

Assessing the Validity of Asthma Associations for Eight Candidate Genes and Age at Diagnosis Effects

María Pino-Yanes^{1,2*}, Almudena Corrales^{1,2}, José Cumplido³, Paloma Poza⁴, Inmaculada Sánchez-Machín⁴, Anselmo Sánchez-Palacios⁵, Javier Figueroa⁵, Orlando Acosta-Fernández⁶, Nisa Buset⁷, José Carlos García-Robaina⁸, Mariano Hernández⁹, Jesús Villar^{1,7,10}, Teresa Carrillo³, Carlos Flores^{1,2,9*}

1 Centro de Investigación Biomédica en Red de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain, **2** Research Unit, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain, **3** Allergy Unit, Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria, Spain, **4** Allergy Management Unit, Hospital del Torax (Ofra), Santa Cruz de Tenerife, Spain, **5** Allergy Unit, Hospital Universitario Insular de Gran Canaria, Las Palmas de Gran Canaria, Spain, **6** Neumology Unit, Hospital Universitario de Canarias, San Cristóbal de La Laguna, Spain, **7** Research Unit, Hospital Universitario Dr. Negrín, Las Palmas de Gran Canaria, Spain, **8** Allergy Unit, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain, **9** Applied Genomics Group (G2A), Genetics Laboratory, Instituto Universitario de Enfermedades Tropicales y Salud Pública de Canarias, Universidad de La Laguna, San Cristóbal de La Laguna, Spain, **10** Keenan Research Center, St. Michael's Hospital, Toronto, Ontario, Canada

Abstract

Background: Before the advent of genome-wide association studies (GWAS), *ADAM33*, *ADRB2*, *CD14*, *MS4A2* (alias *FCER1B*), *IL13*, *IL4*, *IL4R*, and *TNF* constituted the most replicated non-*HLA* candidate genes with asthma and related traits. However, except for the *IL13-IL4* region, none of these genes have been found in close proximity of genome-wide significant hits among GWAS for asthma or related traits. Here we aimed to assess the reproducibility of these asthma associations and to test if associations were more evident considering the effect of age at diagnosis.

Methodology/Principal Findings: We systematically evaluated 286 common single nucleotide polymorphisms (SNPs) of these 8 genes in a sample of 1,865 unrelated Spanish individuals (606 asthmatics and 1,259 controls). We found that variants at *MS4A2*, *IL4R* and *ADAM33* genes demonstrated varying association effects with the age at diagnosis of asthma, with 10 SNPs showing study-wise significance after the multiple comparison adjustment. In addition, *in silico* replication with GWAS data supported the association of *IL4R*.

Conclusions/Significance: Our results support the important role of *MS4A2*, *IL4R* and *ADAM33* genes in asthma and/or atopy susceptibility. However, additional studies in larger samples sets are needed to firmly implicate these genes in asthma susceptibility, and also to identify the causal variation underlying the associations found.

Citation: Pino-Yanes M, Corrales A, Cumplido J, Poza P, Sánchez-Machín I, et al. (2013) Assessing the Validity of Asthma Associations for Eight Candidate Genes and Age at Diagnosis Effects. PLoS ONE 8(9): e73157. doi:10.1371/journal.pone.0073157

Editor: Gualtiero I. Colombo, Centro Cardiologico Monzino IRCCS, Italy

Received: February 13, 2013; **Accepted:** July 18, 2013; **Published:** September 9, 2013

Copyright: © 2013 Pino-Yanes et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funded by grants from the Carlos III Health Institute (<http://www.isciii.es>) and cofunded by the European Regional Development Fund (FEDER) "A way of making Europe": PI081383 and EMER07/001, and by a grant from Fundación Canaria de Investigación y Salud FUNCIS 27/07 (<http://www.funcis.org>) to CF. MPY was funded by a postdoctoral fellowship from Fundación Ramón Areces (<http://www.fundacionareces.es>). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: cflores@ull.edu.es

† Current address: Department of Medicine, University of California San Francisco, San Francisco, California, United States of America

Introduction

Asthma is a complex respiratory disease characterized by chronic inflammation of the airways and frequently associated with atopy, pulmonary obstruction and bronchial hyper-responsiveness against a diversity of stimulus [1]. Its prevalence varies widely among different populations around the world (1–18%) [2]. Familiar clustering [3], twin studies [4], and genetic studies [5,6] support an important genetic component of the disease, with an estimated heritability of 60% [7].

Before the advent of genome-wide association studies (GWAS) [8], almost a thousand candidate-gene association studies for asthma and related traits were published [9]. Considering the gene

as the unit of replication and using a broad definition for asthma, Ober & Hoffjan [5] elegantly summarized the accumulated evidence for candidate-gene association studies from the literature by assessing the consistency of findings [10]. This yielded a ranking of candidate genes based on the number of positive associations between any polymorphism and any asthma trait [5]. As a result, eight non-*HLA* genes were put forward among the most replicated (in >10 independent studies) and, therefore, these genes were suggested as firm candidates for asthma susceptibility [5]. Four of these genes were located in the linked region for asthma on chromosome 5q: interleukin (*IL*) 4 (*IL4*), *IL13*, *CD14* and the β 2-adrenergic receptor (*ADRB2*); one gene was located in the linked region 6p21: the tumor necrosis factor (*TNF*); one was the first

positionally cloned asthma gene, ADAM metallopeptidase domain 33 (*ADAM33*); another was the gene encoding the α chain of the IL-4 and IL-13 receptors (*IL4R*); and finally, the gene encoding the IgE Fc receptor beta-subunit (*MS4A2*, alias *FCER1B*). However, most of them were assessed in studies with limited sample sizes averaging ≈ 200 individuals per group [5,11], and lacked of a systematical analysis in candidate-gene studies by surveying more than few variants (for example, by using tagging SNPs [tSNPs]). To date, despite the fact that more than ten GWAS of asthma have been published, none of these eight firm candidates have been replicated at genome wide significance, nor have been found in close proximity of GWAS hits, except for the *IL13-IL4* region [8,12–21].

Asthma is clinically recognized as an amalgam of several distinct phenotypes [22,23], which blur the complex genetic architecture underlying the disease susceptibility. Among these phenotypes, the age-at-onset of asthma could differentiate asthmatic groups, so that genetic variants might inconsistently associate with childhood and later-onset disease [17,24,25]. Motivated by this evidence, here we aimed to assess the reliability of asthma associations for the eight most replicated non-*HLA* asthma candidate genes and to explore whether effects of risk alleles varied with the disease age at diagnosis.

Methods

Ethics statement

This study was approved by the External Scientific Committee and Advisory Committee of Experts on ethical, economic, environmental, legal and social affairs at the National Bank and the Ethics Committee of Hospital Universitario NS de Candelaria and Hospital Universitario Doctor Negrín. Written informed consent was obtained from all subjects or appropriate surrogates on the behalf of participants under the age of 18.

Study subjects

This study was conducted using a case-control design of 1,878 DNA samples from unrelated individuals, all reporting at least two generations of Spanish descent. Sample details have been described elsewhere [24]. In brief, cases included 607 asthmatic patients aged >5 years and diagnosed by physicians following the Global Initiative for Asthma (GINA) guidelines for diagnosis and classification of asthma severity (<http://www.ginasthma.com>). These samples were collected and characterized for allergic and asthmatic symptoms in Respiratory Medicine and Allergy Departments, as part of the Genetics of Asthma study (GOA) in the Spanish population. Among cases, atopy was defined by the evidence of allergic sensitization to known allergens, reflected by either a positive skin prick test [SPT] or the specific IgE to one or more known allergens in the serum. For simplicity, those cases that had asthma and also atopy will be referred as atopic asthmatics, although we ignored whether or not allergen exposures lead to the asthma symptoms of these patients. Further sample details can be found in Text S1 and in Table S1.

Control group consisted of 1,271 DNA samples from adults self-reporting no personal or familiar medical history of allergic or pulmonary diseases recruited from the Spanish National DNA Biobank. These were collected from branches of the National Blood Service from unrelated individuals residing in Spain. After signed informed consent, by means of personal interviews, each donor was asked to declare general health status, physic activity, commonly used transportation, nutrition habits, type of work and qualification, demographics, tobacco smoke, alcohol consumption, genealogical information, residence and mother tongue, and

personal and familial history of diseases. See <http://www.bancoadn.org> for further information. In addition to the criteria of the Spanish National DNA Biobank to define healthy controls, we added three more criteria to select the controls for this study: 1) Self-reported Spanish ancestry based on having at least two generations of ancestors born in Spain; 2) Complete data on personal and familiar history of disease recorded in the questionnaire, smoking status, place of origin, and area of residence; 3) Absence of self-reported personal or familiar history of pulmonary or allergic disease. Further sample details can be found in Text S1 and in Table S1.

Selection of tagging SNPs

Tagging SNPs (tSNPs) were selected by means of TagIT 3.03 [26], using available re-sequencing data from European samples from different sources (Table 1 and Table S2). The *IL13* and *IL4* genes, which lie in close proximity, were considered as a single region. Similarly, given the strong linkage disequilibrium (LD) between *LTA* and *TNF* genes [27], common variants of the *LTA* gene were also tagged and jointly analyzed with *TNF*. See Text S1 and Table S2 for further details.

Assessment of population stratification

To reduce the risk for false positives due to major population stratification effects, a total of 83 European ancestry informative markers (termed EuroAIMs) were determined in case and control samples. These EuroAIMs allowed to correct for major differences in Spanish populations due to the North African genetic influences observed in this population, with a mean value of 5–9% for mainland populations and 16–20% for Canary Islanders [28]. A principal component analysis (PCA) based on these genetic markers was used to derive the ancestry estimates in cases and controls as scores of the first principal component (PC1), by means of EIGENSOFT [29]. A full list of EuroAIMs used and the genotyping procedures have been detailed elsewhere [24,28].

Genotyping

Genotyping was conducted using the iPLEX[®] Gold assay on MassARRAY[®] system (Sequenom Inc., San Diego, CA) by the Spanish National Genotyping Center, Santiago de Compostela Node (CeGen, <http://www.cegen.org>). Briefly, iPLEX[®] assays were scanned by MALDI-TOF mass spectrometry and individual SNP genotype calls were automatically generated using Sequenom TYPER 3.4[®] software (Sequenom Inc.). Samples from the Coriell Institute for Medical Research (<http://www.coriell.org>) were included on each SpectroCHIP[®] (Sequenom Inc.) to test allele calling reliability samples of this platform. The SNPs that gave poor quality data on this platform were finally determined at the Hospital Universitario N. S. de Candelaria using either SNaP-shot[®] Multiplex kit reactions (Applied Biosystems, Foster City, CA) or KASPar SNP Genotyping System assays (KBiosciences, Hertfordshire, UK). Genotyping was blind to the disease status and $\approx 6\%$ of the samples were genotyped in duplicate to monitor genotyping quality. See Text S1 for further details.

Statistical analysis

Clinical and demographical data were analyzed by means of the χ^2 -test and the Mann-Whitney U-test using R version 2.15 [30]. Departures from Hardy-Weinberg equilibrium (HWE) were evaluated separately for cases and controls using an exact test [31], by means of a custom script for STATISTICA (StatSoft Inc., Tulsa, OK) [32]. However, as deviations in cases have been considered a symptom of disease association [33–35], only those

Table 1. Summary information used for the selection of tagging SNPs (tSNPs) on the candidate genes.

Gene	Chr. (Mb)	Size (kb)	Covered region (kb)	Data sources	Selected tSNPs	Monomorphic	Final tSNPs	Final haplotype r^2
<i>IL13-IL4</i>	5q31.1 (132.0)	12	29.0	SeattleSNPs ^a	10	0	10	1.00
<i>CD14</i>	5q31.1 (140.0)	2	7.0	Innate Immunity ^b	6	1	5	1.00
<i>ADRB2</i>	5q31 (148.2)	2	9.5	SeattleSNPs ^a	8	1	7	1.00
<i>TNF-LTA</i>	6p21.3 (31.5)	6	9.3	SeattleSNPs ^a	11	0	11	0.85
<i>MS4A2</i>	11q13 (59.9)	10	15.3	HapMap/ T1D ^c	7	0	7	0.97
<i>IL4R</i>	16p12.1 (27.3)	51	56.0	SeattleSNPs ^a	21	0	21	0.92
<i>ADAM33</i>	20p13 (3.6)	14	15.2	EGP ^d	19	4	15	1.00
Total		97	141.3		82	6	76	

^aThe National Heart Lung and Blood Institute's (NHLBI) Programs for Genomic Applications (<http://pga.gs.washington.edu>).

^bThe Innate Immunity NHLBI Program for PGA (<https://regepi.bwh.harvard.edu/IIPGA2>).

^cHapMap phase 2 (<http://hapmap.ncbi.nlm.nih.gov>) and data from 96 type 1 diabetes individuals [28].

^dThe NIEHS Environmental Genome Project (<http://egp.gs.washington.edu>).

doi:10.1371/journal.pone.0073157.t001

tSNPs deviating significantly from HWE in the control group were filtered out from further analyses (threshold p -value = $7.0E-04$ after considering the multiple comparisons performed). Individual tSNP associations were tested under an additive model by means of regression analysis with SNPAssoc [36]. For that, PC1 scores were included as a covariate in regression models to adjust associations for population stratification, and allele effects were estimated as odds ratios (ORs) with 95% confidence intervals (CIs).

Additionally, MaCH 1.0 [37] was used to impute untyped SNPs with data from 380 European individuals deposited in The 1000 Genomes Project (1KGP), May 2011 version [38]. Association testing was performed using Mach2dat [37] adjusting for the PC1 scores. This analysis was conducted using allele dosages for those SNPs showing $MAF \geq 10\%$ and $Rsq > 0.3$, ensuring that all SNPs considered for association testing were accurately imputed (with $>90\%$ of SNPs having $Rsq > 0.8$, and with a mean Rsq across all imputed SNPs of 0.95 [IQR: 0.91–0.97]) (Table S3).

For each gene by separate, a conditional regression-based analysis was used to point out the independent association signals of each locus by including all SNPs associated at nominal significance. We then tested if association tests of the SNPs that represented nominal independent associations within each gene improved considering age-at-onset-varying effects, by implementing a sequential addition (SA) of cases [39]. For that, the age at diagnosis was utilized as a proxy for the age-at-onset of the disease, which was not recorded for most patients, and cases were grouped in categories of quartiles of age (14 [n = 155], 26 [n = 291], 39 [n = 427], and 82 years [n = 606]). The age at diagnosis cutoff obtained was next used to select a sub-sample of cases for which associations were tested again, both for tSNPs and imputed SNPs. LD patterns and regional association results were represented using LocusZoom 1.1 based on LD data from hg18 deposited by 1KGP [40].

