rs11029614	26 532 469	T/G	0.37	-2.78	.005	-2.53	.011	-0.54	.591	0.72	0.61	0.86	-3.67	2.10 ⁻⁴
rs12418975	26 532 767	G/A	0.49	-2.09	.036	-2.98	.003	0.35	.728	0.77	0.66	0.90	-3.20	.001
rs10501053	26 533 301	C/T	0.25	-3.00	.003	-3.00	.003	-0.69	.493	0.68	0.57	0.82	-4.17	3.10 ⁻⁵
rs17243440	26 533 544	C/T	0.22	-2.62	.009	-2.74	.006	-0.76	.449	0.70	0.58	0.84	-3.74	2.10 ⁻⁴
rs17309195	26 536 696	A/G	0.12	0.96	.337	-0.41	.679	1.21	.225	1.10	0.88	1.39	0.86	.389
rs17243454	26 538 243	G/A	0.22	-2.70	.007	-2.68	.007	-0.83	.406	0.70	0.58	0.84	-3.79	2.10 ⁻⁴
rs423207	26 539 637	G/A	0.28	-2.91	.004	-2.67	.008	-0.23	.817	0.72	0.61	0.86	-3.71	2.10 ⁻⁴
rs15783	26 543 377	G/A	0.37	-3.16	.002	-2.86	.004	-0.17	.866	0.70	0.59	0.84	-3.94	8.10 ⁻⁵
rs293980	26 543 709	T/C	0.26	-2.88	.004	-2.24	.025	-0.22	.826	0.74	0.62	0.88	-3.43	6.10 ⁻⁴
rs293974	26 546 702	A/G	0.26	-3.23	.001	-2.67	.007	-1.81	.071	0.66	0.55	0.79	-4.53	6.10 ⁻⁶
rs2063278	26 548 553	G/A	0.33	0.19	.851	-0.70	.486	-0.19	.848	0.96	0.81	1.13	-0.50	.617

ORI, Lower bound of the 95% CI of the OR; ORu, upper bound of the 95% CI of the OR; Z, FBAT test statistic.

*The SNPs span 15 kb on each side of rs1050105, the SNP significantly associated with eczema by using FBAT in EGEA sample.

†The position is expressed in base pairs (dbSNP, build 36.3).

‡ORs were computed by using the minor allele as the risk allele.

reduction in sample size. FBAT analysis in sibs without asthma was not possible because of the small sample size. Regression analysis showed that the 11p14 association with eczema did not depend on the sibs' asthma affection status, both in EGEA ($P = .04$, $.05$, and $.01$ in sibs with asthma vs $P = .01$, $.02$, and $.06$ in sibs without asthma for rs10501053, rs15783, and rs293974, respectively) and in the pooled EGEA, SLSJ, and MRCA samples ($P = .0002$, $.002$, and $.0001$ in sibs with asthma vs $P = .02$, $.02$, and $.02$ in sibs without asthma for rs10501053, rs15783, and rs293974, respectively). Although the association was stronger in siblings with asthma than those without asthma in the pooled sample, which can be partly explained by the much higher proportion of sibs with eczema among those with asthma ($592/985 = 60\%$) than among those without asthma ($151/678 = 22\%$), the association signal still remained in sibs without asthma. Moreover, there was homogeneity of the ORs associated with eczema in sibs with and without asthma (eg, for rs10501053, the ORs were equal to 0.66 [95% CI, 0.52-0.82] and 0.68 [95% CI, 0.50-0.92], respectively).

Finally, the 2 eczema-ascertained German samples did not show any association signal (see Table E2 in this article's Online Repository at www.jacionline.org).

DISCUSSION

This study identified genetic variants in *ANO3* and *MUC15* genes associated with eczema in families ascertained through subjects with asthma. These variants are located near the linkage peak on chromosome 11p14 previously reported in the French EGEA families. Evidence for this association rests on the significant results obtained from the large EGEA discovery family sample and replication in French Canadian families plus strong improvement in statistical significance when combining these

2 samples with another dataset from the UK. Replication of the results was obtained not only across samples but also by use of different statistical methods. Logistic regression analysis provided a validation test of the primary FBAT analysis in EGEA because these 2 methods use different types of information. Our results are thus strengthened by replication at both the population and statistical methodology levels.

The stronger replication of EGEA results in the French Canadian SLSJ dataset than in the UK MRCA sample might be partly explained by the shared French genetic background of the EGEA and SLSJ families. Another explanation might be the reduced power in MRCA, which had a sample size 30% to 40% smaller than the EGEA and SLSJ samples.

