

Functional variants of 17q12-21 are associated with allergic asthma but not allergic rhinitis

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Background: Allergic rhinitis (AR) and asthma are common allergic conditions with a shared genetic component to their cause. The 17q12-21 locus includes several genes that have been linked to asthma susceptibility, but the role of this locus in AR is unclear. Asthma and AR in adults of Chinese ethnicity in Singapore are predominately caused by sensitization against house dust mites with a nearly complete penetrance of the allergen, which presents a unique opportunity for accurately identifying genetic associations with allergic diseases.

Objective: We sought to define the functional role of 17q12-21 in patients with AR and allergic asthma.

Methods: We asked whether single nucleotide polymorphisms (SNPs) in the 17q12-21 locus were associated with AR or asthma in a cohort of 3460 ethnic Chinese subjects residing in Singapore (1435 in the discovery phase and 2025 in the validation phase). Full-blood mRNA gene expression data, plasma IgE levels, and immune cell frequencies in peripheral blood were tested against the tag SNP genotypes. Luciferase assays were used to measure the effect of putative promoter SNPs on expression of the asthma-associated orosomucoid-like 3 gene (*ORMDL3*).

Results: Within 17q12-21, only the tag SNP rs8076131 was significantly associated with asthma ($P = 8.53 \times 10^{-10}$; odds ratio, 0.6715), and AR status was independent of SNPs in this region. C-A alleles at rs8076131 resulted in significantly increased *ORMDL3* expression in HEK293 cells *in vitro* relative to T-G alleles. Moreover, subjects with the risk genotype AA exhibited significantly higher total IgE levels and higher blood eosinophil counts than those with the lower-risk genotypes.

Conclusion: The 17q12-21 locus has a strong genetic association with allergic asthma but not with AR. The polymorphic effect of this locus is attributed to the linkage set tagged by rs8076131, which affects the expression of *ORMDL3*, protein phosphatase 1, regulatory inhibitor subunit 1B (*PPP1R1B*), zona pellucida binding protein 2 (*ZPBP2*), and gasdermin B (*GSDMB*) and is correlated with high IgE levels and eosinophil counts in subjects bearing the risk genotype. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *ORMDL3, 17q21, allergic asthma, allergic rhinitis, Singapore Chinese, IgE, eosinophil counts*

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In recent decades, the prevalence of allergic disease has increased steeply across the globe.¹⁻⁴ Environmental changes are an important contributor to this increase,⁵ but the high heritability of allergic conditions also indicates a strong genetic component to their cause.^{6,7} The common co-occurrence of allergic rhinitis (AR) and asthma in the same subject further suggests the existence of shared susceptibility loci⁸; one candidate is chromosome region 17q12-21, a 300-kb stretch of the genome encompassing 12 genes, including the asthma genes of interest orosomucoid-like 3 gene (*ORMDL3*), gasdermin B (*GSDMB*), IKAROS family zinc finger 3 (Aiolos [*IKZF3*]), and zona pellucida binding protein 2 (*ZBPBP2*; Fig 1). These genes have wide-ranging functions and have been implicated in various aspects of the pathogenesis of asthma and allergy: Orm-like proteins (*ORMDL1*, *ORMDL3*, and *ORMDL4*) can mediate sphingolipid homeostasis, which is thought to be dysregulated in asthmatic patients^{9,10}; *GSDMB* is a member of the gasdermin (*Gsdm*) family of genes, which are expressed in epithelia and have been linked with stem cell proliferation^{11,12}; *IKZF3* belongs to the Ikaros family of zinc-finger proteins, which are transcription factors regulating lymphocyte development¹³; and *ZBPBP2* encodes a secreted-type glycoprotein expressed in germ cell tumors, the testis, and brain medulla.¹⁴ The 17q21 locus containing these genes was first linked to pediatric asthma susceptibility by Moffatt et al¹⁵ and has since been confirmed as an asthma-associated locus in various other populations.¹⁶⁻¹⁸ Although some reports have suggested that 17q12-21 is also a susceptibility locus for AR,¹⁹⁻²¹ others have been unable to confirm the association.²²⁻²⁴ This study investigates the role of 17q21 in asthma and AR for the Chinese population.

Abbreviations used

AR:	Allergic rhinitis
eQTL:	Expression quantitative trait loci
GSDMB:	Gasdermin B
HDM:	House dust mite
IKZF3:	IKAROS family zinc finger 3 (Aiolos)
OR:	Odds ratio
ORMDL3:	Orosomucoid-like 3 gene
SNP:	Single nucleotide polymorphism
SPT:	Skin prick test
ZBP2:	Zona pellucida binding protein 2

The majority of cohorts used in genome-wide association studies of allergic conditions unavoidably have high rates of false-negative results because of the diversity of allergens and the heterogeneity of individual responses. This effect can obscure true associations and make accurate interpretation of the results challenging. We previously showed that in the tropical urban environment of Singapore, the typically complex allergen sensitization profile is reduced to monosensitization by house dust mite (HDM).²⁵ Skin prick tests (SPTs) with the 12 most common allergens revealed that 98% of subjects with positive SPT responses react to HDM, with two thirds of the cohort reacting exclusively to this allergen. Moreover, because of a strong environmental penetrance, nearly 80% of the Chinese ethnicity population responds to HDM sensitization, which is associated with a prevalence of AR and asthma of about 40% and 15%, respectively.²⁵ This clearly defined population and environment thus represents a unique framework within which to accurately define the role of genetic polymorphisms in patients with allergic disease. Here we conducted a cohort-based correlation study on approximately 3500 adults of Chinese ethnicity residing in Singapore to reveal the true role of 17q12-21 in susceptibility to AR and asthma.

METHODS**Samples**

The samples used in this study were collected with approval of the appropriate institutional review boards in Singapore. Recruitment was performed in compliance with the Helsinki Declaration. In addition, parental/guardian consent was obtained for all participants less than 21 years of age.²² We used a 2-stage design of discovery and validation, both of which were part of an ongoing epidemiologic study on allergies. All samples were collected by using the same study protocol and in the same ethnic Chinese population in Singapore. Demographics of the samples are provided in Table 1. Volunteers were of Chinese ethnicity and resident in Singapore, and DNA was extracted from mouthwash or whole blood. The ethnicity of the donors was self-reported; however, their Chinese ethnicity was confirmed previously by using principal component analysis because it was similar to the Han Chinese (CHB) ethnicity from the HapMap project.^{22,26} All participants completed an International Study of Asthma and Allergies in Childhood/Allergic Rhinitis and its Impact on Asthma-based questionnaire for allergy and underwent SPTs to determine their sensitization to a panel of allergens commonly found in Singapore, including the HDM allergens from *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, as well as *Elaeis guineensis* and *Curvularia lunata*. An SPT response was considered positive when a wheal of at least 3 mm in diameter was observed 15 minutes after the skin prick. Histamine and saline were used as positive and negative controls, respectively. The AR diagnostic criteria used were 2 or more self-reported symptoms of AR (nasal congestion, rhinorrhea, nasal itching, and sneezing) persisting for 4 or more days a week accompanied by a positive

SPT response for HDM.^{22,27} Allergic asthma was defined based on a self-reported doctor's diagnosis of asthma and a positive SPT response for HDM.^{28,29} ImmunoCAP was used to measure total IgE levels in plasma samples from volunteers.²⁵ Gene expression within the 17q12-21 locus was evaluated by using whole-blood expression quantitative trait loci (eQTL) estimated from a cohort in Singapore. Data were available for a subset of the genotype samples.^{25,28} The various cohorts used in the study are described in Fig E1 in this article's Online Repository at www.jacionline.org.

