

A role of *FCER1A* and *FCER2* polymorphisms in IgE regulation

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Abstract

Background: Both *FCER2* and *FCER1A* encode subunits of IgE receptors. Variants in *FCER1A* were previously identified as major determinants of IgE levels in genome-wide association studies.

Methods: Here we investigated in detail whether *FCER2* polymorphisms affect IgE levels alone and/or by interaction with *FCER1A* polymorphisms. To cover the genetic information of *FCER2*, 21 single-nucleotide polymorphisms (SNPs) were genotyped by Illumina HumanHap300 BeadChip (5 SNPs) and the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS; 14 SNPs) in at least 1303 Caucasian children (651 asthmatics) (ISAAC II/ MAGICs population); genotypes of two SNPs were imputed.

Results: SNP rs3760687 showed the most consistent effect on total serum IgE levels (b [SE] = -0.38 [0.16]; $P = 0.016$), while *FCER2* polymorphisms in general were predominantly associated with mildly-to-moderately increased IgE levels (50th and 66th percentiles). Gene-by-gene interaction analysis suggests that *FCER2* polymorphism rs3760687 influences IgE levels mainly in individuals not homozygous for the risk allele of *FCER1A* polymorphism rs2427837, which belongs to the major IgE-determining tagging bin in the population.

Conclusion: *FCER2* polymorphism rs3760687 affects moderately elevated total serum IgE levels, especially in the absence of homozygosity for the risk allele of *FCER1A* SNP rs2427837.

Immunoglobulin E (IgE) mediates allergic responses through its cellular receptors FcεRI and FcεRII (1). On mast cells and basophils, FcεRI has the form of a heterotetramer ($\alpha\beta\gamma_2$) of which only the α -subunit encoded by the *FCER1A* gene is IgE receptor specific. On B cells, FcεRII is composed of CD23 polypeptides encoded by the *FCER2* gene (2). Both FcεRI and FcεRII affect the synthesis of IgE in a regulatory loop (1). Candidate gene studies and genome-wide association (GWA) studies identified single nucleotide polymor-

phisms (SNPs) in *FCER1A* to be major genetic determinants of IgE levels (2–6). Microsatellite and SNP-based studies on *FCER2* (19p13 locus) suggested that genetic alterations in that gene may also influence IgE levels (7–9). Pharmacogenetic studies found that *FCER2* SNPs increase the risk of asthma exacerbations and influence total serum IgE levels in asthmatics (7–10). A role of these SNPs in gene function was confirmed by molecular biology (7, 9). So far, *FCER1A* and *FCER2* polymorphisms have been analyzed in detail only

separately, and thus, it is not clear whether gene-by-gene interaction between *FCER1A* and *FCER2* may modulate IgE levels. In this study, we (i) fine-mapped and re-genotyped the *FCER2* locus to replicate and extend the findings of previous studies (7, 8) and (ii) investigated interaction effects between *FCER1A* and *FCER2* on IgE levels.

Methods

Polymorphisms in *FCER1A* and *FCER2* were studied in at least 1303 German children (651 asthmatics). These children were recruited from the German part of the International Study of Asthma and Allergy in Childhood, phase II (ISAAC II), and the German Multicenter Asthma Genetic in Childhood Study (MAGICS). This study design has already been used for the first GWAS on asthma (11), and further details of the populations are given in the Supporting Information text and Table S1. SNPs for fine mapping of *FCER2* were selected by a linkage disequilibrium (LD)-based tagging approach combined with a literature search for SNPs changing gene function, as described in the Supporting Information (Figure S1). Fourteen SNPs in *FCER2* were genotyped by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in the MAGICS and ISAAC II populations (11, 12), while five further SNPs were derived from Illumina chip genotyping and two from HapMap II-based imputation (genotyping success rate varied by SNP as described in detail in the Supporting Information).

LD calculations and LD-based tagging procedures were performed using Haploview software, version 4.2 (13). For binary traits, additive genetic effects were modeled by logistic regression, with odds ratios (ORs) as an output. Total serum IgE was measured by the IMMULITE 2000 system (Siemens Healthcare Diagnostics, Eschborn, Germany). IgE values were approximated to normality by logarithmic transformation, and genotype effects were analyzed in an additive model using linear regression with estimates (standard error) showing the magnitude of the effect. Age and gender were covariates used for an adjustment. To investigate SNP effects on average IgE levels as well as on extreme values within the population, we performed an analysis of total IgE percentiles using the 50th (60.4 IU/ml), 66th (115 IU/ml), and 90th (457 IU/ml) percentiles as established in our cohort previously (12). In secondary analyses, associations of *FCER2* SNPs with asthma (atopic and nonatopic asthma), allergic rhinitis, and atopic dermatitis were investigated and phenotype definitions are given in the Supporting Information.

FCER1A and *FCER2* top IgE-associated polymorphisms rs2427837 (see ref. 14) and rs3760687 were analyzed for interactions by logistic regression adjusted for age and gender. Further, subjects were stratified for the presence/absence of the homozygous risk allele of *FCER1A* SNP rs2427837, and in each group, the effect of *FCER2* SNP rs3760687 on IgE levels was analyzed. Similarly, the stratification was performed for homozygous risk allele of *FCER2* SNP rs3760687, and the effects of *FCER1A* SNP rs2427837 were investigated for IgE levels.

Statistics for single SNP analyses were carried out by Plink (version 1.07; <http://pngu.mgh.harvard.edu/~purcell/plink/>) and by R (version 2.14; <http://cran.r-project.org/>) software for interaction analyses. To determine putative functional effects of the IgE-associated *FCER2* SNPs, *in silico* analysis was performed using Human Splicing Finder (version 2.4.1; <http://www.umd.be/HSF/>) for splicing effect, HaploReg (version 2; <http://www.broadinstitute.org/mammals/haploreg/haploreg.php>) based on ENCODE resource for DNase site, and regulatory chromatin states and Genomatix Software Suite (version 2.6; <http://www.genomatix.de/solutions/genomatix-software-suite.html>) for transcription factor prediction.

