

Analysis of the high affinity IgE receptor genes reveals epistatic effects of *FCER1A* variants on eczema risk

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To cite this article: Mahachie John JM, Baurecht H, Rodríguez E, Naumann A, Wagenpfeil S, Klopp N, Mempel M, Novak N, Bieber T, Wichmann H-E, Ring J, Illig T, Cattaert T, Van Steen K, Weidinger S. Analysis of the high-affinity IgE receptor genes reveals epistatic effects of *FCER1A* variants on total IgE levels and eczema risk. *Allergy* 2009; DOI: 10.1111/j.1398-9995.2009.02297.x.

Keywords

atopic dermatitis; genetics; IgE.

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Accepted for publication 14 November 2009

DOI:10.1111/j.1398-9995.2009.02297.x

Edited by: Hans-Uwe Simon

Abstract

Background: High levels of total and allergen-specific IgE levels are a key feature in allergic diseases. The high-affinity receptor for IgE, which is composed of one alpha (*FCER1A*), one beta (*FCER1B*), and two gamma (*FCER1G*) subunits, represents the central receptor of IgE-induced reactions. In a genome-wide association scan, we recently identified associations between functional *FCER1A* variants and total serum IgE levels. Previous studies had reported linkage and association of *FCER1B* variants with IgE and atopic traits. The *FCER1G* gene has not yet been investigated with regard to atopy. Filaggrin (*FLG*) is the strongest known risk gene for eczema, in particular the allergic subtype of eczema.

Methods: We investigated the association of *FCER1A*, *FCER1B*, and *FCER1G* variants with IgE in a large population-based cohort ($n = 4261$) and tested for epistatic effects using the model-based multifactor dimensionality reduction (MB-MDR) method. In addition, we investigated a potential interaction between *FLG* and *FCER1A* variants in a large collection of eczema cases ($n = 1018$) and population controls.

Results: Three strongly correlated *FCER1A* polymorphisms were significantly associated with total and specific IgE levels as well as allergic sensitization. No associations were seen for *FCER1B* and *FCER1G*. After adjustment for *FLG* effects, a significant epistatic effect of the *FCER1A* variants rs10489854 and rs2511211 on eczema risk was detected.

Conclusions: These results suggest that *FCER1A* variants by themselves and in combination influence IgE levels and act synergistically to influence eczema risk.

Atopic diseases such as asthma and eczema are multifactorial disorders with a complex etiology, resulting from the interaction between multiple genes and environmental factors (1, 2). A key feature of these diseases is an IgE-

mediated aberrant immune responsiveness to otherwise harmless environmental allergens, with the majority of patients exhibiting increased total and allergen-specific IgE levels (3). Numerous metabolic networks can contribute to

IgE production, but their complex interactions are not well understood yet (4).

Total IgE is a strongly heritable quantitative trait (5, 6). In contrast to the considerable genetic and phenotypic heterogeneity at the level of allergic diseases, total IgE represents a more defined and quantifiable endophenotype. Although not without controversy, it is commonly thought that it is useful to study such low-level physiological traits associated with the higher-order diseases of interest, because they probably involve fewer genes and fewer interacting levels, and eventually provide simpler clues to an enhanced functional understanding of the genetics of the more complex diseases (7, 8). In addition, intermediate phenotypes might help to cope with epistatic interaction between genes (9).

Despite the high heritability estimates for total IgE, only few genetic variants are known to influence its concentrations. In a recent population-based genome-wide association study (GWAS) involving >11 000 individuals, we demonstrated that functional variants in the gene encoding the alpha chain of the high-affinity receptor for IgE (*FCER1A*) on chromosome 1q23 are strongly associated with total IgE levels (10). Interestingly, earlier studies had suggested linkage of atopy to the gene encoding the beta chain of the high-affinity IgE receptor (*FCER1B*), and there have been several yet conflicting studies regarding *FCER1B* variants and total IgE levels (for references see: (10)). To our best knowledge, the gene encoding the gamma subunit (*FCER1G*) has not been investigated with regard to IgE. Given these observations, the central role of the high-affinity IgE receptor in allergic responses and the biologic interaction between its different subunits, we investigated the association of *FCER1A*, *FCER1B*, and *FCER1G* with IgE and atopy in a large population-based cohort and tested for interaction between these loci.

Null alleles of the gene encoding the key epidermal protein (pro-)filaggrin (*FLG*), which cause a deficiency of biologically effective filaggrin, are the most highly significant and widely replicated genetic risk factor for eczema with an estimated odds ratio of 3 (11). Based on the findings on *FLG*, it has been hypothesized that in eczema, a deficient skin barrier allows an increased entry of allergens and drives stimulation of immunologic mechanisms (12). Thus, the combination of a genetically determined skin barrier deficiency and propensity toward IgE-mediated immune responses ('atopic diathesis') might have a strong impact on eczema. We therefore also investigated a potential interaction between *FLG* mutations and *FCER1A* variants in a large collection of eczema cases and population controls.

Materials and methods

Ethics statement

A detailed description of the recruitment and the applied standardized examinations of the KORA S4 (Cooperative Research in the Region of Augsburg) cohort and the eczema cohort has been described elsewhere (13, 14). All studies were approved by the local ethics committees, all clinical investiga-

tions were conducted according to the principles expressed in the Declaration of Helsinki, and all participants gave written informed consent.

Study population

KORA S4 is an epidemiological study group including 4261 unrelated German adult individuals representative of the population within the age range of 25–74 years in the city and region of Augsburg (Bavaria, Germany); probands were recruited from 1999 to 2001 (15). In addition to demographic data, all subjects had to complete a standardized questionnaire that included the basic allergy questions of the European Community Respiratory Health Survey on respiratory health (16). Total and specific IgE antibodies to aero-allergens (Sx1) were measured using RAST FEIA CAP system (Pharmacia, Freiburg, Germany). Allergic sensitization was defined as specific IgE levels ≥ 0.35 KU/l (CAP class ≥ 1). DNA was available for 4261 individuals (50.9% females), and all subsequent analyses were based on this number.

All German patients with eczema were unrelated and of white origin, with eczema diagnosed on the basis of a skin examination by experienced dermatologists using the UK diagnostic criteria (17). The collection of 1018 individuals (59.5% females) was recruited in the department of dermatology of the University hospitals of Bonn and Munich.

Selection of single nucleotide polymorphisms (SNPs) and genotyping

A set of linkage disequilibrium (LD)-tagging SNPs completely covering each gene region inclusive surrounding up- and downstream sequence for *FCER1A* (chr1:157 514 000–157 547 000; $n = 9$), *FCER1B* (chr11:59 609 000–59 628 000; $n = 3$), and *FCER1G* (chr1:159 446 501–159 458 686; $n = 10$) was selected using the HapMap database (release #22, March 2007, on NCBI B36 assembly, dbSNP b126) and implementing Haploview software (v 3.3, May 2006, <http://www.broad.mit.edu/mpg/haploview/>) for calculation of correlation (pairwise; $r^2 > 0.8$). The minor allele frequency (MAF) was set at a cut-off of 0.05 and Hardy-Weinberg Equilibrium (HWE) P -value was set at a 0.05 significance level. In addition, we selected three IgE-associated *FCER1A* SNPs (rs2427837, rs2251746, and rs2511211) from our previous GWAS (Weidinger S 2008), and one SNP reported to increase transcriptional *FCER1A* activity [rs2427827 (18)]. In *FCER1B*, we supplemented tagging SNPs with variants reported to be strongly associated with specific IgE by Traherne et al. (19), which were not already covered by our tagging SNP selection.

