

ORIGINAL ARTICLE

Different *FCER1A* polymorphisms influence IgE levels in asthmatics and non-asthmatics

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Keywords

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Abstract

Background: Recently, three genome-wide association studies (GWAS) demonstrated *FCER1A*, the gene encoding a ligand-binding subunit of the high-affinity IgE receptor, to be a major susceptibility locus for serum IgE levels. The top association signal differed between the two studies from the general population and the one based on an asthma case-control design. In this study, we investigated whether different *FCER1A* polymorphisms are associated with total serum IgE in the general population and asthmatics specifically.

Methods: Nineteen polymorphisms were studied in *FCER1A* based on a detailed literature search and a tagging approach. Polymorphisms were genotyped by the Illumina HumanHap300Chip (6 polymorphisms) or MALDI-TOF MS (13 polymorphisms) in at least 1303 children (651 asthmatics) derived from the German International Study of Asthma and Allergies in Childhood II and Multicentre Asthma Genetics in Childhood Study.

Results: Similar to two population-based GWAS, the peak association with total serum IgE was observed for SNPs rs2511211, rs2427837, and rs2251746 (mean $r^2 > 0.8$), with the lowest p-value of 4.37×10^{-6} . The same 3 polymorphisms showed the strongest association in non-asthmatics (lowest p = 0.0003). While these polymorphisms were also associated with total serum IgE in asthmatics (lowest p = 0.003), additional polymorphisms (rs3845625, rs7522607, and rs2427829) demonstrated associations with total serum IgE in asthmatics only (lowest p = 0.01).

Conclusions: These data suggest that *FCER1A* polymorphisms not only drive IgE levels in the general population but that specific polymorphisms may also influence IgE in association with asthma, suggesting that disease-specific mechanisms in IgE regulation exist.

Abbreviations

CI, Confidence interval; *FCER1A*, *FcεRIα* gene; *FcεRI(α)*, High-affinity IgE receptor (α -subunit); GWA, study Genome-wide association study; HWE, Hardy-Weinberg equilibrium; IgE, Immunoglobulin E; ISAAC, International Study of Asthma and Allergies in Childhood; LD, Linkage disequilibrium; MAF, Minor allele frequency; MAGICs, Multicentre Asthma Genetics in Childhood Study; MALDI-TOF MS, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; OR, Odds ratio; SNP, Single-nucleotide polymorphism.

In two of three genome-wide association (GWA) studies on total serum IgE, the strongest association signal was derived from a locus on chromosome 1q23 harboring *FCER1A*, the gene encoding the α -subunit (*FcεRIα*) of the high-affinity IgE receptor (*FcεRI*) (1–3). IgE molecules bound to allergen interact with *FcεRI* and induce the release of inflammatory mediators, frequently associated with allergy and atopic

disorders (4–6). FcεRI is present on mast cells and/or basophils as a tetramer composed of three distinct subunits ($\alpha\beta\gamma_2$). Of the three FcεRI subunits, only FcεRIα is FcεRI specific, while β- and γ-subunits are shared with other Fc receptors (4, 7). In humans, FcεRI is also expressed in a trimeric form ($\alpha\gamma_2$) on the surface of antigen-presenting cells where it contributes to antigen uptake and presentation (4, 6, 7).

Comparing the three GWA studies on total serum IgE levels (1–3), some discordance exists in the source of the association signals with the *FCER1A* locus. In the two general population-based GWA studies, the strongest association signal came from the two single-nucleotide polymorphisms (SNPs) being in high pairwise linkage disequilibrium (LD), rs2427837 and rs2251746 (1–3). In the third study cohort, consisting of approximately 50% asthmatics, rs2252226 showed the strongest effect (2). Thus, we speculated that different genetic factors within the *FCER1A* locus might contribute to IgE regulation in the general population and to elevated IgE in asthmatics. We therefore analyzed the *FCER1A* locus in-depth and investigated the association between the *FCER1A* SNPs and total serum IgE levels comparatively in asthmatics and non-asthmatics. Furthermore, considering the obvious links between IgE regulation and atopy, we aimed to explore the role of *FCER1A* genetic variants in specific sensitization and related allergic phenotypes.

Methods

SNP selection

To provide the best coverage of *FCER1A* and its neighborhood, SNPs were selected from two sources, including (i) a LD-based tagging and (ii) a literature search for functional and/or clinically relevant SNPs, as described in more details in the Supplement. In brief, using HapMap resources, a set of 6 tagging SNPs was identified to cover *FCER1A* and its closest surrounding (chr1:157509000..157555000; NCBI B36 assembly). Detailed literature search identified 17 clinically and/or functionally relevant SNPs. Because four literature-identified variants were overlapping with tagging SNPs, the final genotyping list comprised 19 polymorphisms, as listed in Table 1.

Study population and genotyping

In our previous GWA study on childhood asthma, 1422 children of German or Austrian ethnicity (728 asthmatics) were chip-genotyped by Illumina Sentrix HumanHap300 BeadChip and had full information on asthma status available (8, 9). With this data set, we have also contributed to the meta-analysis by the GABRIEL Consortium (2). Most of the asthmatic cases (655) were obtained from the Multicentre Asthma Genetic in Childhood Study (MAGICS) (8, 9). All the controls (694) and 73 asthmatic cases were derived from the large ($n = 5629$) cross-sectional International Study of Asthma and Allergy in Childhood, phase II (ISAAC II) (8, 10), from the same ethnic population source. In subjects belonging to ISAAC II, two *FCER1A* SNPs (rs2427837 and rs2251746) have

been previously analyzed in the replication phase of the study by Weidinger et al. (1). 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 subsequent quality controls and an update of consent in a follow-up study, the number of subjects for whom the chip genotyping results were available for the purposes of this study decreased to 1303 (651 asthmatics). From this data set, the chip genotyping data on 6 of 19 selected *FCER1A* SNPs were extracted. The remaining 13 *FCER1A* SNPs were re-genotyped with the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique (11). For MALDI-TOF MS genotyping, 1454 subjects were available: 1303 from chip genotyping in the asthma project, children recruited later in the study ($n = 30$) and children not included in the previous asthma analysis due to insufficient DNA for chip genotyping ($n = 113$) or unconfirmed asthma status ($n = 8$).

In MAGICS, asthma was diagnosed by pediatric pulmonologists based on physical examination, clinical history, lung function tests, and allergy testing. In ISAAC II, children were classified as having asthma if their parents reported a physician's diagnosis of asthma once, or recurrent spastic or asthmatic bronchitis more than once. Also, diagnosis of allergic rhinitis and atopic dermatitis was based on the report of the relevant doctor's diagnosis in the parental questionnaire.

Total serum IgE levels were measured in both MAGICS and ISAAC II using the IMMULITE 2000 system [Siemens Healthcare Diagnostics, Germany (former DPC Biermann)]. In MAGICS, sensitization to common inhalant allergens, *D. pteronyssinus*, *D. farinae*, cat epithelium, grass pollen, and birch pollen, was assessed by the measurement of specific serum IgE using CLA-1 (Hitachi Chemical Diagnostics, UK) or AllergyScreen (Mediawiss Analytic, Germany) panels. In ISAAC II, atopic sensitization was tested by skin prick test to *D. pteronyssinus*, *D. farinae*, *Alternaria tenuis*, cat dander, mixed grass and mixed tree pollen, and/or by Sx1 panel (ImmunoCAP, Phadia, Germany) covering a mix of the following allergens: local grass pollen, rye pollen, birch pollen, mugwort pollen, *D. pteronyssinus*, cat dander, dog dander, and *Cladosporium herbarum*.

Investigated phenotypes

Asthma, atopic asthma, and non-atopic asthma were analyzed in a case-control design comprising participants of both MAGICS and ISAAC II. Subjects with asthma were compared to those without. Atopic asthma was defined as the presence of asthma and atopy based on the detection of specific IgE against at least one of the allergens tested (RAST/CAP class equivalent of >2) in MAGICS and the positive result of Sx1 testing (RAST/CAP class of >2) in ISAAC II. The presence of asthma with the absence of atopy defined non-atopic asthma. 'Non-atopic' was defined by negative results for all specific IgE tested (RAST/CAP class equivalent of <1) in MAGICS and negative Sx1 (RAST/CAP class of <1) and skin prick tests (a wheal reaction <3 mm after subtraction of the negative control for all tested allergens) in ISAAC II. Both asthma subphenotypes