Results

All 14 SNPs genotyped by MALDI-TOF MS showed a minimum call rate of more than 90% and no deviation from Hardy-Weinberg equilibrium (HWE). Genotypes of five further SNPs derived from Illumina (GWA) genotyping and two additional SNPs that were imputed also passed quality control. Genotyped and imputed SNPs were assigned to 17 tagging bins (BINs) (Figure S2). Only one *FCER2* SNP (rs3760687) was associated with overall serum IgE levels in our cohort (Table 2). However, when we investigated IgE levels according to the population-based percentiles as previously described (12), we found that a number of *FCER2* SNPs were predominantly associated with mild-to-moderate increase in IgE levels (correlating with 50th and 66th percentiles in children) rather than with extremely high IgE levels (90th percentile). Significant associations with IgE levels in nonasthmatics (rs2277991 and rs2277989) could also be seen using 50th percentile as a cutoff (Table 1 and Table S2). No significant association was observed in asthmatics. Investigating IgE levels in atopic and nonatopic subjects from the ISAAC cohort, a clear pattern of association was observed in nonatopics only (Table S3).

Next, we analyzed the combined effect of *FCER2* and *FCER1A* polymorphisms on IgE levels. For this purpose, we investigated interactions between *FCER2* SNP rs3760687 and *FCER1A* SNP rs2427837 showing the most significant association with total IgE in a previous study (14). A borderline significant statistical interaction (P for interaction = 0.044) was observed analyzing IgE levels (Table S4).

In a second step, we examined whether effects of the *FCER2* SNP rs3760687 are specifically present in individuals homozygous or not for the *FCER1A* rs2427837 risk allele. Therefore, we stratified our study population by the presence/absence of the homozygosity for the respective risk allele and investigated the effect of the other receptor polymorphism (Table 2). Interestingly, *FCER2* rs3760687 had almost no effect on IgE levels in subjects carrying two *FCER1A* rs2427837 risk alleles. *FCER2* effects in our population were limited to serum IgE levels. None of the studied SNPs in the *FCER2* gene appeared to be associated with asthma and its subphenotypes, atopic sensitization, allergic rhinitis, or atopic dermatitis (Tables S5 and S6).

In silico analysis showed that the C allele of rs3760687 just upstream of the first untranslated exon of CD23a (a

Table 1 Associations of *FCER2* polymorphisms with total serum IgE levels and the risk of total serum IgE levels above the specified percentiles

SNP	Location of SNPs in <i>FCER2</i>	BIN	Minor allele	MAF	Source	Total IgE in complete cohort (A)			50th percentile (B) (lgE cutoff 60.4 IU/ml)			66th percentile (C) (lgE cutoff 115 IU/ml)			90th percentile (D) (lgE cutoff 457 IU/ml)		
						Estimate	SE	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)		
rs1078625	Downstream	1	G	0.31	CHIP	-0.09	0.07	0.180	0.86 (0.71-1.04)	0.123	0.94 (0.78-1.13)	0.512	0.96 (0.77-1.21)	0.746			
rs2228138	Exon	1	T	0.28	MALDI-TOF	-0.04	0.07	0.570	0.94 (0.78-1.14)	0.534	1.02 (0.85-1.22)	0.863	1.06 (0.85-1.33)	0.597			
rs28364072	Intron	1	G	0.27	MALDI-TOF	-0.02	0.07	0.727	0.96 (0.79-1.16)	0.654	1.04 (0.87-1.25)	0.679	1.06 (0.84-1.32)	0.636			
rs2277991	Intron	1	G	0.28	CHIP	-0.13	0.07	0.072	0.81 (0.67-0.99)	0.041	0.92 (0.76-1.11)	0.378	0.97 (0.76-1.23)	0.791			
rs12971845	Intron	2	C	0.16	Imputation	-0.02	0.09	0.825	0.91 (0.70-1.17)	0.050	0.99 (0.78-1.27)	0.968	1.03 (0.76-1.41)	0.840			
rs11668338	Intron	3	A	0.09	MALDI-TOF	0.12	0.10	0.251	1.05 (0.78-1.41)	0.742	1.12 (0.85-1.49)	0.413	1.39 (1.01-1.91)	0.046			
rs12984649	Intron	4	A	0.19	MALDI-TOF	-0.02	0.08	0.772	0.95 (0.77-1.17)	0.624	0.95 (0.78-1.17)	0.652	0.98 (0.77-1.27)	0.904			
rs2277989	Intron	5	G	0.49	CHIP	-0.08	0.06	0.202	0.82 (0.69-0.98)	0.030	0.95 (0.80-1.12)	0.527	1.00 (0.81-1.24)	0.986			
rs7249360	Intron	6	A	0.21	MALDI-TOF	-0.02	0.08	0.748	0.98 (0.79-1.21)	0.847	1.07 (0.87-1.31)	0.520	1.00 (0.78-1.29)	0.984			
rs7249320	Intron	6	A	0.23	MALDI-TOF	0.04	0.07	0.589	1.01 (0.83-1.24)	0.901	1.12 (0.92-1.35)	0.264	1.15 (0.91-1.45)	0.231			
rs2228137	Exon	7	A	0.12	MALDI-TOF	0.03	0.09	0.749	1.00 (0.77-1.30)	0.984	1.21 (0.94-1.55)	0.143	1.12 (0.83-1.51)	0.461			
rs10415518	Intron	8	A	0.37	Imputation	0.01	0.08	0.901	1.04 (0.83-1.29)	0.765	1.07 (0.87-1.33)	0.522	0.88 (0.68-1.15)	0.349			
rs8110128	Intron	9	A	0.46	MALDI-TOF	0.02	0.06	0.763	1.04 (0.87-1.23)	0.698	1.03 (0.87-1.22)	0.730	1.06 (0.86-1.30)	0.568			
rs2287867	Intron	10	G	0.45	MALDI-TOF	-0.08	0.06	0.174	0.82 (0.69-0.98)	0.026	0.85 (0.72-1.00)	0.047	1.02 (0.83-1.25)	0.867			
rs11672997	Intron	11	A	0.12	MALDI-TOF	0.07	0.09	0.424	1.12 (0.86-1.46)	0.400	1.03 (0.80-1.32)	0.824	1.21 (0.90-1.61)	0.207			
rs1990975	Intron	12	T	0.28	MALDI-TOF	-0.09	0.07	0.192	0.82 (0.68-0.99)	0.036	0.88 (0.73-1.05)	0.154	1.00 (0.80-1.25)	0.994			
rs2303112	Intron	13	G	0.42	CHIP	0.10	0.06	0.142	1.18 (0.98-1.41)	0.073	1.12 (0.94-1.33)	0.194	1.02 (0.83-1.27)	0.839			
rs3760687	Upstream	14	T	0.04	MALDI-TOF	-0.38	0.16	0.017	0.53 (0.34-0.81)	0.003	0.59 (0.38-0.92)	0.020	0.65 (0.36-1.19)	0.164			
rs4804221	Upstream	15	T	0.26	MALDI-TOF	-0.09	0.07	0.183	0.83 (0.69-1.01)	0.063	0.90 (0.75-1.09)	0.276	0.91 (0.72-1.15)	0.433			
rs753733	Upstream	16	A	0.14	CHIP	0.10	0.09	0.284	1.17 (0.90-1.51)	0.242	1.15 (0.90-1.46)	0.259	1.01 (0.75-1.37)	0.933			
rs12611038	Upstream	17	T	0.33	MALDI-TOF	-0.02	0.07	0.786	0.95 (0.79-1.15)	0.624	0.99 (0.83-1.18)	0.917	1.03 (0.83-1.28)	0.816			

Number of individuals with nonmissing phenotype involved in the respective calculations for the different methods of genotyping.

