

Letter to the Editor

IL-1 receptor–associated kinase 3 gene (*IRAK3*) variants associate with asthma in a replication study in the Spanish population

To the Editor:

Asthma is a chronic inflammatory disease associated with genetic and environmental factors. Although the 12q13-24 chromosome region had previously shown linkage to asthma, the IL-1 receptor–associated kinase 3 gene (*IRAK3*) has recently emerged as a susceptibility candidate for asthma as a result of positional cloning of persistent asthma with age of onset less than 13 years in Italian samples.¹ *IRAK3* encodes a protein that negatively regulates Toll-like receptor signaling pathways involved in innate host defense and in the control of adaptive immune responses. However, single nucleotide polymorphisms (SNPs) near *IRAK3* have not reached the stringent significance thresholds in any of the genome-wide association studies (GWASs) performed in asthmatic patients.² Here we aimed to replicate the association of *IRAK3* genetic variants with susceptibility to asthma in a case-control study with unrelated Spanish subjects.

For this purpose, DNA samples from 607 patients aged 5 years or older with physician-diagnosed asthma and fulfilling the Global Initiative for Asthma guidelines (www.ginasthma.com) were collected from respiratory medicine and allergy departments as part of the Genetics of Asthma (GOA) study in the Spanish population. Samples from 1271 nonasthmatic patients without a personal or familial history of allergic or pulmonary diseases were obtained from the Spanish National DNA Biobank (www.bancoadn.org), and these were used as control samples. Further sample details can be found in the Methods section and Table E1 in this article's Online Repository at www.jacionline.org.

Fifteen tagging SNPs (tSNPs) were selected and genotyped, as described elsewhere,³ to comprehensively survey common gene variation. All of them passed our quality control filters and were considered for the analyses (see the Methods section and Table E2 in this article's Online Repository at www.jacionline.org). Additionally, 83 European ancestry informative markers (EuroAIMs; see Table E3 in this article's Online Repository at www.jacionline.org) were genotyped and used for principal component analysis with EIGENSOFT to derive the scores for the first principal component (PC1).⁴ Association of individual tSNPs was tested with the Cochran-Armitage trend test, and then a logistic regression analysis was used to calculate odds ratios (ORs) with 95% CIs and to adjust for population stratification by using the PC1 scores. Additionally, 15 untyped SNPs of the gene were imputed and tested for association. Haplotype associations were also tested to replicate previous findings.¹ A false discovery rate (FDR) threshold of 5% was established to limit the expected proportion of false-positive results incurred in the study when a particular subject's SNP test result was considered significant. Further method details are described in the Methods section in this article's Online Repository.

For asthma, only 1 SNP (rs1168774) was nominally associated but was not considered significant in the context of the multiple tests performed ($P = .038$, FDR = 42%, see Table E4 in this article's Online Repository at www.jacionline.org). However, 4

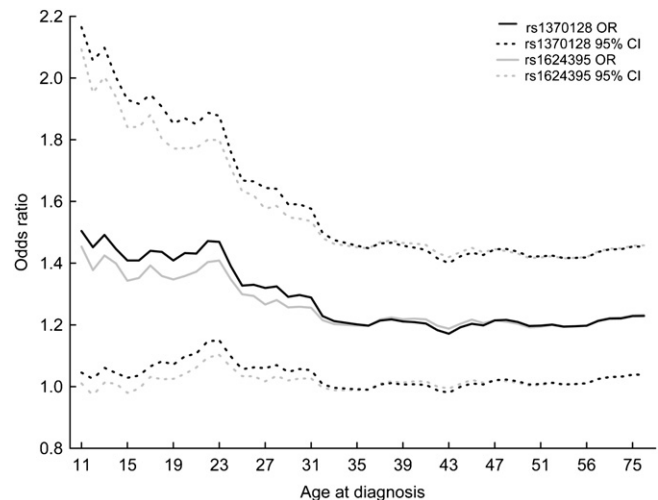


FIG 1. ORs and their 95% CIs for the SNPs rs1624395 and rs1370128 in the sequential addition of atopic asthma cases according to age at diagnosis.

tSNPs showed nominal association with atopic asthma (see Table E4 for unadjusted results), 3 of them remaining significant after adjusting for population stratification: rs1732877 (OR, 1.24; 95% CI, 1.04-1.46; $P = .014$), rs1624395 (OR, 1.22; 95% CI, 1.03-1.45; $P = .019$), and rs1370128 (OR, 1.22; 95% CI, 1.03-1.44; $P = .019$). Testing of untyped variants revealed 8 additional SNPs with nominal association with atopic asthma ($.014 \leq P \text{ value} \leq .048$, see Table E4). All 11 SNPs were considered significantly associated with atopic asthma (FDR = 2%) and showed moderate-to-strong linkage disequilibrium (LD; $0.48 \leq r^2 \leq 1.0$). Three of them replicated previous findings (rs1624395, rs1370128, and rs2293657).¹

A previous study suggested that association of *IRAK3* with asthma varied with the age of onset.¹ Analyzing the tSNPs replicating previous findings (rs1370128 and rs1624395), we observed that the effects were largest for 22 years of age at diagnosis (Fig 1) both for rs1370128 (OR, 1.46; 95% CI, 1.14-1.87; $P = .001$; permuted $P = .021$) and rs1624395 (OR, 1.40; 95% CI, 1.09-1.79; $P = .007$; permuted $P = .043$). Thereafter, considering only those atopic asthma cases diagnosed at 22 years of age or less, we observed nominal associations for 8 tSNPs, all showing larger effects than when considering all atopic asthma cases (see Table E4). After adjusting for population stratification, all these associations remained significant: rs2701653 (OR, 1.51; 95% CI, 1.04-2.19; $P = .030$), rs78503618 (OR, 1.57; 95% CI, 1.15-2.16; $P = .005$), rs1732877 (OR, 1.43; 95% CI, 1.11-1.85; $P = .007$), rs1624395 (OR, 1.37; 95% CI, 1.07-1.76; $P = .013$), rs1370128 (OR, 1.44; 95% CI, 1.12-1.84; $P = .004$), rs1152912 (OR, 1.36; 95% CI, 1.06-1.76; $P = .018$), rs1152913 (OR, 1.48; 95% CI, 1.14-1.91; $P = .003$), and rs1152916 (OR, 1.44; 95% CI, 1.11-1.86; $P = .007$). On the contrary, none of the tSNPs was significantly associated with atopic asthma diagnosed at age greater than 22 years ($.228 \leq P \text{ value} \leq .971$, not shown), suggesting that associations found with atopic asthma were likely to be due to the cases with an age at diagnosis of 22 years or less.