Table 1 Associations of *FCER1A* polymorphisms with total serum IgE levels

A			B			C			D							
SNP	BIN	Minor allele	MAF	Source	Complete cohort		Non-asthmatics		Asthmatics		Atopic asthmatics		Non-atopic asthmatics (stringent definition)		Non-atopic asthmatics (loose definition)	
					Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p	Estimate (SE)	p		
rs2511211	1	G	0.22	MALDI-TOF MS	-0.31 (0.07)	3.45*10 ⁻⁵	-0.27 (0.10)	0.006	-0.31 (0.10)	0.003	-0.24 (0.09)	0.011	-0.18 (0.21)	0.410	-0.25 (0.21)	0.225
rs10489854	2	T	0.06	CHIP	0.27 (0.14)	0.058	0.26 (0.17)	0.132	0.20 (0.21)	0.334	0.12 (0.18)	0.522	0.40 (0.49)	0.415	0.29 (0.45)	0.513
rs2494262	3	C	0.48	MALDI-TOF MS	0.12 (0.06)	0.057	0.12 (0.08)	0.137	0.15 (0.09)	0.086	0.09 (0.07)	0.218	0.24 (0.20)	0.246	0.23 (0.18)	0.209
rs2511214	3	G	0.48	MALDI-TOF MS	0.14 (0.06)	0.027	0.14 (0.08)	0.072	0.16 (0.09)	0.066	0.10 (0.08)	0.197	0.24 (0.20)	0.246	0.23 (0.18)	0.209
rs2427827	3	T	0.44	MALDI-TOF MS	0.11 (0.06)	0.069	0.14 (0.08)	0.084	0.15 (0.09)	0.093	0.06 (0.08)	0.413	0.24 (0.19)	0.216	0.29 (0.18)	0.109
rs2252226	3	C	0.45	MALDI-TOF MS	0.09 (0.06)	0.136	0.09 (0.08)	0.238	0.14 (0.09)	0.104	0.08 (0.08)	0.324	0.19 (0.21)	0.348	0.25 (0.18)	0.177
rs2427836	4	C	0.47	CHIP	-0.15 (0.06)	0.016	-0.16 (0.08)	0.042	-0.16 (0.09)	0.082	-0.07 (0.08)	0.368	0.25 (0.21)	0.238	-0.19 (0.19)	0.314
rs2494251	4	A	0.45	CHIP	-0.16 (0.06)	0.014	-0.14 (0.08)	0.086	-0.20 (0.09)	0.025	-0.12 (0.08)	0.143	0.17 (0.20)	0.409	-0.16 (0.19)	0.395
rs2427837	5	A	0.27	MALDI-TOF MS	-0.32 (0.07)	4.37*10 ⁻⁶	-0.32 (0.09)	0.0003	-0.26 (0.10)	0.006	-0.21 (0.09)	0.019	-0.18 (0.19)	0.348	-0.25 (0.19)	0.186
rs2251746	5	C	0.27	CHIP	-0.30 (0.07)	3.54*10 ⁻⁵	-0.32 (0.09)	0.0004	-0.22 (0.10)	0.037	-0.20 (0.09)	0.031	0.01 (0.21)	0.965	-0.14 (0.21)	0.512
rs12565775	6	C	0.09	MALDI-TOF MS	0.21 (0.11)	0.048	0.26 (0.14)	0.055	0.16 (0.15)	0.278	0.06 (0.12)	0.632	0.01 (0.37)	0.974	-0.18 (0.35)	0.618
rs7548864	6	A	0.09	CHIP	0.23 (0.11)	0.037	0.33 (0.14)	0.019	0.08 (0.16)	0.621	0.06 (0.13)	0.681	-0.05 (0.33)	0.888	-0.21 (0.33)	0.519
rs12119226	6	T	0.08	MALDI-TOF MS	0.18 (0.11)	0.093	0.24 (0.14)	0.096	0.10 (0.15)	0.516	0.02 (0.12)	0.849	-0.10 (0.36)	0.779	-0.24 (0.35)	0.489
rs2427824	7	T	0.26	MALDI-TOF Ms	-0.03 (0.07)	0.622	0.05 (0.09)	0.588	-0.07 (0.10)	0.496	-0.02 (0.08)	0.811	0.00 (0.22)	0.991	-0.05 (0.21)	0.812
rs2494250	7	G	0.28	MALDI-TOF MS	0.02 (0.07)	0.719	0.12 (0.09)	0.155	-0.03 (0.10)	0.792	0.02 (0.08)	0.806	0.02 (0.22)	0.919	-0.04 (0.20)	0.843
rs3845625	8	T	0.15	MALDI-TOF MS	0.15 (0.08)	0.087	-0.03 (0.11)	0.809	0.25 (0.11)	0.031	0.05 (0.10)	0.611	0.51 (0.26)	0.054	0.53 (0.25)	0.034
rs7522607	8	C	0.17	CHIP	0.19 (0.09)	0.028	0.07 (0.11)	0.540	0.30 (0.12)	0.013	0.11 (0.10)	0.283	0.51 (0.28)	0.077	0.52 (0.26)	0.049
rs16842010	9	C	0.06	MALDI-TOF MS	0.15 (0.12)	0.231	0.07 (0.16)	0.675	0.29 (0.18)	0.105	0.10 (0.15)	0.492	0.45 (0.46)	0.335	0.39 (0.40)	0.330
rs2427829	10	A	0.28	MALDI-TOF MS	0.15 (0.07)	0.030	0.06 (0.09)	0.482	0.24 (0.09)	0.010	0.12 (0.08)	0.148	0.28 (0.21)	0.187	0.35 (0.19)	0.078

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

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

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

(D) Total IgE atopic asthmatics: MALDI-TOF MS (438), CHIP (377); Total IgE non-atopic asthmatics (stringent definition): MALDI-TOF MS (96), CHIP (83); Total IgE non-atopic asthmatics (loose definition): MALDI-TOF MS (147), CHIP (127).

All statistical analyses include adjustment for age and sex.

Numbers given in bold correspond to the statistically significant findings ($p \leq 0.05$).

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

were compared to a control group comprised of ISAAC II subjects without asthma and negative for atopy according to the criteria above. Basic comparable characteristics of the both populations are given in Table S1.

Total serum IgE was analyzed in the whole study cohort, in non-asthmatics and asthmatics, the latter also after further stratified for atopy. In addition to 'stringent' definition of non-atopic asthma described above, a more 'loose' criterion of RAST/CAP class of <3 was used.

Sensitization to particular allergens was analyzed in the whole study cohort, in non-asthmatics and asthmatics. It was defined by a positive skin prick test result or RAST/CAP class of >2, in ISAAC II or MAGICs, respectively.

Atopic dermatitis, allergic rhinitis, and atopy in general were studied in the cross-sectional set of subjects randomly selected from ISAAC II, as described above. Subjects having any of these three phenotypes were analyzed in comparison with those negative to atopic dermatitis, allergic rhinitis, or atopy, respectively. Atopy was defined as a positive skin prick test result to any of the allergens tested. Subjects from the MAGIC study were not included in the analysis of atopic dermatitis, allergic rhinitis, and atopy. Since all MAGIC study subjects in this study had asthma (in contrast to the cross-sectional design of ISAAC II), any analysis of the other three atopic phenotypes in a combined data set could have been biased.

Statistical analysis

LD calculations and LD-based tagging procedures were conducted using HAPLOVIEW v. 4.2 software (12). All pairwise LD values reported here correspond to the square of the correlation coefficient (r^2). Statistical analyses were conducted using PLINK software package version 1.07 (13). For binary traits, additive genetic effects were modeled by logistic regression, with odds ratios (ORs) showing the effect size as an output. The genotype effect on total serum IgE concentrations was also analyzed in an additive model using linear regression with estimates [standard error] showing the magnitude of the effect. Before entering the model, IgE values were approximated to normality by ln-transformation [$\ln(\text{IgE})$]. To adjust for age and sex, they were included in linear regression as covariates. *In silico* analysis of SNP effect on transcription factor binding was conducted by ALIBABA 2.1 online software (<http://www.gene-regulation.com/pub/programs/alibaba2/index.html>).

Results

Genotyping results and LD structure

The minimum call rate was 95%. Deviations from Hardy-Weinberg equilibrium (HWE) in the control group were assessed for quality control of genotyping procedures, demonstrating that none of the 19 genotyped SNPs deviated from HWE. Nineteen genotyped SNPs formed ten tagging bins (called further 'BINs') comprising 1–4 SNPs (Fig. 1, Table 1A).

Association of FCER1A SNPs with total serum IgE levels

When the whole study group was analyzed irrespective of asthma status, ten SNPs in seven BINs (BIN-1, -3, -4, -5, -6, -8, and -10) showed an association with total serum IgE levels (Table 1B). The top association signal was observed for two SNPs forming BIN-5, rs2427837, and rs2251746, and SNP rs2511211 (BIN-1), the latter remaining with the former two in LD of 0.76 and 0.72, respectively (Fig. 1). The same three SNPs gave the peak signal when the association with total serum IgE levels was analyzed in non-asthmatics only (Table 1C). Association signal could also be observed in non-asthmatics for a SNP rs7548864 belonging to BIN-6 (Table 1C). Interestingly, in asthmatics, although the effect of BIN-1 and BIN-5 on total serum IgE could be observed, two additional association signals with IgE, BIN-8 (rs3845625 and rs7522607) and BIN-10 (rs2427829), not found in non-asthmatics, were identified (Table 1C). The associations observed for BIN-3 and BIN-4 seemed to be independent of asthma status, and rather an effect of sample size reduction was observed after stratification for asthma (Table 1C). These results suggest that, in addition to genetic effects on total serum IgE levels present in the general population (driven by BIN-5 and BIN-1), also an additional asthma-specific effect of FCER1A SNPs on IgE levels may exist (BIN-8 and BIN-10). For a more detailed analysis of the associations between FCER1A and total serum IgE in asthmatics, asthmatics were stratified by atopy to inhalant allergens (Table 1D). In atopic asthmatics, only BIN-1 and BIN-5 demonstrated association with serum IgE. When a permissive definition of 'non-atopic' asthmatics includes cases with low levels of clinically less relevant sensitization, it became obvious that this group of individuals was driving the association between IgE and BIN-8 and BIN-10. When, for a more stringent definition of non-atopic asthmatics, only asthmatic individuals with absolutely no sensitization were analyzed, the same trends were observed but were no longer significant.