To judge the significance of SNP associations in the context of the multiple comparisons performed, a false discovery rate (FDR) was calculated using QVALUE [41]. A FDR threshold of 5% (p -value ≤ 0.0012) was established to declare study-wise significance to limit the expected proportion of false positives incurred in the study when a particular individual SNP test was called significant. This was assessed considering altogether the p -values from all SNPs analyzed, both genotyped and imputed, the tests from the

SA of cases to obtain the age cutoff at which the allele effects were largest, and all the comparisons performed (i.e. associations with asthma, atopic asthma, and age-of-onset before the cutoffs). Functional annotation of associated SNPs was carried out using the software HaploReg [42].

Results

A total of 13 samples (1 case and 12 controls) were excluded from the analyses because of genotype quality (completion rate $<90\%$). Out of the initial set of 82 tSNPs, 6 were found monomorphic (rs5744440, rs35684381, rs597040, rs8124875, rs614971 and rs17548816) by using both iPLEX[®] and an alternative genotyping method (see the Supplementary methods in Text S1). Only one tSNP (rs12361312 at *MS4A2*) deviated significantly from HWE expectations in the control group and was discarded from further analyses (Table S2). Therefore, a total of 75 tSNPs, which maintained an adequate coverage for all genes ($r^2 \geq 0.85$), and 211 imputed SNPs were considered for association studies in 1,865 samples (606 cases and 1,259 controls) (Table S3). The mean completion rate among the 75 tSNPs was 98.5% (P_{25} – P_{75} = 98.7–100.0%), and the estimated overall genotype discordance rate among duplicated samples was 0.30% (95% CI = 0.08%–1.09%).

Association testing revealed a total of 35 SNPs (16 tSNPs and 19 imputed) that were associated with either asthma or atopic asthma at nominal significance, although these were not considered significant in the context of the multiple comparisons (p -values >0.0012) (Table S3). Based on the premise that incorporating the age-at-onset in the analyses might increase the power to detect association [24,25], we next used SA of asthma patients to estimate the age at diagnosis cutoff maximizing allele effects. For that, among the 35 SNPs that reached nominal significance, we first excluded the redundant SNPs from each gene using conditional logistic regressions for asthma or atopic asthma. We identified the following SNPs as the ones showing independent nominal associations: rs1800925 (–1112 C/T) in *IL13-IL4* (only for atopic asthma), rs2071590 in *LTA-TNF* (only for asthma), rs569108 (Gly237Glu) in *MS4A2*, and rs1805015 (Ser478Pro) in *IL4R* (both for asthma and atopic asthma) and rs2787095 in *ADAM33* (only for asthma) (data not shown). These 5 SNPs represented independent associations for each gene and coincidentally, these

5 SNPs had been associated with asthma or related traits in previous studies, but in this study we extended their association to a Southwestern European population with noticeable North African influences.

SA did not show any age at diagnosis cutoff that significantly maximized the association of rs1800925 (in *IL13-IL4*) with atopic asthma (lowest p -perm = 0.063). In contrast, SA revealed allele effects peaking at the same age at diagnosis, 39 years (number of cases = 427), for SNPs from *MS4A2* and *IL4R*: rs569108 in *MS4A2* (p -perm = 0.005), and rs1805015 in *IL4R* (p -perm = 0.001). However, SA revealed allele effects peaking at a different age at diagnosis for the SNPs from the other two genes: rs2071590 in *LTA-TNF* showing a maximum at 26 years (p -perm = 0.002, number of cases = 291), and rs2787095 in *ADAM33* with a maximum at 14 years (p -perm = 3.0E-04, number of cases = 155). The results obtained from the SA analyses using the quartiles of the distribution of the age at diagnosis were equivalent to those obtained using it as a continuous variable (data not shown).

Testing associations on the sub-sample of cases with the age at diagnosis of asthma before the maxima determined by SA for each gene revealed 18 additional SNPs reaching nominal significance (Table S3). Five of these SNPs ($0.013 \leq p$ -value ≤ 0.050) were located in *IL4R* gene and only one of them constituted a positive finding in previous studies. After conditioning these new associations from *IL4R* to the SNP rs1805015, only one SNP showed independent association (rs3024676, p -value = 0.021). The remaining 13 were all SNPs from *ADAM33* ($3.8E-5 \leq p$ -value ≤ 0.039), and 6 of them have been associated in at least one previous study (Table S3 and Figure 1). After adjusting the association of these 13 SNPs in *ADAM33* that emerged with the age at diagnosis cutoff for the SNP rs2787095, 7 SNPs (rs2787093, rs628965, rs628977, rs630712, rs598418, rs2853209, and rs603112) resulted independently associated from this SNP ($0.012 \leq p$ -value ≤ 0.048). Therefore, the advantages of taking into account the age at diagnosis varying effects for replication studies in asthma were clearly evidenced in *ADAM33*, a gene for which SNP-level replications are scarce in the literature [5,43]. Otherwise, we would have missed >50% of SNPs of this gene that showed association in previous studies. For the *LTA-TNF* and *MS4A2* genes, we only observed subtle increases of effect sizes for the SNPs that were revealed in our previous analyses, but did not evidence more SNPs reaching nominal significance (Table S3 and Figure 1). After a global FDR assessment accounting for all comparisons performed, only 10 SNPs in *MS4A2*, *IL4R* and *ADAM33* genes showed an FDR <5%, which were considered associated at study-wise significance (Table 2). Among these, 7 SNPs were identified to be functional, as they were either predicted to cause missense changes in the protein encoded or had empirically demonstrated regulatory roles as deduced from ENCODE project experimental data [42] (Table S4).

In order to provide evidence for replication at these *loci*, we accessed the GABRIEL data, the largest GWAS meta-analysis in asthma performed in Europeans [17]. There, we were able to allocate 11 out of the 51 SNPs that reached nominal significance with asthma in our study (Table S5). Only the SNP rs1805012, located in *IL4R*, demonstrated *in silico* replication in GABRIEL ($p = 5.7E-04$), showing the same direction of effects as in our study.

Discussion

In this study, we have comprehensively analyzed the association of 286 common variants of eight candidate genes with asthma and atopic asthma in a case-control Spanish sample and found associations for 10 SNPs in three of them (*MS4A2*, *IL4R* and

ADAM33) after considering all tests performed. We additionally provided *in silico* replication for *IL4R* with GWAS data from the GABRIEL study.

It is well known that the age-at-onset of asthma is associated with different phenotypic characteristics [44], and it has recently evidenced that age-varying genetic associations can cause non-replication and, consequently, lead to missing important genetic associations [45]. Therefore, here we re-evaluated the association of these genes by restricting the analysis to case subjects with an age at diagnosis of asthma before a cutoff that maximized allele effects of replicated variants. This allowed us to verify that association improved for certain genes, such as *ADAM33*, as recently supported for other firm candidates [25,46,47], and also to gain insight in the genetic complexity of asthma associations at these candidate genes. Intriguingly, many of their effects peaked in the range of age at diagnosis between 20 and 45 years, coincidental with the age range with the maximum expression of the disease [48,49]. It remains to be solved whether or not true biological mechanisms underlie this and previous observations [17,25,46]. Nevertheless, our results suggest that it will be worth considering the disease age at diagnosis in further studies, as well as in the research of improved asthma treatment and prevention.

To identify firm susceptibility genes and understand the biological processes underlying the development of the disease, replication in independent well-powered studies is essential, regardless of whether the first evidence of association was provided by a GWAS study or a candidate gene survey [50]. Besides, replication efforts allow testing the generalizability of findings in other populations, and discovering novel genetic *loci* contributing to phenotypic trait variability [51]. Particularly, testing the associations in populations of recent African ancestry will likely improve the detection of new risk variants [52], as they may offer the opportunity to refine the signal or to allocate the causal variants [53]. Our study aligns with these considerations, as it was performed in a population with sizeable North African genetic influences [28,54], and with a sample size representing a substantially larger population of cases (>97%) than the vast majority of prior published case-control studies of these genes in unrelated individuals, although still far from optimal to detect weak effects.

Under a simplistic scenario assuming complete LD of associated SNPs with causal variants, the analyzed sample size provided a 70% power to detect a minimum risk of 1.45 for a risk allele frequency of 45% with a two-sided $p = 0.0012$ significance level for the primary outcome (asthma), and ranged from 14.6% to 52.6% for the analyses in subset of cases with atopic asthma and asthma before the age at diagnosis cutoff (Table S6). We acknowledge that risk effects of this range are in the upper bound of those expected for common variants in complex traits [55], which may have contributed to our failure to detect associations for some of the genes tested. Alternatively, our failure to find associations may possibly be attributed to: i) Our impossibility to test their association with more relevant traits or patient sub-samples (e.g. asthma drug responses [56,57], environmental exposures [58]); ii) The use of controls self-reporting no personal or familial history of pulmonary or allergic disease, but without a disease confirmation based on a clinical characterization (e.g. lung function measurements, SPT or specific IgE testing); iii) The lack of a true association with asthma susceptibility, as has been suggested for particularly relevant variants by meta-analyses [56]. Whichever is correct, a recently published study with on a similar sample size showed positive and negative association results fully congruent with ours [59]. In support of our results, we were able to replicate *in silico* the association of a SNP in *IL4R* in the largest GWAS study

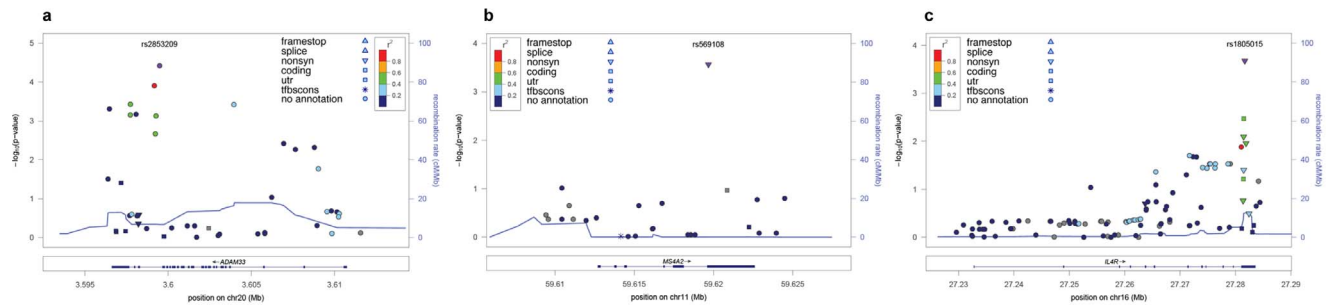


Figure 1. P-values of association by chromosome position with A) asthma ≤ 14 years for *ADAM33*, B) asthma ≤ 39 years for *MS4A2* and, C) asthma ≤ 39 years for *IL4R*. P-values are expressed in $-\log_{10}$ scale. The SNP number shown on the plot denotes the result for the most significant SNP for each gene and the results for the remaining were color coded to reflect their LD with this SNP based on pairwise r^2 values from the 1KGP. Estimated recombination rates (from 1KGP) were also plotted on the right axis to reflect the local LD structure.
doi:10.1371/journal.pone.0073157.g001

published to date that included more than 25,000 Europeans [17]. This SNP from *IL4R*, as well as few others from the same gene that were found associated in our study (rs1801275, rs1805015, and rs3024676), also demonstrated congruent effects and significant association in a recent GWAS of total IgE levels [60]. This evidence supports that, despite the enormous efforts to disentangle asthma genes such as those entailed by the GABRIEL study [17] or the EVE consortium [19], many more asthma susceptibility genes awaits its discovery.

Some recent replication studies focusing on candidate genes have utilized available arrays for genome wide genotyping [61–63] where common variants of many key asthma candidate genes could be insufficiently covered. In this respect, Michel et al. [59] indicated that only 37% of the previously associated SNPs from 14 candidate genes were captured by the array utilized by the same authors on the first GWAS for asthma and, surprisingly, not a single SNP from key asthma genes such as *ADAM33*, *IL4* and *CD14* was contained in their array [8]. Only after extending the study by further genotyping (and by imputation) on the same samples of their GWAS, these authors were able to consistently replicate many of the biological candidates that were missing from their GWAS [59]. We confirmed that the coverage of published

GWAS for asthma performed in European populations to date has been insufficient for *ADAM33* ($<30\%$), even in a best-case scenario using the HapMap phase 2 data as a reference for comparisons (Table S7). If array comparisons were made against the 1KGP sequencing data [38], the coverage would be even lower (Table S7). Besides, it is worth noting that the estimated coverage of these genes might be inflated, as these were implicitly derived for HapMap CEU data and the same data was used to inform the SNP contents of the array, and we have assumed that the 100% of SNPs contained in the array were successfully genotyped. Effects similar to those related to the age-of-onset of asthma, exceptionally explored [17], could have also contributed to find no association for the genes explored here in the published GWAS for asthma.

In conclusion, here we found the association of 10 common variants in three biological candidate genes (*MS4A2*, *IL4R* and *ADAM33*) that attained study-wise significance, and one of them was also supported by *in silico* replication in GWAS data. Therefore, we provided independent support for their role as risk factors for the amalgam of asthma phenotypes. Moreover, our results evidenced the genetic complexity at some of these susceptibility *loci* and the importance of considering age-at-onset effects. Given the low statistical power of the present study,

Table 2. Association summary of the 10 SNPs that resulted significantly associated with asthma after adjustments for the multiple comparisons.

Gene	rs#	Position ^a	Comparison	Allele1/ Allele2	Frequency controls ^b	Frequency cases ^b	OR (95% CI) ^b	p-value
<i>MS4A2</i>	<u>rs569108</u>	59863104	Asthma diagnosed ≤ 39	A/G	0.961	0.985	2.45 (1.39–4.33)	2.7E-04 ^c
<i>IL4R</i>	<u>rs1805015</u>	27374180	Asthma diagnosed ≤ 39	T/C	0.809	0.864	1.45 (1.17–1.80)	2.1E-04 ^c
<i>ADAM33</i>	rs2787093	3648462	Asthma diagnosed ≤ 14	T/C	0.890	0.822	0.56 (0.40–0.78)	4.9E-04
	rs628965	3649713	Asthma diagnosed ≤ 14	G/A	0.619	0.523	0.65 (0.50–0.84)	1.0E-04 ^c
	<u>rs628977</u>	3649721	Asthma diagnosed ≤ 14	C/T	0.617	0.516	0.66 (0.51–0.85)	3.7E-04 ^c
	rs630712	3650066	Asthma diagnosed ≤ 14	A/C	0.892	0.826	0.57 (0.41–0.80)	1.0E-04
	rs597980	3651165	Asthma diagnosed ≤ 14	A/G	0.440	0.326	0.60 (0.46–0.79)	1.2E-04 ^c
	rs598418	3651269	Asthma diagnosed ≤ 14	A/G	0.618	0.523	0.65 (0.50–0.84)	1.0E-04 ^c
	rs2853209	3651472	Asthma diagnosed ≤ 14	A/T	0.482	0.362	0.58 (0.45–0.75)	3.8E-05 ^c
	<u>rs2787095</u>	3655943	Asthma diagnosed ≤ 14	C/G	0.584	0.530	1.51 (1.19–1.91)	3.8E-04 ^c

tSNPs are underlined.

^aAccording to NCBI build 36.3.

^bComputed for allele 1.