(A) Total IgE complete cohort: MALDI-TOF MS (1261), CHIP (1145); (B) 50th complete cohort: MALDI-TOF MS (827 cases/434 controls), CHIP (740 cases/405 controls); (C) 66th complete cohort: MALDI-TOF MS (633 cases/628 controls), CHIP (561 cases/584 controls); (D) 90th complete cohort: MALDI-TOF MS (253 cases/1008 controls), CHIP (221 cases/924 controls).

The *P*-value is adjusted for age and gender; if < 0.05, it is considered to be significant (bold font).

SNP, single nucleotide polymorphism; BIN, tagging bin; MAF, minor allele frequency; SE, standard error; CI, confidence interval.

Table 2 Effects of FCER1A rs2427837 and FCER2 rs3760687 on total serum IgE levels in subpopulations stratified for the respective other risk allele

Effect of	In individuals carrying*	Total IgE in complete cohort (A)		50th percentile (IgE cutoff 60.4 IU/ml)		66th percentile (IgE cutoff 115 IU/ml)		90th percentile (IgE cutoff 457 IU/ml)	
		Estimate	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
FCER2 rs3760687	FCER1A rs2427837 GG n = 662	-0.08 (0.22)	0.721	0.84 (0.44–1.60)	0.598	0.80 (0.44–1.46)	0.476	0.96 (0.47–1.96)	0.900
FCER2 rs3760687	FCER1A rs2427837 GA or AA n = 556	-0.70 (0.23)	0.003	0.32 (0.17–0.62)	7.13*10⁻⁴	0.40 (0.19–0.81)	0.011	0.34 (0.10–1.12)	0.076
FCER1A rs2427837	FCER2 rs3760687 CC n = 1119	-0.28 (0.07)	1.13*10⁻⁴	0.68 (0.56–0.84)	2.07*10⁻⁴	0.71 (0.58–0.86)	5.14*10⁻⁴	0.77 (0.60–0.98)	0.035
FCER1A rs2427837	FCER2 rs3760687 CT or TT n = 89	-0.81 (0.23)	5.80*10⁻⁴	0.31 (0.15–0.65)	0.003	0.30 (0.13–0.70)	0.006	0.28 (0.08–0.99)	0.047

Complete cohort was stratified for FCER1A rs2427837 and FCER2 rs3760687, respectively, to evaluate the SNP effect on IgE in relation to the other.

The statistical analysis was conducted by logistic regression modeling additive effects adjusting for age and sex. P-value < 0.05 is considered to be significant (bold font).

*The given numbers specify the samples for which both genotypes and IgE measurements were available.
CI, confidence interval.

constitutively expressed, B-cell-specific isoform) may create a selective binding motif for different transcription factors.

Discussion

The CD23 molecule (encoded by *FCER2* gene) is considered to be a regulator in biological actions of IgE potentially relevant in asthma and IgE-mediated allergic diseases (7, 10). In our study population, we found *FCER2* rs3760687 to modify total serum IgE levels. This SNP was shown previously to affect the binding of Sp1 and Sp3 transcription factors (15). Additional functional effects of this and other IgE-associated polymorphisms were suggested by our bioinformatic analysis (Table S7). Using a percentile-based approach, we showed that *FCER2* SNP affect mildly to moderately increased IgE levels. Thus, one can conclude that *FCER2* SNPs may not push the IgE system to a limit, but that rather subtle, regulatory effects on IgE steady state exist. When we stratified our cross-sectional study population by atopy status, it became apparent that *FCER2* SNPs affect total serum IgE levels in nonatopic rather than atopic individuals. This may suggest that *FCER2* association with basal serum IgE levels may not be linked directly with atopy development in the general population, which is different from the findings in *FCER1A* (14).

In contrast to these observations, *FCER2* SNPs rs2228138 (former name rs4996974), rs28364072, and rs7249320, previously reported to be associated with serum IgE levels in a US population (7), were not correlated with IgE in our study. SNPs rs28364072 and rs7249320 were also reported to be associated with increased exacerbation rates (7, 10) and a higher risk of asthma (10, 16). No significant association with asthma, atopy, allergic rhinitis, and atopic dermatitis was observed for both these SNPs in our population. Another exonic variant, rs2228137 (R62W), has previously been linked with severe exacerbations in asthmatics (7, 17), and in functional studies, rs2228137 was found to alter the stability of the CD23 molecule in the membrane (9, 18). Additionally, it upregulates IL-4 receptor expression on B lymphocytes so that it may influence IgE class switching (17). However, in our study, no significant association of rs2228137 with IgE levels, asthma, or other studied atopic disorders was identified. These discrepancies could be, among other reasons for a failure of replication, due to differences in ethnicity between study populations. In the US study, no analysis of percentiles was performed, and thus, no direct comparison with our results is possible.

The production and function of IgE is modulated by a network of proteins that include FcεRI(α) and CD23 (FcεRII) (1). Because of the biological relation between FcεRI(α) and FcεRII molecules in IgE regulation (1), we studied gene-by-gene interaction effects. In this analysis, a borderline interaction effect was observed. However, one should interpret these data cautiously with the awareness that the population is small for this kind of studies.

When a more detailed analysis was carried out where stratification by risk allele homozygosity status was applied, it became obvious that *FCER2* SNP rs3760687 only showed effects on serum IgE levels in subjects not carrying two

FCER1A rs2427837 risk alleles. This may indicate that the presence of the homozygosity for the *FCER1A* risk allele overrides any effects of *FCER2* rs3760687 polymorphism. On the other hand, this fact implies that SNPs in both genes should be studied concomitantly in a conditional analysis in the future when genetic determinants of serum IgE levels are investigated. This in-depth analysis may become especially important in population where IgE levels are driven by not only allergy but also challenges such as parasite exposure. It is conceivable that SNPs in *FCER1A* and *FCER2* may contribute differently to IgE regulation in such environments.