Tag single nucleotide polymorphism selection

A total of 10 tag single nucleotide polymorphisms (SNPs) were selected to tag the chromosome 17q12-21 locus, with a minor allele frequency of 5% using the Hap Map Chinese Han in Beijing (CHB) population. A linkage disequilibrium threshold of an r^2 value of 0.8 was used to tag SNPs for association analysis by using the tagger algorithm.³⁰

Genotyping

The amount of DNA isolated from patients' samples was measured in triplicate on a NanoDrop (ND 1000; Thermo Scientific, Wilmington, Del) before use. Genotyping for the discovery phase was performed on purified DNA samples by using the Illumina Bead Xpress Assay (Illumina, San Diego, Calif), according to the manufacturer's recommendations, at the University of Utah Genomics Core Facility (Salt Lake City, Utah). In the validation phase samples were genotyped with the Sequenom platform with MassARRAY and iPLEX technology (Sequenom, San Diego, Calif). Oligonucleotides were designed according to Sequenom guidelines by using MassARRAY Assay Design software. Amplicons containing SNPs of interest underwent multiplex PCR, followed by primer extension reactions. Data were analyzed with Sequenom TYPER software. Extent of clustering of genotype calls was used to select samples for inclusion in the statistical analysis.

Luciferase assay in HEK293T cells

Human embryonic kidney cells (HEK293T) were purchased from the American Type Culture Collection (ATCC, Manassas, Va) and grown in RPMI-1640 medium (Sigma-Aldrich, Singapore) with 2 g/L sodium bicarbonate, 2 mmol/L L-glutamine, and 10% FBS. Cells were grown at 37°C in a 5% CO₂ atmosphere in air in a humidified incubator.

The haplotype effect of selected SNPs on *ORMDL3* gene expression was measured by using the luciferase assay. The region spanning +1906 bp to +3392 bp of *ORMDL3* was cloned into a promoter-less pGL4.10 vector containing a firefly luciferase reporter gene (Promega, Singapore). Plasmid constructs were transiently transfected into HEK293T cells by using Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen, Singapore). Cotransfection with a renilla luciferase construct with HSV-TK promoter (plasmid pGL4.74) was used to control for variations in transfection efficiency; accordingly, the firefly luciferase reading was normalized against that of renilla luciferase. All experiments were performed in triplicate, with the luciferase reading measured at 24 or 48 hours after transfection. An independent samples *t* test was used to calculate the *P* value of the average difference in gene expression level induced by each allele of the SNP.

Whole-blood eQTL data: Singapore Chinese

We analyzed whole-blood gene expression data from 71 Chinese ethnicity volunteers in the context of their whole-genome SNP profile (data from another ongoing study). mRNA was extracted from whole blood collected into tempus RNA tubes (Life Technologies, Carlsbad, Calif), and transcript abundance was measured by using the Illumina HumanHT-12-v4 Expression Bead Chip (Illumina, San Diego, Calif). The Illumina Human Omni5Quad chip was used to determine the genome-wide SNP profile. Only Illumina probes free of any SNPs were used to determine the expression level of the genes to avoid allele-specific artifacts. Probes used for analysis included the following: ILMN_1657095 (StAR-related lipid transfer [START] domain containing 3 [*STARD3*]), ILMN_1662174 (*ORMDL* sphingolipid biosynthesis regulator 3

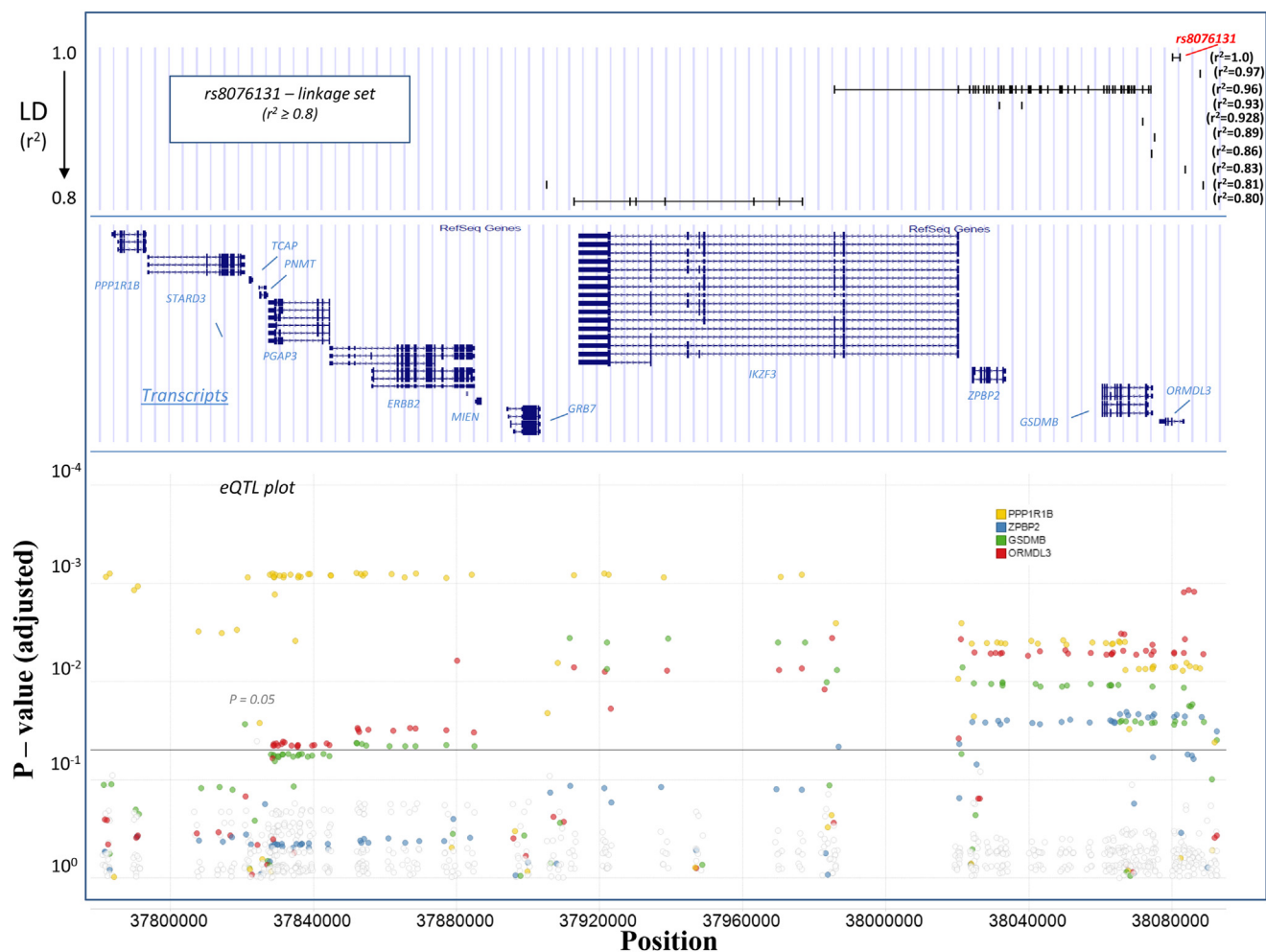


FIG 1. Chromosomal arrangement of genes in the 17q region with corresponding eQTL *P* values from whole-blood mRNA analysis of 71 Singapore Chinese subjects and linkage disequilibrium (LD) plots generated by using ArchiLD software.

TABLE I. Demographics of the Singapore Chinese population

Phenotype	Discovery cohort			Validation cohort	
	Sex	Count	Age (y), median (minimum-maximum)	Age (y), median (minimum-maximum)	Count
Patients with allergic asthma*	Missing	3	—	—	3
	F	339	20 (8.00-57.00)	20 (6.00-46.00)	233
	M	322	22 (6.00-35.00)	21 (6.00-31.00)	335
	Total	718‡	21 (6.00-57.00)	21 (6.00-46.00)	571‡
Patients with AR†	Missing	1	—	—	4
	F	410	21 (8.00-39.00)	20 (6.00-52.00)	569
	M	384	22 (6.00-42.00)	21 (6.00-74.00)	559
	Total	795‡	21 (6.00-42.00)	21 (6.00-74.00)	1132‡
Control subjects§	Missing	—	—	—	3
	F	520	21 (19.00-59.00)	21 (18.00-63.00)	410
	M	197	22 (20.00-56.00)	22 (19.00-56.00)	192
	Total	717	21 (18.00-59.00)	21 (18.00-63.00)	603
	Total (unique)	1893	22 (6.00-59.00)	21 (6.00-74.00)	1948

F, Female; M, male.