Genotyping was performed using the iPLEX Mass Array system (MALDI-TOF MS; Sequenom, San Diego, CA, USA) according to the manufacturers instructions as described previously (10). Primers as well as allele frequencies for all SNPs analyzed in the population-based KORA S4 cohort ($n = 4261$) and in the German cases ($n = 1018$) are shown in Tables S1–S3.

Statistical analysis

Deviation from Hardy–Weinberg equilibrium was tested in the population-based KORA population. For all analyses, only individuals with complete information on the outcome of interest, genotypes for all SNPs, and information on covariates were considered.

Single SNP analysis

Quantitative traits were log-transformed when necessary, and linear regression models were applied. For binary traits, logistic regression models were designed. In the KORA population study, sex and age (centered variable and its quadratic effect) were the confounding factors considered in adjusting the fitted models. For the case–control dataset, the two groups had different age distributions, which was accounted for by categorizing age based on the quartiles computed for cases and controls separately. Thus, the models fitted for the case–control dataset were adjusted for the categorized age variable and sex.

Epistasis analysis

For exploring interaction in high dimensional genomic data we used the Model-Based Multifactor Dimensionality Reduction (MB-MDR) method proposed by (20, 21). Based on 1000 permutations, a maximum statistic approach to MB-MDR significance testing was applied (22). In particular, interest was in detecting second-order interactions. A brief description of the MB-MDR method is presented in Text S1. Interaction effects indicated by MB-MDR were further evaluated using logistic regression models with product interaction terms. For comparison, variable importance values were generated by means of the random forest method. Random forests provide variable importance measures that can be employed to detect variables relevant for predicting the response. The most commonly used variable importance measure is the permutation importance (23).

All statistical analyses were carried out with R 2.8.1 (24), unless otherwise stated.

Table 1 Association results for all *FCER1A*, *FCER1B*, and *FCER1G* variants tested in the KORA cross-sectional cohort ($n = 4261$) and total IgE. Regression models were adjusted for gender as well as linear and quadratic mean-centered age. Every line refers to a single model where only estimates of the genetic variables are displayed

SNP	Gene	Function*	Alleles	MAF	Estimates	95% CI		P-value
rs2511214	<i>FCER1A</i>	Unknown	T>G	0.482	0.11	0.05	0.17	0.00050
rs12119226	<i>FCER1A</i>	Intron	C>T	0.098	0.13	0.02	0.23	0.01611
rs16842010	<i>FCER1A</i>	Unknown	A>G	0.063	0.16	0.04	0.29	0.01241
rs7549785	<i>FCER1A</i>	Untranslated	C>T	0.164	0.07	−0.01	0.15	0.09832
rs10489854	<i>FCER1A</i>	Unknown	C>T	0.057	0.13	−0.01	0.26	0.06077
rs2251746	<i>FCER1A</i>	Intron	T>C	0.264	−0.21	−0.28	−0.14	3.3E-09
rs2427824	<i>FCER1A</i>	Intron	C>T	0.270	0.08	0.01	0.15	0.02084
rs2427837	<i>FCER1A</i>	Near gene	G>A	0.265	−0.20	−0.28	−0.13	1.7E-08
rs2427827	<i>FCER1A</i>	Intron	C>T	0.442	0.12	0.06	0.18	0.00023
rs2511211	<i>FCER1A</i>	Unknown	T>G	0.230	−0.21	−0.28	−0.13	8.9E-08
rs2494262	<i>FCER1A</i>	Unknown	A>C	0.482	0.12	0.06	0.19	0.00016
rs12565775	<i>FCER1A</i>	Intron	A>C	0.102	0.12	0.01	0.22	0.02549
rs3845625	<i>FCER1A</i>	Intron	C>T	0.164	0.09	0.00	0.17	0.04940
rs10797094	<i>FCER1G</i>	Intron	A>G	0.438	−0.08	−0.14	−0.01	0.01742
rs1136207	<i>FCER1G</i>	Coding synonymous	C>T	0.137	0.07	−0.02	0.16	0.10725
rs1136224	<i>FCER1G</i>	Untranslated	A>G	0.146	0.10	0.01	0.19	0.02899
rs11421	<i>FCER1G</i>	Untranslated	T>C	0.156	0.04	−0.05	0.13	0.36410
rs11587213	<i>FCER1G</i>	Near gene	A>G	0.176	0.00	−0.08	0.08	0.94403
rs12094497	<i>FCER1G</i>	Intron	G>A	0.096	−0.04	−0.14	0.07	0.52506
rs2070902	<i>FCER1G</i>	Intron	C>T	0.236	0.05	−0.03	0.12	0.20723
rs4489574	<i>FCER1G</i>	Unknown	C>T	0.300	0.06	0.00	0.13	0.05823
rs4656994	<i>FCER1G</i>	Intron	G>A	0.212	0.05	−0.03	0.13	0.21196
rs12721035	<i>FCER1G</i>	Unknown	G>A	0.058	−0.06	−0.20	0.07	0.35380
rs2847664	<i>FCER1B</i>	Intron	G>A	0.422	−0.01	−0.07	0.06	0.79763
rs513986	<i>FCER1B</i>	Unknown	C>T	0.401	−0.01	−0.08	0.05	0.71832
rs569108	<i>FCER1B</i>	Missense	A>G	0.020	−0.06	−0.27	0.16	0.59381
rs573790	<i>FCER1B</i>	Near gene	C>T	0.385	0.03	−0.04	0.09	0.41569

MAF, minor allele frequency.

*Function retrieved from UCSC genome browser assembly March 2006, dbSNP build 130 (<http://genome.ucsc.edu>): untranslated = variation in transcript but not in coding regional interval; near gene = variation within 2000 bases of gene, but not in transcript; coding synonymous = no change in peptide for allele with respect to reference assembly; missense = change in peptide for allele with respect to reference assembly; and unknown = no known functional classification.

Results

The location of the genotyped *FCER1A*, *FCER1B*, and *FCER1G* SNPs and their frequencies are provided in Table 1. Primer sequences are provided in Table S1. Genotyping success rates exceeded 92.4% for all SNPs. All analyzed SNPs were in Hardy–Weinberg equilibrium.

Significant association of *FCER1A* SNPs with total and specific IgE

First, we examined the effect of individual *FCER1A*, *FCER1B*, and *FCER1G* polymorphisms on IgE levels in the KORA sample (cross-sectional setting). Several variants of the *FCER1A* gene showed strong associations with IgE levels, even after correction for multiple testing (Table 1). In addition to rs2251746 and rs2427837, which had previously been associated with IgE in a GWAS (10), the strongly correlated polymorphism rs2511211 ($r^2 \geq 0.70$) also showed IgE-reducing effects (P -values = 3.3×10^{-9} , 1.7×10^{-8} and 8.9×10^{-8} , respectively). An additional sub-analysis showed slightly stronger effects in individuals with allergic sensitization when compared to nonallergic individuals (Table S5). In contrast, the three variants, rs2511214

($P = 0.0005$), rs2427827 ($P = 0.00023$) and rs2494262 ($P = 0.00016$), which showed strong correlation with each other, but low correlation to rs2251746, rs2427837, and rs2511211, were significantly associated with a mild increase in total IgE levels.