^cSNPs associated in previous studies.

doi:10.1371/journal.pone.0073157.t002

particularly limited in the case subset analyses when considering the age at diagnosis, further studies will be needed to identify causal variants and to unravel if these genes are truly associated with asthma, with atopy or with both.

Supporting Information

Table S1 Relevant demographic and clinical features of GOA samples.

(DOC)

Table S2 Information, completion rates and Hardy-Weinberg equilibrium (HWE) *p*-values for the tSNPs.

(DOC)

Table S3 Association summary of SNPs with asthma, atopic asthma and asthma with age at diagnosis before the cutoff demonstrating the largest effects.

(DOC)

Table S4 Functional annotation of the 10 associated SNPs.

(DOC)

Table S5 *In silico* replication of the associated SNPs contained in the GABRIEL study.

(DOC)

Table S6 Sample sizes and statistical power for each analysis performed in a subset of cases.

References

- Ober C (2005) Perspectives on the past decade of asthma genetics. *J Allergy Clin Immunol* 116: 274–278.
- Braman SS (2006) The global burden of asthma. *Chest* 130: S4–12.
- Holberg CJ, Halonen M, Wright AL, Martinez FD (1999) Familial aggregation and segregation analysis of eosinophil levels. *Am J Respir Crit Care Med* 160: 1604–1610.
- Thomsen SF, van der Sluis S, Kyvik KO, Skytthe A, Backer V (2010) Estimates of asthma heritability in a large twin sample. *Clin Exp Allergy* 40: 1054–1061.
- Ober C, Hoffjan S (2006) Asthma genetics 2006: the long and winding road to gene discovery. *Genes Immun* 7: 95–100.
- Bouzigon E, Forabosco P, Koppelman GH, Cookson WO, Dizier MH, et al. (2010) Meta-analysis of 20 genome-wide linkage studies evidenced new regions linked to asthma and atopy. *Eur J Hum Genet* 18: 700–706.
- Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD (1990) Genetics of asthma and hay fever in Australian twins. *Am Rev Respir Dis* 142: 1351–1358.
- Moffatt MF, Kabisch M, Liang L, Dixon AL, Strachan D, et al. (2007) Genetic variants regulating ORM DL3 expression contribute to the risk of childhood asthma. *Nature* 448: 470–473.
- Swarr DT, Hakonarson H (2010) Unraveling the complex genetic underpinnings of asthma and allergic disorders. *Curr Opin Allergy Clin Immunol* 10: 434–442.
- Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, et al. (2007) Replicating genotype-phenotype associations. *Nature* 447: 655–660.
- Daley D, Lemire M, Akhabir L, Chan-Yeung M, He JQ, et al. (2009) Analyses of associations with asthma in four asthma population samples from Canada and Australia. *Hum Genet* 125: 445–459.
- Hancock DB, Romieu I, Shi M, Sienra-Monge JJ, Wu H, et al. (2009) Genome-wide association study implicates chromosome 9q21.31 as a susceptibility locus for asthma in Mexican children. *PLoS Genet* 5: e1000623.
- Himes BE, Hunninghake GM, Baurley JW, Rafaels NM, Sleiman P, et al. (2009) Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. *Am J Hum Genet* 84: 581–593.
- Mathias RA, Grant AV, Rafaels N, Hand T, Gao L, et al. (2010) A genome-wide association study on African-ancestry populations for asthma. *J Allergy Clin Immunol* 125: 336–346.
- Sleiman PM, Flory J, Imielinski M, Bradfield JP, Annaiah K, et al. (2010) Variants of DENND1B associated with asthma in children. *N Engl J Med* 362: 36–44.
- Li X, Howard TD, Zheng SL, Haselkorn T, Peters SP, et al. (2010) Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol* 125: 328–335.
- Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, et al. (2010) A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 363: 1211–1221.
- Ferreira MA, McRae AF, Medland SE, Nyholt DR, Gordon SD, et al. (2011) Association between ORM DL3, IL1RL1 and a deletion on chromosome 17q21 with asthma risk in Australia. *Eur J Hum Genet* 19: 458–464.
- Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, et al. (2011) Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 43: 887–892.
- Hirota T, Takahashi A, Kubo M, Tsunoda T, Tomita K, et al. (2011) Genome-wide association study identifies three new susceptibility loci for adult asthma in the Japanese population. *Nat Genet* 43: 893–896.
- Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, et al. (2011) Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. *Lancet* 378: 1006–1014.
- Haldar P, Pavord ID, Shaw DE, Berry MA, Thomas M, et al. (2008) Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med* 178: 218–224.
- Henderson J, Granel R, Sterne J (2009) The search for new asthma phenotypes. *Arch Dis Child* 94: 333–336.
- Pino-Yanes M, Sanchez-Machin I, Cumplido J, Figueroa J, Torres-Galvan MJ, et al. (2012) IL-1 receptor-associated kinase 3 gene (IRAK3) variants associate with asthma in a replication study in the Spanish population. *J Allergy Clin Immunol* 129: 573–575.
- Halapi E, Gudbjartsson DF, Jonsdottir GM, Bjornsdottir US, Thorleifsson G, et al. (2010) A sequence variant on 17q21 is associated with age at onset and severity of asthma. *Eur J Hum Genet* 18: 902–908.
- Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, et al. (2005) A single-nucleotide polymorphism tagging set for human drug metabolism and transport. *Nat Genet* 37: 84–89.
- Belfer I, Buzas B, Hipp H, Dean M, Evans C, et al. (2004) Haplotype structure of inflammatory cytokines genes (IL1B, IL6 and TNF/LTA) in US Caucasians and African Americans. *Genes Immun* 5: 505–512.
- Pino-Yanes M, Corrales A, Basaldua S, Hernandez A, Guerra L, et al. (2011) North African influences and potential bias in case-control association studies in the Spanish population. *PLoS One* 6: e18389.
- Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, et al. (2006) Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 38: 904–909.
- R Development Core Team (2008) R: A language and environment for statistical computing; computing RfIs, editor. Vienna, Austria.
- Wigginton JE, Cutler DJ, Abecasis GR (2005) A note on exact tests of Hardy-Weinberg equilibrium. *Am J Hum Genet* 76: 887–893.
- Sun X, Ma SF, Wade MS, Flores C, Pino-Yanes M, et al. (2010) Functional variants of sphingosine-1-phosphate receptor 1 gene associate with asthma susceptibility. *J Allergy Clin Immunol* 126: 241–249.

(DOC)

Table S7 Coverage of candidate genes on commercial arrays used in asthma GWAS in samples of European ancestry.

(DOC)

Text S1 Supplementary methods.

(DOC)

Acknowledgments

The authors want to thank Tobías Felipe for programming, and Maria Torres (CeGen, Santiago de Compostela Node) for her exceptional technical support with iPLEX® Gold assays. In addition, we would like to express our gratitude to the Juvenile Diabetes Research Foundation/Wellcome Trust Diabetes and Inflammation Laboratory (JDRF/WT DIL, Cambridge Institute for Medical Research) for granting the access to the MS442 re-sequencing data, and acknowledge that those who carried out the original analysis and collection of the data bear no responsibility for the analysis or interpretations of this study.

Author Contributions

Conceived and designed the experiments: MPY CF. Performed the experiments: MPY AC JC PP ISM ASP JF OAF NB JCGR JV TC. Analyzed the data: MPY CF. Contributed reagents/materials/analysis tools: CF MH JV. Wrote the paper: MPY MH CF.

33. Chakraborty R, Zhong Y (1994) Statistical power of an exact test of Hardy-Weinberg proportions of genotypic data at a multiallelic locus. *Hum Hered* 44: 1–9.
34. Salanti G, Amountza G, Ntzani EE, Ioannidis JP (2005) Hardy-Weinberg equilibrium in genetic association studies: an empirical evaluation of reporting, deviations, and power. *Eur J Hum Genet* 13: 840–848.
35. Balding DJ (2006) A tutorial on statistical methods for population association studies. *Nat Rev Genet* 7: 781–791.
36. Gonzalez JR, Armengol L, Sole X, Guino E, Mercader JM, et al. (2007) SNPassoc: an R package to perform whole genome association studies. *Bioinformatics* 23: 644–645.
37. Li Y, Willer CJ, Ding J, Scheet P, Abecasis GR (2010) MaCH: using sequence and genotype data to estimate haplotypes and unobserved genotypes. *Genet Epidemiol* 34: 816–834.
38. The 1000 Genomes Project Consortium (2010) A map of human genome variation from population-scale sequencing. *Nature* 467: 1061–1073.
39. Macgregor S, Craddock N, Holmans PA (2006) Use of phenotypic covariates in association analysis by sequential addition of cases. *Eur J Hum Genet* 14: 529–534.
40. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, et al. (2010) LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 26: 2336–2337.
41. Storey JD, Tibshirani R (2003) Statistical significance for genomewide studies. *Proc Natl Acad Sci USA* 100: 9440–9445.
42. Ward LD, Kellis M (2012) HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 40: D930–934.
43. Vercelli D (2008) Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 8: 169–182.
44. Gelfand EW (2008) Is asthma in childhood different from asthma in adults? Why do we need special approaches to asthma in children? *Allergy Asthma Proc* 29: 99–102.
45. Lasky-Su J, Lyon HN, Emilsson V, Heid IM, Molony C, et al. (2008) On the replication of genetic associations: timing can be everything! *Am J Hum Genet* 82: 849–858.
46. Bouzigon E, Corda E, Aschard H, Dizier MH, Boland A, et al. (2008) Effect of 17q21 variants and smoking exposure in early-onset asthma. *N Engl J Med* 359: 1985–1994.
47. Castro-Giner F, de Cid R, Gonzalez JR, Jarvis D, Heinrich J, et al. (2010) Positionally cloned genes and age-specific effects in asthma and atopy: an international population-based cohort study (ECRHS). *Thorax* 65: 124–131.
48. Barbee RA, Kaltenborn W, Lebowitz MD, Burrows B (1987) Longitudinal changes in allergen skin test reactivity in a community population sample. *J Allergy Clin Immunol* 79: 16–24.
49. Boulet LP, Turcotte H, Laprise C, Lavertu C, Bedard PM, et al. (1997) Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy* 27: 52–59.
50. Nicolae DL, Ober C (2009) (Too) great expectations: the challenges in replicating asthma disease genes. *Am J Respir Crit Care Med* 179: 1078–1079.
51. Pulit SL, Voight BF, de Bakker PI (2010) Multiethnic genetic association studies improve power for locus discovery. *PLoS One* 5: e12600.
52. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, et al. (2009) Finding the missing heritability of complex diseases. *Nature* 461: 747–753.
53. Baye TM, Butsch Kovacic M, Biagini Myers JM, Martin LJ, Lindsey M, et al. (2011) Differences in candidate gene association between European ancestry and African American asthmatic children. *PLoS One* 6: e16522.
54. Botigue LR, Henn BM, Gravel S, Maples BK, Gignoux CR, et al. (2013) Gene flow from North Africa contributes to differential human genetic diversity in southern Europe. *Proc Natl Acad Sci USA*, in press.
55. Hindorf LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, et al. (2009) Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci USA* 106: 9362–9367.
56. Contopoulos-Ioannidis DG, Manoli EN, Ioannidis JP (2005) Meta-analysis of the association of beta2-adrenergic receptor polymorphisms with asthma phenotypes. *J Allergy Clin Immunol* 115: 963–972.
57. Finkelstein Y, Bournissen FG, Hutson JR, Shannon M (2009) Polymorphism of the ADRB2 gene and response to inhaled beta-agonists in children with asthma: a meta-analysis. *J Asthma* 46: 900–905.
58. Simpson A, John SL, Jury F, Niven R, Woodcock A, et al. (2006) Endotoxin exposure, CD14, and allergic disease: an interaction between genes and the environment. *Am J Respir Crit Care Med* 174: 386–392.
59. Michel S, Liang L, Depner M, Klopp N, Ruehler A, et al. (2010) Unifying candidate gene and GWAS Approaches in Asthma. *PLoS One* 5: e13894.
60. Granada M, Wilk JB, Tuzova M, Strachan DP, Weidinger S, et al. (2012) A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. *J Allergy Clin Immunol* 129: 840–845.
61. Galanter JM, Torgerson D, Gignoux CR, Sen S, Roth LA, et al. (2011) Cosmopolitan and ethnic-specific replication of genetic risk factors for asthma in 2 Latino populations. *J Allergy Clin Immunol* 128: 37–43.
62. Rogers AJ, Raby BA, Lasky-Su JA, Murphy A, Lazarus R, et al. (2009) Assessing the reproducibility of asthma candidate gene associations, using genome-wide data. *Am J Respir Crit Care Med* 179: 1084–1090.
63. Wu H, Romieu I, Shi M, Hancock DB, Li H, et al. (2010) Evaluation of candidate genes in a genome-wide association study of childhood asthma in Mexicans. *J Allergy Clin Immunol* 125: 321–327.

Table S1. Relevant demographic and clinical features of GOA samples.

Variable	Cases (n = 607)	Controls (n = 1,271)	p-value
Gender (male %)	34.3	59.1	<0.001 ^a
Age at recruitment, median years (P ₂₅ -P ₇₅) ^b	34 (25-44)	40 (32-48)	<0.001 ^c
Smoking habits (ever smokers, %)	27.3	48.1	<0.001 ^a
Clinical features:			
Total IgE levels (IU/mL), median (P ₂₅ -P ₇₅) ^b	202.0 (90.1-396.3)	NA	
Age at diagnosis, median (P ₂₅ -P ₇₅) ^b	14 (26-39)	NA	
FEV1% predicted, median (P ₂₅ -P ₇₅) ^b	83 (68-95)	NA	
Allergic rhinitis (%)	74.2	NA	
Atopic dermatitis (%)	20.2	NA	
SPT (% positive)	76.9	NA	
Specific IgE (% positive)	51.6	NA	
Atopy (%)	76.7	NA	
Asthma Severity (%):			
Severe	28.8		
Moderate	23.9		
Mild	47.3		
Treatment:			
Inhaled corticoids (%)	85.6	NA	
Oral corticoids (%)	4.6	NA	
Long-acting β 2-agonists (%)	53.0	NA	
Short acting β 2 agonists (%)	81.8	NA	
Body mass index kg/m ² , median (P ₂₅ -P ₇₅) ^b	24.6 (21.1-27.5)	NA	

^a χ^2 test; ^bP₂₅, Percentile 25; P₇₅, Percentile 75; ^cMann-Whitney U-test; NA, not available.

Table S2. Information, completion rates and Hardy-Weinberg equilibrium (HWE) *p*-values for the tSNPs.