*Patients with allergic asthma were classified based on a self-reported doctor's diagnosis of asthma with a positive SPT response to one of the allergens tested.

†Patients with AR were defined by 2 or more self-reported rhinitis symptoms and a positive SPT response.

‡A total of 337 samples in the discovery cohort and 358 samples in the validation cohort were from patients with both AR and asthma.

§Control subjects are healthy subjects classified based on absence of symptoms, a history of allergic disease, and a negative SPT response. There were 1320 total control subjects.

TABLE II. Association of tag SNPs in the chromosome 17q21 locus with the allergic asthma phenotype

Chromosome	SNP	BP	Minor allele	MAF in cases	MAF in control subjects	Major allele	CHISQ	P value	P _{cond}	OR	SE	L95	U95	P _{HWE}
17	rs8076131	38,080,912	G	0.2144	0.2787	A	15.93	6.58E-05*	Reference	0.7062	0.08735	0.5951	0.8381	.3746
17	rs2941504	37,830,900	G	0.3312	0.3964	A	13.06	.0003*	1.41E-01	0.7543	0.07811	0.6472	0.8791	.3517
17	rs1058808	37,884,037	C	0.3252	0.3891	G	11.54	.00068*	2.22E-01	0.7565	0.08222	0.6439	0.8888	.6511
17	rs1877031	37,814,080	T	0.3463	0.4007	C	8.843	.0029*	4.61E-01	0.7921	0.07841	0.6793	0.9237	.7378
17	rs876493	37,824,545	T	0.2662	0.3054	C	5.247	.022†	5.34E-01	0.8252	0.08391	0.7001	0.9728	.2844
17	rs1136201	37,879,588	G	0.09687	0.1225	A	4.76	.029†	8.27E-01	0.768	0.1212	0.6056	0.974	.07903
17	rs17608925	38,082,831	C	0.06494	0.08438	T	3.917	.048†	8.93E-01	0.7537	0.1433	0.5692	0.998	.2119
17	rs12601930	37,789,432	T	0.1882	0.1794	C	0.3657	.5454	7.67E-01	1.061	0.09746	0.8763	1.284	.2136
17	rs9532	37,792,613	T	0.00071	0.000705	C	0.0000	.996	9.02E-01	1.007	1.415	0.06293	16.12	1

BP, Base pair on chromosome 17; CHISQ, association statistic χ^2 value for the tag SNP tested for allergic asthma; L95, lower confidence at 95%; MAF, minor allele frequency; Major allele, other allele for the tag SNP; Minor allele, minor allele for the tag SNP for which the association statistics have been calculated; OR, OR for the tag SNP; P, P value for the tag SNP tested for allergic asthma; P_{cond}, P value for the tag SNP conditional to the top associated SNP rs8076131 for allergic asthma association; P_{HWE}, Hardy-Weinberg equilibrium P value for the tag SNP tested in the entire population; SNP, tag SNP tested for association with allergic asthma; U95, upper confidence at 95%.

*P < .005 (Bonferroni-corrected P value).

†P < .05 (nominal P value for association).

[ORMDL3]), ILMN_1666206 (gasdermin B [GSDMB]), ILMN_1669692 (IKAROS family zinc finger 3 [IKZF3]), ILMN_1710027 (phenylethanolamine N-methyltransferase [PNMT]), ILMN_172707 (migration and invasion enhancer 1 [MIEN1]), ILMN_1798582 (growth factor receptor-bound protein 7 [GRB7]), ILMN_1804316 (titin-cap [TCAP]), ILMN_1805636 (post-GPI attachment to proteins 3 [PGAP3]), ILMN_2304495 (protein phosphatase 1, regulatory inhibitor subunit 1B [PPP1R1B]), ILMN_2352131 (Erb-B2 receptor tyrosine kinase 2 [ERBB2]), and ILMN_2383638 (zona pellucida binding protein 2 [ZPBP2]).

Statistical analysis

Genotypes of the tag SNPs were tested for Hardy-Weinberg equilibrium across the entire population by using the χ^2 test. The Cochran-Armitage trend test was used as the primary measure of allelic association with the disease phenotype. All P values and odds ratios (ORs) with 95% CIs were estimated by using the PLINK program (version 1.07). Meta-analysis was performed with both fixed effects and random effects by using the meta-analysis command. For association analysis with functional parameters, such as whole-blood RNA measurements and plasma quantitation for IgE values, a nonparametric Mann-Whitney test was executed in Prism software (GraphPad Software, La Jolla, Calif). Illustrative figures were also generated with Prism software.

RESULTS

The tag SNP rs8076131 within 17q12-21 is significantly associated with allergic asthma

The study was carried out with a discovery and a validation cohort of 1435 and 2025 Singapore residents of Chinese ethnicity, respectively (Table I). The median age was 21 years, and each cohort consisted of roughly equal numbers of healthy control subjects and patients with either allergic asthma or AR.

A total of 10 tag SNPs within the 17q12-21 locus were defined by using DNA samples from the discovery cohort, of which 9 were in Hardy-Weinberg equilibrium; rs2941503 did not meet the criteria and was excluded from further analysis. The association of the remaining 9 tag SNPs with allergic asthma is summarized in Table II. The strongest correlation was detected for rs8076131, where the G allele was associated with asthma at a P value of 6.58×10^{-5} and an OR of 0.7062. Six other SNPs also initially exhibited an asthma association (P < .05). However, after correction for multiple testing (Bonferroni threshold: P < .006) and conditional association (P_{cond}) against rs8076131, none of the associations of the other tag SNPs remained significant. Thus in our

Chinese ethnicity cohort only rs8076131 within 17q12-21 is significantly associated with the manifestation of allergic asthma.

This association of rs8076131 with allergic asthma was confirmed in the validation cohort (Table III). The minor allele was again the G allele, with a P value of 1.83×10^{-6} and an OR of 0.63. A fixed-model meta-analysis approach was used to combine the statistical analyses of the 2 cohorts (Table III), which revealed a combined P value of 8.53×10^{-10} at an OR of 0.6715. Similar association statistics were evident when using the random model, where the nonsignificant heterogeneity Q value at a P value of .389 confirmed the homogeneity of the discovery and validation cohorts. Therefore we can conclude that the genetic association is consistent between the 2 groups.

Tag SNPs within 17q12-21 are not associated with AR manifestation

We next investigated whether any of the 17q12-21 tag SNPs were associated with manifestation of the typical clinical symptoms of AR in either the discovery or validation cohorts.

The discovery cohort consisted of 795 patients with AR and 717 nonallergic control subjects. Because AR often co-occurs with allergic asthma, the analysis was carried out separately for the patients with AR with concurrent asthma. Within the AR patient group of the discovery cohort, 42.4% of subjects also met the asthma diagnostic criteria and were analyzed separately from the nonasthma AR group (Table IV). Neither AR group exhibited any association with the 17q12-21 tag SNPs. Although a nominal significance for the association of rs8076131 with AR and concurrent asthma (P = .0069) was initially indicated, this association was lost on Bonferroni correction (Table IV).

The validation cohort confirmed the lack of association seen in the discovery cohort. Although rs8076131 remained associated with asthma in the presence of AR (P = .00002), it was not linked with AR in the absence of asthma (P = .12, see Table E1 in this article's Online Repository at www.jacionline.org). Thus in our Chinese cohort in Singapore the 17q12-21 locus is not linked to AR.