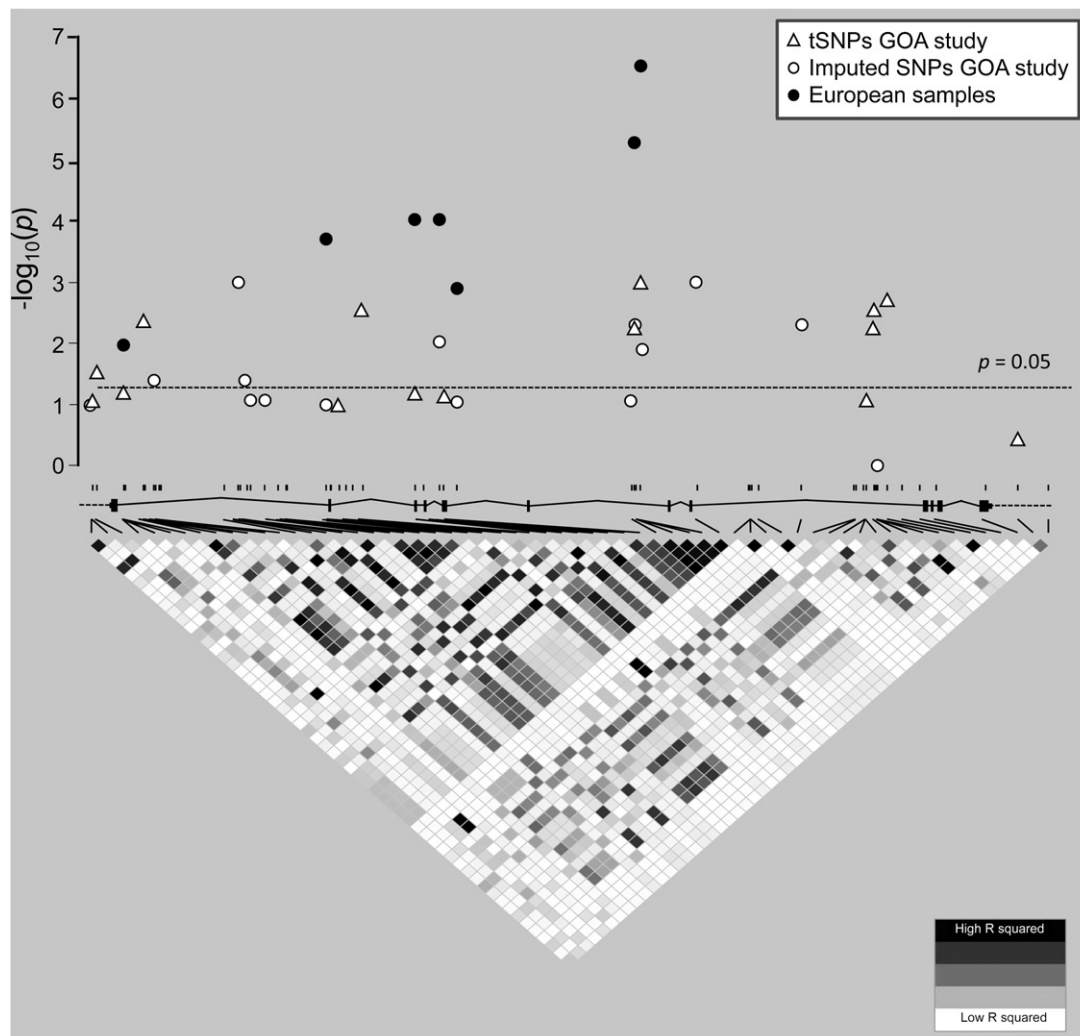


FIG 2. Association P values (in log scale) of *IRAK3* gene SNPs with atopic asthma diagnosed at age 22 years or less and in a meta-analysis across Europeans. An LD plot of r^2 values based on resequencing data is shown for the 58 SNPs of *IRAK3*, as well as their relative position in the gene. Each diamond of the LD plot represents the pairwise r^2 correlation between SNPs.

Testing of untyped variants revealed 8 additional SNPs nominally associated with atopic asthma diagnosed at age 22 years or less ($.001 \leq P \text{ value} \leq .040$, Fig 2 and see Table E4). All 16 SNPs nominally associated with atopic asthma diagnosed at age 22 years or less were considered significant ($\text{FDR} \leq 2\%$), and most of them were in moderate-to-strong LD. The exception were rs2701653 and rs78503618 from the 5' region, which showed weak LD in pairwise comparisons between them ($r^2 = .001$) and with the rest of the SNPs of the gene ($0.001 \leq r^2 \leq .319$). However, only the inclusion of the SNP rs2701653 as a covariate in logistic regression left a significant association for the 2 SNPs that replicated previous findings ($P = .012$ and $P = .007$ for rs1624395 and rs1370128, respectively), indicating the presence of independent associations.

Haplotype testing of a previously associated 3-SNP region (rs11465955, rs1624395 and rs1370128)¹ replicated the association of the protective CGC haplotype ($.001 \leq P \leq .032$, see Table E5 in this article's Online Repository at www.jacionline.org). However, the association of the TAT risk haplotype¹ was not replicated ($P \geq .068$), although it showed a significant effect in logistic

regression (OR, 1.39; 95% CI, 1.07-1.81; $P = .013$). Nevertheless, the CAT haplotype was detected as a risk haplotype for all asthma phenotypes ($P \leq .023$, $1.37 \leq \text{OR} \leq 1.75$; see Table E5). A further exploration suggested that the 3 SNPs included in haplotypes had almost perfect LD (D') with 2 other nearby SNPs (rs2289134 and rs1732877), revealing similar effects when extending the haplotypes to intron 2 of the *IRAK3* gene (see Table E5).