Gene	rs#	Position ^a	Effects and other designations	CR ^b (%)	HWE Controls	HWE Cases
<i>IL13-IL4</i>	rs1800925	132020708	-1112 C/T	97	0.858	0.283
	rs20541	132023863	Gln130Arg	99	0.031	0.637
	rs1295683	132026775		96	0.121	0.607
	rs2243297	132027070		96	0.408	1.000
	rs2243208	132029050		99	1.000	0.428
	rs2243211	132029321		99	0.836	0.394
	rs2243302	132032430		97	0.711	0.704
	rs2070874	132037609	Prom+33	91	0.027	0.026
	rs2227284	132040624	SNP+3017	99	0.667	0.006
	rs2243281	132044294		97	0.341	0.789
<i>LTA-TNF</i>	rs915654	31646476		96	0.010	0.863
	rs2844482	31647746	-753 G/A	98	0.602	1.000
	rs2071590	31647747		97	0.045	0.852
	rs2239704	31648120		96	0.518	0.793
	rs909253	31648292	NcoI Intron 1, 252 A/G	98	0.721	0.684
	rs746868	31648408		99	0.643	0.664
	rs2229092	31648736		99	0.826	1.000
	rs1041981	31648763		94	0.609	0.026
	rs1799724	31650461	-857 C/T	99	0.672	0.117
	rs1800629	31651010	-308 G/A	98	0.612	0.185
	rs1800610	31651806		99	1.000	0.056
<i>CD14</i>	rs2915863	139994561	-1601 intronic C/T	92	0.002	0.223
	rs2569191	139994087		97	0.059	0.621
	rs2569190	139993100	-159 C/T	98	0.231	1.000
	rs4914	139991652	1309 synonymous coding C/G	98	0.214	0.682
	rs2563298	139991499	1462 C/A	93	0.497	0.741
<i>MS4A2</i>	rs12361312	59609172		98	9.9E-06	0.358
	rs513986	59609429		99	0.728	0.799
	rs2583477	59610431		97	0.605	0.679
	rs573790	59611961		98	0.249	0.653
	rs1441586	59612604	-109 C/T	98	0.425	0.680
	rs569108	59619680	Gly237Glu	99	0.433	0.042
	rs2847655	59622247	RsaIex7	99	0.272	0.616
<i>ADRB2</i>	rs12654778	148185934	-654 A/G	98	0.051	0.547
	rs11959427	148186221		94	0.285	0.857
	rs1042713	148186633	Arg16Gly	98	0.129	0.800
	rs1042718	148187110		97	0.126	0.030
	rs1042719	148187640		97	0.319	0.314
	rs1042720	148187826		98	1.000	0.785
	rs4705271	148192184		99	0.091	0.379

<i>ADAM33</i>	rs554743	3610142		98	0.884	0.102
	rs2853213	3609840		98	0.660	0.195
	rs17548962	3609803		98	1.000	1.000
	rs570269 ^c	3607647		98	0.109	0.793
	rs2853210	3606211		97	0.815	0.910
	rs597165	3605804		98	1.000	0.602
	rs2787095	3603943		99	0.449	0.068
	rs511898	3603085	216_F_+1 Intron	99	0.622	0.663
	rs528557	3599742	216_S_2 ORF (Gly717Gly)	97	0.883	0.262
	rs574174	3598694	216_ST_+7 Intron	98	0.924	0.433
	rs2280091	3598234	216_T_1 ORF (Thr764Met)	98	0.320	0.314
	rs2280090	3598205	216_T_2 ORF (Ser774Pro)	99	0.152	0.254
	rs678881	3597803		98	1.000	0.917
	rs628977	3597721		98	0.433	1.000
	rs512625	3596378		98	0.623	0.848
<i>IL4R</i>	rs2057768	27229596	-3223 C/T	98	0.423	1.000
	rs1110470	27243928		98	0.954	0.115
	rs2283563	27253855		98	0.519	0.693
	rs3024537	27260320		99	0.382	0.626
	rs1805010	27263704	Ile50Val	97	0.730	0.184
	rs2301807	27265599		97	0.698	1.000
	rs3024585	27267345		98	0.122	0.624
	rs3024586	27267542		99	0.027	0.572
	rs2283561	27268668		95	0.859	0.800
	rs3024607	27271112		99	0.359	0.759
	rs3024613	27271754		98	0.955	0.461
	rs3024632	27273797		99	0.563	0.239
	rs3024676	27281059		99	0.165	0.175
	rs2234897	27281113		100	1.000	0.224
	rs1805011	27281373	Glu375Ala	99	0.185	0.866
	rs1805012	27281465	Cys406Arg	99	0.156	0.173
	rs1805013	27281481	Ser411Leu	99	0.140	1.000
	rs1805015	27281681	Ser478Pro	98	0.359	0.039
	rs1801275	27281901	Gln551Arg	98	0.026	0.104
	rs1805016	27282428	Ser727Ala	98	0.457	0.715
	rs1049631	27283043		98	0.040	0.032

^aAccording to NCBI build 36.3;^bCompletion rate; ^cNo distinction was made between C and the new T allele for this analysis. Nominal significant deviations in bold.

Table S3. Association summary of SNPs with asthma, atopic asthma and asthma with age-at-diagnosis of the disease before the cutoff demonstrating the largest effects at replicated SNPs.

Gene	rs#	Position ^a	Allele1/Allele2	Rsq ^b	Frequency allele 1				OR ± SE ^c			p-value		
					Controls	Asthma	Atopic asthma	Asthma before age-at-diagnosis cutoff	Asthma	Atopic asthma	Asthma before age-at-diagnosis cutoff	Asthma	Atopic asthma	Asthma before age-at-diagnosis cutoff
<i>IL13-IL4</i>	rs115008099	131991881	C/T	0.79	0.823	0.805	0.792	NA	0.84 ± 0.10	0.78 ± 0.12	NA	0.094	0.035	NA
	rs1881457	131992409	A/C	0.80	0.820	0.801	0.789	NA	0.84 ± 0.10	0.78 ± 0.12	NA	0.092	0.035^d	NA
	<u>rs1800925</u>	131992809	C/T		0.801	0.779	0.764	NA	0.85 ± 0.10	0.78 ± 0.11	NA	0.100	0.037^d	NA
	rs1295687	131994462	G/C	0.37	0.950	0.955	0.948	NA	1.32 ± 0.28	0.89 ± 0.31	NA	0.319	0.697	NA
	rs1295686	131995843	C/T	0.96	0.848	0.843	0.822	NA	0.94 ± 0.10	0.82 ± 0.11	NA	0.513	0.080	NA
	<u>rs20541</u>	131995964	G/A		0.854	0.848	0.826	NA	0.94 ± 0.10	0.83 ± 0.11	NA	0.462	0.066	NA
	rs1295685	131996445	G/A	0.96	0.846	0.841	0.818	NA	0.94 ± 0.10	0.82 ± 0.11	NA	0.505	0.069	NA
	rs848	131996500	C/A	0.96	0.846	0.840	0.818	NA	0.94 ± 0.10	0.82 ± 0.11	NA	0.506	0.069	NA
	rs847	131996669	C/T	0.96	0.845	0.840	0.818	NA	0.94 ± 0.10	0.82 ± 0.11	NA	0.509	0.069	NA
	rs2069757	131998413	G/A	0.97	0.927	0.926	0.913	NA	0.96 ± 0.14	0.82 ± 0.16	NA	0.767	0.197	NA
	<u>rs1295683</u>	131998876	G/A		0.919	0.912	0.899	NA	0.93 ± 0.14	0.83 ± 0.15	NA	0.400	0.109	NA
	<u>rs2243297</u>	131999171	T/A		0.963	0.966	0.956	NA	1.01 ± 0.18	0.80 ± 0.20	NA	0.733	0.398	NA
	rs2243204	131999494	C/T	0.96	0.926	0.925	0.912	NA	0.96 ± 0.14	0.82 ± 0.16	NA	0.786	0.196	NA
	<u>rs2243208</u>	132001151	A/G		0.923	0.914	0.902	NA	0.91 ± 0.13	0.79 ± 0.15	NA	0.367	0.082	NA
	rs2243210	132001386	G/A	0.97	0.925	0.920	0.911	NA	0.92 ± 0.13	0.83 ± 0.15	NA	0.553	0.226	NA
	<u>rs2243211</u>	132001422	C/A		0.926	0.922	0.914	NA	0.93 ± 0.13	0.83 ± 0.15	NA	0.572	0.263	NA
	rs2243217	132001933	A/T	0.91	0.922	0.916	0.906	NA	0.90 ± 0.13	0.80 ± 0.15	NA	0.449	0.147	NA
	rs2243218	132002024	G/A	0.89	0.922	0.916	0.907	NA	0.91 ± 0.14	0.80 ± 0.16	NA	0.470	0.155	NA
	rs2243219	132002125	A/G	0.88	0.921	0.915	0.905	NA	0.91 ± 0.14	0.80 ± 0.16	NA	0.472	0.157	NA
	rs2243221	132002844	C/T	0.87	0.923	0.918	0.908	NA	0.90 ± 0.14	0.80 ± 0.16	NA	0.459	0.156	NA
	rs2243300	132004086	G/T	0.81	0.922	0.917	0.908	NA	0.90 ± 0.14	0.80 ± 0.16	NA	0.469	0.166	NA
	rs2243228	132004363	A/C	0.75	0.915	0.911	0.902	NA	0.92 ± 0.14	0.81 ± 0.17	NA	0.562	0.209	NA
	<u>rs2243302</u>	132004531	G/A		0.866	0.876	0.870	NA	1.09 ± 0.12	1.04 ± 0.14	NA	0.460	0.874	NA
	rs762534	132004756	C/A	0.66	0.913	0.910	0.901	NA	0.92 ± 0.15	0.80 ± 0.18	NA	0.589	0.209	NA
	rs2243248	132008644	T/G	0.59	0.929	0.933	0.932	NA	1.09 ± 0.18	1.08 ± 0.22	NA	0.627	0.719	NA
	rs2243250	132009154	C/T	0.97	0.853	0.855	0.856	NA	1.00 ± 0.10	1.02 ± 0.12	NA	0.990	0.877	NA
	<u>rs2070874</u>	132009710	C/T		0.868	0.867	0.867	NA	1.00 ± 0.10	1.02 ± 0.12	NA	0.816	0.883	NA
	rs734244	132010726	C/T	0.96	0.853	0.854	0.856	NA	1.00 ± 0.10	1.02 ± 0.12	NA	0.987	0.867	NA
	<u>rs2227284</u>	132012725	G/T		0.728	0.734	0.727	NA	1.04 ± 0.08	1.02 ± 0.10	NA	0.771	0.932	NA
	rs2227282	132013179	G/C	0.95	0.739	0.749	0.744	NA	1.04 ± 0.08	1.02 ± 0.10	NA	0.625	0.821	NA
	rs2243263	132013299	G/C	0.97	0.898	0.911	0.907	NA	1.15 ± 0.12	1.10 ± 0.15	NA	0.247	0.504	NA
	rs2243266	132013789	G/A	0.94	0.851	0.852	0.853	NA	0.99 ± 0.10	1.01 ± 0.12	NA	0.927	0.952	NA
	rs2243267	132013886	G/C	0.94	0.851	0.852	0.852	NA	0.99 ± 0.10	1.01 ± 0.12	NA	0.927	0.953	NA
	rs2243268	132013963	A/C	0.94	0.851	0.852	0.852	NA	0.99 ± 0.10	1.01 ± 0.12	NA	0.926	0.954	NA
	rs2243270	132014109	A/G	0.92	0.841	0.838	0.837	NA	0.97 ± 0.10	0.97 ± 0.12	NA	0.724	0.779	NA

	rs2243274	132014832	G/A	0.91	0.841	0.838	0.837	NA	0.97 ± 0.10	0.97 ± 0.12	NA	0.723	0.778	NA
	<u>rs2243281</u>	132016395	T/C		0.901	0.915	0.910	NA	1.15 ± 0.12	1.10 ± 0.15	NA	0.218	0.514	NA
	rs2243282	132016554	C/A	0.93	0.851	0.851	0.852	NA	0.99 ± 0.10	1.01 ± 0.12	NA	0.922	0.959	NA
	rs2243284	132016992	G/A	0.89	0.842	0.838	0.838	NA	0.97 ± 0.10	0.97 ± 0.12	NA	0.744	0.785	NA
	rs2243285	132016993	G/T	0.97	0.900	0.913	0.909	NA	1.16 ± 0.12	1.11 ± 0.15	NA	0.241	0.487	NA
	rs2243288	132017944	A/G	0.89	0.840	0.836	0.836	NA	0.97 ± 0.10	0.97 ± 0.12	NA	0.717	0.767	NA
	rs2243289	132018132	A/G	0.91	0.850	0.850	0.851	NA	0.99 ± 0.10	1.01 ± 0.12	NA	0.918	0.968	NA
	rs2243290	132018169	C/A	0.91	0.850	0.850	0.851	NA	0.99 ± 0.10	1.01 ± 0.12	NA	0.918	0.968	NA
	rs2243291	132018983	G/C	0.89	0.840	0.836	0.836	NA	0.97 ± 0.10	0.97 ± 0.12	NA	0.717	0.767	NA
	rs2243293	132019799	A/G	0.89	0.747	0.760	0.757	NA	1.06 ± 0.09	1.06 ± 0.10	NA	0.467	0.600	NA
CD14	rs2569188	140008419	A/G	0.98	0.483	0.503	0.485	NA	1.07 ± 0.07	1.00 ± 0.09	NA	0.363	0.992	NA
	rs58150262	140008476	C/T	0.87	0.789	0.773	0.768	NA	0.92 ± 0.09	0.88 ± 0.11	NA	0.358	0.235	NA
	rs2563315	140008505	G/A	0.90	0.271	0.276	0.253	NA	1.02 ± 0.08	0.90 ± 0.10	NA	0.808	0.285	NA
	rs7723769	140008880	C/T	0.87	0.789	0.773	0.768	NA	0.92 ± 0.09	0.88 ± 0.11	NA	0.358	0.235	NA
	rs56746017	140008901	T/A	0.87	0.789	0.773	0.768	NA	0.92 ± 0.09	0.88 ± 0.11	NA	0.358	0.235	NA
	rs7728187	140009273	C/G	0.86	0.790	0.774	0.769	NA	0.92 ± 0.09	0.88 ± 0.11	NA	0.358	0.235	NA
	<u>rs2563298</u>	140011315	A/C		0.258	0.263	0.239	NA	1.02 ± 0.08	0.89 ± 0.10	NA	0.774	0.322	NA
	<u>rs4914</u>	140011468	G/C		0.116	0.113	0.105	NA	0.96 ± 0.11	0.90 ± 0.14	NA	0.670	0.411	NA
	<u>rs2569190</u>	140012916	G/A		0.482	0.494	0.487	NA	1.06 ± 0.07	1.00 ± 0.08	NA	0.276	0.883	NA
	rs5744455	140013307	G/A	0.87	0.786	0.771	0.765	NA	0.92 ± 0.09	0.88 ± 0.11	NA	0.357	0.232	NA
	rs5744454	140013567	T/G	0.93	0.778	0.762	0.756	NA	0.92 ± 0.09	0.88 ± 0.10	NA	0.356	0.233	NA
	<u>rs2569191</u>	140013903	T/C		0.484	0.496	0.488	NA	1.06 ± 0.07	1.00 ± 0.08	NA	0.355	0.890	NA
	rs3138078	140014117	C/A	0.91	0.782	0.767	0.761	NA	0.92 ± 0.09	0.88 ± 0.11	NA	0.372	0.239	NA
	<u>rs2915863</u>	140014377	T/C		0.460	0.444	0.460	NA	1.04 ± 0.07	0.99 ± 0.08	NA	0.571	0.918	NA
	rs3138076	140014613	T/C	0.94	0.775	0.759	0.753	NA	0.93 ± 0.09	0.89 ± 0.10	NA	0.385	0.246	NA
	rs2569192	140015208	C/G	0.95	0.258	0.260	0.237	NA	1.01 ± 0.08	0.89 ± 0.10	NA	0.920	0.242	NA
	rs2569193	140015495	A/G	0.95	0.258	0.261	0.237	NA	1.01 ± 0.08	0.89 ± 0.10	NA	0.920	0.242	NA
	rs2563310	140015741	G/A	0.98	0.484	0.502	0.484	NA	1.06 ± 0.07	1.00 ± 0.09	NA	0.429	0.962	NA
	rs3138074	140015932	A/T	0.93	0.775	0.759	0.753	NA	0.93 ± 0.09	0.89 ± 0.10	NA	0.385	0.245	NA
	rs5744441	140016847	G/A	0.90	0.780	0.765	0.759	NA	0.93 ± 0.09	0.89 ± 0.11	NA	0.384	0.245	NA
	rs4912717	140018235	A/G	0.93	0.260	0.262	0.239	NA	1.01 ± 0.08	0.89 ± 0.10	NA	0.923	0.241	NA
	rs4912718	140018378	T/C	0.97	0.485	0.503	0.486	NA	1.06 ± 0.07	1.00 ± 0.09	NA	0.428	0.963	NA
ADRB2	rs11746634	148202668	G/C	0.95	0.628	0.618	0.610	NA	0.96 ± 0.08	0.92 ± 0.09	NA	0.586	0.366	NA
	rs11168067	148202801	G/A	0.95	0.628	0.618	0.610	NA	0.96 ± 0.08	0.92 ± 0.09	NA	0.586	0.365	NA
	rs9325122	148202936	T/C	0.92	0.649	0.641	0.631	NA	0.96 ± 0.08	0.92 ± 0.09	NA	0.611	0.344	NA
	rs11957351	148203104	T/C	0.37	0.370	0.355	0.353	NA	0.84 ± 0.12	0.82 ± 0.14	NA	0.151	0.177	NA
	rs11960649	148203144	C/A	0.87	0.605	0.594	0.587	NA	0.95 ± 0.08	0.92 ± 0.09	NA	0.543	0.357	NA
	rs35684381	148203236	T/C	0.73	0.818	0.823	0.814	NA	1.04 ± 0.11	0.96 ± 0.13	NA	0.720	0.768	NA
	rs1432622	148203762	C/T	0.96	0.628	0.618	0.610	NA	0.96 ± 0.07	0.92 ± 0.09	NA	0.586	0.366	NA
	rs1432623	148204008	T/C	0.96	0.628	0.618	0.610	NA	0.96 ± 0.07	0.92 ± 0.09	NA	0.586	0.365	NA