Taken together, we identified rs3760687 polymorphism in the *FCER2* gene to affect moderately elevated serum IgE levels in our population, especially in the absence of the homozygosity for the more relevant *FCER1A* rs2427837 risk allele.

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Author contributions

VS wrote the first draft of the manuscript, contributed to the design of the experiments, and contributed to data interpretation. SM performed statistical analyses and contributed to

data interpretation; VG contributed to statistical analysis. AF carried out MALDI-TOF MS genotyping; CV, AB, AB, AH, OL, ER, BS, TF, and JG contributed to data collection; DPP and MK developed the study design, participated in data analysis and interpretation, and wrote the final version of the manuscript.

Conflicts of interest

All authors declare that they have no competing financial or personal interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Detailed description of study populations and investigated phenotypes; and Details on polymorphism selection for genotyping.

Figure S1. Selection process of polymorphisms from *FCER2* gene region.

Figure S2. Schematic representation of *FCER2* gene region with location of genotyped polymorphisms (rs numbers), tag-ging bins (in brackets), and pair wise linkage disequilibrium (r^2).

Table S1. Descriptive statistics of MAGICs and ISAAC populations.

Table S2. Associations of *FCER2* polymorphisms with total serum IgE levels.

Table S3. Associations of *FCER2* polymorphisms with total serum IgE levels (in the cross-sectional ISAAC II cohort).

Table S4. Interaction effects of *FCER1A* rs2427837 and *FCER2* rs3760687 on total serum IgE levels.

Table S5. Associations of *FCER2* polymorphisms with asthma and its subphenotypes.

Table S6. Associations of *FCER2* polymorphisms with atopic sensitization, allergic rhinitis and atopic dermatitis.

Table S7. *In silico* analysis of putative functional effects of single nucleotide polymorphisms (SNPs) associated with IgE levels.

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A ROLE OF *FCER1A* AND *FCER2* POLYMORPHISMS IN IGE REGULATION

Supplement

Detailed description of study populations and investigated phenotypes

Subjects investigated in the present study were selected from the Multicenter Asthma Genetic in Childhood Study (MAGICS) and the German leg of the International Study of Asthma and Allergies in Childhood phase II study (ISAAC II) (1, 2).

In MAGICS, 1255 children were recruited between 2001 and 2007 from seven different asthma clinical centers located in Germany or Austria. Asthma was defined in 865 children (mean age 11 years) by pediatric pulmonologists based on physical examination, clinical history, lung function tests, and allergy status. Only children of German descent were included in subsequent genetic analyses.

In ISAAC II a large cross-sectional study conducted in 1995 and 1996, 5629 school children aged between 9–11 years, were recruited in Munich and Dresden (Germany) (3). The methodology of the ISAAC II study has been described in details elsewhere (3, 4). In this study, asthma status was accessed by the parents of the children, who reported a physician's diagnosis of asthma once or recurrent spastic or asthmatic bronchitis more than once. Atopic dermatitis (eczema) and allergic rhinitis were defined by the doctor's diagnostic report in parental questionnaire.

In our previous GWA study on childhood asthma, 1422 children of German/ Austrian ethnicity (728 asthmatics) were chip-genotyped by Illumina Sentrix HumanHap300 BeadChip and had full information on asthma status available (1). With this dataset, we have also

contributed to the meta-analysis by the GABRIEL Consortium (5). Most of the asthmatic cases (655) were obtained from the MAGICS (2). Further 73 asthmatic cases and all age- and sex-matched controls (694) derived from the large (n=5629) ISAAC II (2) from the same ethnic population source. Written informed consent was obtained from the parents of all children from both MAGICS and ISAAC II, and the study protocols were approved by the respective ethics committees.

Due to continuous quality control and an update of consent in a follow up study, the number of subjects for whom chip-genotyping results were available for the purposes of the present study decreased to 1311 (651 asthmatics). However, an additional 151 children, who were recruited after GWAS genotyping was performed or had too little DNA available for GWAS genotyping could be included in genotyping of novel SNPs for fine mapping of the *FCER1A* and *FCER2* loci (6). Thus 1454 subjects (763 asthmatics) were genotyped with matrix-assisted-laser-desorption/ionization-time-of-flight-mass-spectrometry (MALDI-TOF-MS).

Asthma, atopic asthma and non-atopic asthma were compared in a case-control design, including subjects from both MAGICS and ISAAC II. Atopic asthma was equal to the presence of both asthma and atopy, the latter defined by the positive results for specific IgE against at least one of allergens tested (RAST/CAP class equivalent of >2) in MAGICS and the positive specific IgE results based on Sx1 testing (RAST/CAP class of >2) in ISAAC II. The presence of asthma with the absence of atopy was considered to be non-atopic asthma. “Non-atopic” was defined by negative results for all specific IgE tested (RAST/ CAP class equivalent of 0) in MAGICS, and negative skin prick tests (a wheal reaction <3 mm after subtraction of the negative control for all tested allergens) and Sx1 (RAST/CAP class of 0) in ISAAC II.

Allergic sensitization against common inhalant allergens, *D. pteronyssinus*, *D. farinae*, cat epithelium, grass pollen and birch pollen, was tested in MAGICS by measuring specific serum IgE using CLA-1 (Hitachi Chemical Diagnostics, UK) or AllergyScreen (Mediwiss Analytic, Germany) panels. In ISAAC II, atopic sensitization was assessed by skin prick testing against common allergens, such as *D. pteronyssinus*, *D. farinae*, *Alternaria tenuis*, cat dander, mixed grass and mixed tree pollen, and/ or by Sx1 assay (ImmunoCAP, Phadia, Germany) containing a cocktail of antibodies against allergens, including local grass pollen, rye pollen, birch pollen, mugwort pollen, *D. pteronyssinus*, cat dander, dog dander and *Cladosporium herbarum*.