Evidence for association with eczema was only found in families ascertained through asthma, whereas it was lacking in German samples ascertained through eczema. The most striking phenotypic difference between these 2 types of samples was the frequency of asthma that was much higher in the asthma-ascertained samples than in the eczema-ascertained samples. However, we found that the association with eczema was not related to the presence of asthma in the asthma-ascertained samples. Furthermore, we verified that there was no indication of association of 11p14 SNPs with asthma, either in the 3 asthma-ascertained samples (results not shown) or in any published asthma GWAS (see Ober and Yao³). Thus the association detected here appears specific to eczema, but how it might be related to the mode of ascertainment of families through asthma remains unclear. We hypothesized that the ascertainment of samples through subjects with asthma might have induced the presence of genetic factors, environmental factors, or both running in EGEA, SLSJ, and MRCA families and interacting with the 11p14 variants and thus made it possible to detect that locus. Moreover, a recent epidemiologic study demonstrated an association of eczema with

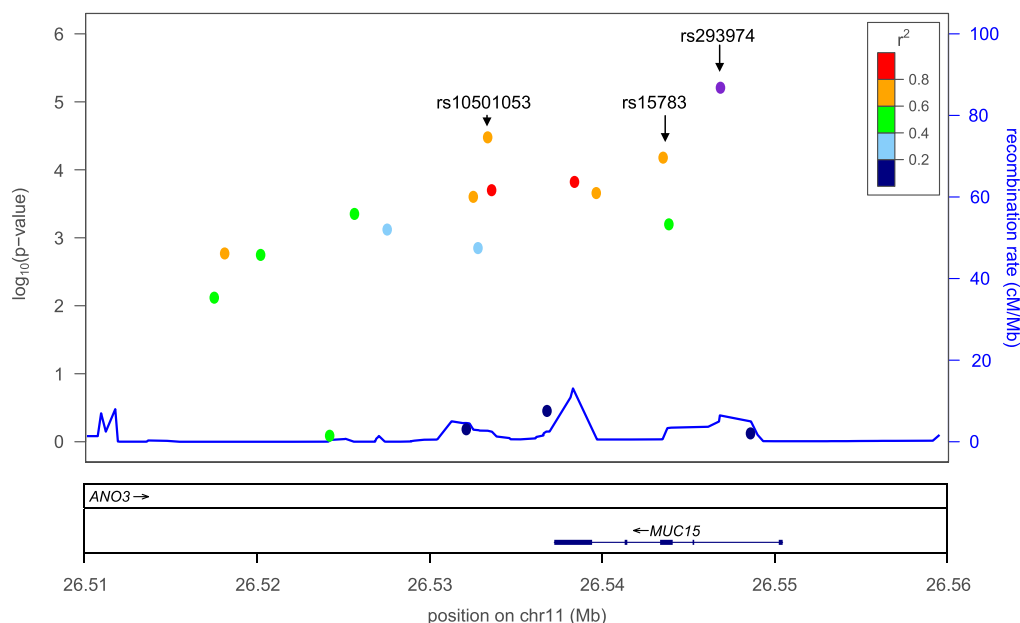


FIG 2. Outcomes of tests of association of eczema with SNPs spanning a 30-kb region on 11p14 (as defined in the Methods section) using logistic regression in the pooled asthma-ascertained family samples (EGEA, SLSJ, and MRCA). The $\log_{10} P$ values are from the Wald tests for imputed SNPs by using allele dosage, as indicated on the left vertical axis. The most significant SNP is colored purple, and the degree of LD between that SNP and the others is indicated by color according to the key (red being the highest degree of LD). The names of the 3 most significant SNPs (rs293974, rs10501053, and rs15783) are shown. The estimated recombination rate is shown by the blue line and indicated on the right vertical axis. The genes in the region and their positions are given underneath the graph. The horizontal line under the name of the gene represents introns (thin parts) and exons (thick parts). The whole *MUC15* gene lies within the *ANO3* intron. The transcribed strand is indicated with an arrow showing that *MUC15* is transcribed from the opposite strand of *ANO3*. Plots were produced with LocusZoom.²⁴

specific types of asthma, which supports the idea that the recruitment scheme of families through probands with asthma might have indeed selected for a specific subgroup of patients with eczema.²⁵

One of the 3 SNPs showing strongest association (rs10501053) is located in the *ANO3* gene, whereas the other 2 SNPs (rs15783 and rs293974) belong to the 2 overlapping *ANO3* and *MUC15* genes. The SNP rs15783 is the only nonsynonymous SNP; it is intronic with respect to *ANO3* (intron 13 of NM_031418.2 transcript), whereas it is exonic regarding *MUC15* and corresponds to a missense substitution (Thr > Ile) at position 229 of *MUC15* isoform b. Although stepwise regression analysis of the pooled data showed statistically significant evidence for the effect of rs293974, this does not make that SNP more likely to be a potential causal variant than the other 2 variants because it is well known that association studies can only pinpoint a locus of interest but need to be further followed up to characterize the causal variants.