All total-IgE-associated *FCER1A* SNPs also consistently showed association with specific IgE levels as well as the binary trait allergic sensitization. Accordingly, rs2251746, rs2427837, and rs2511211 were associated with a reduced risk of sensitization (OR = 0.84, 0.83 and 0.80), and rs2511214, rs2427827, and rs2494262 increased the risk (OR = 1.15, 1.16 and 1.16).

For *FCER1G*, we found one polymorphism (rs10797094) to be associated with reduced IgE levels on a nominal significance level. However, this association was not robust to correction for multiple testing. No associations were seen for the *FCER1B* polymorphisms.

Because the number of eczema cases in KORA was too low ($n = 210$, 5%), to examine potential gene–gene interaction effects on eczema with sufficient power, both the 1018 eczema cases and the KORA cohort were analyzed jointly as a case–control study. In this case–control setting, the IgE-reducing effect of the *FCER1A* variants rs2251746, rs2427837, and rs2511211 observed in the KORA cross-sectional cohort could be confirmed with a roughly similar magnitude (Table S4). Furthermore, a weak but not significant association with eczema was observed confirming previous observations (10). The known association of *FLG* variants with eczema could be confirmed with an OR of 3.89 for the combined genotype (95%CI = 3.10–4.89, $P = 9.1 \times 10^{-32}$) (Table 2).

Table 2 Association results for all *FCER1A* and *FLG* variants tested in the case–control cohort and eczema. Regression models were adjusted for gender as well as categorized age. Every line refers to a single model where only estimates of the genetic variables are displayed

SNP	OR	95% CI		P-value
rs10489854	0.94	0.73	1.20	0.6011
rs2251746	0.96	0.85	1.10	0.5938
rs2427824	1.02	0.90	1.16	0.7489
rs2427837	1.01	0.89	1.16	0.8455
rs2427827	0.97	0.86	1.09	0.5999
rs2511211	0.89	0.77	1.02	0.0946
rs2494262	0.99	0.89	1.11	0.9048
rs12565775	0.94	0.78	1.14	0.5439
rs3845625	0.97	0.83	1.14	0.7326
R501X	3.90	2.66	5.70	2.3E-12
2282del4	4.04	3.07	5.32	2.1E-23
<i>FLG</i> (combined genotype)	3.89	3.10	4.89	9.1E-32

Interaction of *FCER1A* variants on IgE levels

Potential interaction effects of *FCER1A*, *FCER1B*, and *FCER1G* polymorphisms were examined in the population-based cross-sectional KORA cohort using the MB-MDR method. Results indicated strong epistasis between the two *FCER1A* variants rs2251746 and rs16842010 (P -value < 0.001). These variants were found to be weakly correlated ($r^2 = 0.003$, $D' = 0.328$) and can thus be regarded as uncorrelated. The significant interaction observed from the MB-MDR indicated that these two SNPs jointly have a strong effect on total IgE (Table 3). However, it must be taken into account that this effect appeared to be driven by a strong main effect of rs2251746 (Table 1).

Table 3 Results for epistatic effects in KORA and the case–control cohort

Trait	Method	SNPs		Risk	P-value
log(IgE)	MB-MDR	rs2251746	rs16842010	Low risk	<0.001*
Eczema†	MB-MDR	rs2511211	rs10489854	Low risk	0.0460
Eczema†	Regression‡	rs2511211	rs10489854	OR = 0.19 [0.07–0.55]	0.0020

MB-MDR, model-based multifactor dimensionality reduction.

* P -value for MB-MDR method was calculated by a permutation test.

†Adjusted for filaggrin.

‡Regression model with product interaction terms adjusted for main SNP effects.

These findings were also supported by results from the random forest method where both polymorphisms showed high positive variable importance values (Table 4).

Interaction of two *FCER1A* variants decreases risk for eczema

Potential interaction effects of *FCER1A* variants and *FLG* mutations on eczema risk were examined in the case-control setting. The MB-MDR indicated strong epistasis between *FLG* haploinsufficiency and several SNPs of the *FCER1A* gene. Because of their known and confirmed remarkably strong effect on eczema risk, we additionally performed the algorithm with adjustment for *FLG* effects to determine

whether these results were attributed to genuine epistasis. After this correction, the interaction between *FLG* and *FCER1A* polymorphisms vanished, but epistasis between the two *FCER1A* SNPs rs10489854 and rs2511211 could be detected (permutation $P = 0.046$; Table 3). Concerning linkage disequilibrium, the two variants of the *FCER1A* gene showed weak correlation ($r^2 = 0.01$, $D' = 0.80$). These findings were also supported by results from the random forest method where both polymorphisms showed positive variable importance values (Table 4). In contrast to random forests the bagging method, which does not use a random set of predictors at each run, was not able to detect the variable importance of rs10489854 emphasizing this higher-order interaction with rs2511211. Finally, the MB-MDR finding of

Table 4 Results for bagging and random forests with, respectively, five and four randomly preselected variables in each split for the analysis of *FCER1A*, *FCER1B*, *FCER1C*, and filaggrin (*FLG*) in KORA and *FCER1A* and *FLG* in the case-control setting. The average permutation importance ± 2 standard errors (SE) of the mean over 100 iterations are displayed for each variable. High positive values indicate a high variable importance. Small positive or negative values indicate that a variable is irrelevant for predicting the response

Variable	<i>FCER1A*FCER1B*FCER1G</i> for log(IgE)				<i>FCER1A*FLG</i> for eczema			
	Bagging		Random forest		Bagging		Random forest	
	Mean – 2 SE	Mean + 2 SE	Mean – 2 SE	Mean + 2 SE	Mean – 2 SE	Mean + 2 SE	Mean – 2 SE	Mean + 2 SE
Sex	0.10329	0.10433	0.07411	0.07489	0.00225	0.00230	0.00143	0.00148
Age*	0.01328	0.01397	0.00730	0.00772	0.06313	0.06329	0.05116	0.05138
Age ²	0.00623	0.00681	0.00208	0.00254				
rs2511214 (<i>FCER1A</i>)	0.00648	0.00687	0.00376	0.00418				
rs12119226 (<i>FCER1A</i>)	0.00056	0.00081	0.00088	0.00116				
rs16842010 (<i>FCER1A</i>)	0.00721	0.00757	0.00300	0.00326				
rs7549785 (<i>FCER1A</i>)	0.00090	0.00125	–0.00011	0.00017				
rs10489854 (<i>FCER1A</i>)	0.00003	0.00023	0.00075	0.00097	–0.00003	0.00000	0.00044	0.00047
rs2251746 (<i>FCER1A</i>)	0.02139	0.02225	0.01003	0.01061	0.00020	0.00024	0.00017	0.00021
rs2427824 (<i>FCER1A</i>)	0.00110	0.00146	0.00057	0.00096	0.00033	0.00037	0.00007	0.00011
rs2427837 (<i>FCER1A</i>)	0.00743	0.00799	0.01976	0.02073	0.00104	0.00107	0.00031	0.00035
rs2427827 (<i>FCER1A</i>)	0.00664	0.00708	0.00548	0.00594	0.00181	0.00187	0.00093	0.00099
rs2511211 (<i>FCER1A</i>)	0.00487	0.00545	0.00929	0.00992	0.00195	0.00199	0.00081	0.00085
rs2494262 (<i>FCER1A</i>)	0.00221	0.00245	0.00369	0.00411	0.00222	0.00228	0.00088	0.00094
rs12565775 (<i>FCER1A</i>)	0.00065	0.00088	0.00111	0.00141	0.00042	0.00046	0.00047	0.00051
rs3845625 (<i>FCER1A</i>)	0.00053	0.00080	0.00017	0.00045	0.00042	0.00046	0.00005	0.00008
rs10797094 (<i>FCER1G</i>)	0.00157	0.00194	0.00084	0.00120				
rs1136207 (<i>FCER1G</i>)	0.00476	0.00511	0.00287	0.00317				
rs1136224 (<i>FCER1G</i>)	0.00198	0.00236	0.00118	0.00151				
rs11421 (<i>FCER1G</i>)	0.00302	0.00344	0.00129	0.00157				
rs11587213 (<i>FCER1G</i>)	–0.00009	0.00027	–0.00045	–0.00012				
rs12094497 (<i>FCER1G</i>)	–0.00027	0.00006	–0.00149	–0.00123				
rs2070902 (<i>FCER1G</i>)	0.00474	0.00509	0.00142	0.00173				
rs4489574 (<i>FCER1G</i>)	0.00705	0.00757	0.00331	0.00369				
rs4656994 (<i>FCER1G</i>)	0.00771	0.00817	0.00402	0.00436				
rs12721035 (<i>FCER1G</i>)	–0.00170	–0.00146	–0.00120	–0.00100				
rs2847664 (<i>FCER1B</i>)	0.00331	0.00375	0.00028	0.00058				
rs513986 (<i>FCER1B</i>)	0.00368	0.00411	0.00119	0.00149				
rs569108 (<i>FCER1B</i>)	0.00594	0.00617	0.00183	0.00196				
rs573790 (<i>FCER1B</i>)	0.00280	0.00329	–0.00072	–0.00034				
FLG _{combGtyp} (<i>FLG</i>)	–0.00207	–0.00187	–0.00119	–0.00102	0.01351	0.01361	0.01171	0.01181