Details on polymorphism selection for genotyping

To provide the best coverage of *FCER2* and its neighborhood, we adopted a combined approach comprising linkage disequilibrium (LD)-based tagging and a detailed literature search for functional and/or trait-associated single-nucleotide polymorphisms (SNPs). Tagging for the region covering *FCER2* gene within its upstream and downstream surrounding (Chromosome 19: 7654000-7678500) was conducted on the data obtained from HapMap database (Data Phase III, release #3, May 2010, NCBI B36 assembly, dbSNP b126; http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap3r3_B36/) using Haploview software (version 4.2; <http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview>) (7) and the following criteria: minor allele frequency (MAF) ≥ 0.1 , Hardy-Weinberg Equilibrium (HWE) P -value > 0.05 and $r^2 > 0.8$. A set of 14 tagging SNPs covering for 21 variants was identified. Since three tagging variants were overlapping with SNPs previously chip-genotyped by Illumina Sentrix HumanHap300 BeadChip for the purposes of our genome-wide

association (GWA) study on asthma (1, 2), they were excluded from the current round of genotyping. Detailed literature search identified five SNPs, which have been previously demonstrated to be functional or associated with IgE levels, asthma exacerbations and/or lung cancer (8-12). Out of 16 SNPs to be genotyped by matrix-assisted-laser-desorption/ionization-time-of-flight-mass-spectrometry MALDI-TOF-MS technique (13), 14 were successfully genotyped. For the two SNPs that could not be genotyped due to technical reasons, data from imputation based on chip-genotyping and HapMap II data were used (2). Finally, genotypes of five additional SNPs were extracted from the GWA study dataset (1, 2) including the three variants overlapping with tagging SNPs (*see above*) and two additional polymorphisms not selected by tagging and literature-based approaches but present in our GWA data of the targeted chromosome region. In this way a total of 21 SNPs were selected for statistical analysis (Suppl. Figure 1). Details on the methodology of genotyping, including primers and all the additional procedures of handling the samples, could be obtained from the authors on request.

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Supplementary Table I. Descriptive statistics of MAGICS and ISAAC populations

	MAGICS	ISAAC II
Male sex, n* (%)	457/691 (66.1)	386/763 (50.6)
Age, mean (SD)	10.99 (2.90)	9.63 (0.59)
Asthmatic, n* (%)	691/691 (100)	72/755 (9.54)
Atopic sensitization, n* (%)	505/598 (84.4)	194/651 (29.8)
Total serum IgE levels, median (Q1-Q3)	260 (91-611)	69 (23-188)

*Number affected/number with data available

Supplementary Table II. Associations of *FCER2* polymorphisms with total serum IgE levels

SNP	BIN	Complete cohort (A)		Non-asthmatics (B)		Asthmatics (B)		Atopic asthmatics (C)		Non-atopic asthmatics (C)	
		Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value
rs1078625	1	-0.09 (0.07)	0.180	-0.16 (0.09)	0.065	-0.01 (0.10)	0.939	0.00 (0.09)	0.983	0.20 (0.22)	0.366
rs2228138	1	-0.04 (0.07)	0.570	-0.15 (0.09)	0.093	0.03 (0.10)	0.780	0.02 (0.08)	0.810	0.27 (0.20)	0.179
rs28364072	1	-0.02 (0.07)	0.727	-0.14 (0.09)	0.119	0.04 (0.10)	0.705	0.01 (0.08)	0.950	0.30 (0.20)	0.142
rs2277991	1	-0.13 (0.07)	0.072	-0.19 (0.09)	0.029	-0.03 (0.10)	0.761	-0.01 (0.09)	0.892	0.21 (0.22)	0.348
rs12971845	2	-0.02 (0.09)	0.825	-0.02 (0.11)	0.831	0.03 (0.14)	0.845	-0.10 (0.12)	0.407	-0.53 (0.30)	0.087
rs11668338	3	0.12 (0.10)	0.251	-0.07 (0.14)	0.625	0.21 (0.14)	0.124	0.10 (0.12)	0.409	0.28 (0.30)	0.346
rs12984649	4	-0.02 (0.08)	0.772	-0.04 (0.10)	0.657	-0.02 (0.11)	0.871	0.02 (0.10)	0.870	0.30 (0.21)	0.162
rs2277989	5	-0.08 (0.06)	0.202	-0.19 (0.08)	0.019	-0.06 (0.09)	0.526*	-0.04 (0.08)	0.622*	0.53 (0.21)	0.017
rs7249360	6	-0.02 (0.08)	0.748	-0.18 (0.10)	0.061	0.09 (0.11)	0.411	0.00 (0.09)	0.975	0.33 (0.21)	0.120
rs7249320	6	0.04 (0.07)	0.589	-0.15 (0.09)	0.104	0.16 (0.10)	0.107	0.10 (0.09)	0.264	0.34 (0.21)	0.105
rs2228137	7	0.03 (0.09)	0.749	-0.07 (0.13)	0.586	0.03 (0.13)	0.819	0.04 (0.11)	0.680	-0.02 (0.28)	0.933
rs10415518	8	0.01 (0.08)	0.901	0.09 (0.10)	0.365	-0.02 (0.12)	0.854	0.07 (0.10)	0.462	-0.19 (0.23)	0.413
rs8110128	9	0.02 (0.06)	0.763	0.01 (0.08)	0.859	0.00 (0.09)	0.987	0.01 (0.08)	0.924	-0.18 (0.18)	0.327
rs2287867	10	-0.08 (0.06)	0.174	-0.05 (0.08)	0.504	-0.11 (0.09)	0.187	0.00 (0.08)	0.951	-0.26 (0.21)	0.217
rs11672997	11	0.07 (0.09)	0.424	-0.01 (0.13)	0.913	0.05 (0.12)	0.708	0.08 (0.11)	0.456	0.13 (0.25)	0.618
rs1990975	12	-0.09 (0.07)	0.192	-0.06 (0.09)	0.492	-0.09 (0.09)	0.366	0.02 (0.08)	0.792	-0.38 (0.21)	0.069
rs2303112	13	0.10 (0.06)	0.142	0.07 (0.08)	0.355	0.07 (0.09)	0.466	-0.03 (0.08)	0.742	0.25 (0.23)	0.281
rs3760687	14	-0.38 (0.16)	0.017	-0.22 (0.19)	0.235	-0.35 (0.26)	0.178	-0.24 (0.23)	0.310	0.06 (0.53)	0.915
rs4804221	15	-0.09 (0.07)	0.183	-0.06 (0.09)	0.524	-0.14 (0.10)	0.155	0.00 (0.08)	0.956	-0.32 (0.21)	0.138
rs753733	16	0.10 (0.09)	0.284	0.17 (0.11)	0.117	0.06 (0.14)	0.647	0.06 (0.12)	0.617	-0.04 (0.27)	0.872
rs12611038	17	-0.02 (0.07)	0.786	0.02 (0.09)	0.802	-0.04 (0.09)	0.694	0.06 (0.08)	0.477	-0.30 (0.22)	0.172

Number of individuals with non-missing phenotype involved in the respective calculations for the different rounds of genotyping.