Because our results suggested that atopic asthma cases diagnosed at age 22 years or less accounted for the discovered associations, we explored whether these associations had directionally consistent effects with previous findings by performing a meta-analysis with data from candidate-gene case-control association studies of unrelated patients with childhood and early-onset asthma (see the Methods section in this article's Online Repository for further details).^{1,5} This analysis included 3384 samples (736 cases and 2648 control subjects) and compared 7, 3, and 5 SNPs overlapping between our samples and Sardinian, Italian, and Japanese subjects, respectively. This joint analysis only evidenced allele and effect-wise replication among Europeans (Fig 2 and see Table E6 in this article's Online Repository

at www.jacionline.org), with 1 SNP associated with suggestive significance at a genome-wide level (rs1370128; OR, 1.54; 95% CI, 1.31-1.82; $P = 2.9 \times 10^{-7}$). Congruent with these results, a study of 39 candidate genes in non-Hispanic white nuclear families with asthma⁶ reported a nominal association for 2 *IRAK3* SNPs: rs1821777, which shows an r^2 value of 1 with rs2289134 associated into haplotypes of 5 SNPs in our study, and rs1152912, which is also associated in our study. However, a large-scale asthma GWAS among Europeans reported a P value of .07 for 1 *IRAK3* SNP not tested in this study (rs17826057) in a meta-analysis combining cases with childhood-onset, later-onset, severe, and occupational asthma, showing nonsignificant results also when the analysis was restricted to asthma cases with onset at less than 16 years.⁷ Similarly, *IRAK3* was not associated with childhood asthma among Japanese or Mexican nuclear families in which most cases were atopic (90%).^{5,8} Although more studies need to be accomplished, the nontransferability of the *IRAK3* association with asthma between studies could be due to differences in LD between populations, genetic and environmental interactions, genetic heterogeneity, or the inadequate gene coverage of previous studies (see Table E7 in this article's Online Repository at www.jacionline.org for gene coverage in Europeans in commercial arrays).⁸ Thus, despite the fact that SNPs from the *IRAK3* gene are not among the GWAS hits for asthma to date,² our results support the importance of this gene in patients with atopic asthma.

In this study we performed the first independent replication of *IRAK3* association with asthma,¹ examining extensively the most common variation of the gene. Our results replicated the association of *IRAK3* at the SNP and haplotype levels and evidenced that other variants of the gene had independent effects in atopic asthma. These results were robust to population stratification and to the differences in age and sex between cases and control subjects (not shown). Strikingly, the association of haplotypes was not restricted to atopic asthma diagnosed at age 22 years or less but also was found among all asthma cases. However, the TAT risk haplotype was not replicated, which could be motivated by our limited power to detect association on the basis of previous findings¹ (<60% for risks ≥ 1.2 at a 2-sided P value of .05 and a variant frequency of 0.35 to 0.40) or the potential presence of private risk variants in Sardinians. Moreover, we could not distinguish the causal variant because all but a few associated SNPs were in moderate-to-strong LD. Despite the fact that the tested trait was not exactly the same as that analyzed among Italian samples,¹ because the persistency of asthma symptoms was not recorded among GOA samples, the fact that we replicated the same SNPs and the protective haplotype supports that these results are unlikely to be spurious.

In conclusion, our results confirm the importance of the *IRAK3* gene in asthma pathogenesis in European populations, supporting the hypothesis of shared risk factors among complex diseases³ and bolstering the relevance of the hygiene hypothesis in asthma development.⁹ Further research is needed to discern whether this association is due to rare gene variants,¹⁰ to the biological function of *IRAK3*, or to LD with nearby genes, as well as its relevance in non-European populations.

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METHODS

Ethics statement

This study was approved by the ethics committees at participating hospitals, and written informed consent was obtained from all subjects.

Study subjects

This study was conducted by using a case-control design of DNA samples from unrelated subjects reporting at least 2 generations of Spanish descent. Case samples that fulfilled the Global Initiative for Asthma guidelines for the diagnosis and classification of asthma (www.ginasthma.com) were collected from respiratory medicine and allergy departments as part of the GOA study in the Spanish population. This sample included 607 patients with physician-diagnosed asthma with active specific medication for asthmatic symptoms and age of 5 years or older. We recorded basic demographic data (age, sex, and smoking habits), age at diagnosis of the disease, severity of the illness, family history of allergic disease, allergic symptoms (rhinitis, atopic dermatitis, and food and drug allergy), basal FEV₁, and symptomatic treatment. Atopy was confirmed by a positive skin prick test response (a wheal with a diameter 3 mm greater than that elicited by the saline control) to one of 7 common allergens, including dust mite, epithelium, pollen, fungi, food, latex, and others, or by specific serum IgE levels of greater than 0.35 IU/mL. Allergens evaluated for specific IgE levels were dust mite (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Glycyphagus* species, *Blomia tropicalis*, *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, and *Euroglyphus maynei*), epithelium (*Felis domesticus*, *Canis familiaris*, *Equus caballus*, and *Oryctolagus cuniculus*), pollen (*Olea europaea*, *Salsola* species, *Lolium perenne*, *Artemisia vulgaris*, *Parietaria* species, *Platanus* species, *Chenopodium* species, *Plantago* species, *Rumex* species, and *Cupressus* species), fungi (*Penicillium notatum*, *Alternaria alternate*, *Aspergillus fumigatus*, and *Cladosporium herbarum*), food (cow's milk, hen's egg, peanut, soybean, wheat, fish, shrimp, crabs, lobster, clams, oysters, mussels, banana, chestnut, and kiwi), latex, and cockroach (*Blattella germanica*).