	rs11168068	148204121	T/C	0.96	0.628	0.618	0.610	NA	0.96 ± 0.07	0.92 ± 0.09	NA	0.586	0.365	NA
	rs17778257	148204577	T/A	0.98	0.402	0.395	0.377	NA	0.98 ± 0.07	0.91 ± 0.09	NA	0.726	0.248	NA
	rs2400706	148204864	C/T	0.96	0.773	0.777	0.768	NA	1.02 ± 0.09	0.97 ± 0.10	NA	0.835	0.732	NA
	rs2895795	148204966	T/A	0.96	0.773	0.777	0.768	NA	1.02 ± 0.09	0.97 ± 0.10	NA	0.836	0.732	NA
	rs2400707	148205052	G/A	0.97	0.628	0.618	0.610	NA	0.96 ± 0.07	0.92 ± 0.09	NA	0.586	0.365	NA
	rs2053044	148205372	G/A	0.97	0.628	0.618	0.610	NA	0.96 ± 0.07	0.92 ± 0.09	NA	0.586	0.365	NA
	<u>rs12654778</u>	148205741	A/G		0.399	0.388	0.371	NA	0.96 ± 0.07	0.89 ± 0.09	NA	0.565	0.184	NA
	rs11168070	148205927	C/G	0.97	0.641	0.633	0.623	NA	0.96 ± 0.07	0.92 ± 0.09	NA	0.617	0.349	NA
	<u>rs11959427</u>	148206028	T/C		0.639	0.641	0.633	NA	0.97 ± 0.07	0.93 ± 0.09	NA	0.979	0.706	NA
	rs1042711	148206348	T/C	0.97	0.642	0.635	0.624	NA	0.97 ± 0.07	0.92 ± 0.09	NA	0.673	0.374	NA
	rs1801704	148206375	T/C	0.98	0.642	0.636	0.626	NA	0.97 ± 0.07	0.93 ± 0.09	NA	0.691	0.409	NA
	<u>rs1042713</u>	148206440	A/G		0.416	0.414	0.393	NA	0.99 ± 0.07	0.92 ± 0.09	NA	0.872	0.250	NA
	rs1042714	148206473	C/G	0.96	0.644	0.635	0.626	NA	0.96 ± 0.08	0.92 ± 0.09	NA	0.600	0.371	NA
	rs1042717	148206646	G/A	0.97	0.773	0.779	0.771	NA	1.03 ± 0.09	0.99 ± 0.10	NA	0.721	0.891	NA
	<u>rs1042718</u>	148206917	C/A		0.816	0.813	0.807	NA	0.97 ± 0.09	0.95 ± 0.11	NA	0.703	0.552	NA
	<u>rs1042719</u>	148207447	G/C		0.687	0.716	0.709	NA	1.16 ± 0.08	1.13 ± 0.09	NA	0.086	0.265	NA
	<u>rs1042720</u>	148207633	G/A		0.638	0.653	0.647	NA	1.10 ± 0.08	1.08 ± 0.09	NA	0.418	0.657	NA
	rs7702861	148211858	C/G	0.55	0.776	0.779	0.777	NA	1.04 ± 0.11	1.02 ± 0.14	NA	0.721	0.896	NA
	<u>rs4705271</u>	148211991	C/A		0.801	0.797	0.792	NA	0.97 ± 0.09	0.94 ± 0.10	NA	0.793	0.636	NA
LTA-TNF	rs2009658	31538244	C/G	0.96	0.838	0.805	0.802	0.795	0.80 ± 0.09	0.78 ± 0.11	0.74 ± 0.12	0.015	0.022	0.013
	<u>rs915654</u>	31538497	A/T		0.350	0.391	0.387	0.387	1.19 ± 0.07	1.16 ± 0.09	1.17 ± 0.10	0.025	0.085	0.132
	<u>rs2844482</u>	31539767	C/T		0.837	0.804	0.802	0.794	0.80 ± 0.09	0.78 ± 0.11	0.74 ± 0.12	0.020^d	0.033^d	0.018^d
	<u>rs2071590</u>	31539768	G/A		0.622	0.673	0.676	0.699	1.20 ± 0.07	1.25 ± 0.09	1.31 ± 0.10	0.013^d	0.013^d	0.003^d
	rs1800683	31540071	A/G	0.99	0.276	0.284	0.274	0.273	1.02 ± 0.08	0.98 ± 0.10	0.95 ± 0.10	0.854	0.829	0.642
	<u>rs2239704</u>	31540141	C/A		0.575	0.615	0.624	0.625	1.17 ± 0.07	1.23 ± 0.09	1.24 ± 0.10	0.055	0.029	0.071
	<u>rs909253</u>	31540313	G/A		0.274	0.279	0.268	0.270	1.02 ± 0.08	0.98 ± 0.10	0.96 ± 0.10	0.978	0.694	0.651
	<u>rs746868</u>	31540429	G/C		0.578	0.621	0.627	0.633	1.17 ± 0.07	1.23 ± 0.09	1.24 ± 0.10	0.037	0.025	0.038
	rs2229094	31540556	T/C	0.99	0.699	0.662	0.645	0.639	0.85 ± 0.08	0.79 ± 0.09	0.77 ± 0.10	0.035	0.007	0.007
	<u>rs2229092</u>	31540757	A/C		0.932	0.940	0.942	0.943	1.15 ± 0.07	1.18 ± 0.09	1.16 ± 0.10	0.360	0.354	0.402
	<u>rs1041981</u>	31540784	A/C		0.270	0.278	0.266	0.265	1.02 ± 0.08	0.98 ± 0.10	0.96 ± 0.10	0.841	0.774	0.660
	rs1799964	31542308	T/C	0.84	0.786	0.753	0.744	0.733	0.81 ± 0.09	0.76 ± 0.11	0.71 ± 0.12	0.024^d	0.011^d	0.004^d
	rs1800630	31542476	C/A	0.98	0.838	0.803	0.799	0.792	0.79 ± 0.09	0.77 ± 0.11	0.74 ± 0.12	0.012^d	0.016^d	0.010^d
	<u>rs1799724</u>	31542482	C/T		0.888	0.912	0.921	0.913	1.30 ± 0.12	1.42 ± 0.15	1.27 ± 0.16	0.021^d	0.009^d	0.068
	<u>rs1800629</u>	31543031	G/A		0.870	0.855	0.846	0.861	0.91 ± 0.10	0.83 ± 0.12	0.96 ± 0.14	0.321	0.123	0.797
	<u>rs1800610</u>	31543827	G/A		0.889	0.916	0.920	0.911	1.31 ± 0.12	1.45 ± 0.15	1.29 ± 0.16	0.014	0.016	0.127
MS4A2	<u>rs513986</u>	59852853	C/T		0.570	0.595	0.597	0.589	1.11 ± 0.07	1.11 ± 0.09	1.07 ± 0.08	0.138	0.194	0.347
	rs1286165	59853010	A/G	0.99	0.570	0.593	0.594	0.585	1.11 ± 0.07	1.11 ± 0.09	1.07 ± 0.08	0.169	0.233	0.420
	rs2847659	59853852	A/G	0.99	0.570	0.593	0.594	0.585	1.11 ± 0.07	1.11 ± 0.09	1.07 ± 0.08	0.169	0.233	0.420
	<u>rs2583477</u>	59853855	T/C		0.519	0.553	0.553	0.550	1.15 ± 0.07	1.15 ± 0.09	1.14 ± 0.08	0.038	0.102	0.097
	rs10792255	59854313	T/C	0.81	0.233	0.251	0.247	0.250	1.15 ± 0.09	1.10 ± 0.11	1.14 ± 0.10	0.136	0.372	0.222

	rs524748	59854572	C/T	0.99	0.571	0.594	0.596	0.586	1.11 ± 0.07	1.11 ± 0.09	1.07 ± 0.08	0.169	0.232	0.420
	<u>rs573790</u>	59855385	T/C		0.333	0.351	0.342	0.349	1.09 ± 0.07	1.05 ± 0.09	1.08 ± 0.08	0.306	0.648	0.449
	<u>rs1441586</u>	59856028	T/C		0.534	0.543	0.527	0.551	1.06 ± 0.07	1.00 ± 0.09	1.10 ± 0.08	0.636	0.722	0.398
	rs2583476	59857581	G/A	0.98	0.561	0.561	0.549	0.563	1.00 ± 0.07	0.95 ± 0.09	1.00 ± 0.08	0.943	0.547	0.958
	rs2847663	59858036	C/G	0.98	0.561	0.561	0.549	0.563	1.00 ± 0.07	0.95 ± 0.09	1.01 ± 0.08	0.946	0.550	0.954
	rs2847664	59858497	G/A	0.98	0.561	0.561	0.549	0.563	1.00 ± 0.07	0.95 ± 0.09	1.01 ± 0.08	0.955	0.558	0.945
	rs556917	59858712	A/T	0.98	0.522	0.536	0.527	0.547	1.06 ± 0.07	1.02 ± 0.09	1.10 ± 0.08	0.422	0.839	0.221
	rs2847666	59859576	A/G	0.98	0.567	0.572	0.558	0.577	1.02 ± 0.07	0.96 ± 0.09	1.04 ± 0.08	0.829	0.636	0.665
	rs2847667	59859609	C/T	0.98	0.567	0.572	0.558	0.577	1.02 ± 0.07	0.96 ± 0.09	1.04 ± 0.08	0.826	0.639	0.662
	rs502581	59860178	G/T	0.98	0.521	0.536	0.528	0.547	1.06 ± 0.07	1.02 ± 0.09	1.11 ± 0.08	0.390	0.780	0.200
	rs2583471	59861814	G/A	0.98	0.560	0.561	0.550	0.564	1.00 ± 0.07	0.96 ± 0.09	1.01 ± 0.08	0.998	0.614	0.893
	rs2070970	59861983	C/T	0.98	0.560	0.561	0.550	0.564	1.00 ± 0.07	0.96 ± 0.09	1.01 ± 0.08	0.992	0.620	0.887
	rs2847668	59862261	A/T	0.98	0.560	0.562	0.551	0.564	1.00 ± 0.07	0.96 ± 0.09	1.01 ± 0.08	0.985	0.626	0.880
	<u>rs569108</u>	59863104	A/G		0.961	0.976	0.977	0.985	1.62 ± 0.21	1.76 ± 0.27	2.45 ± 0.29	0.012^d	0.027^d	2.7E-04^d
	rs1290426	59864279	C/G	0.78	0.309	0.338	0.330	0.337	1.17 ± 0.09	1.13 ± 0.10	1.17 ± 0.10	0.058	0.235	0.107
	<u>rs2847655</u>	59865671	T/C		0.566	0.572	0.559	0.578	1.02 ± 0.07	0.97 ± 0.09	1.04 ± 0.08	0.818	0.685	0.619
	rs502419	59866175	G/A	0.99	0.520	0.536	0.529	0.547	1.07 ± 0.07	1.04 ± 0.09	1.12 ± 0.08	0.342	0.657	0.167
	rs2855017	59866309	C/T	0.99	0.559	0.560	0.551	0.564	1.00 ± 0.07	0.97 ± 0.09	1.02 ± 0.08	0.971	0.677	0.823
	rs17528859	59867379	T/C	0.98	0.559	0.560	0.551	0.564	1.00 ± 0.07	0.97 ± 0.09	1.02 ± 0.08	0.970	0.677	0.822
	rs574704	59867913	A/G	0.98	0.521	0.538	0.531	0.549	1.07 ± 0.07	1.04 ± 0.09	1.12 ± 0.08	0.330	0.654	0.157
<i>IL4R</i>	<u>rs2057768</u>	27322095	T/C		0.306	0.309	0.313	0.311	1.01 ± 0.08	1.03 ± 0.09	1.03 ± 0.09	0.869	0.710	0.775
	rs2107356	27323404	C/T	0.90	0.601	0.603	0.593	0.588	1.01 ± 0.08	0.97 ± 0.09	0.95 ± 0.08	0.887	0.748	0.546
	rs8060938	27324881	C/A	0.93	0.556	0.562	0.551	0.550	1.03 ± 0.07	0.99 ± 0.09	0.99 ± 0.08	0.654	0.882	0.894
	rs12927172	27325021	A/G	0.93	0.399	0.408	0.409	0.407	1.04 ± 0.08	1.05 ± 0.09	1.04 ± 0.09	0.586	0.620	0.668
	rs12927543	27325023	A/G	0.90	0.897	0.895	0.901	0.897	0.97 ± 0.12	1.04 ± 0.15	0.99 ± 0.14	0.814	0.798	0.940
	rs34872711	27326206	A/C	0.89	0.849	0.850	0.862	0.860	1.00 ± 0.10	1.11 ± 0.13	1.09 ± 0.12	0.991	0.416	0.495
	rs7499292	27326268	G/T	0.93	0.399	0.408	0.409	0.407	1.04 ± 0.08	1.05 ± 0.09	1.04 ± 0.08	0.584	0.615	0.671
	rs8052962	27326842	T/G	0.93	0.399	0.408	0.409	0.407	1.04 ± 0.08	1.05 ± 0.09	1.04 ± 0.08	0.584	0.615	0.671
	rs11645013	27327116	A/G	0.95	0.554	0.564	0.554	0.551	1.05 ± 0.07	1.01 ± 0.09	1.00 ± 0.08	0.505	0.936	0.980
	rs8060025	27327214	T/G	0.93	0.399	0.408	0.409	0.407	1.04 ± 0.08	1.05 ± 0.09	1.04 ± 0.08	0.583	0.613	0.672
	rs8044444	27328543	C/T	0.88	0.845	0.845	0.856	0.858	0.99 ± 0.10	1.09 ± 0.13	1.09 ± 0.12	0.932	0.507	0.459
	rs7190969	27329118	A/G	0.90	0.897	0.895	0.900	0.897	0.97 ± 0.12	1.04 ± 0.15	0.99 ± 0.14	0.814	0.800	0.942
	rs1076238	27331030	G/A	0.91	0.608	0.610	0.600	0.594	1.01 ± 0.07	0.97 ± 0.09	0.94 ± 0.08	0.924	0.722	0.486
	rs6498011	27331894	A/G	0.95	0.400	0.411	0.411	0.409	1.05 ± 0.07	1.05 ± 0.09	1.04 ± 0.08	0.545	0.596	0.652
	rs6498012	27331974	C/G	0.95	0.400	0.411	0.411	0.409	1.05 ± 0.07	1.05 ± 0.09	1.04 ± 0.08	0.545	0.595	0.652
	rs7187471	27332334	A/G	0.95	0.400	0.411	0.411	0.409	1.05 ± 0.07	1.05 ± 0.09	1.04 ± 0.08	0.545	0.595	0.652
	rs34213105	27333381	A/C	0.92	0.851	0.854	0.865	0.864	1.01 ± 0.10	1.11 ± 0.13	1.09 ± 0.12	0.937	0.400	0.451
	rs35092916	27335002	G/C	0.92	0.851	0.854	0.865	0.864	1.01 ± 0.10	1.11 ± 0.13	1.09 ± 0.12	0.936	0.399	0.451
	<u>rs1110470</u>	27336427	G/A		0.547	0.558	0.549	0.542	1.04 ± 0.07	1.00 ± 0.09	1.00 ± 0.08	0.505	0.863	0.889
	rs78288118	27336437	C/T	0.97	0.552	0.561	0.551	0.549	1.04 ± 0.07	1.00 ± 0.09	1.00 ± 0.08	0.566	1.000	0.965