*In KORA, age was centered and for the case-control study, age was categorized based on quartiles computed for cases and controls separately.

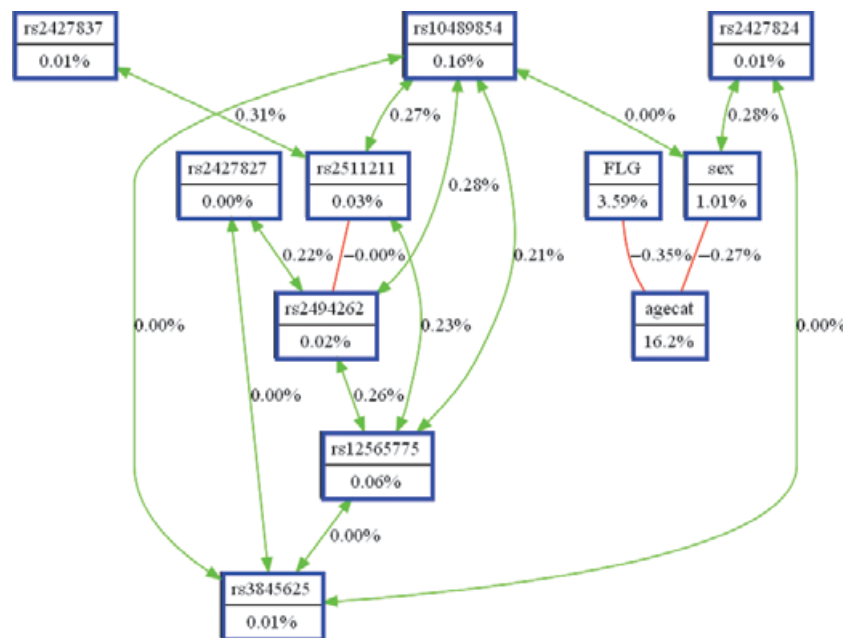


Figure 1 Entropy-based interaction graph for eczema. Values in the boxes represent the individual information gain and the positive values along the arrows represent positive interaction. The negative

values can be explained as negative interaction/redundancy i.e. the amount of information shared by the attributes.

the epistatic effect of rs2511211 and rs10489854 for eczema was also suggested by a logistic regression model with product interaction terms showing an OR of 0.19 (95%CI = 0.07–0.55, $P = 0.002$, Table 3).

To visualize the amount of uncertainty eliminated by each SNP or two-way interaction for the eczema outcome, an entropy-based interaction graph (Fig. 1) was created using the Orange machine learning software that is freely available at <http://www.aillab.si/orange/>. In analogy to the MB-MDR and the random forest models, the graph also pointed toward the interaction between SNPs rs10489854 and rs2511211. Both variants were seen to eliminate 0.16% and 0.03% by themselves, respectively. Jointly, the two SNPs could eliminate an additional 0.27% of the uncertainty.

Discussion

IgE is commonly regarded a key player in allergic diseases because elevated serum IgE levels have been closely associated with atopy and allergic asthma (25). In a recent population-based GWAS, we demonstrated that common functional variants within the gene encoding the alpha chain of the high-affinity IgE receptor (*FCER1A*) are strongly associated with total IgE levels (10). In a subsequent independent replication study on two German birth cohorts, we could show that these variants strongly influence basal IgE production independently from environmental stimuli and at different stages of life, operating as early as during the fetus period (26). The high-affinity receptor for IgE (FCERI), which consists of a tetramer of a ligand-binding alpha chain (FCER1A), a signal-augmenting beta subunit (FCER1B),

and a signal-transducing gamma chain dimer (FCER1G), plays a key role in the induction and maintenance of allergic responses, in that it controls the activation of mast cells and basophils through IgE and participates in IgE-mediated antigen presentation (27). Previous studies had suggested the linkage of atopy to the *FCER1B* gene (28), and there have been several studies that reported an association of *FCER1B* variants and atopy-related traits but conflicting results for total IgE [for references see: (10)]. In a more recent study, in analogy to our results, no association between *FCER1B* tag-SNPs and IgE levels was observed (29). So far, no studies on *FCER1G* and atopic traits have been reported.

This analysis is the most comprehensive study on the genes encoding the three FCER1 subunits and atopy performed to date. However, it has to be pointed out that our approach did not consider the potential biologic function of the individual genetic variants investigated and comprises the application of different but complementary approaches. First, we searched for individual effects of single polymorphisms in *FCER1A*, *FCER1B*, and *FCER1G* on total or specific IgE levels. Whereas in analogy to previous studies (10, 26), a strong association with three tightly linked *FCER1A* variants was detected, no associations were seen for *FCER1B* and *FCER1G*. Next, we investigated SNP–SNP interaction. We found a significant interaction for the IgE outcome without adjusting for any main effects. However, it must be taken into account that this effect might have been driven by the strong main effect of rs221746 (Table 1). In such cases, the interaction will not be genuine epistasis. Finally, following the hypothesis that eczema (atopic dermatitis) is caused by an inherited disturbance of both the epidermal and immunologic skin barrier, we evaluated interac-

tion between null alleles of the *FLG* gene, the strongest known risk gene for eczema causing an epidermal barrier disruption, and *FCER1A* variants influencing IgE production. After adjusting for the *FLG* main effect, no significant epistasis between *FLG* and *FCER1A*, but between two *FCER1A* variants was observed. The fact that epistasis between *FLG* and *FCER1A* SNPs could not be detected after adjusting for the main effect of *FLG* (which was highly significant) implies that these interactions are most probably not 'genuine'. Hence, one should bear in mind that if individual SNPs exert a strong main effect, results from analysis for epistasis might be driven by this main effect; thus, adjustment is necessary.