Allelic state at rs8076131 correlates with differential expression of multiple genes within 17q12-21

17q12-21 spans a region of nearly 300,000 bp and contains a total of 12 genes (Fig 1). The tag SNP rs8076131 itself is part of a

TABLE III. Meta-analysis of the association between rs8076131 and allergic asthma phenotype

Chromosome	BP	SNP	Minor allele	Major allele	MAF	OR	SE	L95	U95	<i>P</i> _{allele}	
Discovery cohort											
17	38,080,912	rs8076131	G	A	0.24655	0.7062	0.08735	0.5951	0.8381	6.58E-05*	
Chromosome	BP	SNP	Minor allele	Major allele	MAF	OR	SE	L95	U95	<i>P</i> _{allele}	
Validation cohort											
17	38,080,912	rs8076131	G	A	0.2484	0.6311	0.09683	0.522	0.763	1.83E-06*	
Chromosome	BP	SNP	Minor allele	Major allele	No.	<i>P</i> value	<i>P</i> (R)	OR	OR(R)	Q	I
Meta-analysis											
17	38,080,912	rs8076131	G	A	2	8.53E-10*	8.53E-10	0.6715	0.6715	0.389	0

BP, Base pair; *I*, *I*² heterogeneity index (0-100); *Major allele*, other allele for rs8076131; *Minor allele*, minor allele for rs8076131 for which the association statistics have been calculated; *No.*, number of studies for the meta-analysis; *OR*, OR calculated by using the fixed effect model; *OR(R)*, OR calculated by using the random effect model; *P*_{allele}, *P* value from the allele test in the discovery and validation populations; *P*(R), *P* value calculated by using the random effect model; *P* value, *P* value calculated by using the fixed effect model; *Q*, *P* value for Cochran Q statistic.

**P*_{allele} < .05.

TABLE IV. Association of tag SNPs in the chromosome 17q21 locus to AR phenotype

Chromosome	SNP	BP	Minor allele	MAF in cases	MAF in control subjects	Major allele	CHISQ	<i>P</i> value	OR	SE	L95	U95	<i>P</i> _{HWE}
Association with AR in patients with AR with asthma against control subjects													
17	rs8076131	38,080,912	G	0.2232	0.2787	A	7.298	.0069†	0.7437	0.1098	0.5996	0.9223	.3746
17	rs2941504	37,830,900	G	0.3578	0.3964	A	2.863	.09066	0.8485	0.09717	0.7013	1.026	.3517
17	rs1058808	37,884,037	C	0.3467	0.3891	G	3.176	.07471	0.8329	0.1026	0.6812	1.019	.6511
17	rs1877031	37,814,080	T	0.3688	0.4007	C	1.892	.169	0.8739	0.098	0.7212	1.059	.7378
17	rs876493	37,824,545	T	0.2809	0.3054	C	1.278	.2583	0.8883	0.1048	0.7233	1.091	.2844
17	rs1136201	37,879,588	G	0.09697	0.1225	A	2.906	.08823	0.769	0.1544	0.5681	1.041	.07903
17	rs17608925	38,082,831	C	0.0638	0.08438	T	2.707	.09991	0.7395	0.184	0.5156	1.061	.2119
17	rs12601930	37,789,432	T	0.1982	0.1794	C	1.049	.3058	1.131	0.12	0.8938	1.43	.2136
17	rs9532	37,792,613	T	0.00152	0.00071	C	0.3073	.5794	2.15	1.415	0.1343	34.43	1
Association with AR in patients with AR without asthma against control subjects													
17	rs8076131	38,080,912	G	0.2632	0.2787	A	0.6789	.41	0.9243	0.09559	0.7663	1.115	.3746
17	rs2941504	37,830,900	G	0.3654	0.3964	A	2.254	.1333	0.877	0.08744	0.7389	1.041	.3517
17	rs1058808	37,884,037	C	0.3584	0.3891	G	2.116	.1458	0.8771	0.0902	0.7349	1.047	.6511
17	rs1877031	37,814,080	T	0.3814	0.4007	C	0.86	.3537	0.922	0.0876	0.7765	1.095	.7378
17	rs876493	37,824,545	T	0.2783	0.3054	C	1.946	.163	0.8769	0.09417	0.7291	1.055	.2844
17	rs1136201	37,879,588	G	0.11	0.1225	A	0.8337	.3612	0.8851	0.1338	0.6809	1.15	.07903
17	rs17608925	38,082,831	C	0.08315	0.08438	T	0.01096	.9166	0.9841	0.1529	0.7293	1.328	.2119
17	rs12601930	37,789,432	T	0.2051	0.1794	C	2.375	.1233	1.18	0.1077	0.9558	1.458	.2136
17	rs9532	37,792,613	T	0.00222	0.00071	C	0.976	.3232	3.149	1.225	0.2851	34.78	1

BP, Base pair on chromosome 17; *CHISQ*, association statistics χ^2 value for the tag SNP tested for allergic asthma; *L95*, lower confidence at 95%; *MAF*, effect allele frequency; *Major allele*, other allele for the tag SNP; *Minor allele*, effect allele for the tag SNP for which the association statistics have been calculated; *OR*, OR for the tag SNP; *P*_{HWE}, Hardy-Weinberg equilibrium *P* value for the tag SNP tested in the entire population; *P* value, *P* value for the tag SNP tested for allergic asthma; *SNP*, tag SNP tested for association with allergic asthma; *U95*, upper confidence at 95%.

**P* < .005 (Bonferroni-corrected *P* value).

†*P* < .05 (nominal *P* value for association).

set of 2 other perfectly linked SNPs (rs4065275 and rs12603332) located on the extreme right end of 17q12-21 proximal to the *ORMDL3* promoter region (Fig 1, upper panel). The complete set of 82 SNPs tagged to rs8076131 ($r^2 \geq 0.8$) in subjects of Chinese ethnicity extends further, covering the entire right half of 17q12-21. The entire set of SNPs with reference IDs with linkage to rs8076131 has been provided in Fig E2 in this article's Online Repository at www.jacionline.org. Of these 82, 62 are organized in a perfectly linked block that in turn is very closely linked to rs8076131 ($r^2 = 0.96$). Thus in addition to *ORMDL3*, the rs8076131 linkage set has the potential to affect expression of the genes *IKZF3*, *ZBP2*, and *GSDMB* (Fig 1, middle panel).

To determine which genes might be functionally affected by the allelic state at 17q12-21 SNPs, we carried out a genome-wide

eQTL analysis (Fig 1, lower panel). Blood samples from 71 healthy subjects of Chinese ethnicity in Singapore (unpublished data set) underwent genotyping and gene expression analysis. This revealed a significant eQTL effect of the SNPs on expression of 4 of the 12 genes within 17q12-21: *PPP1R1B*, *ZBP2*, *GSDMB*, and *ORMDL3* (Fig 1, lower panel). The expression level of each of these genes was also directly affected by the allelic state of the rs8076131 linkage set (Fig 2, A). Interestingly, this suggests that rs8076131 can affect the expression of both distal and proximal genes within 17q12-21: *PPP1R1B* ($P_{\text{adj}} = .0074$) is located at the extreme left side of the locus, whereas *ZBP2* ($P_{\text{adj}} = .023$) and *GSDMB* ($P_{\text{adj}} = .026$) are proximal to *ORMDL3* ($P_{\text{adj}} = .0051$). Thus the allelic states of the rs8076131 linkage set can affect expression levels of not only *ORMDL3* but