We investigated whether associations with total serum IgE levels in our cohort were driven by a modest general effect of SNPs on IgE levels in many individuals or whether the associations were based on a small subset of individuals with specifically high serum IgE values. For this purpose, we performed an analysis by total IgE percentiles using the 50th (60.4 IU/ml), 66th (115 IU/ml), and 90th (457 IU/ml) percentiles as previously established (11). The association observed with the 50th and 66th percentiles (Table S2A–B) was very similar to the one obtained when IgE was analyzed as a continuous trait (Table 1B–C). Only BIN-1 (rs2511211) and BIN-5 (rs2427837 and rs2251746) were significantly associated with serum IgE at the 90th percentile (Table S2C). In summary, these results suggest that SNP effects are not driven by major effects derived from extremes, but by general, more modest effects on the whole population.

To check whether associations with total serum IgE are driven by the major signal from BIN-5 only, we conducted conditional analyses. SNP rs2251746, which has known functional properties (14), was used as an adjusting factor. While most of the associations observed in general analysis (Table 1)

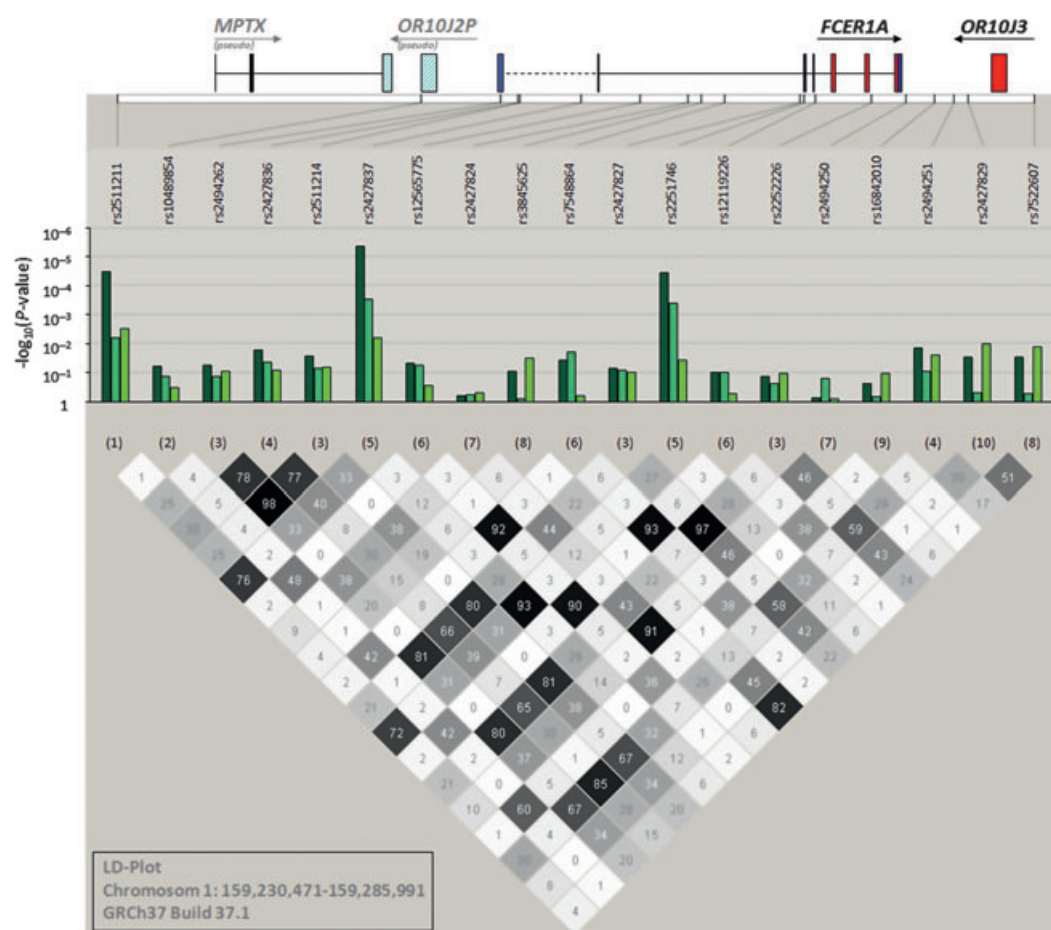


Figure 1 Schematic representation of *FCER1A* gene region with location of genotyped polymorphisms (rs numbers), significance level of the associations with total serum IgE levels observed for each genetic variant, tagging bins (in brackets), and pairwise linkage disequilibrium (r^2). In the gene scheme, red boxes correspond to coding exons, blue boxes to untranslated regions, and green oblique-striped boxes to pseudogenes. With regard to significance level plot, dark green columns (left) correspond to the whole study group, moderate green (middle) to non-asthmatics, and light green (right) to asthmatics. In r^2 plot, numbers represent r^2 values expressed as a percentage of its maximal value (1.0); shading of the squares represents r^2 magnitude with a black-to-white gradient reflecting highest (1.0) to lowest (0.0) r^2 value.

disappeared due to lack of independence with the adjusting SNP, rs7522607 from BIN-8 still presented a significant association with IgE in asthmatics, while rs3845625 from the same BIN and rs2427829 from BIN-10 demonstrated a tendency toward an association in that subgroup (Table S3). This analysis suggests that asthma-specific total IgE signals, especially those from BIN-8, are independent of a major serum IgE determinant in the population from BIN-5. In addition, a previously unseen signal, most probably masked by BIN-5 in the general analysis (Table 1), appeared for BIN-7 in the whole study cohort, which showed also a tendency toward an association with IgE in asthmatics only (Table S3).

Association of *FCER1A* SNPs with asthma

The effect of *FCER1A* SNPs on asthma *per se*, also stratified with regard to atopy to inhalant allergens, was analyzed in a

case-control design. While no association with asthma or non-atopic asthma was observed, all SNPs of BIN-1 and BIN-5, which were also associated with IgE levels, showed an association with atopic asthma (Table 2). In addition, SNP rs3845625 (BIN-8) and SNP rs2427829 (BIN-10), selectively driving total serum IgE signal in asthmatics (Table 1C), also showed an association with atopic asthma (Table 2). For all SNPs, the same alleles associated with elevated IgE also increased the risk for atopic asthma (Tables 1B–C, 2B).

Association of *FCER1A* SNPs with atopic dermatitis, allergic rhinitis, and atopy

Associations between *FCER1A* SNPs and atopic dermatitis, allergic rhinitis, and atopy were analyzed in the cross-sectional ISAAC II population only (Table 3). No SNP showed an association with atopic dermatitis (Table 3C), while SNPs

Table 2 Associations of *FCER1A* polymorphisms with asthma and its subphenotypes

SNP	BIN	A		B			
		Asthma		Atopic asthma		Non-atopic asthma	
		Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
rs2511211	1	0.88 (0.73–1.05)	0.158	0.76 (0.61–0.95)	0.017	0.92 (0.64–1.32)	0.636
rs10489854	2	1.06 (0.76–1.48)	0.728	1.12 (0.73–1.72)	0.606	1.14 (0.59–2.22)	0.694
rs2494262	3	1.04 (0.89–1.21)	0.614	1.16 (0.97–1.40)	0.110	1.02 (0.75–1.39)	0.906
rs2511214	3	1.05 (0.90–1.21)	0.566	1.18 (0.98–1.42)	0.084	0.99 (0.73–1.35)	0.961
rs2427827	3	0.99 (0.85–1.15)	0.867	1.11 (0.92–1.33)	0.293	0.98 (0.72–1.34)	0.904
rs2252226	3	1.00 (0.86–1.16)	0.978	1.11 (0.92–1.34)	0.260	0.96 (0.70–1.31)	0.796
rs2427836	4	0.99 (0.85–1.16)	0.937	0.89 (0.74–1.07)	0.209	1.06 (0.77–1.45)	0.723
rs2494251	4	0.97 (0.83–1.13)	0.694	0.86 (0.71–1.03)	0.108	1.15 (0.84–1.58)	0.376
rs2427837	5	0.92 (0.78–1.08)	0.302	0.78 (0.64–0.96)	0.021	0.87 (0.62–1.22)	0.404
rs2251746	5	0.93 (0.78–1.10)	0.404	0.81 (0.65–1.00)	0.050	0.91 (0.64–1.29)	0.581
rs12565775	6	0.95 (0.74–1.23)	0.695	1.06 (0.77–1.45)	0.735	0.93 (0.54–1.59)	0.790
rs7548864	6	1.03 (0.79–1.34)	0.832	1.15 (0.83–1.59)	0.413	1.17 (0.69–1.97)	0.557
rs12119226	6	1.00 (0.77–1.30)	0.976	1.13 (0.81–1.56)	0.475	1.10 (0.65–1.85)	0.737
rs2427824	7	0.92 (0.78–1.09)	0.348	0.89 (0.72–1.10)	0.277	1.10 (0.79–1.55)	0.567
rs2494250	7	0.96 (0.81–1.13)	0.626	0.99 (0.81–1.22)	0.934	1.15 (0.83–1.60)	0.409
rs3845625	8	1.21 (0.98–1.49)	0.076	1.33 (1.03–1.71)	0.030	0.84 (0.54–1.31)	0.435
rs7522607	8	1.13 (0.92–1.40)	0.242	1.25 (0.97–1.61)	0.082	0.78 (0.49–1.23)	0.284
rs16842010	9	0.95 (0.71–1.29)	0.759	1.05 (0.72–1.52)	0.806	0.79 (0.41–1.52)	0.478
rs2427829	10	1.09 (0.93–1.29)	0.292	1.24 (1.01–1.51)	0.043	1.03 (0.74–1.45)	0.856