A) Total IgE complete cohort: MALDI-TOF MS (1261), CHIP (1145)

B) Total IgE non-asthmatics: MALDI-TOF MS (651), CHIP (620); Total IgE asthmatics: MALDI-TOF MS (602), CHIP (517)

C) Total IgE atopic asthmatics: MALDI-TOF MS (438), CHIP (377); Total IgE non-atopic asthmatics: MALDI-TOF MS (96), CHIP (83);

All statistical analyses include adjustment for age and sex.

Abbreviations: SNP, single-nucleotide polymorphism; BIN, tagging bin; MAF, minor allele frequency; SE, standard error.

* A is the minor allele

Supplementary Table III. Associations of *FCER2* polymorphisms with total serum IgE levels (in the cross-sectional ISAAC II cohort)

SNP	BIN	complete ISAAC II cohort (A)		Atopics (B)		Non-Atopics (C)	
		Estimate (SE)	P-value	Estimate (SE)	P-value	Estimate (SE)	P-value
rs1078625	1	-0.14 (0.08)	0.101	0.05 (0.13)	0.710	-0.22 (0.09)	0.013
rs2228138	1	-0.13 (0.09)	0.113	0.04 (0.13)	0.759	-0.20 (0.09)	0.029
rs28364072	1	-0.12 (0.09)	0.167	0.00 (0.13)	0.987	-0.17 (0.09)	0.064
rs2277991	1	-0.18 (0.09)	0.034	0.07 (0.13)	0.613	-0.26 (0.09)	0.005
rs12971845	2	-0.08 (0.11)	0.459	-0.18 (0.18)	0.330	0.03 (0.12)	0.799
rs11668338	3	-0.05 (0.13)	0.679	0.05 (0.21)	0.815	-0.12 (0.14)	0.416
rs12984649	4	-0.02 (0.10)	0.818	-0.04 (0.15)	0.788	-0.09 (0.11)	0.414
rs2277989	5	-0.18 (0.08)	0.024	-0.03 (0.13)	0.795*	-0.25 (0.08)	0.003
rs7249360	6	-0.14 (0.09)	0.134	0.04 (0.14)	0.806	-0.21 (0.10)	0.040
rs7249320	6	-0.12 (0.09)	0.170	0.03 (0.14)	0.816	-0.20 (0.10)	0.036
rs2228137	7	-0.01 (0.12)	0.929	0.23 (0.17)	0.181	-0.21 (0.13)	0.116
rs10415518	8	0.06 (0.10)	0.524	-0.10 (0.16)	0.537	0.08 (0.10)	0.469
rs8110128	9	0.07 (0.08)	0.396	0.16 (0.12)	0.182	-0.01 (0.08)	0.930
rs2287867	10	-0.03 (0.08)	0.687	0.06 (0.12)	0.633	-0.10 (0.08)	0.201
rs11672997	11	-0.04 (0.12)	0.724	-0.19 (0.18)	0.295	-0.06 (0.13)	0.647
rs1990975	12	-0.03 (0.08)	0.762	0.16 (0.13)	0.216	-0.10 (0.09)	0.287
rs2303112	13	0.07 (0.08)	0.370	0.04 (0.12)	0.738	0.08 (0.08)	0.336
rs3760687	14	-0.15 (0.18)	0.400	-0.07 (0.27)	0.791	-0.28 (0.20)	0.159
rs4804221	15	0.00 (0.09)	0.983	0.01 (0.14)	0.961	-0.03 (0.10)	0.755
rs753733	16	0.12 (0.11)	0.282	-0.23 (0.18)	0.209	0.28 (0.11)	0.013
rs12611038	17	0.06 (0.08)	0.443	-0.05 (0.13)	0.678	0.05 (0.09)	0.596

Number of individuals with non-missing phenotype involved in the respective calculations for the different rounds of genotyping.

A) Total IgE in complete ISAAC II cohort: MALDI-TOF MS (726), CHIP (693)

B) Total IgE in Atopics: MALDI-TOF MS (185), CHIP (176)

C) Total IgE in Non-Atopics: MALDI-TOF MS (507), CHIP (486)

General IgE levels in complete ISAAC II cohort: median=69.1; Q1=23.45; Q3=187.75

General IgE levels in Atopics: median=227; Q1=113; Q3=528

General IgE levels in Non-Atopics: median=43.5; Q1=18.15; Q3=99.05

* A is the minor allele

Supplementary Table IV. Interaction effects of *FCER1A* rs2427837 and *FCER2* rs3760687 on total serum IgE levels

	Total IgE in complete cohort (A)		50 th percentile (IgE cutoff 60.4 IU/mL)		66 th percentile (IgE cutoff 115 IU/mL)		90 th percentile (IgE cutoff 457 IU/mL)	
Interaction	Estimate	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
rs3760687 x rs2427837	-0.48 (0.24)	0.044	0.48 (0.23-1.01)	0.052	0.45 (0.20-1.02)	0.057	0.37 (0.10-1.33)	0.128

The statistical analysis was conducted by logistic regression modeling statistical interaction of additive effects adjusted for age and sex

P-value < 0.05 is considered to be significant

Abbreviation: CI, confidence interval

Supplementary Table V. Associations of *FCER2* polymorphisms with asthma and sub-phenotypes