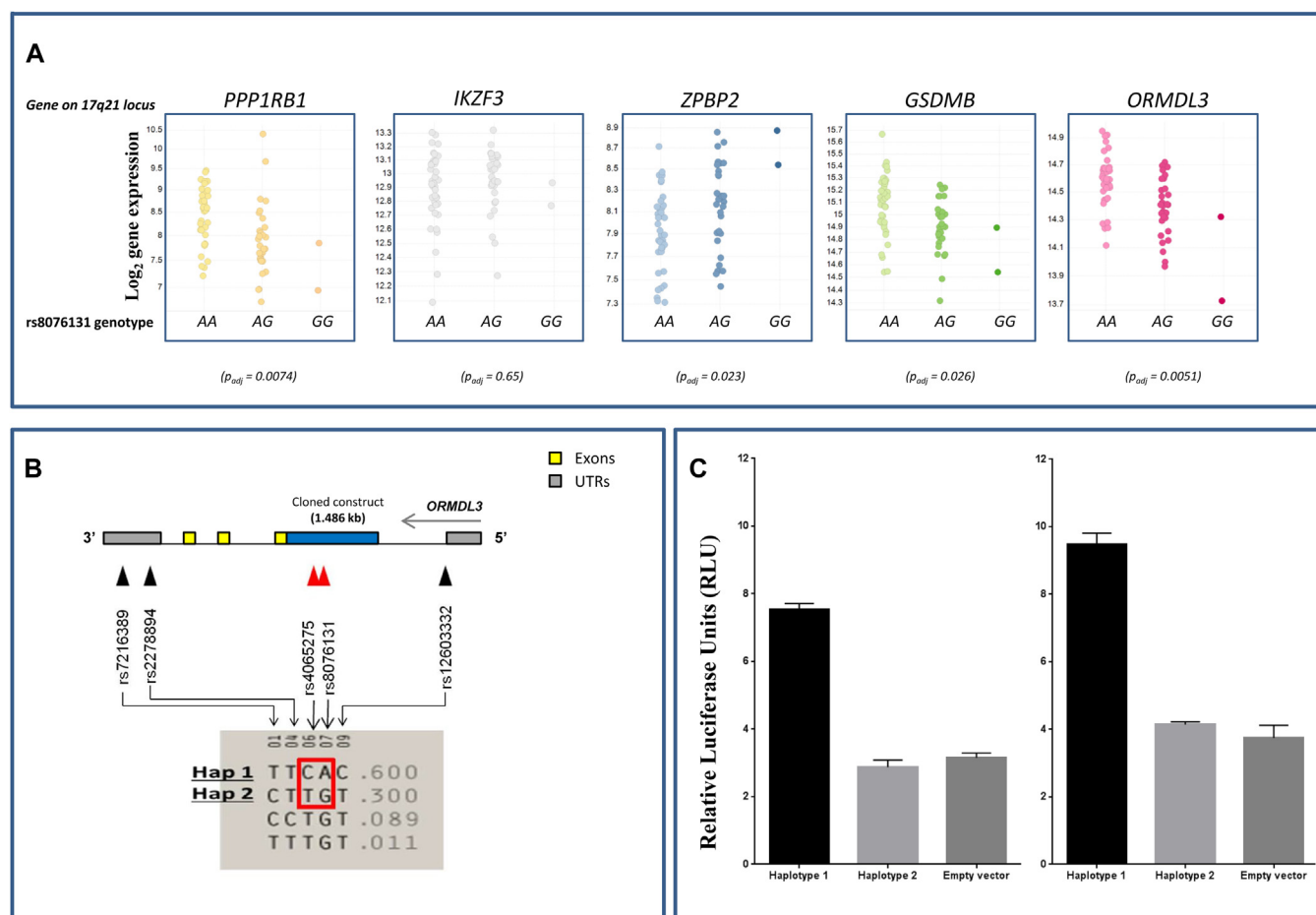


FIG 2. Effect of genetic polymorphisms on gene expression. **A**, Gene expression of 17q genes stratified by rs8076131 genotype with P_{adj} values from the whole-blood eQTL cohort of 71 subjects described in Fig E1 ($n = 39$ for the AA genotype, $n = 30$ for the AG genotype, and $n = 2$ for the GG genotype). P_{adj} values were calculated by adjusting for a number of genes tested in the chromosome 17q region. **B**, Schematic illustration showing the region of the *ORMDL3* gene along with the location of SNPs and haplotype frequencies used for the *in vitro* luciferase assay. **C**, Relative luciferase units stratified by the haplotypes previously mentioned. The 2 common haplotypes (haplotype 1 at 0.6 and haplotype 2 at 0.3 frequencies) from HapMap CHB data were selected for the luciferase assay.

also a whole set of genes distributed throughout the 17q12-21 locus.

The observed gene expression profile of the Chinese cohort is in good agreement with the profile reported for the whole blood of white subjects. A prior study³¹ focusing only on the right part of the 17q12-21 locus also detected an eQTL effect for *ZBP2*, *GSDMB*, and *ORMDL3* but not for *IKZF3*. Moreover, a large-scale whole-blood eQTL data set consisting of 5416 white subjects confirmed rs8076131 as a strong eQTL for *ORMDL3* and *GSDMB* (Table V).³² No significant eQTL effect for this SNP was reported for *PPP1RB* or *ZBP2*. Although *PPP1RB* was identified in our eQTL data from Chinese subjects, it was not detected in the eQTL meta-analysis of white subjects, which might be attributed to quality control issues or to differences in the SNP architecture of white and Chinese subjects.

Allelic state at rs8076131 directly affects *ORMDL3* promoter activity

The broad effect of the rs8076131 linkage set on expression of multiple genes within 17q12-21 suggests that several SNPs of the

set are directly functional, that the allelic effect has a global effect on the region, or both. In line with the latter, a prior study³¹ found that the allelic state of common SNPs at the 17q12-21 locus alters its chromatin structure. The SNP is part of the cluster of 62 perfectly linked SNPs that are closely linked to rs8076131 ($r^2 = 0.96$) and presumably controlling chromatin opening through an allele-specific interaction with CCCTC-binding factor, a transcriptional repressor that regulates chromatin structure. Here we used an *in vitro* luciferase reporter assay to investigate whether the rs8076131 SNP cluster can act as a promoter SNP for *ORMDL3* expression. *ORMDL3* was selected for analysis because the rs8076131 cluster contains 3 perfectly linked SNPs (rs4065275, rs12603332, and rs8076131), with rs8076131 and rs4065275 being located within the first intron of this gene (Fig 2, B).

Using the HapMap CHB database, we first identified the 2 most common haplotypes of the rs4065275-rs8076131 pair in the Chinese population. We then generated constructs including 1.5-kb fragments containing the C-A (haplotype 1) and T-G (haplotype 2) alleles amplified directly from the genomic DNA of donors and linked to the luciferase reporter gene. These constructs

TABLE V. Effect of variation in genotype of the chromosome 17q21 SNP tag SNP rs8076131 on whole-blood gene expression

	ILMN_1662174 (<i>ORMDL3</i>)	ILMN_1666206 (<i>GSDMB</i>)
EGCUT	−19.2105	−11.972899
SHIP-TREND	−10.9646	−8.2973795
Groningen-HT12	−14.6488	−17.745834
Groningen-H8v2	−11.0384	—
Rotterdam	−15.509	−13.969234
Fehrman	−14.6282	−14.566276
InCHIANTI	−12.8349	−14.741316
HVH-HT12v3	−2.68673	−0.22281553
Meta <i>z</i> score*	−37.13	−32.7455
Meta <i>P</i> value*	$P = 9.81 \times 10^{-198}$	$P = 9.81 \times 10^{-198}$

Data from a previous large-scale eQTL study of peripheral blood samples from 5311 subjects (Westra et al³²) were used to analyze the potential association of *ORMDL3* SNP genotypes with transcript expression levels. This whole-genome eQTL meta-analysis was performed over 9 data sets from 7 cohorts: EGCUT (*n* = 891), InCHIANTI (*n* = 611), Rotterdam study (*n* = 762), Fehrman (*n* = 1240 on the Illumina HT12v3 platform and *n* = 229 on Illumina H8v2), HVH (*n* = 43 on Illumina HT12v3, *n* = 63 on Illumina HT12v4), SHIP-TREND (*n* = 963), and DILGOM (*n* = 509). The sample preparation and processing method and the eQTL analysis have been reported previously.³² For the current study, we used summary statistic eQTL data from the original meta-analysis for rs8076131 and *ORMDL3* and *GSDMB* genes.

*The meta *z* score and meta *P* value are the combined *z* score and *P* value obtained in the eQTL meta-analysis when analyzing all the data sets together by using data from Westra et al.³² A positive *z* score (>0) implies an increase in expression for the allele assessed, whereas a negative *z* score implies the allele assessed associates with a reduction in gene expression in comparison with the other allele.

were transfected into HEK293T cells, where the promoter activity of the rs4065275-rs8076131 haplotype could be measured by the extent of luciferase expression. At both 24 and 48 hours after transfection, luciferase activity was significantly higher in cells transfected with plasmids bearing rs4065275-rs8076131 haplotype 1 than haplotype 2 (Fig 2, C). These data are in line with the increased expression of *ORMDL3* detected in whole blood of subjects with the A allele of rs8076131 that we observed in the eQTL analysis (Fig 2, A).