A total of 1271 DNA samples from subjects self-reporting at least 2 generations of ancestors born in Spain and without personal or familial history of allergic or pulmonary diseases were used as control samples. These were obtained from the Spanish National DNA Biobank (www.bancoadn.org), a member of the Public Population Project in Genomics Consortium (www.p3gobservatory.org). Briefly, samples were collected from branches of the National Blood Service from unrelated subjects residing in Spain. After signing an informed consent form, donors self-declared general health status, physical activity, transportation and nutrition habits, employment and qualification, demographics, tobacco smoke, alcohol consumption, genealogical information, residence and mother tongue, and personal and familial history of diseases, including infectious, cancerous, blood and circulatory, endocrine, mental and behavioral, respiratory (including asthma symptoms), immunologic (including allergies), bone, congenital, skin and digestive, as well as eye and hearing disorders. No information from medical records was incorporated or revised, and no medical testing was performed on donors.

Selection of tSNPs

tSNPs were selected by using TagIT 3.03^{E1} with published resequencing data from 22.6 kb in 32 unrelated healthy Spanish subjects and forcing the inclusion of previously associated SNPs^{E2,E3} and all potential functional variants, as predicted by PupaSuite^{E4} or previously established by us.^{E3} A final set of 15 tSNPs was selected (Table E2) and tested for performance by using an SNP-dropping-with-resampling method,^{E1} satisfying a haplotype r^2 value of 1.0 in the resequencing data.

Genotyping of tSNPs

DNA was extracted from blood (GFX kit; GE Healthcare, Little Chalfont, United Kingdom) or saliva (Oragene DNA; DNA Genotek, Inc, Kanata, Ontario, Canada) and used for whole-genome amplification with the Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare). Dilutions of this product were quantified by using an SYBR Green I–based DNA quantification

method (Molecular Probes, Eugene, Ore) and genotyped in 2 reactions containing 7 and 8 tSNPs by means of the SNaPshot Multiplex kit (Applied Biosystems, Foster City, Calif), as described elsewhere.^{E3} Electrophoresis was performed on an ABI 310 Genetic Analyzer (Applied Biosystems), and genotypes were automatically assigned by using GeneMapper v3.2 (Applied Biosystems). Genotyping was blind to disease status. Approximately 6% of the samples were genotyped in duplicate to monitor genotyping quality, yielding an estimated overall discordance rate among duplicated samples of 0.07% (95% CI, 0.02% to 0.19%). The genotyping completion rate was greater than 97.7% across all 15 tSNPs (see Table E2).

Assessment of population stratification

Ninety-three EuroAIMs were genotyped in case and control samples to reduce the risk for false-positive results, allowing correcting for major population stratification effects among European and Spanish populations.^{E5,E6} Genotyping of EuroAIMs was conducted by using the iPLEX Gold assay on the MassARRAY system, and genotype calls were automatically generated by using all data from the study simultaneously with TYPER 3.4 software (Sequenom, San Diego, Calif) by the Spanish National Genotyping Center, Santiago de Compostela Node (CeGen, www.cegen.org). Of 93 EuroAIMs, 83 markers were successfully genotyped and considered for the final analysis (see Table E3). Our previous studies have demonstrated that a subset of as few as 65 of these EuroAIMs effectively controlled false-positive results in Spanish case-control association studies.^{E6} On the basis of these markers, principal component analysis was used to derive the scores for the PC1 as ancestry estimates in cases and control subjects. This analysis was performed with EIGEN-SOFT,^{E7} as described elsewhere.^{E6}

Statistical analysis

Clinical and demographic data were analyzed by using χ^2 and Mann-Whitney U tests with SPSS 15.0 software (SPSS, Inc, Chicago, Ill). Departures from Hardy-Weinberg equilibrium were evaluated separately for cases and control subjects by using an exact test.^{E8} Because none of the tSNPs deviated significantly from Hardy-Weinberg equilibrium in the control group (threshold for significance after considering the multiple comparisons performed was a P value of .003), all tSNPs were retained for the association analyses. Further details can be found in Table E2.

Individual tSNP associations were tested by using the Cochran-Armitage trend test, assuming an additive model, and the empiric P value was obtained by using 1000 permutations with a custom script for STATISTICA (StatSoft, Inc, Tulsa, Okla).^{E9} Logistic regression analysis was used to calculate allelic effects as ORs with 95% CIs, to adjust for population stratification by using the PC1 scores as a covariate, and to test the independence of associations between SNPs. These analyses were performed with SPSS 15.0 software (SPSS, Inc).

Because a previous study evidenced an increase of allelic effects among cases with onset at less than 13 years, we explored whether the allelic effects varied with the age at diagnosis to estimate the trait cutoff at which the allelic effects were largest. For that, a previously described algorithm was used.^{E10} Briefly, individual SNP associations were computed as above but repeatedly for each group of cases resulting from the sequential incorporation of atopic asthmatic patients for every increase in 1 year in the age of diagnosis (starting from the age of 11 years, for which the study accumulated >50 cases). The empiric P value, adjusted for the multiple tests done, was then obtained from the comparison of the smallest nominal P value from all tests done with P values calculated from permuting genotypes 1000 times among the whole sample (by reshuffling labels of cases and controls) as the proportion of permutation replicates that yielded P values smaller than that observed in the actual dataset.

Untyped SNPs with multilocus LD values of .7 or greater^{E11} or with a minor allele frequency (MAF) of 10% or greater were imputed from tSNPs and tested for association by means of TUNA version 1.1^{E11} to limit the imputation error,^{E3} allowing us to analyze 15 additional SNPs of the gene. Haplotypes from the resequencing data were estimated with PHASE 2.1^{E12} to be used as a reference for the underlying LD across the gene. An FDR was assessed with QVALUE^{E13} considering all 30 SNPs tested (15 tSNPs and 15 imputed

SNPs) to judge the SNP associations in the context of the multiple comparisons performed. An omnibus test overall haplotype frequency distribution was first obtained with WHAP to determine whether there were haplotype associations.^{E14} Then the statistical significance for the association of specific haplotypes was assessed by using logistic regression with HAPSTAT,^{E15} and ORs and 95% CIs were obtained by using THESIAS,^{E16} with the latter using the most common haplotype as a reference.