Allergic diseases as many other complex disorders are thought to be the result of a complicated network of numerous susceptibility loci, many of which exert additive or synergistic effects, but have only a small role when considered in isolation (30, 31). Among other reasons, failures to identify and replicate individual genetic risk factors for complex diseases have been attributed to epistasis obscuring the effect of single loci (32, 33). Designing suitable analytical strategies to identify multi-SNP interactions in addition to factors that exhibit an independent effect is also one of the main challenges for GWASs.

A number of multi-locus approaches have been developed to detect epistatic effects e.g. MDR (34) and MB-MDR (20, 21). In our current study, we searched for possible genetic interactions evaluating combinations of identified risk-modifying variants using the MB-MDR method.

Though both methods, MDR and MB-MDR, are designed to compress high-dimensional genetic data into a single dimension, the MB-MDR approach was preferred because unlike the MDR, it is applicable to any kind of outcome and one can adjust for main effects and for confounding factors. (for more information, see Supporting Information). This MB-MDR seems to be a promising method to analyze complex interactions in large datasets taking into account any confounding factors.

In conclusion, we report further evidence that *FCER1A* influences atopy-related traits by individual and synergistic effects of common polymorphisms. However, our results also demonstrate that the genetic susceptibility to atopic traits is complex and that most individual genetic factors are of low effect size. Thus, although our findings were significant after permutation testing, further studies are needed that replicate our findings and take into account also other genetic factors and their functional consequences, as well as their interaction with environmental influences.

Acknowledgments

This work was supported by grants of the DFG (WE 2678/6-1), the German Ministry of Education and Research (BMBF)

References

1. Brown SJ, McLean WH. Eczema genetics: current state of knowledge and future goals. *J Invest Dermatol* 2009;129:543–552.
2. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008;8:169–182.
3. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nat Rev Immunol* 2008;8:205–217.

as part of the National Genome Research Network (NGFN), and the Christiane Kühne Center for Allergy Research and Education (<http://www.ck-care.ch/>). Jestinah M. Mahachie John is supported by the Belgian Network BIOMAGNET, funded by the Interuniversity Attraction Poles Programme initiated by the Belgian State, Science Policy Office. Stephan Weidinger is supported by a Heisenberg fellowship of the DFG (WE 2678/4-1), a grant from the Wilhelm-Vaillant-Stiftung and grant C47-08 of the University Hospital Rechts der Isar, Technische Universität München. Natalija Novak is supported by a Heisenberg-Professorship of the DFG (NO454/-52).

The KORA Augsburg studies were financed by the Helmholtz Zentrum Munich, German Research Center for Environmental Health, Neuherberg, Germany and supported by grants from the German Federal Ministry of Education and Research (BMBF). The KORA study group consists of H-E. Wichmann (speaker), A. Peters, C. Meisinger, T. Illig, R. Holle, J. John, and co-workers who are responsible for the design and conduct of the KORA studies.

We are extremely grateful to all the patients who took part in this study, the professionals who helped in recruiting them, and the KORA team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

Supporting Information

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

Supplementary Methods.

Table S1. *FCER1A* variants, primers, call rates/number of individuals typed successfully, and minor allele frequencies (MAFs).

Table S2. *FCER1B*, *FCER1G* and *FLG* variants, primers, call rates/number of individuals typed successfully, and minor allele frequencies (MAFs).

Table S3. Primers, call rates/number of individuals typed successfully, and minor allele frequencies (MAFs) of *FCER1A* and *FLG* variants in the case control cohort.

Table S4. Association results for all *FCER1A* and *FLG* variants tested in the cross-sectional case-control cohort.

Table S5. Subanalyses of the association of *FCER1A* variants and IgE levels in atopic ($n = 1116$) and a nonatopic ($n = 3012$) individuals of the KORA cross-sectional cohort ($n = 4261$).

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4. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. *Nat Rev Immunol* 2003;**3**:721–732.
5. Jacobsen HP, Herskind AM, Nielsen BW, Husby S. IgE in unselected like-sexed monozygotic and dizygotic twins at birth and at 6 to 9 years of age: high but dissimilar genetic influence on IgE levels. *J Allergy Clin Immunol* 2001;**107**:659–663.
6. Strachan DP, Wong HJ, Spector TD. Concordance and interrelationship of atopic diseases and markers of allergic sensitization among adult female twins. *J Allergy Clin Immunol* 2001;**108**:901–907.
7. Gibson G. Decanalization and the origin of complex disease. *Nat Rev Genet* 2009;**10**:134–140.
8. Gieger C, Geistlinger L, Altmaier E, Hrabec de Angelis M, Kronenberg F, Meitinger T et al. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genet* 2008;**4**:e1000282.
9. Frankel WN, Schork NJ. Who's afraid of epistasis? *Nat Genet* 1996;**14**:371–373.
10. Weidinger S, Gieger C, Rodriguez E, Baurrecht H, Mempel M, Klopp N et al. Genome-wide scan on total serum IgE levels identifies *FCER1A* as novel susceptibility locus. *PLoS Genet* 2008;**4**:e1000166.
11. Rodriguez E, Baurecht H, Herberich E, Wagenpfeil S, Brown SJ, Cordell HJ et al. Meta-analysis of filaggrin polymorphisms in eczema and asthma: robust risk factors in atopic disease. *J Allergy Clin Immunol* 2009;**123**:1361–1370 e7.
12. Bieber T. Atopic dermatitis. *N Engl J Med* 2008; **358**:1483–1494.
13. Esparza-Gordillo J, Weidinger S, Folster-Holst R, Bauerfeind A, Ruschendorf F, Patone G et al. A common variant on chromosome 11q13 is associated with atopic dermatitis. *Nat Genet* 2009;**41**:596–601.
14. Wichmann HE, Gieger C, Illig T. KORA-gen – resource for population genetics, controls and a broad spectrum of disease phenotypes. *Gesundheitswesen* 2005;**67**(Suppl. 1):S26–S30.
15. Holle R, Happich M, Lowel H, Wichmann HE. KORA – a research platform for population based health research. *Gesundheitswesen* 2005;**67**(Suppl. 1):S19–S25.
16. Burney PG, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;**7**:954–960.
17. Williams HC, Burney PG, Hay RJ, Archer CB, Shipley MJ, Hunter JJ et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. *Br J Dermatol* 1994;**131**:383–396.
18. Bae JS, Kim SH, Ye YM, Yoon HJ, Suh CH, Nahm DH et al. Significant association of FcεpsilonRIα promoter polymorphisms with aspirin-intolerant chronic urticaria. *J Allergy Clin Immunol* 2007;**119**:449–456.
19. Traherne JA, Hill MR, Hysi P, D'Amato M, Broxholme J, Mott R et al. LD mapping of maternally and non-maternally derived alleles and atopy in FcεpsilonRI-β. *Hum Mol Genet* 2003;**12**:2577–2585.
20. Calle ML, Urrea V, Vellalta G, Malats N, Steen KV. Improving strategies for detecting genetic patterns of disease susceptibility in association studies. *Stat Med* 2008;**27**:6532–6546.
21. Calle ML, Urrea V, Mallats N, van Steen K. MB-MDR: model-based multifactor dimensionality reduction for detecting interactions in high-dimensional genomic data. Vic: University of Vic, 2008. Available at <http://www.recercat.net/handle/2072/5001>.
22. Westfall PH, Young SS. Resampling-based Multiple Testing. New York: Wiley, 1993.
23. Strobl C, Boulesteix AL, Zeileis A, Hothorn T. Bias in random forest variable importance measures: illustrations, sources and a solution. *BMC Bioinformatics* 2007;**8**:25.
24. Team RDC. R: a language and environment for statistical computing. Vienna, Team RDC, 2009.
25. Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med* 1989;**320**:271–277.
26. Chen CM, Weidinger S, Klopp N, Sausenthaler S, Bischof W, Herbarth O et al. Common variants in *FCER1A* influence total serum IgE levels from cord blood up to six years of life. *Allergy* 2009;**64**:1327–1332.
27. Kraft S, Kinet JP. New developments in FcεpsilonRI regulation, function and inhibition. *Nat Rev Immunol* 2007;**7**:365–378.
28. Cookson WO, Young RP, Sandford AJ, Moffatt MF, Shirakawa T, Sharp PA et al. Maternal inheritance of atopic IgE responsiveness on chromosome 11q. *Lancet* 1992;**340**:381–384.
29. Maier LM, Howson JM, Walker N, Spickett GP, Jones RW, Ring SM et al. Association of IL13 with total IgE: evidence against an inverse association of atopy and diabetes. *J Allergy Clin Immunol* 2006;**117**:1306–1313.
30. Moore JH. A global view of epistasis. *Nat Genet* 2005;**37**:13–14.
31. Phillips PC. Epistasis – the essential role of gene interactions in the structure and evolution of genetic systems. *Nat Rev Genet* 2008;**9**:855–867.
32. Carlborg O, Haley CS. Epistasis: too often neglected in complex trait studies? *Nat Rev Genet* 2004;**5**:618–625.
33. Moore JH. The ubiquitous nature of epistasis in determining susceptibility to common human diseases. *Hum Hered* 2003;**56**:73–82.
34. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet* 2001;**69**:138–147.