rs71388093	27339024	C/T	0.94	0.307	0.323	0.332	0.322	1.08 ± 0.08	1.13 ± 0.10	1.07 ± 0.09	0.341	0.199	0.464
rs35004258	27339027	C/T	0.94	0.307	0.323	0.332	0.322	1.08 ± 0.08	1.13 ± 0.10	1.07 ± 0.09	0.341	0.199	0.464
rs9673499	27339145	C/T	0.94	0.307	0.323	0.332	0.322	1.08 ± 0.08	1.13 ± 0.10	1.07 ± 0.09	0.341	0.199	0.464
rs58653621	27339762	G/T	0.94	0.465	0.479	0.475	0.465	1.06 ± 0.07	1.05 ± 0.09	1.00 ± 0.08	0.443	0.613	0.971
rs6498013	27340735	C/T	0.93	0.309	0.322	0.331	0.321	1.07 ± 0.08	1.12 ± 0.10	1.06 ± 0.09	0.420	0.231	0.546
rs4787948	27341059	G/A	0.96	0.301	0.315	0.325	0.314	1.07 ± 0.08	1.12 ± 0.10	1.06 ± 0.09	0.402	0.220	0.528
rs7190472	27341553	C/T	0.97	0.305	0.320	0.329	0.318	1.08 ± 0.08	1.13 ± 0.09	1.06 ± 0.09	0.355	0.201	0.503
rs34943813	27342379	A/G	0.93	0.852	0.856	0.866	0.864	1.02 ± 0.10	1.12 ± 0.13	1.10 ± 0.12	0.824	0.366	0.444
rs78224329	27342381	T/C	0.92	0.854	0.858	0.868	0.867	1.03 ± 0.10	1.13 ± 0.13	1.10 ± 0.12	0.797	0.355	0.434
rs12925861	27342596	A/T	0.97	0.305	0.320	0.330	0.318	1.08 ± 0.08	1.13 ± 0.09	1.06 ± 0.09	0.355	0.200	0.504
rs2107355	27343479	G/A	0.90	0.314	0.331	0.343	0.328	1.09 ± 0.08	1.16 ± 0.10	1.07 ± 0.09	0.309	0.130	0.463
rs2107354	27343515	C/T	0.97	0.305	0.320	0.330	0.318	1.08 ± 0.08	1.13 ± 0.09	1.06 ± 0.09	0.354	0.199	0.504
rs4787951	27343963	C/T	0.96	0.301	0.317	0.328	0.314	1.08 ± 0.08	1.14 ± 0.09	1.06 ± 0.09	0.347	0.157	0.525
rs35630199	27344199	T/G	0.96	0.843	0.846	0.860	0.855	1.01 ± 0.10	1.14 ± 0.12	1.08 ± 0.11	0.886	0.293	0.515
rs3916997	27345114	G/A	0.98	0.464	0.477	0.473	0.463	1.06 ± 0.07	1.04 ± 0.09	1.00 ± 0.08	0.449	0.628	0.995
rs1981551	27345352	T/C	0.96	0.301	0.317	0.328	0.314	1.08 ± 0.08	1.14 ± 0.09	1.06 ± 0.09	0.347	0.156	0.525
<u>rs2283563</u>	27346354	C/T		0.672	0.703	0.708	0.700	1.16 ± 0.08	1.18 ± 0.09	1.14 ± 0.09	0.041	0.059	0.091
rs12708699	27348165	T/C	0.97	0.846	0.849	0.861	0.859	1.01 ± 0.10	1.12 ± 0.12	1.09 ± 0.12	0.912	0.351	0.460
rs12708700	27348322	T/C	0.97	0.846	0.849	0.861	0.859	1.01 ± 0.10	1.12 ± 0.12	1.09 ± 0.12	0.911	0.351	0.460
rs12445247	27348630	G/A	0.95	0.557	0.567	0.560	0.553	1.05 ± 0.07	1.02 ± 0.09	0.99 ± 0.08	0.495	0.830	0.923
rs3785356	27349168	T/C	0.96	0.301	0.316	0.328	0.313	1.08 ± 0.08	1.14 ± 0.09	1.06 ± 0.09	0.351	0.159	0.528
rs9940480	27349438	C/T	0.97	0.457	0.469	0.466	0.457	1.05 ± 0.07	1.04 ± 0.09	1.01 ± 0.08	0.459	0.635	0.943
rs9929928	27349493	G/T	0.98	0.847	0.851	0.862	0.860	1.02 ± 0.10	1.12 ± 0.12	1.09 ± 0.11	0.858	0.340	0.475
rs2382720	27350012	T/C	0.98	0.463	0.474	0.471	0.462	1.05 ± 0.07	1.04 ± 0.09	1.00 ± 0.08	0.500	0.665	0.994
rs3024530	27350687	G/A	0.95	0.471	0.478	0.476	0.467	1.03 ± 0.07	1.02 ± 0.09	0.99 ± 0.08	0.671	0.784	0.887
rs2057767	27351338	G/A	0.95	0.471	0.477	0.476	0.467	1.03 ± 0.07	1.02 ± 0.09	0.99 ± 0.08	0.674	0.788	0.885
rs3024536	27352713	C/T	0.99	0.848	0.852	0.864	0.860	1.02 ± 0.10	1.13 ± 0.12	1.09 ± 0.12	0.813	0.311	0.465
<u>rs3024537</u>	27352819	G/A		0.847	0.852	0.863	0.860	1.02 ± 0.10	1.13 ± 0.12	1.09 ± 0.11	0.855	0.355	0.480
rs3024543	27353230	G/A	0.99	0.848	0.853	0.864	0.861	1.03 ± 0.10	1.14 ± 0.12	1.09 ± 0.12	0.802	0.303	0.460
rs3024544	27353357	C/T	0.99	0.848	0.853	0.864	0.861	1.03 ± 0.10	1.14 ± 0.12	1.09 ± 0.12	0.799	0.301	0.457
rs3024546	27354067	C/A	0.99	0.847	0.853	0.864	0.861	1.03 ± 0.10	1.14 ± 0.12	1.09 ± 0.12	0.772	0.283	0.436
rs3024547	27354361	C/T	0.99	0.847	0.853	0.864	0.861	1.03 ± 0.10	1.14 ± 0.12	1.09 ± 0.12	0.768	0.281	0.433
rs3024548	27354531	G/C	0.98	0.462	0.471	0.468	0.459	1.04 ± 0.07	1.03 ± 0.09	0.99 ± 0.08	0.612	0.775	0.917
rs3024550	27354947	T/C	0.99	0.847	0.853	0.865	0.861	1.03 ± 0.10	1.15 ± 0.12	1.10 ± 0.12	0.758	0.274	0.425
rs3024552	27355004	C/G	0.98	0.458	0.466	0.463	0.456	1.04 ± 0.07	1.03 ± 0.09	1.00 ± 0.08	0.592	0.765	0.993
rs3024554	27355285	G/T	0.98	0.847	0.853	0.865	0.861	1.03 ± 0.10	1.15 ± 0.12	1.10 ± 0.12	0.751	0.270	0.419
rs3024555	27355349	A/G	0.98	0.847	0.853	0.865	0.861	1.03 ± 0.10	1.15 ± 0.12	1.10 ± 0.12	0.745	0.267	0.415
rs3024556	27355362	A/G	0.97	0.304	0.318	0.327	0.316	1.07 ± 0.08	1.12 ± 0.09	1.05 ± 0.09	0.423	0.235	0.559
<u>rs1805010</u>	27356203	G/A		0.458	0.447	0.429	0.433	1.03 ± 0.07	1.02 ± 0.09	0.99 ± 0.08	0.478	0.180	0.199
rs2074572	27356359	T/C	0.96	0.364	0.387	0.395	0.386	1.10 ± 0.07	1.15 ± 0.09	1.10 ± 0.08	0.197	0.130	0.268