Under the principle that if SNPs had consistent effects (ie, allele and effect wise) among different studies, a joint analysis would improve the results obtained from the studies by using a separate analysis, a meta-analysis of association for the SNPs overlapping across our study and the previous ones^{E2,E17} was performed. For this purpose, fixed and random effects meta-analyses were examined with the R library rmeta version 2.16.^{E18} The patterns of LD, as r^2 and D' values, were explored with Haploview 3.32.^{E19}

Finally, an evaluation of the array coverage of SNPs from the *IRAK3* gene with MAFs of 5% or greater in the GWAS performed in samples of European ancestry to date was assessed.^{E20-E26} For that, the information of SNPs contained in the arrays was downloaded from the UCSC Genome Bioinformatics Web page (<http://genome.ucsc.edu>) for the region delimited by our resequencing survey. Gene coverage was then assessed as the proportion of SNPs that were captured by the array both directly and indirectly (with a multimarker $r^2 \geq 0.8$) with respect to the total number of SNPs with MAFs of 5% or greater in Utah residents with ancestry from northern and western Europe from HapMap release 28 and in our resequencing data.

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- E26. 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.

TABLE E1. Relevant demographic and clinical features of samples

Variable	Cases (n = 607)	Control subjects (n = 1271)	P value
Sex (% males)	23.3 (567)	59.1 (1270)	<.001*
Age (y), mean, P ₂₅ -P ₇₅	37, 25-44 (569)	40, 32-48 (1270)	<.001†
Smoking habits (% ever smokers)	27.3 (344)	48.1 (1122)	<.001*
Clinical features			
Serum IgE levels (IU/mL), mean, P ₂₅ -P ₇₅	478.1, 89.3-394.5 (259)	NA	
Age at diagnosis (y), mean, P ₂₅ -P ₇₅	28, 14-39 (570)	NA	
FEV ₁ (% predicted), mean, P ₂₅ -P ₇₅	82, 68-95 (231)	NA	
Rhinitis (% positive)	74.2 (485)	NA	
Dermatitis (% positive)	20.2 (480)	NA	
SPT response (% positive)	76.6 (445)	NA	
Specific IgE (% positive)	51.2 (287)	NA	
Atopy (% positive)	76.7 (464)	NA	
Treatment			
Inhaled corticoids (%)	85.6 (250)	NA	
Oral corticoids (%)	4.6 (305)	NA	
Long-acting β_2 -agonists (%)	53.0 (317)	NA	
Short-acting β_2 -agonists (%)	81.8 (231)	NA	
Body mass index (kg m ⁻²), mean, P ₂₅ -P ₇₅	24.6, 21.1-27.5 (227)	NA	

Numbers in parentheses indicate the total number of subjects with available data.

NA, Not available; P₂₅, percentile 25; P₇₅, percentile 75.

* χ^2 Test.

†Mann-Whitney U test.

TABLE E2. Information, CRs, and HWE *P* values for *IRAK3* tSNPs

rs No.	Position*	SNP (effect)	CR (%)	HWE <i>P</i> value			Diagnosed at ≤22 y
				Control subjects	Asthmatic patients	Atopic asthmatic patients	
rs1732887	64867883	−1464A>G	98.7	.004	.579	.779	.404
rs2701653	64868152	−1195C>T	98.1	.312	.276	.570	.754
rs1732886	64870029	c.133+550A>G	98.1	.014	.912	.888	.579
rs78503618	64871319	c.133+1841_1853delGGAAGTGGTCAGT	98.7	.278	.534	.368	.201
rs2289134	64884954	c.316+1014T>C	98.3	.307	.654	.909	1.000
rs1732877	64885411	c.316+1471T>C	97.7	.642	.616	.157	.515
rs11465955	64889666	c.381+99C>T	98.2	.250	.721	.909	.907
rs1152888	64891495	c.439G>A (p.Val147Ile)	98.0	.434	.139	.766	.289
rs1624395	64904483	c.654−2287G>A	98.9	.593	1.000	.519	.513
rs1370128	64904905	c.654−1865C>T	98.5	.522	.457	.164	.589
rs11176095	64919952	c.888−4581T>C	98.7	.005	.168	.421	.034
rs1152912	64920175	c.888−4358G>A	98.3	.280	.808	.245	.750
rs1152913	64920682	c.888−3851T>A	98.2	.865	.060	.031	.139
rs1152916	64921571	c.888−2962A>T	98.9	1.000	.056	.152	.072
rs10506481	64930378	*2157T>C	98.3	.114	.011	.218	.003

Nominally significant associations are shown in boldface.

CR, Completion rate; HWE, hardy-Weinberg equilibrium.

*According to National Center for Biotechnology Information build 36.3.