Analysis of the high affinity IgE receptor genes reveals epistatic effects of FCER1A variants on total IgE levels and eczema risk

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Supplementary Information

Supplementary Methods

MB-MDR: model-based multifactor dimensionality reduction

MB-MDR is a dimensionality reduction method proposed by Calle *et al.* (1) for exploring interactions in high dimensional genomic data. This method maintains the main idea under MDR(2) of merging multi-locus genotypes in order to overcome the dimensionality problem and increase the power to detect gene interactions associated with disease or respective binary outcomes. With MDR, multi-locus genotypes are assigned to high-risk and low-risk groups in order to reduce the initial model dimension to one.

Although combining cells may potentially increase the power to detect significant gene interactions, Calle *et al.* (1) showed how the specific dimension-reduction strategy followed by the MDR methodology conveys some limitations. Among them, are that the MDR method may miss some important interactions, it cannot adjust for main effects (which can result in false positive interactions) and for confounding factors and that the outcome is only allowed to be a binary variable.

MB-MDR was proposed as a (potentially) parametric extension of MDR, which overcomes the aforementioned limitations. The principal difference between these two methods is that MB-MDR combines the multi-locus genotypes in three different categories: high risk, low risk and no evidence. With MB-MDR, only those genotypes exhibiting some significant evidence of high or low risk will be merged in the high or low categories, while those cells that either show no evidence of association or have no sufficient sample size will be combined in a third category of not enough evidence. For further details concerning the MB-MDR algorithm we refer to (3).

Random Forest

Random forests, and the related method bagging, are ensemble methods where a set of classification or regression trees is aggregated for prediction (4, 5). Random forests provide variable importance measures that can be employed to detect variables relevant for

1
2
3 predicting the response. The most commonly used variable importance measure is the
4
5 permutation importance (6).
6

7 For variable selection purposes the advantage of the random forest permutation accuracy
8
9 importance measure as compared to univariate screening methods is that it covers the
10
11 impact of each predictor variable individually as well as in multivariate interactions with other
12
13 predictor variables. For example, Lunetta et al. (7) demonstrated that genetic markers
14
15 relevant in interactions with other markers or environmental variables can be detected more
16
17 efficiently by means of random forests than by means of univariate screening methods like
18
19 Fisher's exact test (7). Here the random forest implementation cforest from the package
20
21 party(8, 9) in the R system for statistical computing (10) is used, because it guarantees
22
23 unbiased variable selection for predictor variables of different scales of measurement (6).
24
25 Predictor variables considered here were age (centered and its quadratic form), gender and
26
27 respective SNPs from the *FCER1A*, *FCER1B* and *FCER1G* genes for the KORA cross-
28
29 sectional population based cohort. For the case-control setting we used categorized age
30
31 based on quartiles computed for cases and controls separately, gender, SNPs from the
32
33 *FCER1A* gene and the *FLG* combined genotype as predictor variables (Table 5 in the main
34
35 manuscript).
36
37
38
39

40 To assess stability of results 100 random forests with 500 trees each were fitted with the
41
42 configuration guaranteeing unbiased variable selection suggested by Strobl et al. (6). The
43
44 random forests were built with 5 randomly pre-selected variables in each split (argument
45
46 mtry=5) in the KORA population-based cross sectional cohort and with 4 randomly pre-
47
48 selected variables in each split (argument mytry=4) in the case-control setting. In contrast the
49
50 method bagging is contained in random forests as a special case in which the number of
51
52 randomly pre-selected variables is equal to the number of available variables with the price
53
54 of lower diversity of the trees to be employed. On the whole, high positive values of the
55
56 importance measure indicate a high variable importance. Small positive or even negative
57
58 values indicate that a variable is irrelevant for predicting the response
59
60

Supplementary Tables

Table S1: *FCER1A* variants, primers, call rates/number of individuals typed successfully and minor allele frequencies (MAFs)