Allelic state at rs8076131 correlates with IgE levels and eosinophil frequencies in blood

The IgE pathway is central to the pathology of allergic disease both in general and specifically in our cohort of patients with allergic asthma in Singapore.²⁵ Therefore we asked whether allelic state at rs8076131 was linked with total IgE titers in peripheral blood. Indeed, subjects with the risk genotype AA had significantly higher plasma levels of total IgE: the median total IgE titer for the AA group was 245.5 kU/L compared with 163.5 kU/L in the combined AG/GG group (*P* = .006; Fig 3, A). A similar trend was observed for the HDM-specific IgE titers, with the AA group having a median titer of 24 kU/L of HDM-specific IgE, whereas the other genotypes had a median of only 11.70 kU/L (*P* = .0397, data not shown).

The frequency of eosinophils in circulating blood is also linked to the manifestation of asthma.³³ The median frequency of eosinophils in whole blood in the AA group was 0.86 compared with 0.54 in the AG/GG group (*P* = .026; Fig 3, B). Therefore subjects bearing the AA genotype have both significantly more total IgE and a significantly higher frequency

of eosinophils in peripheral blood than those bearing AG or GG at rs8076131.

DISCUSSION

The common co-occurrence of AR and allergic asthma in affected subjects suggests a component of shared genetic susceptibility, but identifying the gene loci involved using traditional approaches has proved problematic. Recent reports suggested the 17q12-21 chromosome region as a likely candidate in the manifestation of both conditions,²⁰⁻²⁴ but one of the largest allergy studies to date did not find an association with self-reported AR.²⁴ In this study we confirmed the association of this locus with allergic asthma susceptibility but did not replicate any significant association with AR in our population.

Our approach used a large population of subjects of Chinese ethnicity resident in the relatively uniform tropical urban environment of Singapore. Uniquely, this environment gives rise to almost complete penetrance of a single dominant allergen, HDM,²⁵ which effectively eliminates the abundant false-negative results that typically reduce the accuracy of environment-dependent genome-wide association studies. By using a 2-stage design with discovery and validation populations and by separately analyzing subjects with single morbidities versus comorbidities, we were able to obtain sufficient statistical power to detect the true association between SNP allelic state within the 17q12-21 locus and allergic airway conditions. In agreement with previous studies,^{15,16} we found a strong association between 17q12-21 and asthma manifestation. However, in agreement with previous reports in other populations, we also found a stronger association between 17q12-21 and AR when asthma was also present.^{19,24} Because of the large number of SNPs linked to our significant tag SNP, a single causative SNP is unlikely. More than 80 SNPs are linked to rs8076131 with an *r*² value of 0.8 or greater: within this set, 1 SNP (rs12936231) has been identified as part of a chromatin-regulating network,³¹ and our own study indicates that the rs8076131/rs4065275 haplotype directly controls the transcriptional level of *ORMDL3*. It is entirely possible that these SNPs are not the only important functional variants but are rather part of a set of SNPs that regulate 17q12-21 expression in a coordinated fashion. In fact, Berlivet et al³⁴ have shown that 2 of the SNPs of the rs8076131 set exhibit functionality independently of each other.

Although the association of this locus with asthma is clear, it is less obvious which gene is actually mediating the effect. In our cohort the strongest association was between allelic state at rs8076131 and asthma manifestation, which was linked with differences in expression levels of 4 genes (*ORMDL3*, *PPP1R1B*, *ZBP2*, and *GSDMB*) within the 17q12-21 locus in cells from peripheral blood. Considering that in other asthma-associated tissues additional genes can be affected by this polymorphism, it remains an open question as to which gene or combination of genes is important in driving the disease. Studies in mice genetically deficient in individual gene candidates might well prove informative in this regard.

Interestingly, although the effect of the SNPs was most obvious within the 17q12-21 locus, we also observed increased total plasma IgE levels in subjects carrying the rs8076131 risk genotype AA. Especially in the tropical environment of Singapore, asthma incidence is strongly correlated with the titer

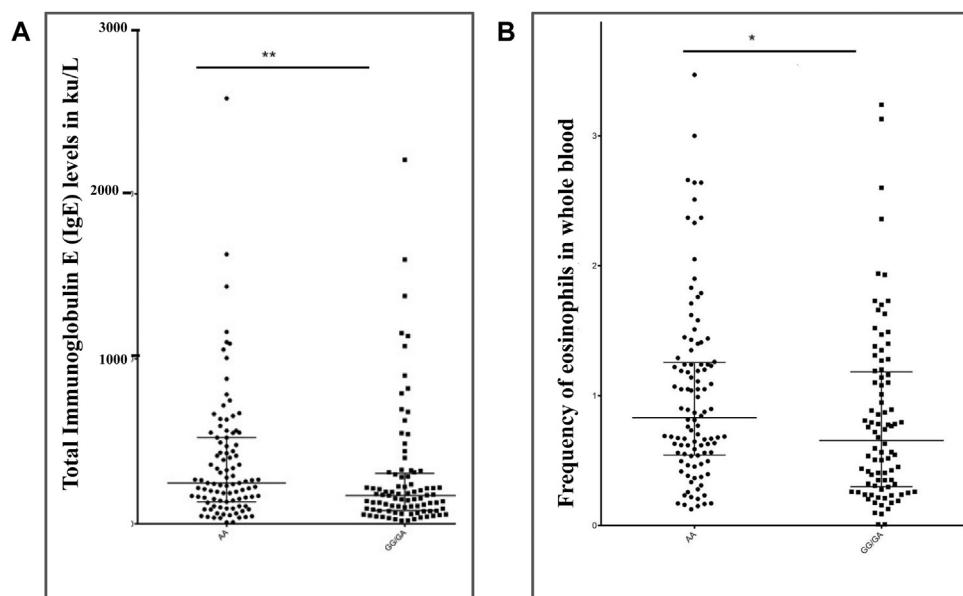


FIG 3. Clinical relevance of the rs8076131 genotype. **A**, Total immunoglobulin levels detected in plasma in kilounits per liter from 180 subjects described in Fig E1. **B**, Percentage of eosinophils in whole blood stratified by rs8076131 alleles from 196 Singapore Chinese subjects (Fig E1). *P* values were estimated by using a nonparametric Mann-Whitney test, **P* < .05 and ***P* < .01.

of total and HDM-specific IgE in peripheral blood.²⁵ Although correlation of the rs8076131 allelic state with IgE titers clearly confirms the role this polymorphism plays in the manifestation of asthma, it remains unclear whether the abundance of IgE is caused by a trans effect of the SNP or is rather a reflection of the manifestation of asthma. Likewise, the increased numbers of eosinophils in peripheral blood observed here are typical in asthmatic patients,^{35,36} and a recent report has suggested a mechanistic link between *ORMDL3* and eosinophil tracking, recruitment, and degranulation.³⁷ Thus it is possible that the increased eosinophil numbers in blood from rs8076131 AA subjects might be a direct consequence of the increased expression of *ORMDL3* driven by the polymorphism.

As a limitation of our study, we cannot delineate whether the association to IgE levels is dependent or independent of the asthma status. Because of the near-complete penetrance with HDM allergens, virtually all asthmatic patients in Singapore are associated with HDM-specific IgE. However, studying other populations of asthmatic patients without IgE in other regions might help to shed light on this question.

In summary, we have confirmed the role of 17q12-21 as a susceptibility locus for allergic asthma and have defined rs8076131 as the tag SNP in this region that is closely associated with both disease manifestation and key markers of allergic reactions (IgE and eosinophil counts). Because the allelic state of the rs8076131 linkage set affects not only *ORMDL3* but also *PPP1R1B*, *ZBP2*, and *GSDMB*, the identification of the gene or genes ultimately driving the disease manifestation of asthma remains an ongoing challenge.