SNP	BIN	Asthma (A)		Atopic asthma (B)		Non-atopic asthma (C)	
		Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
rs1078625	1	1.05 (0.89-1.25)	0.542	1.09 (0.89-1.34)	0.411	1.12 (0.80-1.56)	0.502
rs2228138	1	1.14 (0.97-1.35)	0.116	1.14 (0.93-1.40)	0.211	1.19 (0.87-1.64)	0.284
rs28364072	1	1.16 (0.98-1.37)	0.082	1.18 (0.96-1.45)	0.114	1.22 (0.88-1.68)	0.230
rs2277991	1	1.06 (0.90-1.27)	0.480	1.06 (0.86-1.31)	0.598	1.09 (0.77-1.53)	0.640
rs12971845	2	0.91 (0.72-1.14)	0.397	0.99 (0.75-1.31)	0.956	0.61 (0.37-1.01)	0.052
rs11668338	3	1.16 (0.89-1.50)	0.270	1.26 (0.92-1.73)	0.149	1.32 (0.80-2.18)	0.273
rs12984649	4	1.01 (0.84-1.22)	0.918	1.02 (0.81-1.28)	0.899	0.97 (0.66-1.41)	0.858
rs2277989	5	1.07 (0.91-1.24)	0.428	1.13 (0.93-1.36)	0.221	0.98 (0.72-1.34)	0.906
rs7249360	6	1.05 (0.87-1.27)	0.602	1.07 (0.85-1.35)	0.550	1.09 (0.76-1.56)	0.641
rs7249320	6	1.15 (0.97-1.37)	0.115	1.20 (0.97-1.49)	0.091	1.21 (0.86-1.70)	0.266
rs2228137	7	1.23 (0.98-1.55)	0.081	1.22 (0.92-1.61)	0.160	1.30 (0.85-2.01)	0.231
rs10415518	8	0.92 (0.75-1.12)	0.390	0.89 (0.70-1.13)	0.320	1.02 (0.69-1.51)	0.931
rs8110128	9	1.03 (0.88-1.19)	0.747	1.00 (0.83-1.21)	0.998	0.90 (0.67-1.22)	0.506
rs2287867	10	1.01 (0.87-1.18)	0.853	1.02 (0.85-1.22)	0.865	1.17 (0.87-1.58)	0.299
rs11672997	11	1.17 (0.94-1.47)	0.164	1.31 (0.99-1.74)	0.061	1.40 (0.91-2.16)	0.131
rs1990975	12	0.99 (0.84-1.16)	0.859	0.96 (0.78-1.17)	0.686	1.10 (0.79-1.52)	0.586
rs2303112	13	1.06 (0.90-1.24)	0.485	1.04 (0.86-1.25)	0.723	1.08 (0.78-1.49)	0.645
rs3760687	14	0.79 (0.54-1.16)	0.233	0.67 (0.42-1.05)	0.080	0.61 (0.27-1.37)	0.230
rs4804221	15	1.04 (0.88-1.23)	0.675	1.02 (0.83-1.25)	0.878	1.23 (0.88-1.73)	0.223
rs753733	16	0.83 (0.66-1.04)	0.102	0.86 (0.65-1.12)	0.261	0.78 (0.50-1.23)	0.292
rs12611038	17	1.02 (0.87-1.20)	0.774	1.02 (0.84-1.24)	0.862	1.12 (0.80-1.55)	0.511

Number of individuals with non-missing phenotype involved in the respective calculations for the different rounds of genotyping

A) Asthma: MALDI-TOF MS (763 cases / 683 controls), CHIP (651 cases / 652 controls)

B) Atopic asthma: MALDI-TOF MS (548 cases / 426 controls), CHIP (468 cases / 408 controls)

C) Non-atopic asthma: MALDI-TOF MS (113 cases / 426 controls), CHIP (98 cases / 408 controls)

P-value < 0.05 is considered to be significant

Abbreviation: CI, confidence interval

Supplementary Table VI. Associations of *FCER2* polymorphisms with atopic sensitization, allergic rhinitis and atopic dermatitis

SNP	BIN	Atopy (A)		Allergic rhinitis (B)		Atopic dermatitis (C)	
		Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)	P-value
rs1078625	1	1.09 (0.84-1.40)	0.520	1.27 (0.90-1.81)	0.176	1.15 (0.86-1.54)	0.362
rs2228138	1	1.04 (0.80-1.34)	0.798	1.30 (0.91-1.85)	0.150	1.14 (0.85-1.54)	0.384
rs28364072	1	1.05 (0.81-1.36)	0.719	1.32 (0.93-1.89)	0.119	1.16 (0.86-1.56)	0.325
rs2277991	1	1.01 (0.77-1.31)	0.964	1.20 (0.83-1.71)	0.330	1.19 (0.88-1.60)	0.252
rs12971845	2	0.92 (0.65-1.31)	0.661	1.04 (0.64-1.70)	0.861	0.93 (0.62-1.40)	0.742
rs11668338	3	1.12 (0.75-1.68)	0.578	1.27 (0.74-2.19)	0.386	1.49 (0.96-2.31)	0.073
rs12984649	4	1.25 (0.93-1.67)	0.141	0.96 (0.62-1.47)	0.844	1.08 (0.77-1.53)	0.656
rs2277989	5	1.08 (0.85-1.37)	0.535	1.23 (0.87-1.72)	0.240	1.08 (0.82-1.43)	0.569
rs7249360	6	1.03 (0.78-1.38)	0.820	1.19 (0.80-1.76)	0.401	1.11 (0.80-1.54)	0.519
rs7249320	6	1.07 (0.82-1.41)	0.610	1.33 (0.92-1.93)	0.129	1.07 (0.78-1.47)	0.686
rs2228137	7	1.16 (0.81-1.65)	0.423	1.50 (0.94-2.38)	0.086	0.78 (0.50-1.23)	0.284
rs10415518	8	1.02 (0.75-1.39)	0.897	0.71 (0.46-1.09)	0.116	1.12 (0.78-1.59)	0.545
rs8110128	9	0.95 (0.75-1.21)	0.685	1.04 (0.74-1.44)	0.837	1.12 (0.85-1.47)	0.436
rs2287867	10	1.11 (0.88-1.41)	0.371	1.35 (0.97-1.87)	0.076	1.00 (0.77-1.32)	0.975
rs11672997	11	1.29 (0.91-1.83)	0.161	1.26 (0.78-2.05)	0.342	1.28 (0.86-1.91)	0.225
rs1990975	12	0.97 (0.75-1.27)	0.838	1.20 (0.84-1.71)	0.322	0.84 (0.62-1.14)	0.261
rs2303112	13	0.96 (0.75-1.22)	0.716	0.90 (0.64-1.26)	0.536	1.08 (0.82-1.43)	0.571
rs3760687	14	1.11 (0.64-1.93)	0.711	1.37 (0.67-2.81)	0.390	0.95 (0.49-1.84)	0.882
rs4804221	15	1.05 (0.80-1.38)	0.730	1.30 (0.91-1.87)	0.152	0.90 (0.66-1.24)	0.525
rs753733	16	0.85 (0.60-1.20)	0.346	0.62 (0.36-1.07)	0.084	0.84 (0.56-1.25)	0.397
rs12611038	17	1.20 (0.93-1.54)	0.170	1.31 (0.93-1.86)	0.124	1.06 (0.79-1.43)	0.684

Number of individuals with non-missing phenotype involved in the respective calculations for the different rounds of genotyping

A) Atopy: MALDI-TOF MS (192 cases / 536 controls), CHIP (183 cases / 515 controls)

B) Allergic rhinitis: MALDI-TOF MS (80 cases / 665 controls), CHIP (77 cases / 636 controls)

C) Atopic dermatitis: MALDI-TOF MS (131 cases / 603 controls), CHIP (123 cases / 578 controls)

P-value < 0.05 is considered to be significant

Abbreviation: CI, confidence interval

Supplementary Table VII. *In-silico* analysis of putative functional effects of single-nucleotide polymorphisms (SNPs) associated with IgE levels