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

Numbers given in bold correspond to the statistically significant findings ($p \leq 0.05$).

see Table 1; CI, confidence interval.

rs7548864 (BIN-6) and rs2427837 (BIN-5) were associated with atopy as defined by skin prick test (Table 3A). Three SNPs forming BIN-6, rs12565775, rs7548864, and rs12119226, and a SNP rs10489854 (BIN-2) were associated with allergic rhinitis (Table 3B). These data show a partial overlap between association signals for atopy and those for allergic rhinitis (rs7548864).

In the whole study group (ISAAC II and MAGICS) as well as after stratification for asthma status, we also analyzed the effect of *FCER1A* SNPs on sensitization against specific allergens (Table S4–6). Genetic variants in *FCER1A* were not at all associated with specific sensitization to house dust mites (*D. pteronyssinus* or *D. farinae*) (Table S4) and weakly associated with tree pollen sensitization. In contrast, strong and persistent associations with grass pollen as well as cat dander sensitization were observed (Table S5–6).

Discussion

We used a combination of tagging and literature-based approaches for SNP selection to investigate the association between *FCER1A* locus and total serum IgE levels in-depth in asthmatics, non-asthmatics, and in the complete cohort. We found different patterns of association between asthmatics and

non-asthmatics, and we explored the effects of *FCER1A* variants on atopy and ‘allergic disorders’, such as asthma, atopic dermatitis, or allergic rhinitis. Our observation of the association between *FCER1A* SNPs and total serum IgE levels confirms and extends the previous findings of GWA-based (1–3) and candidate gene studies (15–21).

In two general population-based GWA studies (1, 3), the top association with total serum IgE had been observed for chromosome 1q23/*FCER1A*. In those two studies, the peak association signal within this region (rs2427837/rs2251746) differed from the one observed in the third GWA study (rs2252226) (2), where asthmatics accounted for about half of the study population. In the present study, SNPs rs2427837 and rs2251746 (BIN-5), and rs2511211 (BIN-1) being in close LD with the former two, were most strongly associated with total serum IgE in the complete cohort and in non-asthmatics. Of the three top-associated SNPs, rs2251746 is the best candidate to functionally explain the observed association with total serum IgE levels, as it was previously shown to functionally affect *FCER1A* expression (14, 22). The minor allele of SNP rs2251746 disrupts a binding site for GATA-1, leading to a lower activity of the *FCER1A* proximal promoter (14, 22). The two other *FCER1A* SNPs rs2427827 and rs2494262, both belonging in our study together with rs2252226 to BIN-3, were

Table 3 Associations of *FCER1A* polymorphisms with atopic sensitization, allergic rhinitis or atopic dermatitis

SNP	BIN	A		B		C	
		Atopy (SPT reactivity)		Allergic rhinitis		Atopic dermatitis	
		Odds ratio (95% CI)	p	Odds ratio (95% CI)	p	Odds ratio (95% CI)	p
rs2511211	1	0.82 (0.61–1.10)	0.181	0.77 (0.50–1.18)	0.225	0.75 (0.53–1.06)	0.106
rs10489854	2	1.54 (0.95–2.49)	0.077	1.83 (1.01–3.33)	0.048	0.59 (0.29–1.19)	0.142
rs2494262	3	1.13 (0.89–1.44)	0.321	0.98 (0.70–1.37)	0.896	0.95 (0.72–1.26)	0.731
rs2511214	3	1.16 (0.92–1.48)	0.213	1.08 (0.78–1.52)	0.636	0.96 (0.73–1.26)	0.779
rs2427827	3	1.09 (0.86–1.39)	0.467	1.04 (0.74–1.46)	0.822	1.02 (0.77–1.34)	0.900
rs2252226	3	1.06 (0.83–1.34)	0.650	0.96 (0.69–1.35)	0.822	0.96 (0.73–1.26)	0.755
rs2427836	4	0.82 (0.65–1.05)	0.115	0.85 (0.61–1.19)	0.354	1.08 (0.82–1.42)	0.610
rs2494251	4	0.84 (0.66–1.07)	0.160	0.79 (0.56–1.11)	0.173	1.05 (0.79–1.38)	0.747
rs2427837	5	0.75 (0.57–0.99)	0.043	0.79 (0.54–1.17)	0.243	0.86 (0.63–1.17)	0.326
rs2251746	5	0.77 (0.58–1.02)	0.069	0.86 (0.58–1.26)	0.428	0.82 (0.60–1.13)	0.221
rs12565775	6	1.43 (0.97–2.09)	0.068	1.68 (1.04–2.71)	0.033	0.86 (0.53–1.37)	0.521
rs7548864	6	1.52 (1.03–2.24)	0.034	1.82 (1.13–2.94)	0.015	0.79 (0.48–1.31)	0.363
rs12119226	6	1.47 (0.99–2.18)	0.054	1.74 (1.07–2.84)	0.026	0.90 (0.55–1.46)	0.670
rs2427824	7	0.94 (0.72–1.24)	0.672	0.71 (0.48–1.07)	0.103	0.98 (0.72–1.33)	0.882
rs2494250	7	1.03 (0.79–1.33)	0.851	0.85 (0.59–1.24)	0.410	0.95 (0.71–1.29)	0.758
rs3845625	8	1.08 (0.77–1.51)	0.664	1.23 (0.78–1.92)	0.372	1.05 (0.72–1.54)	0.803
rs7522607	8	1.06 (0.76–1.47)	0.742	1.27 (0.82–1.96)	0.287	1.01 (0.69–1.48)	0.961
rs16842010	9	1.26 (0.80–2.00)	0.323	1.59 (0.88–2.87)	0.124	0.82 (0.46–1.46)	0.500
rs2427829	10	1.11 (0.86–1.44)	0.427	1.18 (0.83–1.68)	0.364	0.94 (0.69–1.27)	0.692

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

(A) Atopy sensitization: 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).

Numbers given in bold correspond to the statistically significant findings ($p \leq 0.05$).

see Tables I and II; SPT, skin prick testing.

also reported to functionally affect transcriptional activity as described in more details elsewhere (21–23).

The association between SNPs rs2427837, rs2251746, and rs2511211 was also present in asthmatics. However, additional and strong serum IgE level association signals of BIN-8 (rs3845625 and rs7522607) and BIN-10 (rs2427829) were found in asthmatics only. No functional properties of these SNPs are known. All three SNPs are non-coding and were subjected to *in silico* analysis of their potential influence on transcription factor binding (Fig. 1). No such effect was predicted for SNP rs3845625 located upstream of the *FCER1A* proximal promoter (Fig. 1) (14). Interestingly, the minor allele of SNP rs7522607 was found to remove the binding site for GATA-1. SNP rs7522607 is located about 1.5 kb upstream of *OR10J3* gene (with regard to the transcription direction) (Fig. 1). A minor allele of SNP rs2427829 was found to create a binding site for C/EBP α transcription factor, for which an effect on *FCER1A* and/or *OR10J3* is difficult to predict because the SNP is located downstream of both genes. Other potential functional effects of those three SNPs, that is, on splicing or mRNA stability, are rather not expected due to their location far from exon/intron borders (rs3845625) or simply outside of the genes (rs7522607 or rs2427829). It is also possible that those SNPs exert their effects on IgE by their hypothetical LD proxies not analyzed in this study and/or in combination with other

functional genetic variants. Nevertheless, SNPs rs3845625, rs7522607 and rs2427829 may represent novel, asthma-specific factors involved in serum IgE regulation. Interestingly, further substratification with regard to atopic status revealed that associations of BIN-8 and BIN-10 with total serum IgE levels were observed in non-atopic asthmatics. The minor alleles that were associated with elevation of total IgE levels in non-atopic asthmatics increased the risk of atopic asthma but were not associated with IgE levels in atopic asthmatics. This may indicate that the SNP effects are primarily independent of atopy but may drive the subsequent development of atopic asthma when present. Indeed these data may fit to a current hypothesis proposed by Patrick Holt (24–27), where pulmonary virus infections during childhood (wheeze bronchitis and early virus-induced asthma) may turn on unspecific IgE production, which then can push atopy development in those early asthmatics. Fc ϵ RI plays a central role in this hypothesis, as it is expressed also on bone marrow-derived myeloid precursor cells including immature dendritic cells that are recruited to the airways, for example, after viral infections of the lung. These 'Fc ϵ RI-armed' dendritic cells (24–27) have been suggested to trigger Th2-polarized immune reactions to allergens. It seems possible that SNPs in BIN-8 and BIN-10 are genetic risk factors for exactly those mechanisms recently described (24–27) in the sense that these specific SNPs

dysregulate primary FcεRI expression. This may in turn lead to elevated IgE levels in children who have non-atopic asthma, which is most commonly equivalent to virus-induced asthma and wheeze during childhood. According to the Holt hypothesis, some of these children will become atopic, and at this point, Th2 immunity is the main determinant for IgE production. Thus, the SNPs initially contributing to the induction of the switch will no longer be associated with levels of total IgE in those patients who became atopic asthmatics. Indeed, this is how our data could be interpreted, but further testing of this hypothesis is needed, for example, in birth cohorts.