TABLE E3. Summary statistics for EuroAIMs successfully genotyped in this study

rs No.	Alleles	Chromosome	Position*	CR (%)	MAF		HWE <i>P</i> values	
					Control subjects	Asthmatic patients	Control subjects	Asthmatic patients
rs1157492	C/T	1	211627299	97.9	0.430	0.397	.908	.549
rs11807062	A/C	1	3176394	97.5	0.171	0.179	.070	.089
rs1416467	A/G	1	80470749	98.5	0.491	0.469	.910	.162
rs1890131	C/T	1	164329131	98.3	0.303	0.293	.893	.694
rs2236876	A/G	1	169749143	97.2	0.315	0.325	.427	.347
rs2419063	A/G	1	186988492	97.8	0.105	0.122	1.000	.702
rs495347	C/T	1	17909228	98.6	0.304	0.300	.592	.285
rs725974	G/T	1	167344909	96.7	0.187	0.215	1.000	.275
rs7552548	A/G	1	82775867	96.5	0.320	0.310	.030	.177
rs10496610	C/G	2	123757682	99.2	0.230	0.222	.749	1.000
rs1448314	A/G	2	223811705	98.1	0.087	0.089	.101	.307
rs1517407	A/G	2	63401982	98.2	0.474	0.469	.046	.323
rs3769005	C/G	2	136437098	98.2	0.482	0.523	.255	.805
rs4832640	C/T	2	19025771	97.1	0.255	0.272	.200	.214
rs6432110	A/T	2	10688616	98.1	0.431	0.458	.017	.620
rs6745653	C/T	2	97748515	98.8	0.116	0.124	.679	.709
rs1879558	C/T	3	155137053	98.7	0.188	0.176	.307	.122
rs2596834	C/T	3	10795067	95.4	0.495	0.470	.013	.561
rs4686497	A/G	3	190124209	98.3	0.168	0.205	.042	.209
rs822759	G/T	3	22948116	98.6	0.324	0.356	.846	.214
rs9290675	C/T	3	180489335	99.1	0.131	0.120	.135	.562
rs9861816	C/G	3	195649797	98.2	0.259	0.240	.235	.501
rs10516982	G/T	4	96963279	98.1	0.272	0.289	.566	.135
rs1373557	G/T	4	167601812	98.9	0.202	0.199	.332	.201
rs17443616	A/G	4	41063093	98.5	0.306	0.311	.640	.182
rs1873195	C/T	4	38713739	98.5	0.301	0.267	.788	.021
rs2014303	A/C	4	10262125	97.8	0.221	0.224	.869	.289
rs2251432	A/G	4	191063741	97.7	0.208	0.190	.195	.503
rs4555709	C/T	4	53867889	99.2	0.073	0.074	.035	.559
rs9328764	A/G	4	2143685	98.6	0.160	0.174	.599	.398
rs974020	C/T	4	117865302	98.6	0.296	0.270	.153	.470
rs16891982	C/G	5	33987450	99.3	0.201	0.253	.429	.452
rs33706	C/G	5	87540920	98.6	0.284	0.305	.036	.290
rs3822616	A/G	5	94828145	98.4	0.192	0.183	.143	.338
rs10484547	C/G	6	29560753	99.4	0.071	0.083	.828	.592
rs2187684	C/T	6	32872697	98.0	0.360	0.341	.902	.273
rs2804756	C/T	6	698586	99.1	0.086	0.079	.151	.253
rs756147	A/G	6	141672412	99.5	0.055	0.077	.009	.773
rs10486207	A/G	7	7884020	98.4	0.282	0.273	.184	.917
rs17864053	A/G	7	89801957	98.6	0.209	0.210	.229	.806
rs1922086	C/T	7	155515702	97.7	0.394	0.414	.006	.312
rs2097884	A/C	7	4125658	97.4	0.251	0.257	.172	.747
rs2219248	C/T	7	113965977	98.7	0.355	0.350	1.000	.059
rs2367191	A/G	7	141571268	98.3	0.413	0.405	.412	.671
rs2905347	A/G	7	22393559	96.4	0.435	0.449	.640	.677
rs10504924	C/T	8	94157437	97.7	0.092	0.123	.864	.704
rs920590	C/T	8	19695441	98.2	0.372	0.366	.114	.479
rs10512122	C/G	9	81715524	98.6	0.175	0.165	.170	.458
rs1408794	A/G	9	12641340	94.8	0.477	0.523	.011	.004
rs2086085	A/C	9	1680761	99.1	0.106	0.120	.455	.019
rs1045873	A/C	10	25177778	98.6	0.301	0.281	.105	.268
rs10508372	A/G	10	9012024	99.6	0.087	0.092	.723	.216
rs10509384	A/G	10	78693188	97.3	0.298	0.284	.276	.611
rs10509954	A/G	10	113658378	98.4	0.109	0.115	.143	.105
rs379773	C/G	10	109905342	95.4	0.353	0.354	.704	.470
rs7908825	C/G	10	75224547	97.4	0.324	0.312	1.000	.104
rs1560569	A/G	11	8896463	97.2	0.344	0.340	.344	.781
rs2847502	C/T	11	119623708	98.9	0.306	0.304	.206	.847
rs4938377	G/T	11	116801502	98.1	0.063	0.076	1.000	.141
rs923031	A/C	11	15777602	98.3	0.468	0.449	.138	.741
rs1003306	A/T	12	2624458	98.0	0.339	0.372	.447	.005

(Continued)

TABLE E3. (Continued)

rs No.	Alleles	Chromosome	Position*	CR (%)	MAF		HWE <i>P</i> values	
					Control subjects	Asthmatic patients	Control subjects	Asthmatic patients
rs1582398	A/G	12	78624019	97.5	0.129	0.140	.377	.732
rs3809125	C/T	12	55130616	98.3	0.367	0.369	.112	.724
rs7965049	A/G	12	7537044	97.5	0.245	0.253	.357	.227
rs998401	A/G	12	53641500	98.0	0.074	0.074	.058	1.000
rs7997100	C/T	13	67988075	98.4	0.349	0.348	.010	.719
rs986642	C/G	13	56944263	98.8	0.239	0.268	.160	.009
rs10483853	A/G	14	72826052	98.7	0.234	0.257	.344	.236
rs10519269	A/C	15	77705433	96.5	0.106	0.130	.095	.586
rs1129038	C/T	15	26030454	98.4	0.331	0.341	.898	.927
rs7163907	C/T	15	73632152	98.9	0.303	0.328	.254	.164
rs1107820	C/T	17	41491195	97.4	0.274	0.242	1.000	.120
rs1476162	A/G	17	8031808	98.6	0.135	0.146	.544	1.000
rs2003092	A/G	17	59516509	97.7	0.274	0.314	1.000	.337
rs959260	C/T	17	70881017	98.3	0.218	0.215	.934	.716
rs2418844	A/G	18	26037261	97.8	0.489	0.503	.253	.806
rs4892082	A/C	18	69051672	97.5	0.123	0.131	1.000	.721
rs523776	C/T	18	7554299	98.4	0.242	0.220	1.000	.722
rs959763	G/T	18	56797826	98.0	0.363	0.386	.854	.796
rs103294	C/T	19	59489660	98.7	0.162	0.156	.835	.643
rs202546	C/T	20	1611539	97.3	0.429	0.438	.449	.675
rs477627	A/G	20	47613465	97.6	0.193	0.217	.647	.904
rs969539	A/G	22	24942341	97.2	0.374	0.362	.626	.211

CR, Completion rate; HWE, Hardy-Weinberg equilibrium.

*According to National Center for Biotechnology Information build 35.