SNP	Minor allele	Location of SNPs in <i>FCER2</i> [†]	Putative functional impact
rs2277991	G	Intron	EIEs site broken in 'C' allele but not in 'T' allele also IIEs new site is found with 'C' allele but not with 'T' allele. DNase site in B-lymphocyte, lymphoblastoid and urothelial cells
rs11668338	A	Intron	Potential splice sites in 'A' allele but not in 'G' allele and also EIE site is broken in 'A' allele but not in 'G' allele
rs2277989	G	Intron	EIEs site broken in 'G' allele but not in 'A' allele. Regulatory chromatin states (1_active_promoter in B-lymphocyte and lymphoblastoid cells)
rs2287867	G	Intron	EIEs site broken in 'G' allele but not in 'A' allele also IIEs new site is found with 'G' allele but not with 'A' allele. Regulatory chromatin states (4_strong_enhancer in B-lymphocyte, and lymphoblastoid) and DNase site in B-lymphocyte and lymphoblastoid cells
rs1990975	T	Intron	EIEs site broken in 'T' allele but not in 'C' allele also IIEs new site is found with 'T' allele but not with 'C' allele. Regulatory chromatin states (4_strong_enhancer in B-lymphocyte and lymphoblastoid cells)
rs3760687	T	Upstream	Zinc finger with KRAB and SCAN domains 3; TGFbeta-inducible early gene (TIEG) / Early growth response gene alpha (EGRAalpha); Kidney-enriched kruppel-like factor, KLF15 and Sterol regulatory element binding protein binds to 'C' allele and not 'T' allele

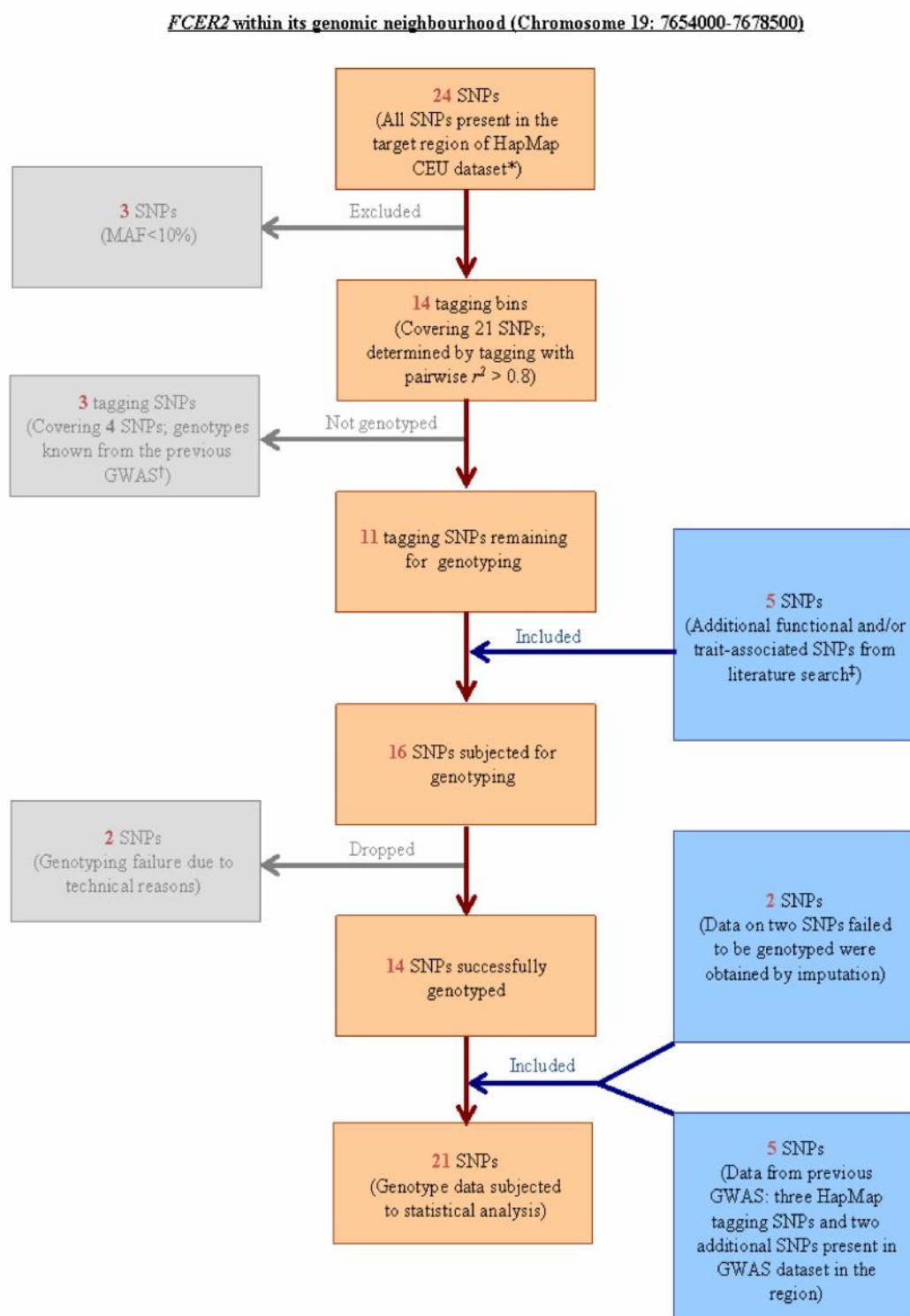
† The location of the SNPs is based on dbSNP database for *FCER2* gene (encodes CD23 molecule). The search was done on July 31th 2013 (<http://www.ncbi.nlm.nih.gov/projects/SNP/>)

EIEs are exon-identity elements and IIEs are intron-identity elements whose prediction is based on methods of Zhang et al., 2008 (14)

The information of DNase site and regulatory chromatin states is based on ENCODE resource (15)

Figure E I. Selection process of polymorphisms from *FCER2* gene region

Abbreviations: SNP, single-nucleotide polymorphisms; MAF, minor allele frequency; GWAS,



genome wide association study

* The international Hapmap 3 Consortium. Nature 2010 (13)

† Previous genotyping or imputed in our cohort include: our genome-wide association study on childhood asthma (1)

‡ The functionally important SNPs were taken from Tantisira *et al.*, 2007 (8); Koster *et al.*, 2011 (11); Meng *et al.*, 2007 (10); Shen *et al.*, 2009 (12); Potaczek *et al.*, 2009 (9)