SNPs in BIN-5 (rs2427837) and BIN-6 (rs7548864) showed an association with atopy in our cross-sectional analysis. Several *FCER1A* variants including rs2427837 have already previously been shown to be associated with atopic sensitization (1, 20). The association observed for rs2427837 seems to be driven by a strong total serum IgE signal. SNP rs7548864 was associated not only with atopy but also with allergic rhinitis. When specific IgE against common allergens was analyzed (Table S4–6), a major involvement of *FCER1A* SNPs was found for grass and cat sensitization, but not for house dust mite and tree pollen sensitization. Interestingly, these associations were also present in asthmatics and/or observed for SNPs in BIN-8, which were also found to drive serum IgE levels in an asthma-specific manner. Since the variability in the prevalence of sensitization against different allergens exists between different countries, regions, and ethnicities (28), our results might potentially differ from those reported elsewhere.

Several SNPs in BIN-6 (rs12565775, rs7548864, and rs12119226) and BIN-2 (rs10489854) were associated with allergic rhinitis in our study, although no such association had been reported previously (1, 29). Our observation seems to fit well with the clinical observation that IgE-related mechanisms seem to be more directly involved in allergic rhinitis than in atopic dermatitis or asthma (30, 31). Studies conducted so far provided no clear evidence of an association between *FCER1A* SNPs and atopic dermatitis (1, 19, 20, 32, 33), and also in our cross-sectional population, we did not observe any association with atopic dermatitis. The associations we observed with allergic rhinitis may at least partly result from their overlap with atopic sensitization. However, considering the limited number of patients with allergic rhinitis and/or atopic dermatitis in our cross-sectional population derived from ISAAC II available for the present study, these results need to be interpreted with caution.

Our study population is much smaller than the large consortia-based GWA studies on IgE. We did not correct for multiple testing as we investigated already established associations in more detail in a more uniform cohort. However, this reasoning does not apply to investigations into other phenotypes than IgE and asthma, and thus, limitations in the interpretation of these associations lacking further replication are acknowledged.

The major novel finding of the present study is the identification of genetic variants in *FCER1A* that are specific markers of IgE levels in asthmatics, additional to genetic determinants of 'basal' IgE levels also present in the same gene, previously identified by GWA studies. The data may suggest

that *FCER1A*/FcεRI(α) variants and IgE-related mechanisms could be involved in switch from non-atopic (infection induced) asthma to atopic asthma according to the hypothesis recently suggested by Patrick Holt (24–27). However, identification of those patients and the underlying mechanism may prove to be difficult in asthma patients as basal IgE levels, which are also genetically determined (at least in part), may not be related to disease development. To unmask asthma-related IgE mechanism from basal IgE levels is not trivial, but it may help to explain why IgE levels on the population level are related to asthma in Western societies but not to other settings and why on the individual level this relationship is less clear and not useful for clinical assessment. Considering recent clinical and experimental evidence suggesting the involvement of *FCER1A*/FcεRI(α) in the pathogenesis of (atopic) asthma (24–27), further functional and genetic investigation is necessary to better understand and potentially prevent mechanisms leading to atopic asthma in those genetically susceptible individuals.

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Authors' contributions

Daniel P. Potaczek contributed to study design and execution, participated in data interpretation, and drafted the first version of the manuscript; Sven Michel contributed to genotyping and performed statistical analyses, data interpretation, and manuscript preparation; Vishwas Sharma contributed to study execution and data interpretation; Sonja Zeilinger and Thomas Illig performed the MALDI-TOF MS genotyping; Christian Vogelberg, Andrea von Berg, Albrecht Buße, Andrea Heinzmann, Otto Laub, Ernst Rietschel, Burkhard Simma, Thomas Frischer, and Jon Genuneit contributed to data collection; Michael Kabesch developed the study design, participated in data collection, analysis, and interpretation, and wrote the final version of the manuscript.

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Conflict of interest statement

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

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Supporting Information

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

Data S1. Selection of polymorphisms for genotyping.

Table S1. Characteristics of the population used for the analyses.

Table S2. Associations between FCER1A polymorphisms and the risk of having total serum IgE levels above the certain percentiles.

Table S3. Associations of FCER1A polymorphisms with total serum IgE levels conditioned on rs2251746.

Table S4. Associations of FCER1A polymorphisms with atopic sensitization to house dust mites.

Table S5. Associations of FCER1A polymorphisms with atopic sensitization to tree and grass pollen.

Table S6. Associations of FCER1A polymorphisms with atopic sensitization to cat dander.

DIFFERENT *FCER1A* POLYMORPHISMS INFLUENCE IGE LEVELS IN ASTHMATICS AND NON-ASTHMATICS

Supplementary materials – selection of polymorphisms for genotyping

To provide the best coverage of *FCER1A* and its neighbourhood with functional and/ or clinically relevant single-nucleotide polymorphisms (SNPs), we applied a combined approach, comprising linkage disequilibrium (LD)-based tagging and profound literature search. Tagging was conducted for the region encompassing full *FCER1A* gene with upstream and downstream surrounding (chr1:157509000..157555000) (1; 2), based on the data obtained from the 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/). A set of 6 tagging SNPs was identified by Haploview software [version 4.2; <http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview> (3); minor allele frequency (MAF) >0.1, Hardy-Weinberg Equilibrium (HWE) *P*-value >0.05, r^2 >0.8, pairwise tagging]. Detailed literature search identified 17 relevant SNPs, including those associated with total and/ or specific serum IgE (4-12), aspirin induced urticaria (9), breast cancer (13), monocyte chemoattractant protein-1 (14) or lipid metabolism (15), and/or functionally affecting *FCER1A*/ FcεRIα expression (9; 16-18). Because four literature-identified variants were overlapping with tagging SNPs, the final genotyping list comprised only 19 polymorphisms, as listed in Table 1-A of the main document. Of those 19 SNPs, 6 were previously chip-genotyped (19) and the remaining 13 were re-genotyped for the purposes of the present study using MALDI-TOF-MS technique (20). Detailed information on the genotyping methodology, including used oligonucleotides, and all the adjacent procedures of handling the samples are available from the authors upon request.

The data obtained by chip-genotyping have been included in the meta-analysis by the GABRIEL Consortium (4). SNPs rs2427837 and rs2251746, genotyped in the ISAAC II subjects, have been previously analyzed in the replication phase of the study by Weidinger *et al* (5).

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Supplementary Table 1 Characteristics of the population used for the analyses

	MAGICS	ISAAC
Male sex, n* (%)	457/691 (65.1)	386/763 (50.6)
Age, mean (SD)	10.99 (2.90)	9.63 (0.59)
Asthmatic, n* (%)	691/691 (100)	70/755 (9.7)
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.

[#]Stringent atopy definition was used

Supplementary Table 2 Associations between *FCER1A* polymorphisms and the risk of having total serum IgE levels above the certain percentiles