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Key messages

- Genetic variants of the chromosome 17q12-21 locus are associated with allergic asthma but not AR in an ethnic Chinese population from Singapore.
- These functional allergic asthma-relevant polymorphisms are associated with key biological markers, such as differential gene expression, total IgE levels, and eosinophil counts.

REFERENCES

- Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol* 2005;116:377-83.
- Pawankar R, Baena-Cagnani CE, Bousquet J, Canonica GW, Cruz AA, Kaliner MA, et al. State of world allergy report 2008: allergy and chronic respiratory diseases. *World Allergy Org J* 2008;1(suppl):S4-17.
- Pawankar R, Bunnag C, Chen Y, Fukuda T, Kim YY, Le LT, et al. Allergic rhinitis and its impact on asthma update (ARIA 2008)—western and Asian-Pacific perspective. *Asian Pac J Allergy Immunol* 2009;27:237-43.
- Pawankar R, Bunnag C, Khaltayev N, Bousquet J. Allergic Rhinitis and Its Impact on Asthma in Asia Pacific and the ARIA Update 2008. *World Allergy Org J* 2012; 5(suppl 3):S212-7.
- Strachan DP. Family size, infection and atopy: the first decade of the “hygiene hypothesis.” *Thorax* 2000;55(suppl 1):S2-10.
- Fagnani C, Annesi-Maesano I, Brescianini S, D’Ippolito C, Medda E, Nistico L, et al. Heritability and shared genetic effects of asthma and hay fever: an Italian study of young twins. *Twin Res Hum Genet* 2008;11:121-31.
- Willemsen G, van Beijsterveldt TC, van Baal CG, Postma D, Boomsma DI. Heritability of self-reported asthma and allergy: a study in adult Dutch twins, siblings and parents. *Twin Res Hum Genet* 2008;11:132-42.
- Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. *Immunol Rev* 2011;242:10-30.
- Hjelmqvist L, Tuson M, Marfany G, Herrero E, Balcells S, González-Duarte R. *ORMDL* proteins are a conserved new family of endoplasmic reticulum membrane proteins. *Genome Biol* 2002;3:1-16.

10. Breslow DK, Collins SR, Bodenmiller B, Aebersold R, Simons K, Shevchenko A, et al. Orm family proteins mediate sphingolipid homeostasis. *Nature* 2010;463:1048-53.
11. Saeki N, Usui T, Aoyagi K, Kim DH, Sato M, Mabuchi T, et al. Distinctive expression and function of four GSDM family genes (GSDMA-D) in normal and malignant upper gastrointestinal epithelium. *Genes Chromosomes Cancer* 2009;48:261-71.
12. Tamura M, Tanaka S, Fujii T, Aoki A, Komiyama H, Ezawa K, et al. Members of a novel gene family, Gsdm, are expressed exclusively in the epithelium of the skin and gastrointestinal tract in a highly tissue-specific manner. *Genomics* 2007;89:618-29.
13. Morgan B, Sun L, Avital N, Andrikopoulos K, Ikeda T, Gonzales E, et al. Aiolos, a lymphoid restricted transcription factor that interacts with Ikaros to regulate lymphocyte differentiation. *EMBO J* 1997;16:2004-13.
14. Katoh M, Katoh M. Identification and characterization of human ZBPB-like gene in silico. *Int J Mol Med* 2003;12:399-404.
15. Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, et al. Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 2007;448:470-3.
16. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;363:1211-21.
17. Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 2011;43:887-92.
18. Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, Willis-Owen SA, et al. Variants of DENND1B associated with asthma in children. *N Engl J Med* 2010;362:36-44.
19. Ferreira MA, Matheson MC, Tang CS, Granell R, Ang W, Hui J, et al. Genome-wide association analysis identifies 11 risk variants associated with the asthma with hay fever phenotype. *J Allergy Clin Immunol* 2014;133:1564-71.
20. Tomita K, Sakashita M, Hirota T, Tanaka S, Masuyama K, Yamada T, et al. Variants in the 17q21 asthma susceptibility locus are associated with allergic rhinitis in the Japanese population. *Allergy* 2013;68:92-100.
21. Fuertes E, Soderhall C, Acevedo N, Becker A, Brauer M, Chan-Yeung M, et al. Associations between the 17q21 region and allergic rhinitis in 5 birth cohorts. *J Allergy Clin Immunol* 2015;135:573-6.
22. Andiappan AK, Wang de Y, Anantharaman R, Parate PN, Suri BK, Low HQ, et al. Genome-wide association study for atopy and allergic rhinitis in a Singapore Chinese population. *PLoS One* 2011;6:e19719.
23. Ramasamy A, Curjuric I, Coin LJ, Kumar A, McArdle WL, Imboden M, et al. A genome-wide meta-analysis of genetic variants associated with allergic rhinitis and grass sensitization and their interaction with birth order. *J Allergy Clin Immunol* 2011;128:996-1005.
24. Hinds DA, McMahon G, Kiefer AK, Do CB, Eriksson N, Evans DM, et al. A genome-wide association meta-analysis of self-reported allergy identifies shared and allergy-specific susceptibility loci. *Nat Genet* 2013;45:907-11.
25. Andiappan AK, Puan KJ, Lee B, Nardin A, Poidinger M, Connolly J, et al. Allergic airway diseases in a tropical urban environment are driven by dominant mono-specific sensitization against house dust mites. *Allergy* 2014;69:501-9.
26. Andiappan AK, Anantharaman R, Nilkanth PP, Wang de Y, Chew FT. Evaluating the transferability of Hapmap SNPs to a Singapore Chinese population. *BMC Genet* 2010;11:36.
27. Bousquet J, Khaltayev N, Cruz AA, Denburg J, Fokkens WJ, Togias A, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008;63(suppl 86):8-160.
28. Andiappan AK, Narayanan S, Myers RA, Lee B, Nieuwenhuis MA, Nardin A, et al. Genetic variants of inducible costimulator are associated with allergic asthma susceptibility. *J Allergy Clin Immunol* 2015;135:556-8.
29. Andiappan AK, Parate PN, Anantharaman R, Suri BK, Wang de Y, Chew FT. Genetic variation in BDNF is associated with allergic asthma and allergic rhinitis in an ethnic Chinese population in Singapore. *Cytokine* 2011;56:218-23.
30. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet* 2005;37:1217-23.
31. Verlaan DJ, Berlivet S, Hunninghake GM, Madore AM, Lariviere M, Moussette S, et al. Allele-specific chromatin remodeling in the ZBPB2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet* 2009;85:377-93.
32. Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, et al. Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* 2013;45:1238-43.
33. Szeffler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. *J Allergy Clin Immunol* 2005;115:233-42.
34. Berlivet S, Moussette S, Ouimet M, Verlaan DJ, Koka V, Al Tuwaijri A, et al. Interaction between genetic and epigenetic variation defines gene expression patterns at the asthma-associated locus 17q12-q21 in lymphoblastoid cell lines. *Hum Genet* 2012;131:1161-71.
35. Prussin C, Metcalfe DD. 4. IgE, mast cells, basophils, and eosinophils. *J Allergy Clin Immunol* 2003;111(suppl):S486-94.
36. Adamko DJ, Odemuyiwa SO, Vethanayagam D, Moqbel R. The rise of the phoenix: the expanding role of the eosinophil in health and disease. *Allergy* 2005;60:13-22.
37. Ha SG, Ge XN, Bahaie NS, Kang BN, Rao A, Rao SP, et al. ORMDL3 promotes eosinophil trafficking and activation via regulation of integrins and CD48. *Nat Commun* 2013;4:2479.