SNP		primer	extension primer	Allele		Callrate	MAF
rs2511214	2nd 1st	ACGTTGGATGGTGGTGCTGTTGACACTTTG ACGTTGGATGAACACAGAAGACCATCTCCC	GAAACTGCTAATTTTTGTTGT	T>G	4093	0.9606	0.482
rs12119226	2nd 1st	ACGTTGGATGTCCCATCACTTCTGCTTTC ACGTTGGATGAAAGACAAGGCAAGGTGGAG	ccccTCACTTCTGCTTTCTAATGAG	C>T	4097	0.9615	0.098
rs16842010	2nd 1st	ACGTTGGATGATTCTGAGCCTTAGTGCCTG ACGTTGGATGGTGATCTCTAAGGTCCCTTG	ccCCTTAGTGCCTGGATAATT	A>G	4110	0.9646	0.063
rs7549785	2nd 1st	ACGTTGGATGGAGCACTTACTATTACATGCC ACGTTGGATGAGAAACGTCTGTGCTCAAGG	CTTACTATTACATGCCACTTAAC	C>T	4094	0.9608	0.164
rs10489854	2nd 1st	ACGTTGGATGATACCCAGACTATGACTGAC ACGTTGGATGCAAGGAGGTCAAGAATGCCC	ccGGTTTTTTGCCAATAGCA	C>T	4105	0.9634	0.057
rs2251746	2nd 1st	ACGTTGGATGAGGCACAGCTGATGGGTTAA ACGTTGGATGCTGGAGAGATCTAAGGCTTC	ggcgGATGGGTAAACCAGATATGA	T>C	4121	0.9671	0.264
rs2427824	2nd 1st	ACGTTGGATGAGGGTTGTTCTGTGTTTGGC ACGTTGGATGTGTTGTTTCAGAGACAGGTGG	tCCTTGTGTCAGTTCCTT	C>T	4083	0.9582	0.270
rs2427837	2nd 1st	ACGTTGGATGCTCTCTGTGTTACTACCTGG ACGTTGGATGCAGGTGCCCTCAAGGATTAT	gggtcATTGACTTTCTCCCCATGATG	G>A	4115	0.9657	0.265
rs2427827	2nd 1st	ACGTTGGATGCACCTGGCATATGTTTGGTA ACGTTGGATGCAACTTAGAAAAGTGGGATGC	cccgcTGCTGCTGTTTTATTCTGC	C>T	3999	0.9385	0.442
rs2511211	2nd 1st	ACGTTGGATGCAGTGTCAAAGTTGTATACAG ACGTTGGATGCTCAACTGATCTAACCCAGG	gggagACAGAATAGCTTTCTGTAAGTAC	T>G	3936	0.9237	0.230
rs2494262	2nd 1st	ACGTTGGATGAGCCTTCAGGTTCTACCTTC ACGTTGGATGCCAAGTCTCCTGAGATCATC	ctacaTTCCTTACCTCTTAGTAGCATG	A>C	3987	0.9357	0.482
rs12565775	2nd 1st	ACGTTGGATGACTTGGTTGATTTCTCCCCC ACGTTGGATGGCTGTAATAACTGGGTAGAC	cccaCAATTCTAGTACTCCCTTAAAT	A>C	4002	0.9392	0.102
rs3845625	2nd 1st	ACGTTGGATGGAAGGAAGAGGATAGTGG ACGTTGGATGTATACCAGCTGACAAATGAC	ATAGTGGAATATCTGTTTACTTT	C>T	4001	0.9390	0.164

Table S2: *FCER1B*, *FCER1G* and *FLG* variants, primers, call rates/number of individuals typed successfully and minor allele frequencies (MAFs)

SNP		primer	extension primer	Allele	N	Callrate	MAF
rs10797094 <i>FCER1G</i>	2nd 1st	ACGTTGGATGAGCGTGACACCGTCTCAAAA ACGTTGGATGCCACACCCAACCCAGTTTTTC	AAACCCGAATCTCTTACTA	A>G	4102	0.9627	0.438
rs1136207 <i>FCER1G</i>	2nd 1st	ACGTTGGATGAAAACCTCAACCAAGGTCAG ACGTTGGATGATGCAAGATCAAGGCTCCTG	gCTGGGATTGATTCTTACCAGATG	C>T	4106	0.9636	0.137
rs1136224 <i>FCER1G</i>	2nd 1st	ACGTTGGATGGTTCCTCACTGGAAATTGGC ACGTTGGATGACATGCACAGAAAGCCTGAC	GTGTGTGTGTGTATGTTT	A>G	4084	0.9585	0.146
rs11421 <i>FCER1G</i>	2nd 1st	ACGTTGGATGTGCTCAGATGCTGTTTACGG ACGTTGGATGTTAGCTGGAGTTGGGAATGG	GCTGTTTACGGATATTTATATTC	T>C	4086	0.9589	0.156
rs11587213 <i>FCER1G</i>	2nd 1st	ACGTTGGATGCAATGACCAACTCCTAACCC ACGTTGGATGGGAGTGGAAAATGGCAGATG	ttctcACCCACAGAATGAGGAGACT	A>G	4099	0.9620	0.176
rs12094497 <i>FCER1G</i>	2nd 1st	ACGTTGGATGGGTAGGACTCTGTTCTTGTG ACGTTGGATGCCCATAGAAACAGCAATCCC	gAAGGCAAGGATATAGCCAA	G>A	4116	0.9660	0.096
rs2070902 <i>FCER1G</i>	2nd 1st	ACGTTGGATGGGAAGGCCAGAGAGAAACAG ACGTTGGATGTGGTACTCAGAGGAGAGAAG	AACAGAATTTCTTCCCTTAGA	C>T	4043	0.9488	0.236
rs4489574 <i>FCER1G</i>	2nd 1st	ACGTTGGATGAAACCTCAGGAGATGCTGCC ACGTTGGATGAAGCTTGGAGTCTCTAGACC	gGCTGCCAGTCCTACT	C>T	4123	0.9676	0.300
rs4656994 <i>FCER1G</i>	2nd 1st	ACGTTGGATGTCTCCTTAGTGAAGGCTATG ACGTTGGATGTGTCATCCATAAGCCCAAGG	GGCTATGCCACATTCA	G>A	4097	0.9615	0.212
rs12721035 <i>FCER1G</i>	2nd 1st	ACGTTGGATGAAAGAAAGCTTCCCAAGGTG ACGTTGGATGGTCTTTTATTGTGGGAAAGGG	ccctTGGCCCTCCTTTCCCTTCT	G>A	4118	0.9664	0.058
rs2847664 <i>FCER1B</i>	2nd 1st	ACGTTGGATGCTTCCAGGTTCAAACAAGCC ACGTTGGATGAAAAAATTAGCTGAGTGTG	tgGTAGCTGGGATTACAGG	G>A	4125	0.9681	0.422
rs513986 <i>FCER1B</i>	2nd 1st	ACGTTGGATGCAAAACCACGATGAGATACC ACGTTGGATGGCCAGCATCTGTTTCAAGTTTC	TACCATCTTGCGCCA	C>T	4084	0.9585	0.401
rs569108 <i>FCER1B</i>	2nd 1st	ACGTTGGATGTTACAGTGAGTTGGAAGACC ACGTTGGATGCTTGGCTGTGAATCAGAGTG	agaggTGAAGACCCAGGGG	A>G	4107	0.9639	0.020
rs573790 <i>FCER1B</i>	2nd 1st	ACGTTGGATGCCCATGAGCAAATCTAAACAG ACGTTGGATGATGATCACATGGTTTAGCCC	agcagTGGATTGAGTAGTATGCCTAA	C>T	4083	0.9582	0.385

Table S3: Primers, call rates/number of individuals typed successfully, and minor allele frequencies (MAFs) of *FCER1A* and *FLG* variants in the case control cohort