SNP	BIN	A. 50 th percentile						B. 66 th percentile						C. 90 th percentile					
		Complete cohort		Non-asthmatics		Asthmatics		Complete cohort		Non-asthmatics		Asthmatics		Complete cohort		Non-asthmatics		Asthmatics	
		Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P
		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)	
rs2511211	1	0.67 (0.55-0.82)	0.0001	0.62 (0.47-0.82)	0.001	0.73 (0.51-1.04)	0.080	0.73 (0.60-0.89)	0.002	0.69 (0.51-0.93)	0.014	0.79 (0.58-1.06)	0.120	0.72 (0.56-0.94)	0.014	0.84 (0.51-1.37)	0.475	0.70 (0.51-0.96)	0.027
rs10489854	2	1.51 (1.00-2.28)	0.048	1.50 (0.91-2.48)	0.108	1.43 (0.66-3.10)	0.360	1.73 (1.18-2.54)	0.005	1.88 (1.15-3.07)	0.013	1.51 (0.80-2.85)	0.203	1.08 (0.68-1.72)	0.748	1.41 (0.67-2.97)	0.371	0.86 (0.46-1.59)	0.628
rs2494262	3	1.14 (0.96-1.36)	0.148	1.14 (0.90-1.43)	0.276	1.24 (0.91-1.70)	0.179	1.06 (0.89-1.25)	0.521	1.06 (0.83-1.35)	0.646	1.10 (0.85-1.42)	0.461	1.07 (0.87-1.31)	0.540	1.01 (0.67-1.53)	0.958	1.12 (0.87-1.43)	0.391
rs2511214	3	1.16 (0.97-1.38)	0.098	1.16 (0.93-1.46)	0.194	1.26 (0.92-1.72)	0.155	1.08 (0.92-1.27)	0.360	1.09 (0.86-1.39)	0.473	1.12 (0.87-1.44)	0.390	1.10 (0.90-1.35)	0.347	1.09 (0.73-1.64)	0.668	1.14 (0.88-1.46)	0.318
rs2427827	3	1.12 (0.94-1.33)	0.206	1.13 (0.90-1.43)	0.282	1.26 (0.92-1.72)	0.157	1.04 (0.88-1.23)	0.627	1.04 (0.82-1.33)	0.726	1.13 (0.88-1.46)	0.340	1.07 (0.87-1.31)	0.540	1.15 (0.77-1.71)	0.495	1.09 (0.85-1.40)	0.513
rs2252226	3	1.09 (0.91-1.29)	0.341	1.08 (0.86-1.35)	0.500	1.24 (0.90-1.70)	0.194	1.00 (0.85-1.18)	0.964	0.99 (0.78-1.25)	0.924	1.08 (0.84-1.40)	0.543	1.04 (0.85-1.27)	0.704	1.00 (0.67-1.49)	0.996	1.11 (0.86-1.43)	0.417
rs2427836	4	0.85 (0.71-1.01)	0.064	0.86 (0.69-1.08)	0.190	0.77 (0.55-1.06)	0.110	0.87 (0.73-1.03)	0.094	0.85 (0.67-1.08)	0.187	0.84 (0.65-1.10)	0.212	0.91 (0.74-1.12)	0.380	0.80 (0.53-1.19)	0.261	0.96 (0.74-1.24)	0.733
rs2494251	4	0.85 (0.71-1.01)	0.066	0.89 (0.71-1.11)	0.290	0.72 (0.52-1.00)	0.047	0.88 (0.74-1.04)	0.133	0.87 (0.68-1.10)	0.248	0.85 (0.65-1.11)	0.227	0.90 (0.73-1.11)	0.318	0.82 (0.55-1.23)	0.346	0.91 (0.70-1.18)	0.473
rs2427837	5	0.65 (0.53-0.78)	7.02*10⁻⁴	0.60 (0.46-0.78)	0.0001	0.68 (0.49-0.96)	0.028	0.67 (0.56-0.81)	2.73*10⁻⁵	0.62 (0.47-0.82)	0.001	0.71 (0.54-0.94)	0.017	0.74 (0.59-0.94)	0.014	0.69 (0.43-1.11)	0.128	0.78 (0.59-1.04)	0.096
rs2251746	5	0.66 (0.54-0.80)	3.56*10⁻⁵	0.64 (0.49-0.82)	0.001	0.68 (0.48-0.97)	0.035	0.69 (0.57-0.83)	0.0001	0.66 (0.50-0.87)	0.003	0.73 (0.54-0.98)	0.037	0.77 (0.60-0.98)	0.037	0.67 (0.42-1.09)	0.109	0.83 (0.61-1.13)	0.239
rs12565775	6	1.38 (1.01-1.88)	0.043	1.44 (0.97-2.12)	0.067	1.41 (0.79-2.53)	0.242	1.49 (1.12-1.99)	0.006	1.71 (1.15-2.53)	0.008	1.40 (0.88-2.21)	0.152	1.12 (0.80-1.57)	0.517	1.45 (0.80-2.64)	0.224	1.00 (0.65-1.53)	0.999
rs7548864	6	1.62 (1.16-2.25)	0.004	1.78 (1.18-2.68)	0.006	1.37 (0.76-2.50)	0.297	1.56 (1.16-2.10)	0.004	1.91 (1.28-2.86)	0.002	1.21 (0.76-1.93)	0.417	1.08 (0.76-1.55)	0.660	1.49 (0.80-2.75)	0.209	0.89 (0.57-1.41)	0.627
rs12119226	6	1.40 (1.02-1.92)	0.039	1.49 (0.99-2.24)	0.058	1.29 (0.74-2.26)	0.368	1.49 (1.11-1.99)	0.008	1.73 (1.15-2.61)	0.009	1.29 (0.82-2.02)	0.267	1.03 (0.73-1.47)	0.862	1.21 (0.63-2.34)	0.572	0.94 (0.61-1.44)	0.762
rs2427824	7	0.96 (0.79-1.16)	0.649	1.11 (0.86-1.43)	0.437	0.80 (0.57-1.13)	0.209	0.91 (0.75-1.09)	0.300	1.03 (0.79-1.35)	0.820	0.82 (0.61-1.08)	0.158	0.97 (0.77-1.21)	0.765	1.16 (0.75-1.77)	0.509	0.94 (0.71-1.25)	0.669
rs2494250	7	1.05 (0.87-1.27)	0.635	1.22 (0.96-1.56)	0.109	0.88 (0.63-1.24)	0.461	0.99 (0.83-1.19)	0.928	1.19 (0.92-1.54)	0.190	0.85 (0.64-1.12)	0.253	1.00 (0.80-1.25)	0.993	1.23 (0.81-1.85)	0.331	0.96 (0.73-1.27)	0.780
rs3845625	8	1.15 (0.91-1.47)	0.248	0.85 (0.62-1.17)	0.327	1.89 (1.16-3.06)	0.010	1.11 (0.89-1.39)	0.343	0.80 (0.56-1.13)	0.207	1.50 (1.05-2.14)	0.026	1.12 (0.85-1.46)	0.429	0.83 (0.46-1.50)	0.537	1.16 (0.84-1.61)	0.362
rs7522607	8	1.16 (0.91-1.47)	0.237	0.94 (0.69-1.28)	0.696	1.82 (1.11-2.98)	0.018	1.10 (0.88-1.38)	0.390	0.91 (0.65-1.26)	0.558	1.43 (0.99-2.07)	0.055	1.23 (0.94-1.62)	0.126	1.10 (0.65-1.88)	0.713	1.29 (0.92-1.81)	0.135
rs16842010	9	1.30 (0.90-1.87)	0.158	1.24 (0.79-1.94)	0.357	1.73 (0.83-3.59)	0.144	1.44 (1.03-2.01)	0.034	1.58 (1.00-2.48)	0.050	1.46 (0.84-2.52)	0.178	1.09 (0.73-1.62)	0.686	0.91 (0.41-2.05)	0.829	1.21 (0.74-1.98)	0.448
rs2427829	10	1.09 (0.90-1.31)	0.387	0.94 (0.74-1.20)	0.634	1.48 (1.03-2.13)	0.036	1.10 (0.92-1.31)	0.315	1.01 (0.78-1.31)	0.940	1.23 (0.93-1.63)	0.150	1.16 (0.94-1.45)	0.168	1.07 (0.70-1.63)	0.751	1.23 (0.94-1.61)	0.127

50th (60.4 IU/ mL), 66th (115 IU/ mL) and 90th (457 IU/ mL) percentile as estimated in 3063 children from ISAAC II, a sample representative for German pediatric population [Schedel et al., J Allergy Clin Immunol 2004;114:1100-5].

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

A) 50th complete cohort: MALDI-TOF MS (824 cases / 437 controls), CHIP (737 cases / 408 controls); 50th non-asthmatics: MALDI-TOF MS (323 / 328), CHIP (308 / 312); 50th asthmatics: MALDI-TOF MS (496 / 106), CHIP (424 / 93)

B) 66th complete cohort: MALDI-TOF MS (632 cases / 629 controls), CHIP (560 cases / 585 controls); 66th non-asthmatics: MALDI-TOF MS (217 / 434), CHIP (205 / 415); 66th asthmatics: MALDI-TOF MS (412 / 190), CHIP (352 / 165)

C) 90th complete cohort: MALDI-TOF MS (253 cases / 1008 controls), CHIP (221 cases / 924 controls); 90th non-asthmatics: MALDI-TOF MS (57 / 594), CHIP (54 / 566); 90th asthmatics: MALDI-TOF MS (196 / 406), CHIP (167 / 350)

Supplementary Table 3 Associations of *FCER1A* polymorphisms with total serum IgE levels conditioned on rs2251746