The *ANO3* gene (also known as *TMEM16C*) belongs to a gene family encoding proteins that are involved in calcium-activated chloride channels, which perform many important functions in cell physiology, including secretion of fluids from acinar cells of secretory glands, amplification of olfactory transduction, regulation of cardiac and neuronal excitability, and regulation of vascular tone.²⁶ A study of expression of *TMEM16* paralogs during murine embryogenesis detected *TMEM16C* expression in the suprabasal layers of the skin.²⁷

The *MUC15* gene belongs to the family of mucin genes, which encode large epithelial glycoproteins that are major constituents

of mucus covering the surfaces of epithelial tissues and provide a physical barrier protecting the underlying epithelium. Mucins have emerged as important molecules in promoting or protecting against inflammation and cancer. They have recently been shown to play a role in both benign and malignant skin conditions.²⁸ *MUC15* was found to be overexpressed in patients with metastatic melanoma,^{28,29} and structural motifs in *MUC15* have been identified as being epidermal growth factor receptor.²⁹ Interestingly, a recent study of gene expression in canine AD has shown a significant increase in *MUC15* expression in lesional skin from dogs with AD when compared with that seen in healthy control animals.³⁰ All these findings make *MUC15* a strong candidate for eczema. It should be noted that variants of another mucin gene, *MUC5B*, which is located on 11p15, were recently found to be associated with pulmonary fibrosis through a positional cloning study, as in the present study.³¹ Additionally, 2 other mucin genes, *MUC5AC* and *MUC2*, which are tightly linked to *MUC5B* on 11p15, have been found to be differently expressed in bronchial cells of asthmatic versus nonasthmatic subjects.³² Further exploration of *MUC5B* in the susceptibility to other lung diseases, including asthma, and the study of a possible relationship of *MUC15* with other mucin genes, including *MUC5B*, *MUC5AC*, and *MUC2*, in the susceptibility to asthma and eczema is warranted.

In conclusion, the present study highlights that positional cloning represents a powerful alternative strategy to GWASs to identify new genes in multifactorial diseases. It also shows that the mode of ascertainment of the datasets is a critical feature to facilitate the identification of genes that are involved in complex

networks underlying associated diseases. Further exploration of the 11p14 locus is needed to bring better insight into the role of this locus in eczema and its relationship with respect to allergic asthma.

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Key messages

- Positional cloning is a powerful alternative strategy to GWASs to identify disease genes.
- Well-defined phenotypes and the mode of ascertainment of the datasets are critical features for gene identification.

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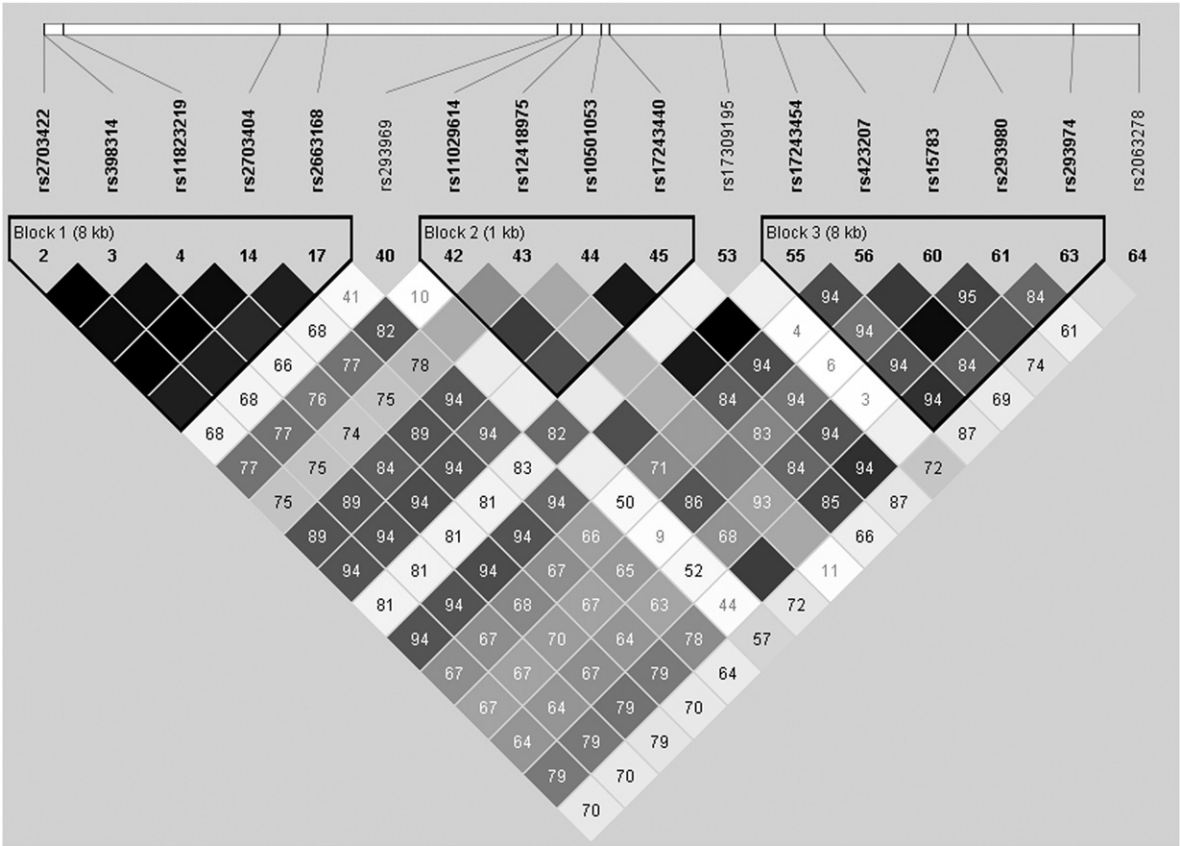