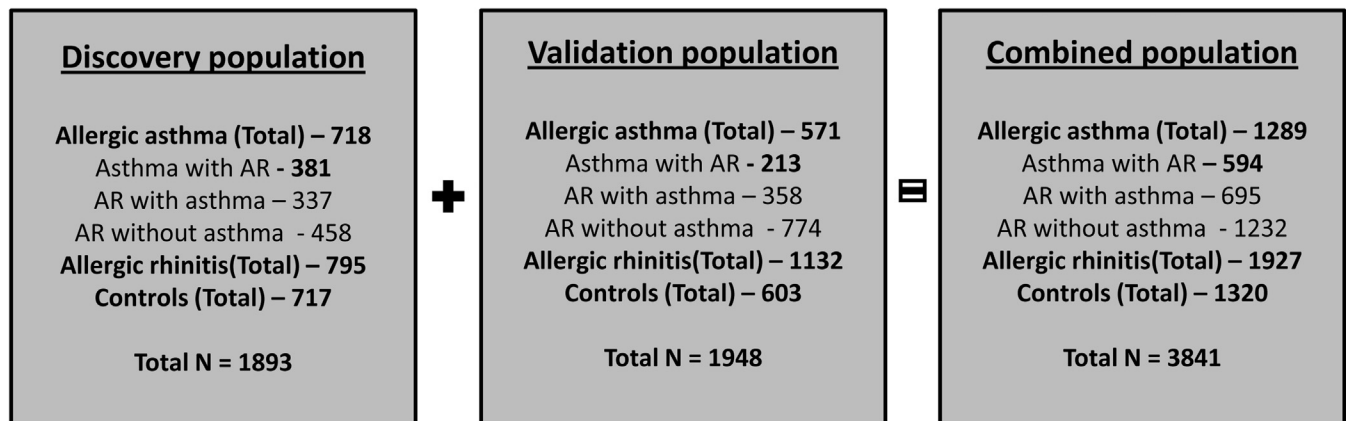
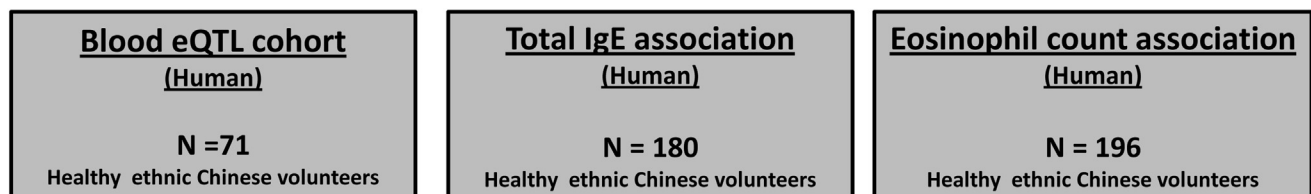
GENETIC ASSOCIATION COHORTS**FUNCTIONAL ASSOCIATION COHORTS**

FIG E1. Design of current study describing the various genetic association cohorts and functional association cohorts.

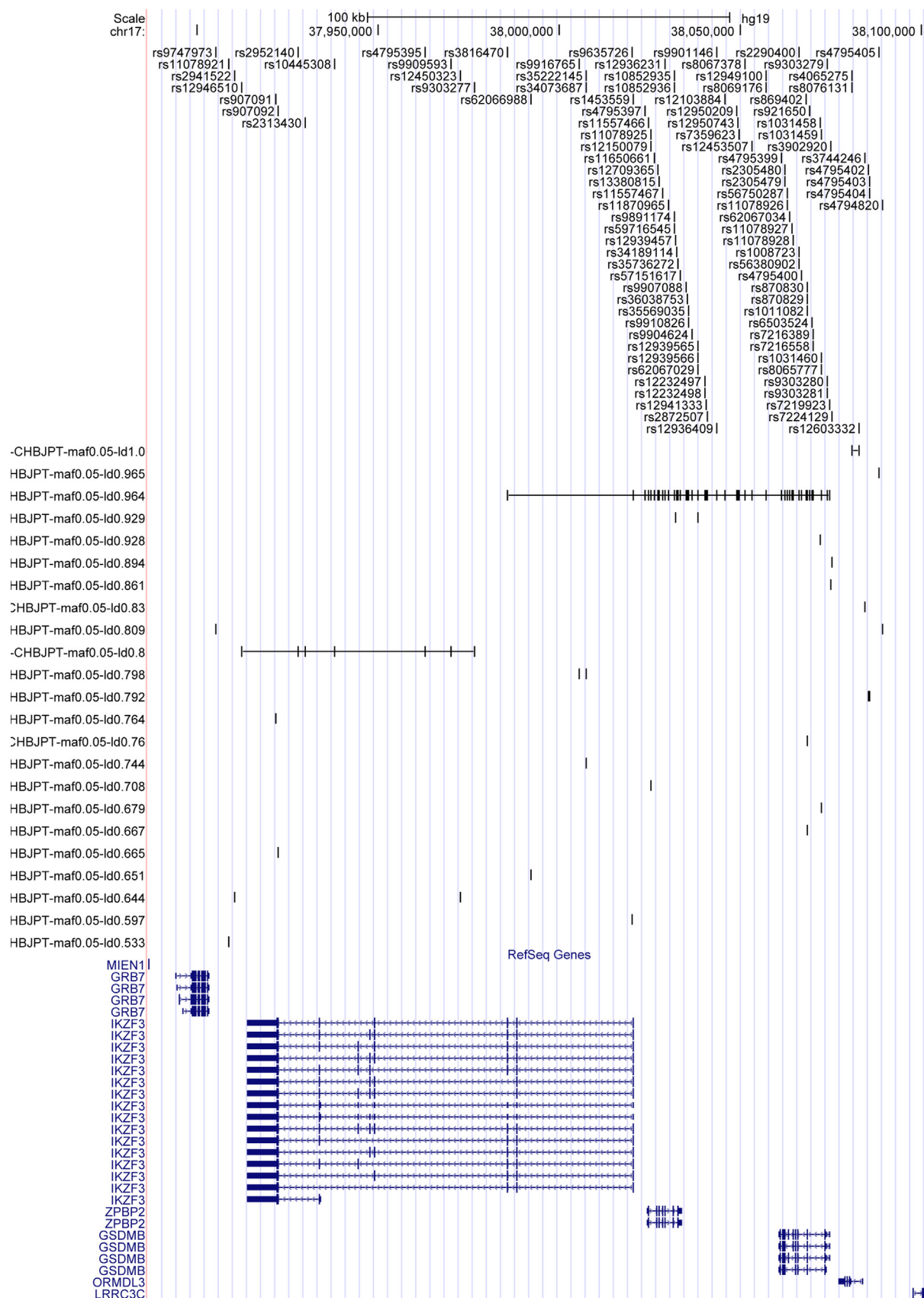


FIG E2. Complete set of linked SNPs with reference to tag SNP rs8076131 at the chromosome 17q21 locus. This figure was generated with ArchiLD software by using a minor allele frequency of 0.05 and including singletons (<http://archild.sign.a-star.edu.sg/>; accessed March 28, 2015).

TABLE E1. Association of tag SNP rs8076131 to AR phenotype in the validation population

Chromosome	SNP	BP	Minor allele	MAF in cases	MAF in control subjects	Major allele	CHISQ	P value	OR	SE	L95	U95	P _{HWE}
Patients with AR and asthma against control subjects													.428
17	rs8076131	38,080,912	G	0.2039	0.294	A	17.98	2.24E-05	0.6151	0.1152	0.4908	0.7708	
Patients with AR without asthma against control subjects													
17	rs8076131	38,080,912	G	0.2665	0.294	A	2.339	.1262	0.8724	0.08927	0.7324	1.039	

BP, Base pair on chromosome 17; CHISQ, association statistics χ^2 value for the tag SNP tested for allergic asthma; L95, lower confidence at 95%; Minor allele, effect allele for the tag SNP for which the association statistics have been calculated; MAF, effect allele frequency; Major allele, other allele for the tag SNP; OR, OR for the tag SNP; P_{HWE}, Hardy-Weinberg equilibrium P value for the tag SNP tested in the entire population; P value, P value for the tag SNP tested for allergic asthma; SNP, tag SNP tested for association to allergic asthma; U95, upper confidence at 95%.