SNP	BIN	Complete cohort		Non-asthmatics		Asthmatics	
		Estimate (SE)	P	Estimate (SE)	P	Estimate (SE)	P
rs2511211	1	-0.07 (0.15)	0.651	-0.03 (0.17)	0.861	-0.21 (0.24)	0.375
rs10489854	2	0.19 (0.14)	0.174	0.16 (0.17)	0.341	0.16 (0.21)	0.457
rs2494262	3	-0.08 (0.08)	0.335	-0.07 (0.10)	0.468	-0.01 (0.11)	0.956
rs2511214	3	-0.05 (0.08)	0.547	-0.03 (0.10)	0.734	0.01 (0.11)	0.948
rs2427827	3	-0.05 (0.08)	0.508	-0.03 (0.10)	0.798	0.03 (0.11)	0.788
rs2252226	3	-0.09 (0.08)	0.271	-0.09 (0.10)	0.328	0.02 (0.11)	0.843
rs2427836	4	0.02 (0.08)	0.777	0.04 (0.10)	0.682	-0.07 (0.12)	0.572
rs2494251	4	-0.01 (0.08)	0.937	0.06 (0.10)	0.562	-0.14 (0.11)	0.194
rs2427837	5	-0.31 (0.28)	0.269	-0.29 (0.30)	0.330	-0.19 (0.59)	0.753
rs2251746	5	-	-	-	-	-	-
rs12565775	6	0.16 (0.11)	0.160	0.20 (0.14)	0.152	0.10 (0.16)	0.539
rs7548864	6	0.16 (0.11)	0.157	0.24 (0.14)	0.087	0.03 (0.16)	0.845
rs12119226	6	0.14 (0.12)	0.234	0.18 (0.15)	0.209	0.05 (0.16)	0.775
rs2427824	7	-0.19 (0.08)	0.020	-0.11 (0.10)	0.291	-0.19 (0.11)	0.095
rs2494250	7	-0.15 (0.08)	0.063	-0.04 (0.10)	0.708	-0.17 (0.11)	0.123
rs3845625	8	0.08 (0.09)	0.353	-0.07 (0.12)	0.556	0.19 (0.13)	0.133
rs7522607	8	0.12 (0.09)	0.183	-0.02 (0.11)	0.848	0.26 (0.12)	0.035
rs16842010	9	0.17 (0.13)	0.190	0.09 (0.16)	0.600	0.32 (0.19)	0.099
rs2427829	10	0.06 (0.07)	0.449	-0.05 (0.09)	0.606	0.21 (0.11)	0.057

Number of individuals with non-missing phenotype involved in the respective calculations for the different methods of genotyping.
Complete cohort: MALDI-TOF MS (1261), CHIP (1145)
Non-asthmatics: MALDI-TOF MS (651), CHIP (620);
Asthmatics: MALDI-TOF MS (602), CHIP (517)

Supplementary Table 4 Associations of *FCER1A* polymorphisms with atopic sensitization to house dust mites

SNP	BIN	A. <i>D. farinae</i>						B. <i>D. pteronyssinus</i>					
		Complete cohort		Non-asthmatics		Asthmatics		Complete cohort		Non-asthmatics		Asthmatics	
		Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P
		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)	
rs2511211	1	0.97 (0.79-1.19)	0.761	0.80 (0.45-1.45)	0.467	1.10 (0.85-1.42)	0.488	1.00 (0.82-1.23)	0.986	0.96 (0.58-1.59)	0.869	1.11 (0.86-1.43)	0.431
rs10489854	2	1.04 (0.71-1.52)	0.856	1.77 (0.79-3.95)	0.164	0.88 (0.53-1.44)	0.603	1.00 (0.68-1.46)	0.991	1.71 (0.83-3.51)	0.144	0.79 (0.48-1.29)	0.345
rs2494262	3	0.96 (0.81-1.14)	0.659	1.13 (0.71-1.79)	0.621	0.90 (0.73-1.11)	0.332	1.01 (0.85-1.19)	0.939	0.94 (0.62-1.43)	0.778	1.01 (0.82-1.24)	0.952
rs2511214	3	0.99 (0.84-1.17)	0.929	1.24 (0.78-1.97)	0.355	0.92 (0.75-1.14)	0.450	1.04 (0.88-1.23)	0.670	1.02 (0.68-1.54)	0.917	1.03 (0.83-1.27)	0.778
rs2427827	3	0.94 (0.79-1.12)	0.485	1.41 (0.88-2.25)	0.158	0.88 (0.71-1.09)	0.237	1.00 (0.85-1.19)	0.985	1.15 (0.76-1.74)	0.518	1.00 (0.81-1.24)	0.996
rs2252226	3	0.96 (0.81-1.14)	0.657	1.23 (0.78-1.95)	0.373	0.92 (0.75-1.14)	0.459	1.02 (0.86-1.20)	0.865	1.04 (0.69-1.57)	0.857	1.04 (0.84-1.28)	0.737
rs2427836	4	1.08 (0.91-1.28)	0.387	0.76 (0.47-1.21)	0.245	1.19 (0.96-1.49)	0.118	1.02 (0.86-1.21)	0.853	0.91 (0.61-1.37)	0.648	1.05 (0.84-1.30)	0.683
rs2494251	4	1.01 (0.85-1.21)	0.881	0.72 (0.44-1.16)	0.175	1.12 (0.89-1.39)	0.336	0.95 (0.80-1.13)	0.551	0.86 (0.57-1.29)	0.457	0.97 (0.78-1.21)	0.809
rs2427837	5	1.03 (0.85-1.24)	0.801	0.87 (0.52-1.48)	0.618	1.14 (0.89-1.44)	0.302	1.03 (0.85-1.24)	0.765	1.00 (0.63-1.59)	0.988	1.10 (0.87-1.40)	0.418
rs2251746	5	1.09 (0.89-1.32)	0.415	0.81 (0.47-1.41)	0.460	1.25 (0.97-1.62)	0.086	1.05 (0.86-1.27)	0.664	0.90 (0.57-1.44)	0.662	1.15 (0.89-1.49)	0.276
rs12565775	6	1.06 (0.79-1.42)	0.694	1.19 (0.55-2.55)	0.665	1.08 (0.75-1.56)	0.675	1.05 (0.79-1.41)	0.722	1.01 (0.49-2.08)	0.974	1.11 (0.77-1.60)	0.581
rs7548864	6	1.01 (0.75-1.37)	0.948	1.28 (0.59-2.75)	0.536	0.91 (0.63-1.32)	0.623	1.01 (0.74-1.36)	0.965	1.03 (0.50-2.12)	0.933	0.95 (0.66-1.38)	0.803
rs12119226	6	1.05 (0.78-1.41)	0.732	1.13 (0.50-2.55)	0.776	1.05 (0.73-1.50)	0.806	1.03 (0.76-1.38)	0.857	0.97 (0.45-2.09)	0.945	1.04 (0.73-1.49)	0.829
rs2427824	7	0.84 (0.69-1.03)	0.088	1.13 (0.68-1.89)	0.631	0.81 (0.64-1.04)	0.097	0.93 (0.77-1.13)	0.481	1.19 (0.76-1.87)	0.441	0.92 (0.72-1.17)	0.503
rs2494250	7	0.91 (0.76-1.10)	0.326	1.37 (0.85-2.20)	0.196	0.85 (0.67-1.07)	0.160	0.97 (0.81-1.17)	0.760	1.27 (0.83-1.95)	0.275	0.93 (0.74-1.18)	0.570
rs3845625	8	1.13 (0.89-1.42)	0.315	1.20 (0.64-2.26)	0.569	1.01 (0.76-1.35)	0.926	1.08 (0.85-1.36)	0.528	0.90 (0.48-1.67)	0.733	1.02 (0.76-1.36)	0.896
rs7522607	8	1.12 (0.88-1.41)	0.353	0.98 (0.51-1.87)	0.943	1.10 (0.81-1.48)	0.543	1.08 (0.86-1.37)	0.508	0.89 (0.50-1.59)	0.689	1.08 (0.80-1.46)	0.600
rs16842010	9	1.06 (0.75-1.48)	0.749	1.39 (0.61-3.16)	0.433	1.06 (0.69-1.63)	0.794	1.02 (0.73-1.44)	0.891	1.41 (0.68-2.94)	0.355	0.98 (0.64-1.51)	0.923
rs2427829	10	1.11 (0.93-1.34)	0.253	1.37 (0.85-2.19)	0.194	1.06 (0.84-1.34)	0.619	1.12 (0.93-1.34)	0.235	1.23 (0.80-1.89)	0.350	1.09 (0.86-1.37)	0.493

Number of individuals with non-missing phenotype involved in the respective calculations for the different methods of genotyping.
A) *D. farinae* complete cohort: MALDI-TOF MS (402 cases / 972 controls), CHIP (344 cases / 895 controls); *D. farinae* non-asthmatics: MALDI-TOF MS (40 / 610), CHIP (38 / 584); *D. farinae* asthmatics: MALDI-TOF MS (361 / 355), CHIP (305 / 304)
B) *D. pteronyssinus* complete cohort: MALDI-TOF MS (403 cases / 973 controls), CHIP (351 cases / 890 controls); *D. pteronyssinus* non-asthmatics: MALDI-TOF MS (51 / 599), CHIP (51 / 571); *D. pteronyssinus* asthmatics: MALDI-TOF MS (351 / 367), CHIP (299 / 312)

Supplementary Table 5 Associations of *FCER1A* polymorphisms with atopic sensitization to tree and grass pollen