TABLE E4. Association summary, including unadjusted effects and *P* values, of *IRAK3* SNPs with asthma and atopic asthma

rs No.	Position*	SNP† (effect)	Risk allele	Minor allele frequency				OR (95% CI)‡			P values§		
				Control subjects	Asthmatic patients	Atopic asthmatic patients	Diagnosed at ≤22 y	Asthmatic patients	Atopic asthmatic patients	Diagnosed at ≤22 y	Asthmatic patients	Atopic asthmatic patients	Diagnosed at ≤22 y
rs1732888	64867853	−1494T>C	T	0.273	0.258	0.269	0.228				.344	.819	.098
rs1732887	64867883	−1464A>G	A	0.258	0.243	0.254	0.210	1.09 (0.93-1.27)	1.02 (0.85-1.23)	1.28 (0.96- 1.72)	.309	.819	.082
rs2701653	64868152	−1195C>T	T	0.094	0.104	0.106	0.134	1.14 (0.91-1.42)	1.15 (0.88-1.51)	1.49 (1.03-2.14)	.307	.355	.034
rs1732886	64870029	c.133+550A>G	A	0.256	0.242	0.247	0.205	1.08 (0.92-1.25)	1.04 (0.87-1.27)	1.32 (0.98-1.75)	.368	.610	.061
rs78503618	64871319	c.133+1841_1853delG GAAGTGG TCAGT	<i>ins</i>	0.267	0.269	0.231	0.192	.99 (0.85-1.16)	1.22 (1.00-1.49)	1.54 (1.12-2.13)	.866	.057	.004
rs56156535	64872409	c.133+2931T>C	C	0.402	0.422	0.439	0.463				.181	.046	.040
rs1168774	64877754	c.134−6004A>G	G	0.429	0.458	0.472	0.483				.038	.014	.001
rs4762088	64877849	c.134−5909G>C	C	0.386	0.407	0.425	0.449				.167	.048	.039
rs17767286	64878509	c.134−5249C>A	A	0.379	0.394	0.411	0.433				.325	.111	.071
rs17767298	64879508	c.134−4250A>G	G	0.362	0.377	0.393	0.413				.316	.108	.072
rs1882200	64883968	c.316+28C>T	T	0.331	0.347	0.365	0.384				.343	.084	.105
rs2289134	64884954	c.316+1014T>C	C	0.334	0.347	0.365	0.385	1.06 (0.91-1.22)	1.14 (0.96-1.36)	1.24 (0.96-1.59)	.410	.117	.095
rs1732877	64885411	c.316+1471T>C	C	0.417	0.445	0.470	0.488	1.13 (0.98-1.29)	1.24 (1.04-1.46)	1.46 (1.13-1.89)	.113	.008	.003
rs11465955	64889666	c.381+99C>T	T	0.334	0.350	0.368	0.392	1.07 (0.93-1.24)	1.16 (0.97-1.37)	1.28 (0.99-1.64)	.293	.082	.058
rs2293657	64891273	c.437−220A>T	T	0.286	0.310	0.327	0.348				.067	.018	.010
rs1152888	64891495	c.439G>A (p.Val147Ile)	A	0.080	0.091	0.100	0.112	1.17 (0.92-1.49)	1.29 (0.97-1.72)	1.46 (0.98-2.17)	.270	.085	.070
rs1821777	64892495	c.588+851C>T	T	0.343	0.350	0.369	0.390				.677	.177	.088
rs55715236	64904334	c.654−2436C>T	T	0.329	0.338	0.359	0.383				.589	.158	.068
rs1623665	64904449	c.654−2321G>T	T	0.415	0.442	0.466	0.489				.124	.023	.005
rs1624395	64904483	c.654−2287G>A	A	0.392	0.419	0.442	0.475	1.12 (0.98-1.29)	1.23 (1.04-1.45)	1.40 (1.09-1.79)	.101	.013	.007
rs1370127	64904584	c.654−2186G>A	A	0.392	0.419	0.442	0.475				.106	.022	.012
rs1370128	64904905	c.654−1865C>T	T	0.415	0.443	0.466	0.489	1.12 (0.98-1.29)	1.22 (1.04-1.44)	1.46 (1.14-1.87)	.107	.011	.001
rs10716217	64908730	c.887+313delA	<i>delA</i>	0.415	0.442	0.466	0.489				.125	.017	.001
rs1152908	64915771	c.887+7354T>A	A	0.399	0.423	0.444	0.484				.126	.015	.005
rs11176095	64919952	c.888−4581T>C	C	0.062	0.066	0.074	0.088	1.10 (0.84-1.44)	1.24 (0.90-1.70)	1.48 (0.96-2.28)	.618	.235	.086
rs1152912	64920175	c.888−4358G>A	A	0.479	0.483	0.499	0.561	1.02 (0.88-1.17)	1.10 (0.93-1.30)	1.40 (1.09-1.81)	.856	.273	.006
rs1152913	64920682	c.888−3851T>A	A	0.496	0.492	0.463	0.401	1.05 (0.92-1.20)	1.18 (1.00-1.39)	1.51 (1.17-1.94)	.515	.057	.003
rs1152915	64920925	c.888−3608A>C	C	0.304	0.312	0.318	0.304				.605	.428	.986
rs1152916	64921571	c.888−2962A>T	A	0.443	0.425	0.402	0.353	1.08 (0.94-1.23)	1.18 (1.00-1.40)	1.46 (1.13-1.89)	.310	.043	.002
rs10506481	64930378	*2157T>C	T	0.123	0.120	0.124	0.104	1.03 (0.84-1.27)	1.00 (0.78-1.28)	1.20 (0.81-1.79)	.767	.986	.362

tSNPs are underlined. Nominally significant associations are shown in boldface.

*According to National Center for Biotechnology Information (NCBI) build 36.3.

†According to the sequence with NCBI accession no. NC_000012.10.

‡Calculated only for the genotyped SNPs.

§Obtained from 1000 permutations.