rs2072130	27356398	T/C	0.97	0.368	0.391	0.396	0.391	1.10 ± 0.07	1.13 ± 0.09	1.10 ± 0.08	0.202	0.165	0.256
rs3024560	27356667	G/T	0.96	0.367	0.393	0.399	0.393	1.12 ± 0.07	1.15 ± 0.09	1.12 ± 0.08	0.135	0.116	0.181
<u>rs2301807</u>	27358098	C/A		0.962	0.974	0.971	0.976	1.41 ± 0.18	1.34 ± 0.22	1.50 ± 0.22	0.053	0.208	0.043
rs3024575	27358132	T/C	0.96	0.381	0.415	0.420	0.417	1.15 ± 0.07	1.18 ± 0.09	1.16 ± 0.08	0.065	0.058	0.080
rs3024577	27358203	G/A	0.98	0.475	0.500	0.505	0.501	1.11 ± 0.07	1.13 ± 0.09	1.11 ± 0.08	0.157	0.156	0.181
rs2283562	27358763	C/T	0.96	0.472	0.491	0.490	0.483	1.08 ± 0.07	1.08 ± 0.09	1.05 ± 0.08	0.284	0.396	0.581
rs2239348	27358948	G/A	0.98	0.566	0.577	0.574	0.568	1.05 ± 0.07	1.04 ± 0.09	1.02 ± 0.08	0.485	0.650	0.840
rs2239347	27359021	C/A	0.96	0.467	0.485	0.485	0.479	1.08 ± 0.07	1.08 ± 0.09	1.05 ± 0.08	0.275	0.371	0.521
rs2239346	27359061	T/A	0.96	0.467	0.485	0.485	0.479	1.08 ± 0.07	1.08 ± 0.09	1.05 ± 0.08	0.274	0.369	0.519
<u>rs3024585</u>	27359844	A/G		0.473	0.496	0.499	0.504	1.10 ± 0.07	1.13 ± 0.09	1.11 ± 0.08	0.218	0.192	0.266
<u>rs3024586</u>	27360041	G/A		0.956	0.963	0.951	0.966	1.16 ± 0.07	0.89 ± 0.09	1.28 ± 0.08	0.384	0.550	0.226
rs3024589	27360627	G/A	0.88	0.499	0.514	0.519	0.511	1.07 ± 0.08	1.10 ± 0.09	1.06 ± 0.08	0.338	0.295	0.479
rs3024590	27360628	C/T	0.88	0.493	0.509	0.515	0.508	1.08 ± 0.07	1.10 ± 0.09	1.07 ± 0.08	0.330	0.274	0.427
<u>rs2283561</u>	27361167	C/A		0.424	0.455	0.462	0.458	1.11 ± 0.07	1.15 ± 0.09	1.11 ± 0.08	0.095	0.080	0.117
<u>rs3024607</u>	27363611	G/A		0.916	0.929	0.932	0.939	1.16 ± 0.13	1.25 ± 0.17	1.34 ± 0.16	0.257	0.179	0.050
rs3024610	27364158	C/T	0.99	0.497	0.514	0.514	0.511	1.06 ± 0.07	1.07 ± 0.09	1.05 ± 0.08	0.413	0.466	0.571
rs3024611	27364200	C/A	0.97	0.844	0.864	0.877	0.878	1.18 ± 0.10	1.32 ± 0.13	1.32 ± 0.12	0.108	0.029	0.020
<u>rs3024613</u>	27364253	C/T		0.500	0.482	0.486	0.514	1.06 ± 0.07	1.07 ± 0.09	1.05 ± 0.08	0.397	0.538	0.563
rs3024619	27364806	A/G	0.98	0.342	0.379	0.391	0.389	1.16 ± 0.07	1.24 ± 0.09	1.21 ± 0.08	0.041	0.017	0.021
rs3024622	27365453	G/C	0.98	0.342	0.379	0.391	0.389	1.16 ± 0.07	1.24 ± 0.09	1.21 ± 0.08	0.042^d	0.017^d	0.021^d
rs3024624	27365614	A/G	0.98	0.500	0.519	0.517	0.515	1.07 ± 0.07	1.07 ± 0.09	1.06 ± 0.08	0.321	0.429	0.501
<u>rs3024632</u>	27366296	T/C		0.921	0.926	0.931	0.936	1.05 ± 0.13	1.17 ± 0.17	1.21 ± 0.16	0.820	0.430	0.237
rs3024634	27366562	A/G	0.88	0.855	0.870	0.883	0.884	1.14 ± 0.11	1.31 ± 0.14	1.31 ± 0.13	0.227	0.053	0.035
rs4787423	27367334	T/C	0.94	0.850	0.866	0.879	0.880	1.14 ± 0.11	1.30 ± 0.13	1.30 ± 0.12	0.208	0.046	0.036
rs3024644	27367826	G/C	0.94	0.844	0.860	0.874	0.876	1.14 ± 0.10	1.30 ± 0.13	1.30 ± 0.12	0.202	0.043	0.030
rs3024647	27367972	A/G	0.94	0.844	0.860	0.874	0.876	1.14 ± 0.10	1.30 ± 0.13	1.30 ± 0.12	0.203	0.044	0.029
rs2891057	27368790	T/A	0.93	0.849	0.865	0.878	0.880	1.14 ± 0.11	1.30 ± 0.13	1.30 ± 0.12	0.217	0.050	0.036
rs2891058	27368833	A/G	0.93	0.844	0.860	0.874	0.875	1.14 ± 0.10	1.30 ± 0.13	1.31 ± 0.12	0.210	0.046	0.029
rs3024656	27369609	G/A	0.70	0.737	0.736	0.736	0.732	0.98 ± 0.10	0.99 ± 0.12	0.95 ± 0.11	0.801	0.911	0.642
rs3024660	27371158	T/C	0.93	0.844	0.860	0.874	0.875	1.14 ± 0.10	1.30 ± 0.13	1.31 ± 0.12	0.211	0.047	0.029
rs3024662	27371397	G/A	0.95	0.503	0.520	0.517	0.514	1.07 ± 0.07	1.06 ± 0.09	1.04 ± 0.08	0.385	0.508	0.610
rs3024666	27371571	A/G	0.93	0.844	0.860	0.873	0.875	1.14 ± 0.10	1.29 ± 0.13	1.31 ± 0.12	0.216	0.049	0.029
<u>rs3024676</u>	27373558	C/A		0.794	0.814	0.827	0.833	1.16 ± 0.09	1.25 ± 0.11	1.29 ± 0.10	0.121	0.054	0.013
<u>rs2234897</u>	27373612	C/T		0.981	0.979	0.983	0.979	1.11 ± 0.07	0.91 ± 0.09	1.14 ± 0.10	0.667	0.767	0.653
<u>rs1805011</u>	27373872	A/C		0.849	0.860	0.869	0.867	1.13 ± 0.10	1.20 ± 0.12	1.20 ± 0.12	0.306	0.195	0.172
rs2234898	27373915	G/T	0.96	0.853	0.870	0.878	0.878	1.17 ± 0.10	1.24 ± 0.13	1.25 ± 0.12	0.124	0.088	0.061
<u>rs1805012</u>	27373964	T/C		0.874	0.900	0.906	0.907	1.27 ± 0.11	1.33 ± 0.13	1.37 ± 0.13	0.018^d	0.019^d	0.008^d
rs2234900	27373972	T/C	0.97	0.848	0.878	0.882	0.888	1.31 ± 0.10	1.35 ± 0.13	1.43 ± 0.12	0.010^d	0.020^d	0.003^d
<u>rs1805013</u>	27373980	C/T		0.960	0.968	0.972	0.975	1.28 ± 0.07	1.43 ± 0.13	1.60 ± 0.13	0.188	0.131	0.040^d
<u>rs1805015</u>	27374180	T/C		0.809	0.846	0.859	0.864	1.28 ± 0.09	1.38 ± 0.12	1.45 ± 0.11	0.004^d	0.002^d	2.1E-04^d

	<u>rs1801275</u>	27374400	A/G		0.765	0.793	0.803	0.807	1.20 ± 0.09	1.29 ± 0.11	1.33 ± 0.10	0.044^d	0.034^d	0.011^d
	<u>rs1805016</u>	27374927	T/G		0.938	0.940	0.944	0.948	1.20 ± 0.16	1.35 ± 0.21	1.45 ± 0.20	0.822	0.582	0.321
	<u>rs1049631</u>	27375542	G/A		0.468	0.475	0.478	0.476	1.03 ± 0.07	1.05 ± 0.08	1.05 ± 0.08	0.771	0.663	0.778
	rs8832	27375787	A/G	0.96	0.462	0.471	0.474	0.473	1.04 ± 0.07	1.05 ± 0.09	1.05 ± 0.08	0.628	0.563	0.564
	rs1029489	27376217	A/G	0.97	0.390	0.408	0.415	0.413	1.08 ± 0.07	1.12 ± 0.09	1.11 ± 0.08	0.282	0.216	0.223
	rs2382721	27376637	G/A	0.93	0.346	0.368	0.382	0.380	1.11 ± 0.08	1.18 ± 0.09	1.17 ± 0.09	0.173	0.067	0.067
	rs3024685	27376910	C/T	0.94	0.392	0.412	0.420	0.417	1.09 ± 0.07	1.13 ± 0.09	1.12 ± 0.08	0.245	0.178	0.185
ADAM33	<u>rs512625</u>	3648378	G/A		0.719	0.687	0.691	0.658	0.86 ± 0.08	0.88 ± 0.09	0.75 ± 0.13	0.047	0.147	0.031
	rs2787093	3648462	T/C	0.94	0.890	0.871	0.882	0.822	0.83 ± 0.11	0.92 ± 0.14	0.56 ± 0.17	0.080	0.537	4.9E-04
	rs614971	3648850	G/C	0.92	0.692	0.677	0.679	0.677	0.94 ± 0.08	0.94 ± 0.10	0.94 ± 0.14	0.436	0.538	0.670
	rs517155	3648877	G/A	0.75	0.899	0.904	0.906	0.894	1.09 ± 0.14	1.12 ± 0.17	0.92 ± 0.23	0.547	0.521	0.714
	rs2787094	3649161	G/C	0.86	0.808	0.784	0.792	0.762	0.85 ± 0.09	0.89 ± 0.11	0.73 ± 0.15	0.070	0.302	0.039^d
	rs677044	3649431	A/G	0.93	0.754	0.746	0.738	0.744	0.95 ± 0.08	0.91 ± 0.10	0.94 ± 0.14	0.556	0.358	0.677
	rs543749	3649679	G/T	0.96	0.892	0.879	0.873	0.912	0.88 ± 0.11	0.82 ± 0.13	1.27 ± 0.22	0.248	0.145	0.272
	rs628965	3649713	G/A	0.99	0.619	0.604	0.606	0.523	0.93 ± 0.07	0.94 ± 0.09	0.65 ± 0.13	0.299	0.485	0.001^d
	<u>rs628977</u>	3649721	C/T		0.617	0.600	0.603	0.516	0.93 ± 0.07	0.94 ± 0.09	0.66 ± 0.13	0.231	0.455	3.7E-04^d
	<u>rs678881</u>	3649803	G/C		0.729	0.730	0.721	0.700	1.00 ± 0.08	0.96 ± 0.10	0.85 ± 0.13	0.966	0.664	0.249
	rs630712	3650066	A/C	0.97	0.892	0.874	0.886	0.826	0.84 ± 0.11	0.94 ± 0.14	0.57 ± 0.17	0.102	0.620	0.001
	rs2280089	3650127	G/A	0.99	0.873	0.869	0.877	0.847	0.99 ± 0.10	1.05 ± 0.13	0.83 ± 0.17	0.884	0.692	0.277
	<u>rs2280090</u>	3650205	G/A		0.876	0.876	0.887	0.857	0.99 ± 0.10	1.05 ± 0.13	0.83 ± 0.17	0.816	0.390	0.440
	<u>rs2280091</u>	3650234	A/G		0.868	0.858	0.859	0.841	0.99 ± 0.10	1.05 ± 0.13	0.83 ± 0.17	0.547	0.616	0.258
	<u>rs574174</u>	3650694	C/T		0.818	0.807	0.797	0.829	0.94 ± 0.09	0.88 ± 0.11	1.08 ± 0.16	0.443	0.203	0.585
	rs597980	3651165	A/G	0.96	0.440	0.415	0.422	0.326	0.90 ± 0.07	0.93 ± 0.09	0.60 ± 0.14	0.143	0.399	1.2E-04^d
	rs44707	3651226	T/G	0.95	0.573	0.550	0.549	0.483	0.90 ± 0.07	0.90 ± 0.09	0.68 ± 0.13	0.146	0.231	0.002^d
	rs598418	3651269	A/G	0.98	0.618	0.603	0.605	0.523	0.93 ± 0.07	0.94 ± 0.09	0.65 ± 0.13	0.305	0.478	0.001^d
	rs2853209	3651472	A/T	0.93	0.482	0.465	0.474	0.362	0.93 ± 0.07	0.97 ± 0.09	0.58 ± 0.13	0.321	0.719	3.8E-05^d
	<u>rs528557</u>	3651742	C/G		0.737	0.721	0.722	0.728	0.93 ± 0.08	0.93 ± 0.10	0.99 ± 0.14	0.411	0.476	0.926
	rs612709	3652207	G/A	0.96	0.868	0.850	0.844	0.878	0.87 ± 0.10	0.82 ± 0.12	1.12 ± 0.19	0.165	0.106	0.555
	rs3918395	3653149	C/A	0.97	0.869	0.868	0.877	0.850	1.02 ± 0.10	1.09 ± 0.13	0.89 ± 0.17	0.842	0.504	0.499
	rs2280092	3653587	C/T	0.97	0.869	0.868	0.877	0.850	1.02 ± 0.10	1.09 ± 0.13	0.89 ± 0.17	0.843	0.504	0.499
	rs2280094	3653709	C/A	0.92	0.768	0.770	0.753	0.772	1.00 ± 0.09	0.91 ± 0.10	0.99 ± 0.15	0.956	0.354	0.966
	rs2271511	3654433	C/T	0.93	0.802	0.788	0.798	0.780	0.94 ± 0.09	0.99 ± 0.11	0.92 ± 0.15	0.489	0.908	0.570
	rs2485700	3654993	T/C	0.87	0.838	0.826	0.829	0.831	0.92 ± 0.10	0.94 ± 0.12	0.97 ± 0.17	0.410	0.586	0.873
	<u>rs511898</u>	3655085	T/C		0.641	0.625	0.632	0.373	1.06 ± 0.07	1.04 ± 0.09	1.05 ± 0.13	0.480	0.730	0.806
	<u>rs2787095</u>	3655943	C/G		0.584	0.542	0.568	0.530	1.16 ± 0.07	1.06 ± 0.09	1.51 ± 0.12	0.027^d	0.485	3.8E-04^d
	rs17548927	3657387	G/A	0.69	0.858	0.856	0.849	0.863	0.97 ± 0.12	0.90 ± 0.14	1.06 ± 0.21	0.814	0.461	0.799
	rs568251	3657803	G/A	0.95	0.867	0.868	0.858	0.859	1.02 ± 0.11	0.93 ± 0.13	0.95 ± 0.18	0.858	0.538	0.777
	<u>rs597165</u>	3657804	A/T		0.864	0.864	0.855	0.854	1.02 ± 0.11	0.93 ± 0.12	0.95 ± 0.18	0.956	0.551	0.738
	<u>rs2853210</u>	3658211	C/T		0.760	0.763	0.772	0.709	1.02 ± 0.09	1.07 ± 0.11	0.76 ± 0.15	0.720	0.494	0.092
	rs487377	3658931	T/C	0.70	0.190	0.209	0.206	0.249	1.19 ± 0.11	1.16 ± 0.13	1.66 ± 0.18	0.109	0.258	0.004

<u>rs570269</u> ^e	3659647	G/C		0.824	0.809	0.818	0.717	0.85 ± 0.12	0.93 ± 0.14	0.57 ± 0.19	0.086	0.287	0.005
rs603112	3660789	C/T	0.67	0.853	0.843	0.847	0.804	0.87 ± 0.12	0.93 ± 0.15	0.59 ± 0.19	0.255	0.595	0.005
rs2853211	3660947	C/G	0.54	0.279	0.279	0.277	0.294	0.99 ± 0.11	0.98 ± 0.13	1.13 ± 0.18	0.954	0.896	0.488
rs2595584	3661029	C/G	0.55	0.409	0.426	0.423	0.459	1.15 ± 0.10	1.12 ± 0.12	1.48 ± 0.16	0.136	0.327	0.017
rs2853212	3661550	A/G	0.70	0.535	0.546	0.540	0.562	1.09 ± 0.09	1.04 ± 0.10	1.20 ± 0.15	0.333	0.687	0.212
<u>rs17548962</u>	3661803	C/T		0.967	0.960	0.962	0.951	0.82 ± 0.19	0.85 ± 0.23	0.67 ± 0.30	0.289	0.534	0.205
<u>rs2853213</u>	3661840	C/G		0.655	0.667	0.664	0.658	1.08 ± 0.08	1.05 ± 0.09	1.06 ± 0.13	0.319	0.580	0.779
<u>rs554743</u>	3662142	T/C		0.733	0.724	0.724	0.701	0.95 ± 0.08	0.95 ± 0.10	0.86 ± 0.13	0.442	0.566	0.215
rs553863	3662235	A/C	0.81	0.606	0.618	0.618	0.630	1.08 ± 0.08	1.07 ± 0.10	1.16 ± 0.14	0.327	0.484	0.293
rs2853214	3662272	C/T	0.79	0.613	0.626	0.626	0.639	1.10 ± 0.08	1.09 ± 0.10	1.19 ± 0.14	0.274	0.399	0.238
rs8124875	3663553	T/G	0.67	0.352	0.354	0.354	0.343	1.02 ± 0.09	1.01 ± 0.11	0.95 ± 0.15	0.846	0.908	0.746

tSNPs are underlined. Nominal significant associations in bold. NA: not available because the gene where it resides did not show any SNP at nominal significance or was not followed in this analysis.

^aAccording to NCBI build 36.3; ^bSquared correlation between true genotypes and estimated allelic dosage of imputed SNPs. Note that the measure is not reported for tSNPs; ^cComputed for allele 1; ^dSNPs associated in previous studies; ^eNo distinction was made between C and the new T allele for this analysis.