SNP	BIN	A. Tree pollen						B. Grass pollen					
		Complete cohort		Non-asthmatics		Asthmatics		Complete cohort		Non-asthmatics		Asthmatics	
		Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P	Odds ratio	P
		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)		(95% CI)	
rs2511211	1	0.89 (0.71-1.13)	0.350	0.90 (0.52-1.56)	0.702	0.95 (0.72-1.25)	0.698	0.80 (0.65-0.98)	0.031	0.95 (0.64-1.41)	0.814	0.78 (0.60-1.01)	0.063
rs10489854	2	1.09 (0.72-1.66)	0.689	1.08 (0.43-2.72)	0.869	1.10 (0.65-1.84)	0.728	1.32 (0.92-1.88)	0.131	1.85 (1.04-3.29)	0.037	1.14 (0.69-1.88)	0.612
rs2494262	3	1.17 (0.96-1.41)	0.113	1.18 (0.76-1.84)	0.452	1.16 (0.93-1.45)	0.199	1.09 (0.93-1.29)	0.286	0.89 (0.64-1.23)	0.479	1.19 (0.96-1.46)	0.117
rs2511214	3	1.18 (0.98-1.43)	0.082	1.17 (0.76-1.81)	0.478	1.18 (0.95-1.48)	0.141	1.13 (0.96-1.33)	0.140	0.96 (0.70-1.32)	0.796	1.21 (0.98-1.50)	0.073
rs2427827	3	1.07 (0.89-1.30)	0.469	1.07 (0.68-1.68)	0.780	1.10 (0.88-1.37)	0.411	1.09 (0.92-1.28)	0.324	0.95 (0.68-1.31)	0.744	1.19 (0.96-1.47)	0.110
rs2252226	3	1.06 (0.87-1.28)	0.573	1.07 (0.69-1.65)	0.763	1.07 (0.85-1.34)	0.576	1.07 (0.91-1.26)	0.417	0.85 (0.61-1.17)	0.312	1.20 (0.97-1.49)	0.092
rs2427836	4	0.89 (0.74-1.09)	0.262	1.02 (0.65-1.58)	0.947	0.85 (0.68-1.08)	0.181	0.86 (0.72-1.01)	0.073	0.92 (0.67-1.27)	0.612	0.80 (0.64-1.00)	0.048
rs2494251	4	0.83 (0.68-1.01)	0.063	0.93 (0.60-1.46)	0.758	0.79 (0.63-1.01)	0.057	0.81 (0.69-0.96)	0.017	0.92 (0.66-1.26)	0.593	0.74 (0.59-0.93)	0.009
rs2427837	5	0.93 (0.75-1.16)	0.533	0.90 (0.55-1.48)	0.683	0.99 (0.77-1.28)	0.934	0.83 (0.69-1.00)	0.047	0.85 (0.59-1.22)	0.380	0.85 (0.67-1.08)	0.173
rs2251746	5	0.92 (0.74-1.16)	0.486	0.94 (0.57-1.56)	0.807	0.95 (0.73-1.25)	0.730	0.82 (0.67-0.99)	0.042	0.88 (0.61-1.27)	0.505	0.81 (0.62-1.04)	0.100
rs12565775	6	1.09 (0.78-1.50)	0.622	0.90 (0.40-2.01)	0.793	1.16 (0.80-1.70)	0.436	1.20 (0.91-1.58)	0.209	1.54 (0.93-2.55)	0.092	1.13 (0.78-1.62)	0.521
rs7548864	6	1.27 (0.92-1.75)	0.154	1.31 (0.63-2.70)	0.473	1.26 (0.85-1.85)	0.253	1.30 (0.98-1.73)	0.071	1.75 (1.06-2.89)	0.028	1.15 (0.79-1.69)	0.464
rs12119226	6	1.11 (0.80-1.55)	0.520	0.97 (0.43-2.18)	0.942	1.17 (0.80-1.71)	0.435	1.21 (0.91-1.60)	0.196	1.61 (0.96-2.71)	0.071	1.11 (0.77-1.60)	0.589
rs2427824	7	0.91 (0.73-1.13)	0.378	0.80 (0.47-1.34)	0.393	0.97 (0.75-1.25)	0.812	0.93 (0.77-1.12)	0.448	0.79 (0.54-1.14)	0.207	1.02 (0.80-1.30)	0.849
rs2494250	7	0.94 (0.77-1.16)	0.584	0.85 (0.52-1.40)	0.523	0.98 (0.77-1.26)	0.900	0.99 (0.83-1.18)	0.911	0.84 (0.59-1.20)	0.332	1.08 (0.86-1.36)	0.521
rs3845625	8	1.17 (0.91-1.51)	0.230	1.13 (0.60-2.11)	0.712	1.10 (0.81-1.49)	0.536	1.10 (0.87-1.37)	0.430	0.88 (0.54-1.43)	0.607	1.11 (0.83-1.48)	0.489
rs7522607	8	1.31 (1.01-1.69)	0.039	1.37 (0.78-2.42)	0.276	1.26 (0.92-1.72)	0.144	1.25 (0.99-1.56)	0.056	1.18 (0.77-1.80)	0.448	1.27 (0.94-1.71)	0.120
rs16842010	9	1.09 (0.75-1.60)	0.645	1.06 (0.44-2.53)	0.898	1.17 (0.74-1.84)	0.498	1.04 (0.75-1.45)	0.818	1.47 (0.83-2.62)	0.191	0.93 (0.60-1.44)	0.755
rs2427829	10	1.20 (0.98-1.46)	0.086	1.18 (0.75-1.88)	0.474	1.19 (0.93-1.52)	0.166	1.13 (0.95-1.35)	0.171	1.01 (0.71-1.42)	0.964	1.16 (0.92-1.46)	0.211

Number of individuals with non-missing phenotype involved in the respective calculations for the different methods of genotyping.
A) Grass pollen complete cohort : MALDI-TOF MS (446 cases / 929 controls), CHIP (392 cases / 849 controls); Grass pollens non-asthmatics: MALDI-TOF MS (92 / 558), CHIP (89 / 533); grass pollens asthmatics: MALDI-TOF MS (352 / 365), CHIP (301 / 310)
B) Tree pollen complete cohort : MALDI-TOF MS (287 cases / 1089 controls), CHIP (246 cases / 995 controls); Tree pollens non-asthmatics: MALDI-TOF MS (45 / 605), CHIP (42 / 580); tree pollens asthmatics: MALDI-TOF MS (242 / 476), CHIP (204 / 407)

Supplementary Table 6 Associations of *FCER1A* polymorphisms with atopic sensitization to cat dander

SNP	BIN	Complete cohort		Non-asthmatics		Asthmatics	
		OR (L95-U95)	P	OR (L95-U95)	P	OR (L95-U95)	P
rs2511211	1	0.96 (0.75-1.22)	0.722	0.89 (0.53-1.50)	0.655	1.04 (0.79-1.39)	0.763
rs10489854	2	1.42 (0.95-2.13)	0.088	1.88 (0.91-3.87)	0.087	1.29 (0.77-2.17)	0.333
rs2494262	3	1.09 (0.90-1.33)	0.370	1.18 (0.77-1.83)	0.450	1.05 (0.83-1.33)	0.657
rs2511214	3	1.14 (0.93-1.38)	0.201	1.22 (0.80-1.87)	0.350	1.10 (0.87-1.39)	0.434
rs2427827	3	1.14 (0.93-1.38)	0.210	1.38 (0.90-2.13)	0.141	1.09 (0.87-1.38)	0.451
rs2252226	3	1.11 (0.91-1.35)	0.301	1.08 (0.71-1.64)	0.722	1.14 (0.90-1.44)	0.289
rs2427836	4	0.82 (0.67-1.00)	0.050	0.74 (0.48-1.14)	0.169	0.83 (0.65-1.05)	0.126
rs2494251	4	0.79 (0.65-0.97)	0.028	0.83 (0.54-1.28)	0.397	0.77 (0.61-0.99)	0.042
rs2427837	5	0.93 (0.75-1.17)	0.538	0.75 (0.45-1.23)	0.250	1.05 (0.80-1.37)	0.725
rs2251746	5	0.86 (0.68-1.09)	0.222	0.75 (0.45-1.24)	0.258	0.93 (0.70-1.23)	0.622
rs12565775	6	1.12 (0.81-1.57)	0.486	1.70 (0.91-3.20)	0.099	0.99 (0.66-1.48)	0.961
rs7548864	6	1.03 (0.73-1.46)	0.875	1.46 (0.75-2.86)	0.265	0.88 (0.58-1.33)	0.542
rs12119226	6	1.07 (0.76-1.50)	0.712	1.53 (0.78-3.02)	0.219	0.95 (0.63-1.42)	0.790
rs2427824	7	0.87 (0.69-1.09)	0.230	0.71 (0.43-1.17)	0.180	0.95 (0.73-1.24)	0.716
rs2494250	7	0.90 (0.73-1.12)	0.365	0.80 (0.50-1.28)	0.352	0.95 (0.73-1.23)	0.691
rs3845625	8	1.43 (1.11-1.85)	0.006	1.63 (0.95-2.79)	0.076	1.32 (0.97-1.79)	0.078
rs7522607	8	1.48 (1.14-1.91)	0.004	1.59 (0.94-2.70)	0.083	1.42 (1.03-1.95)	0.031
rs16842010	9	1.13 (0.77-1.66)	0.534	1.09 (0.48-2.45)	0.836	1.20 (0.75-1.91)	0.447
rs2427829	10	1.21 (0.98-1.49)	0.078	1.37 (0.89-2.11)	0.152	1.14 (0.89-1.48)	0.295

Number of individuals with non-missing phenotype involved in the respective calculations for the different methods of genotyping.
Cat dander complete cohort: MALDI-TOF MS (256 cases / 1112 controls), CHIP (228 cases / 1012 controls); cat dander complete non-asthmatics: MALDI-TOF MS (49 / 601), CHIP (47 / 575); cat dander asthmatics: MALDI-TOF MS (207 / 503), CHIP (181 / 429)