TABLE E5. Analysis of haplotypes of 3 or 5 SNPs

Haplotype*	Frequencies				P value			OR (95% CI); P value†		
	Control subjects	Asthmatic patients	Atopic asthmatic patients	Diagnosed at ≤22 y	Asthmatic patients	Atopic asthmatic patients	Diagnosed at ≤22 y	Asthmatic patients	Atopic asthmatic patients	Diagnosed at ≤22 y
CGC	0.582	0.545	0.525	0.481	.032	.006	.001	Reference	Reference	Reference
<u>TAT</u>	.329	.340	.361	.383	.494	.113	.068	1.09 (0.94-1.27); .267	1.18 (0.98-1.41); .077	1.39 (1.07-1.81); .013
<u>CAT</u>	0.062	0.082	0.084	0.091	.029	.043	.066	1.37 (1.05-1.80); .020	1.46 (1.05-2.02); .023	1.75 (1.12-2.74); .015
<u>CGT</u>	0.022	0.022	0.027	0.036	.984	.957	.154	1.05 (0.65-1.68); .854	0.96 (0.52-1.80); .909	1.89 (0.94-3.83); .076
Omnibus					.071	.035	.010			
TTCGC	0.578	0.542	0.522	0.477	.077	.011	.003	Reference	Reference	Reference
CCTAT	0.327	0.336	0.358	0.376	.417	.118	.093	1.08 (0.93-1.26); .288	1.15 (0.97-1.39); .111	1.35 (1.03-1.77); .031
TCCAT	0.060	0.082	0.085	0.093	.016	.019	.035	1.37 (1.05-1.80); .022	1.43 (1.03-1.98); .032	2.01 (1.27-3.19); .003
TCCGT	0.021	0.021	0.022	0.035	.973	.941	.199	1.04 (0.64-1.70); .877	1.02 (0.55-1.88); .958	1.36 (0.62-3.00); .439
Omnibus					.084	.035	.019			

Nominally significant associations are shown in boldface. SNPs included in the haplotypes studied by Balaci et al^{E2} are underlined.

*Haplotypes are defined by 3 tSNPs (rs11465955, rs1624395, and rs1370128) or 5 SNPs (rs2289134, rs1732877, rs11465955, rs1624395, and rs1370128).

†P value from regression logistic model by using the most common haplotype as reference.

TABLE E6. Meta-analysis of *IRAK3* SNPs in unrelated case-control samples

		Minor allele frequency								Association tests					
		Spanish		Sardinian		Italian		Japanese		Individual studies OR (95% CI)*; <i>P</i> value				Meta-analysis OR (95% CI)*; <i>P</i> value	
Rs no.	Risk allele	Control subjects (n = 1271)	Cases† (n = 139)	Control subjects (n = 460)	Cases (n = 139)	Control subjects (n = 278)	Cases (n = 67)	Control subjects (n = 639)	Cases (n = 391)	Spanish‡	Sardinian	Italian	Japanese§	Europeans	All samples§
rs1732887	A	0.26	0.21					0.10	0.09	1.28 (0.96-1.72); .082			1.06 (0.78-1.43); .725		1.18 (0.95-1.46); .1420
rs2701653	T	0.09	0.13					0.41	0.43	1.49 (1.03-2.14); .034			1.05 (0.87-1.25); .620		1.11 (0.95-1.31); .1791
rs1732886	A	0.26	0.21	0.21	0.16					1.32 (0.98-1.75); .061	1.40 (0.98-2.00); .106			1.36 (1.08-1.72); .0105	
rs1882200	T	0.33	0.38	0.34	0.46					1.25 (0.97-1.61); .105	1.65 (1.26-2.17); .001			1.42 (1.18-1.71); .0002	
rs11465955	T	0.33	0.39	0.35	0.46	0.34	0.43			1.28 (0.99-1.64); .058	1.56 (1.18-2.04); .001	1.48 (1.01-2.18); .029		1.42 (1.2-1.68); .0001	
rs2293657	T	0.29	0.35	0.34	0.46			0.12	0.15	1.32 (1.01-1.71); .010	1.65 (1.26-2.17); .001		1.07 (0.82-1.39); .180	1.47 (1.22-1.77); .0001	1.31 (1.13-1.53); .0004
rs1152888	A	0.08	0.11					0.40	0.40	1.46 (0.98-2.17); .07			0.96 (0.81-1.15); .839		1.03 (0.87-1.21); .3719
rs1821777	T	0.34	0.39	0.36	0.46					1.22 (0.94-1.57); .088	1.54 (1.17-2.02); .001			1.36 (1.13-1.63); .0013	
rs1624395	A	0.39	0.48	0.42	0.51	0.40	0.53			1.40 (1.09-1.79); .007	1.46 (1.11-1.91); .002	1.68 (1.15-2.46); .004		1.47 (1.25-1.74); 5.7×10^{-6}	
rs1370128	T	0.42	0.49	0.43	0.54	0.41	0.55			1.46 (1.14-1.87); .001	1.53 (1.16-2.00); .001	1.76 (1.21-2.58); .002		1.54 (1.31-1.82); 2.9×10^{-7}	
rs10716217	delA	0.42	0.49					0.54	0.55	1.50 (1.17-1.92); .001			1.04 (0.87-1.24); .685		1.17 (1.02-1.36); .0295

*From an additive model.

†Atopic asthma diagnosed at 22 years or younger.

‡From Table E4.

§Only childhood cases from the Japanese study were considered in this analysis.

TABLE E7. Coverage of SNPs from the *IRAK3* gene with MAFs of 5% or greater on commercial arrays used in asthma GWASs in samples of European ancestry*

SNP array	Coverage (%)	
	HapMap CEU	Resequencing
Affymetrix 500K	63	29
Affymetrix Genome-Wide Human SNP Array 6.0	93	71
Illumina HumanHap300†	77	19
Illumina HumanHap550‡	83	35
Illumina HumanHap650Y	83	42
Illumina 1M-Duo	97	65

CEU, Ancestry from northern and western Europe.

*Includes studies described in Moffatt et al, 2007^{E20}; Himes et al, 2009^{E21}; Sleiman et al, 2009^{E22}; Li et al, 2010^{E23}; Moffatt et al, 2010^{E24}; Ferreira et al, 2011^{E25}; and Torgerson et al, 2011^{E26}

†Including the Illumina HumanCNV370-Duo as it was built on the Illumina HumanHap300 BeadChip (317,000 SNPs) with an additional 52,000 markers aimed at detecting copy number variations.

‡Including the Illumina Human610 because it is similar to the Illumina HumanHap550 with the addition of 60,000 markers to target regions with copy number variations.