FIG E1. Pairwise LD r^2 coefficient (estimated from HapMap CEU population) among SNPs in the eczema-associated region (15 kb on each side of rs10501053).

TABLE E1. Distribution of families according to the number of genotyped offspring affected with eczema in the EGEA sample

	No. of families with n affected and genotyped sibs				
	n = 1	n = 2	n = 3	n = 4	n = 5
No.	150	39	6	1	1

TABLE E2. Association analysis of eczema by using the genotyped and imputed SNPs in the 2 German replication samples (family and case-control samples)

SNP*	Position†	Minor/ major allele	Frequency of minor allele	FBAT (270 families)		Logistic regression (915 cases vs 975 control subjects)				
				Z	P value	OR‡	ORI	ORu	Z	P value
rs2703422	26 517 496	T/C	0.30	−1.56	.119	0.74	0.84	1.13	−0.30	.762
rs398314	26 517 526	A/C	0.30	−1.47	.141	0.73	0.84	1.13	−0.31	.757
rs11823219	26 518 069	A/T	0.30	−1.47	.141	0.76	0.84	1.14	−0.28	.778
rs420143	26 520 228	T/G	0.30	−1.56	.119	0.79	0.85	1.14	−0.23	.823
rs2703404	26 524 212	C/T	0.30	−1.56	.119	0.90	0.85	1.15	−0.11	.913
rs2663168	26 525 542	A/G	0.33	−0.67	.504	1.04	0.87	1.16	0.04	.969
rs440645	26 527 384	T/C	0.32	−1.24	.215	0.75	0.82	1.16	−0.29	.772
rs293969	26 532 067	T/C	—	—	—	—	—	—	—	—
rs11029614	26 532 469	T/G	—	—	—	—	—	—	—	—
rs12418975	26 532 767	G/A	—	—	—	—	—	—	—	—
rs10501053	26 533 301	C/T	0.26	−0.84	.401	1.01	0.87	1.15	0.12	.909
rs17243440	26 533 544	C/T	0.24	−0.68	.495	1.01	0.86	1.16	0.12	.905
rs17309195	26 536 696	A/G	—	—	—	—	—	—	—	—
rs17243454	26 538 243	G/A	0.24	−0.68	.499	1.04	0.89	1.22	0.5	.610
rs423207	26 539 637	G/A	0.26	−0.28	.779	1.06	0.91	1.23	0.71	.471
rs15783	26 543 377	G/A	0.34	−1.12	.263	0.98	0.85	1.14	0.24	.805
rs293980	26 543 709	T/C	0.29	0.19	.848	1.06	0.92	1.23	0.79	.431
rs293974	26 546 702	A/G	0.27	−1.60	.110	1.05	0.91	1.21	0.62	.539
rs2063278	26 548 553	G/A	—	—	—	—	—	—	—	—

—, No result is shown for those SNPs because the imputed data did not fulfill either of the following QC criteria: (1) average maximum posterior probability of a given genotype of 0.9 or greater when using the best-imputed genotype in FBAT analysis or (2) quality score (rsq) of 0.9 or greater when using the imputed allele dosage in logistic regression analysis.

ORI, Lower bound of the 95% CI of the OR; ORu, upper bound of the 95% CI of the OR; Z, FBAT test statistic.

*The SNPs span 15 kb on each side of rs1050105, the SNP significantly associated with eczema by using FBAT in the EGEA sample.

†The position is expressed in base pairs (dbSNP, build 36.3).

‡ORs were computed by using the minor allele as the risk allele.