SNP		primer	extension primer	Allele	Cases		KORA		
					N	Callrate	N	Callrate	MAF
rs10489854	2nd 1st	ACGTTGGATGATACCCAGACTATGACTGAC ACGTTGGATGCAAGGAGGTCAAGAATGCCC	ccGGTTTTTTGCCAATAGCA	C>T	1000	0.9823	4105	0.9634	0.057
rs2251746	2nd 1st	ACGTTGGATGAGGCACAGCTGATGGGTTAA ACGTTGGATGCTGGAGAGATCTAAGGCTTC	ggcgGATGGGTTAACCAGATATGA	T>C	1002	0.9843	4121	0.9671	0.264
rs2427824	2nd 1st	ACGTTGGATGAGGGTTGTCTGTGTTTGGC ACGTTGGATGTGTTGTTTCAGAGACAGGTGG	tCCTTGTGTCAGTTCCTT	C>T	1003	0.9853	4083	0.9582	0.270
rs2427837	2nd 1st	ACGTTGGATGCTCTCTGTGTTACTACCTGG ACGTTGGATGCAGGTGCCCTCAAGGATTAT	gggtcATTGACTTTCTCCCCATGATG	G>A	994	0.9764	4115	0.9657	0.265
rs2427827	2nd 1st	ACGTTGGATGCACCTGGCATATGTTTGGTA ACGTTGGATGCAACTTAGAAAAGTGGGATGC	cccgcTGCTGCTGTTTTATTCTGC	C>T	1006	0.9882	3999	0.9385	0.442
rs2511211	2nd 1st	ACGTTGGATGCAGTGTCAAAGTTGTATACAG ACGTTGGATGCTCAACTGATCTAACCCAGG	gggagACAGAATAGCTTTCTGTAAGTAC	T>G	964	0.9470	3936	0.9237	0.230
rs2494262	2nd 1st	ACGTTGGATGAGCCTTCAGGTTCTACCTTC ACGTTGGATGCCAAGTCTCCTGAGATCATC	ctacaTTCCTTACCTCTTAGTAGCATG	A>C	1001	0.9833	3987	0.9357	0.482
rs12565775	2nd 1st	ACGTTGGATGACTTGGTTGATTTCTCCCCC ACGTTGGATGGCTGTAATAACTGGGTAGAC	cccaCAATTCTAGTACTTCCCTTAAAT	A>C	998	0.9804	4002	0.9392	0.102
rs3845625	2nd 1st	ACGTTGGATGGAAAAGGAAGAGGATAGTGG ACGTTGGATGTATACCAGCTGACAAATGAC	ATAGTGGAATATCTGTTTACTTT	C>T	838	0.8232	4001	0.9390	0.164
R501X FLG	2nd 1st	ACGTTGGATGATGGTGTCTGACCCTCTTG ACGTTGGATGCTGGAGGAAGACAAGGATCG	ATGCCTGGAGCTGTCTC	C>T	971	0.9538	3948	0.9265	0.013
2282del4 FLG	2nd 1st	ACGTTGGATGTCTTGGTGGCTCTGCTGATG ACGTTGGATGGGGACATTCAGAAGACTCAG	CATGGCCTGACACTG	ACTG> del	963	0.9460	3995	0.9376	0.025

Table S4: Association results for all *FCER1A* and *FLG* variants tested in the cross sectional case-control cohort. Regression models were adjusted for gender as well as categorized age. Every line refers to a single model where only estimates of the genetic variables are displayed.

SNP	log(IgE)			
	est	95% CI		p-value
rs10489854 <i>FCER1A</i>	0.09	-0.06	0.23	0.2529
rs2251746 <i>FCER1A</i>	-0.21	-0.28	-0.13	2.3E-07
rs2427824 <i>FCER1A</i>	0.07	-0.01	0.15	0.0856
rs2427837 <i>FCER1A</i>	-0.18	-0.26	-0.11	3.6E-06
rs2427827 <i>FCER1A</i>	0.09	0.02	0.16	0.0088
rs2511211 <i>FCER1A</i>	-0.21	-0.29	-0.12	9.9E-07
rs2494262 <i>FCER1A</i>	0.09	0.02	0.16	0.0130
rs12565775 <i>FCER1A</i>	0.06	-0.06	0.17	0.3429
rs3845625 <i>FCER1A</i>	0.08	-0.02	0.17	0.1053
r501x <i>FLG</i>	0.73	0.47	0.98	3.0E-08
2282del4 <i>FLG</i>	0.63	0.45	0.82	2.9E-11
<i>FLG</i> _{combGtype}	0.66	0.51	0.8	8.9E-18

Table S5: Subanalyses of the association of FCER1A variants and IgE levels in atopic (n=1116) and a non-atopic* (n=3012) individuals of the KORA cross-sectional cohort (n=4261). Regression models were adjusted for gender as well as centered age and quadratic age. Every line refers to a single model where only estimates of the genetic variables are displayed.

SNP	Non-atopic*				atopic*				additional adjusted for sensitization **			
	est	95% CI		p-value	est	95% CI		p-value	est	95% CI		p-value
rs2511214	0.07	0.01	0.14	0.02893	0.07	-0.04	0.18	0.19400	0.07	0.02	0.13	0.00954
rs12119226	0.09	-0.02	0.19	0.12017	0.22	0.04	0.39	0.01417	0.13	0.03	0.22	0.00729
rs16842010	0.12	-0.01	0.26	0.06811	0.29	0.07	0.51	0.00956	0.17	0.06	0.29	0.00303
rs7549785	0.02	-0.07	0.10	0.73986	0.06	-0.08	0.20	0.38453	0.03	-0.05	0.11	0.42956
rs10489854	0.08	-0.06	0.23	0.24831	0.25	0.02	0.48	0.03417	0.14	0.02	0.26	0.02717
rs2251746	-0.14	-0.22	-0.07	0.00016	-0.23	-0.35	-0.10	0.00034	-0.17	-0.23	-0.10	2.6E-07
rs2427824	0.07	0.00	0.15	0.05836	0.04	-0.08	0.16	0.52148	0.06	0.00	0.13	0.04729
rs2427837	-0.13	-0.21	-0.06	0.00059	-0.22	-0.34	-0.09	0.00061	-0.16	-0.22	-0.09	1.9E-06
rs2427827	0.08	0.01	0.14	0.02479	0.08	-0.03	0.19	0.17461	0.08	0.02	0.14	0.00733
rs2511211	-0.12	-0.20	-0.04	0.00224	-0.21	-0.34	-0.07	0.00238	-0.15	-0.21	-0.08	2.9E-05
rs2494262	0.08	0.01	0.15	0.01967	0.08	-0.03	0.19	0.14613	0.08	0.02	0.14	0.00516
rs12565775	0.08	-0.03	0.19	0.13340	0.17	-0.01	0.35	0.05988	0.11	0.02	0.20	0.01994
rs3845625	0.03	-0.06	0.12	0.53076	0.09	-0.05	0.24	0.19965	0.05	-0.03	0.13	0.21032

*atopic/ non atopic individuals were defined by allergic sensitization
** these models for each SNP are adjusted for gender, centered age, quadratic age and additional for allergic sensitization

References

1. Calle ML, Urrea V, Mallats N, van Steen K. MB-MDR: model-based multifactor dimensionality reduction for detecting interactions in high-dimensional genomic data. <http://www.recercat.net/handle/2072/5001>. In: Vic: University de Vic; 2007.
2. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet* 2001;69(1):138-47.
3. Calle ML, Urrea V, Vellalta G, Malats N, Steen KV. Improving strategies for detecting genetic patterns of disease susceptibility in association studies. *Stat Med* 2008;27(30):6532-46.
4. Breiman L. Arcing classifiers. *The Annals of Statistics* 1998;26:801-49.
5. Breiman L. Random forests. *Machine Learning* 2001;45:801-49.
6. Strobl C, Boulesteix AL, Zeileis A, Hothorn T. Bias in random forest variable importance measures: illustrations, sources and a solution. *BMC Bioinformatics* 2007;8:25.
7. Lunetta KL, Hayward LB, Segal J, Van Eerdewegh P. Screening large-scale association study data: exploiting interactions using random forests. *BMC Genet* 2004;5(1):32.
8. Hothorn T, Hornik K, Zeileis A. A laboratory for recursive part(y)itioningR package version 0.9-0. In: Munich; 2006.
9. Hothorn T, Hornik K, Zeileis A. Party: A laboratory for recursive part(y)itioningR package version 0.9-0. In; 2006.
10. Team RDC. R: A Language and Environment for Statistical Computing. In: Vienna; 2009.