Table S4. Functional annotation of the 10 associated SNPs

Gene	rs#	dbSNP annotation	Proteins bound	Promoter histone marks	DNAse positive
<i>MS4A2</i>	rs569108	Missense	USF1	-	-
<i>IL4R</i>	rs1805015	Missense	-	-	-
<i>ADAM33</i>	rs2787093	3' UTR	-	-	-
	rs628965	Intronic	-	-	-
	rs628977	Intronic	-	-	-
	rs630712	Intronic	-	-	-
	rs597980	Intronic	CTCF	-	Fibrobl
	rs598418	Intronic	CTCF,RAD21	HepG2	28 cell types ^a
	rs2853209	Intronic	CTCF	HepG2	Caco-2
	rs2787095	Intronic	ZNF263	-	-

^aH1-hESC, HSMM, HSMMtube, HUVEC, LNCaP, MCF-7, 8988T, Fibrobl, GM12891, Gliobla, Ishikawa, Melano, Myometr, Osteobl, PanIsletD, Stellate, Urothelia, AG09309, BE2_C, H7-hESC, HCT-116, HFF, HFF-Myc, HMF, NHDF-Ad, NHDF-neo, NT2-D1, WI-38.

Table S4. *In silico* replication of the 11 associated SNPs contained in the GABRIEL study (10,365 cases and 16,110 controls).

Gene	SNP	position	Reference allele	OR (95% CI)	<i>p</i> -value
<i>LTA-TNF</i>	rs2009658	31646223	C	1.00 (0.94-1.06)	0.863
	rs2844482	31647746	C	0.99 (0.94-1.05)	0.754
	rs2229094	31648535	C	1.03 (0.99-1.08)	0.170
<i>IL4R</i>	rs2283563	27253855	C	0.96 (0.92-1.00)	0.064
	rs3024619	27272307	A	0.97 (0.93-1.01)	0.107
	rs1805012	27281465	C	1.12 (1.05-1.19)	5.73E-04
	rs1805015	27281681	C	1.04 (0.98-1.09)	0.181
	rs1801275	27281901	A	0.97 (0.93-1.02)	0.244
	rs3024660	27278659	C	1.02 (0.97-1.08)	0.402
<i>ADAM33</i>	rs512625	3596378	A	0.97 (0.93-1.01)	0.105
	rs487377	3606931	C	1.03 (0.98-1.08)	0.240

Table S5. Sample sizes and statistical power for each analysis performed in a subset of cases.

Subset of analysis	Number of cases	Statistical power	Statistical power
		MAF=0.20 ^a	MAF=0.45 ^b
Asthma	606	48.4%	69.6%
Atopic	356	29.3%	44.1%
Asthma with age-at-diagnosis ≤ 14	155	10.8%	14.6%
Asthma with age-at-diagnosis ≤ 26	291	23.4%	35.0%
Asthma with age-at-diagnosis ≤ 39	427	35.4%	52.6%

^aAssessed to detect a minimum risk of 1.45 and a two-sided $p=0.0012$ significance level for a minor allele frequency of 20%^a and a minor allele frequency of 45%^b.

Table S6. Coverage of candidate genes on commercial arrays used in asthma GWAS in samples of European ancestry.^a

Gene	SNP Array	HapMap CEU				1KGP CEU			
		SNPs MAF>5%	SNPs in array	# tagged SNPs ^b	Coverage (%)	SNPs MAF>5%	SNPs in array	# tagged SNPs ^b	Coverage (%)
<i>IL13-IL4</i>	Affymetrix 500K	34	11	30	88	47	7	36	77
	Affymetrix Genome-Wide Human SNP Array 6.0	34	11	30	88	47	10	37	79
	Illumina HumanHap 300 ^c	34	5	14	41	47	4	17	36
	Illumina HumanHap 550 ^d	34	7	24	71	47	7	32	68
	Illumina HumanHap 650	34	12	28	82	47	9	32	68
	Illumina HumanHap I1M-Duo	34	33	34	100	47	40	40	85
<i>CD14</i>	Affymetrix 500K	6	2	5	83	15	2	13	87
	Affymetrix Genome-Wide Human SNP Array 6.0	6	3	5	83	15	3	13	87
	Illumina HumanHap 300 ^c	6	0	0	0	15	0	0	0
	Illumina HumanHap 550 ^d /650	6	1	3	50	15	1	4	27
	Illumina HumanHap I1M-Duo	6	2	3	50	15	2	4	27
<i>ADRB2</i>	Affymetrix 500K, Affymetrix Genome-Wide Human SNP Array 6.0	10	2	7	70	27	18	17	63
	Illumina HumanHap 300 ^c	10	3	7	70	27	3	4	15
	Illumina HumanHap 550 ^d	10	4	7	70	27	5	21	78
	Illumina HumanHap 650	10	5	9	90	27	7	23	85
	Illumina HumanHap I1M-Duo	10	10	10	100	27	18	25	93
<i>LTA-TNF</i>	Affymetrix 500K, Affymetrix Genome-Wide Human SNP Array 6.0	13	1	1	8	25	1	1	4
	Illumina HumanHap 300 ^c /550 ^d /650	13	5	8	62	25	5	14	56
	Illumina HumanHap I1M-Duo	13	13	13	100	25	20	20	80
<i>MS4A2</i>	Affymetrix 500K	21	2	20	95	34	2	24	71
	Affymetrix Genome-Wide Human SNP Array 6.0	21	3	20	95	34	3	24	71
	Illumina HumanHap 300 ^c	21	0	0	0	34	0	0	0
	Illumina HumanHap 550 ^d /650	21	3	20	95	34	3	24	71
	Illumina HumanHap I1M	21	9	21	100	34	8	31	91
<i>IL4R</i>	Affymetrix 500K	55	11	19	35	143	10	56	39
	Affymetrix Genome-Wide Human SNP Array 6.0	55	20	39	71	143	24	110	77
	Illumina HumanHap 300 ^c	55	12	35	64	143	12	86	60
	Illumina HumanHap 550 ^d	55	20	51	93	143	18	115	80
	Illumina HumanHap 650	55	26	53	96	143	22	128	90
	Illumina HumanHap I1M-Duo	55	47	54	98	143	81	138	97

<i>ADAM33</i>	Affymetrix 500K Affymetrix Genome-Wide Human SNP Array 6.0	17	2	4	24	50	2	4	8
	Illumina HumanHap 300 ^c	17	3	3	18	50	3	3	6
	Illumina HumanHap 550 ^d /650	17	5	5	29	50	5	9	18
	Illumina HumanHap I1M-Duo	17	11	13	76	50	11	14	28

^aIncludes studies described in Moffatt et al. 2007, Himes et al. 2009, Sleiman et al. 2009, Li et al. 2010, Moffatt et al. 2010, Ferreira et al. 2011, Torgerson et al. 2011.

^bSNPs captured by the array both directly and indirectly (with a multi-marker $r^2 \geq 0.8$)

^cIncluding the Illumina HumanCNV370-Duo as it was built upon the Illumina HumanHap300 BeadChip (317K SNPs) with additional 52K markers aimed at detecting CNVs.

^dThe Illumina Human610 SNP content is similar to the Illumina HumanHap550 with the addition of 60K markers to target regions with CNVs.

Text S1. Supplementary methods

Study subjects

This study was conducted using a case-control design of 1,878 DNA samples from unrelated individuals, all reporting at least two generations of Spanish descent. Sample details have been described elsewhere [1].

Cases included 607 asthmatic patients aged >5 years and diagnosed by physicians following the Global Initiative for Asthma (GINA) guidelines for diagnosis and classification of asthma severity (<http://www.ginasthma.com>). These samples were collected and characterized for allergic and asthmatic symptoms in Respiratory Medicine and Allergy Departments, as part of the Genetics of Asthma study (GOA) in the Spanish population. Among cases, atopy was defined by the evidence of allergic sensitization to known allergens, reflected by either a positive skin prick test [SPT] or the specific IgE to one or more known allergens in the serum. These tests evaluated seven common allergen collections: dust mites (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Glycyphagus* spp, *Blomia tropicalis*, *Acarus siro*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Euroglyphus maynei*), epithelia (*Felis domesticus*, *Canis familiaris*, *Equus caballus*, *Oryctolagus cuniculus*), pollen (*Olea europaea*, *Salsola* spp, *Lolium perenne*, *Artemisia vulgaris*, *Parietaria* spp, *Platanus* spp, *Chenopodium* spp, *Plantago* spp, *Rumex* spp, *Cupressus* spp), fungi (*Penicillium notatum*, *Alternaria alternate*, *Aspergillus fumigatus*, *Cladosporium herbarum*), food (Cow's milk, hen's egg, peanut, soybean, wheat, fish, crustaceans [shrimp, crabs, and lobster], mollusks [clams, oysters, and mussels], banana, chestnut, kiwi), latex, and others (*Blattella germanica*). The SPT was considered positive when the individual had a wheal with a diameter 3

mm greater than the saline control. In addition, specific IgE levels were measured in asthmatic individuals, allowing to classify them in those with a positive result to, at least, one of seven common allergens (specific IgE levels ≥ 0.35 UI mL⁻¹) and those with a negative test (<0.35 UI mL⁻¹). For simplicity, those cases that had asthma and also atopy will be referred as atopic asthmatics, although we ignored whether or not allergen exposures lead to the asthma symptoms of these patients. Further sample details can be found in Table S1.

Control group consisted of 1,271 DNA samples from adults self-reporting no personal or familiar medical history of allergic or pulmonary diseases from the Spanish National DNA Biobank. These were collected from branches of the National Blood Service from unrelated individuals residing in Spain. After signed informed consent, by means of personal interviews, each donor was asked to declare general health status, physic activity, commonly used transportation, nutrition habits, type of work 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 or behavioral, nervous, vision, hearing, respiratory (including asthma symptoms), immunological (including allergies), bone, congenital, skin and digestive. No immediate family (parents, brothers or sisters, children) that has donated samples to the Spanish National DNA Biobank could be a DNA sample donor. The Spanish National DNA Biobank criteria for sample classification as healthy was based on self-reported absence of cardiovascular, renal, pulmonary, hepatic, hematological illnesses or any other chronic conditions which require continuous treatment, hepatitis B, C infections or acquired immunodeficiency syndrome (AIDS), However, no clinical characterization was performed on any subject, no information from medical record

was incorporated, and no medical testing was performed on these individuals. For further information visit <http://www.bancoadn.org>. In addition to the criteria of the Spanish National DNA Biobank to define healthy, we added three more criteria to select the controls for this study: 1) Self-reported Spanish ancestry based on having at least two generations of ancestors born in Spain; 2) Complete data on personal and familiar history of disease recorded in the questionnaire, smoking status, place of origin, and area of residence; 3) Absence of self-reported personal or familiar history of pulmonary or allergic disease.

Selection of tagging SNPs

Tagging SNPs (tSNPs) were selected by means of TagIT 3.03 [2], using available re-sequencing data from European samples from different sources (Table 1 and Table S2). It is worth noting that the re-sequencing data for the *MS4A2* gene was unavailable from any public database when the study started. Thus, for this gene, tSNP selection was done using data from 60 unrelated samples from Utah residents with ancestry from northern and western Europe (CEU) from HapMap [3] and tagging performance was verified against re-sequencing data from 96 type 1 diabetes individuals of white European ancestry (v0.1, accessed on 19th March 2008) [4]. The *IL13* and *IL4* genes, which lie in close proximity, were considered as a single region. Similarly, given the strong linkage disequilibrium (LD) between *LTA* and *TNF* genes [5], common variants of the *LTA* gene were also tagged and jointly analyzed with *TNF*. In all cases, CEU genotypes were also used to complement the set of tSNPs selected from all genes to ensure adequate gene coverage. In addition, previously associated SNPs and all potential functional variants as predicted by PupaSuite [6] were forced to be included as tSNPs. The final tSNPs set was further tested for

tagging performance using a SNP-dropping-with-resampling method [2], satisfying a haplotype $r^2 \geq 0.85$ in the reference genotype data. See Table S2 for further details.

Genotyping

DNA was extracted from blood or saliva and used for whole genome amplification with Illustra *GenomiPhi* V2 DNA Amplification Kit (GE Healthcare, Little Chalfont, UK) as described elsewhere [1]. Genotyping was conducted using the iPLEX[®] Gold assay on MassARRAY[®] system (Sequenom Inc., San Diego, CA) by the Spanish National Genotyping Center, Santiago de Compostela Node (CeGen, <http://www.cegen.org>). Briefly, iPLEX[®] assays were scanned by MALDI-TOF mass spectrometry and individual SNP genotype calls were automatically generated using Sequenom TYPER 3.4[®] software (Sequenom Inc.). Samples from the Coriell Institute for Medical Research (<http://www.coriell.org>) were included on each SpectroCHIP[®] (Sequenom Inc.) to test allele calling reliability samples of this platform.

The SNPs that gave poor quality data on iPLEX[®] Gold assay on MassARRAY[®] system were finally determined at the Hospital Universitario N. S. de Candelaria using either SNaPshot[®] Multiplex kit reactions (Applied Biosystems, Foster City, CA) or KASPar SNP Genotyping System assays (KBiosciences, Hertfordshire, UK). For that, we alternatively utilized an ABI 3500 Genetic Analyzer (Applied Biosystems), automatically assigning genotypes using GeneMapper v3.2 (Applied Biosystems), or a 7500 Fast Real-Time PCR System (Applied Biosystems), with automated calls generated using the 7500 software 2.0.1 based on discriminating plots with 95% confidence, respectively. Genotyping was blind to the disease status and $\approx 6\%$ of the samples were genotyped in duplicate to monitor genotyping quality.

Contrary to the dbSNPs database, that defined the SNP rs570269 in *ADAM33* gene as a C/G change, this SNP was detected as a triallelic *locus* during genotyping

with iPLEX[®] Gold assays. This information has been submitted to NCBI (pending on the submission number) as it was confirmed by sequencing of individuals showing the new T allele (0.4% of controls) by both strands with BigDye[®] v3.1 Terminator Cycle Sequencing kit (Applied Biosystems) under the manufacturer's recommendations and using the amplification primers. Sequencing products were ethanol precipitated and run on an ABI 3500 Genetic Analyzer. However, no distinction was made between T and C alleles during the statistical analysis of association.

Supplementary references

1. Pino-Yanes M, Sanchez-Machin I, Cumplido J, Figueroa J, Torres-Galvan MJ, et al. (2012) IL-1 receptor-associated kinase 3 gene (*IRAK3*) variants associate with asthma in a replication study in the Spanish population. *J Allergy Clin Immunol* 129: 573-575 e510.
2. Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, et al. (2005) A single-nucleotide polymorphism tagging set for human drug metabolism and transport. *Nat Genet* 37: 84-89.
3. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, et al. (2007) A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449: 851-861.
4. Smyth DJ, Howson JM, Payne F, Maier LM, Bailey R, et al. (2006) Analysis of polymorphisms in 16 genes in type 1 diabetes that have been associated with other immune-mediated diseases. *BMC Med Genet* 7: 20.
5. Belfer I, Buzas B, Hipp H, Dean M, Evans C, et al. (2004) Haplotype structure of inflammatory cytokines genes (*IL1B*, *IL6* and *TNF/LTA*) in US Caucasians and African Americans. *Genes Immun* 5: 505-512.
6. Conde L, Vaquerizas JM, Dopazo H, Arbiza L, Reumers J, et al. (2006) PupaSuite: finding functional single nucleotide polymorphisms for large-scale genotyping purposes. *Nucleic Acids Res* 34: W621-625.