

Genome-Wide Scan on Total Serum IgE Levels Identifies *FCER1A* as Novel Susceptibility Locus

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Abstract

High levels of serum IgE are considered markers of parasite and helminth exposure. In addition, they are associated with allergic disorders, play a key role in anti-tumoral defence, and are crucial mediators of autoimmune diseases. Total IgE is a strongly heritable trait. In a genome-wide association study (GWAS), we tested 353,569 SNPs for association with serum IgE levels in 1,530 individuals from the population-based KORA S3/F3 study. Replication was performed in four independent population-based study samples (total $n = 9,769$ individuals). Functional variants in the gene encoding the alpha chain of the high affinity receptor for IgE (*FCER1A*) on chromosome 1q23 (rs2251746 and rs2427837) were strongly associated with total IgE levels in all cohorts with P values of 1.85×10^{-20} and 7.08×10^{-19} in a combined analysis, and in a post-hoc analysis showed additional associations with allergic sensitization ($P = 7.78 \times 10^{-4}$ and $P = 1.95 \times 10^{-3}$). The “top” SNP significantly influenced the cell surface expression of *FCER1A* on basophils, and genome-wide expression profiles indicated an interesting novel regulatory mechanism of *FCER1A* expression via GATA-2. Polymorphisms within the *RAD50* gene on chromosome 5q31 were consistently associated with IgE levels (P values 6.28×10^{-7} – 4.46×10^{-8}) and increased the risk for atopic eczema and asthma. Furthermore, *STAT6* was confirmed as susceptibility locus modulating IgE levels. In this first GWAS on total IgE *FCER1A* was identified and replicated as new susceptibility locus at which common genetic variation influences serum IgE levels. In addition, variants within the *RAD50* gene might represent additional factors within cytokine gene cluster on chromosome 5q31, emphasizing the need for further investigations in this intriguing region. Our data furthermore confirm association of *STAT6* variation with serum IgE levels.

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Author Summary

High levels of serum IgE are considered markers of parasite and helminth exposure. In addition, they are associated with allergic disorders, play a key role in anti-tumoral defence, and are crucial mediators of autoimmune diseases. There is strong evidence that the regulation of serum IgE levels is under a strong genetic control. However, despite numerous loci and candidate genes linked and associated with atopy-related traits, very few have been associated consistently with total IgE. This study describes the first large-scale, genome-wide scan on total IgE. By examining >11,000 German individuals from four independent population-based cohorts, we show 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. In addition, our data confirm association of *STAT6* variation with serum IgE levels, and suggest that variants within the *RAD50* gene might represent additional factors within cytokine gene cluster on chromosome 5q31, emphasizing the need for further investigations in this intriguing region.

Introduction

High levels of IgE have been considered for many years as markers of parasite and helminth exposure to which they confer resistance [1]. In Western lifestyle countries with less contact, however, elevated IgE levels are associated with allergic disorders [2]. Only recently, it has been established that IgE antibodies also play a key role in anti-tumoral defence [3] and are crucial mediators of autoimmune diseases [4], thus challenging the traditional Th1/Th2 dogma.

High total serum IgE levels are closely correlated with the clinical expression and severity of asthma and allergy [5,6]. The regulation of serum IgE production is largely influenced by familial determinants, and both pedigree- and twin-based studies provided evidence of a strong genetic contribution to the variability of total IgE levels [7,8]. Genetic susceptibility of IgE-responsiveness is likely to be caused by a pattern of polymorphisms in multiple genes regulating immunologic responses [9], but so far only very few loci could be established consistently and robustly, most notable *FCER1B*, *IL-13* and *STAT6* [10,11].

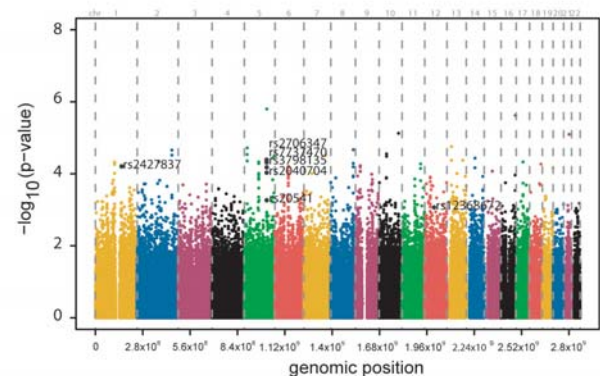
Family and case-control studies indicated that total serum IgE levels are largely determined by genetic factors that are independent of specific IgE responses and that total serum IgE levels are under stronger genetic control than atopic disease [8,12,13,14]. An understanding of the genetic mechanisms regulating total serum IgE levels might also aid in the dissection of the genetic basis of atopic diseases. In an attempt to identify novel genetic variants that affect total IgE levels, we conducted a genome-wide association study (GWAS) in 1,530 German adults and replicated the top signals in altogether 9,769 samples of four independent study populations.

Results

Genome-wide Association Scan

For the GWAS 1,530 individuals from the population-based KORA S3/F3 500 K study with available total IgE levels were typed with the Affymetrix 500 K Array Set. For statistical analysis, we selected SNPs by including only high-quality genotypes to reduce the number of false positive signals. A total of 353,569 SNPs passed all quality control measures and were tested for associations with IgE levels. Figure 1 summarizes the results of the

A



B

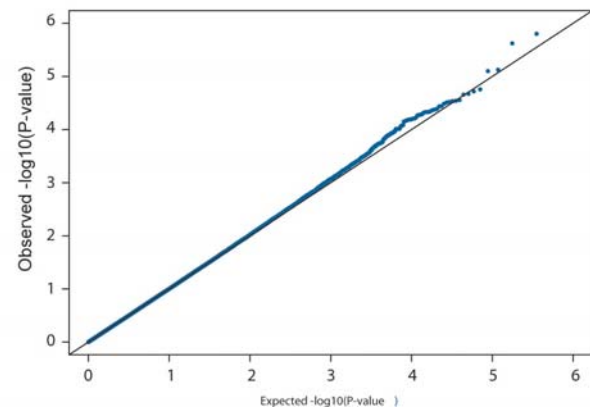


Figure 1. Results of the KORA S3/F3 500 K analysis. a) Genome-wide association study of chromosomal loci for IgE levels: the analysis is based on a population-based sample of 1530 persons. The x-axis represents the genomic position of 353,569 SNPs, and the y-axis shows $-\log_{10}(P \text{ value})$. b) Quantile-quantile plot of P values: Each black dot represents an observed statistic (defined as the $-\log_{10}(P \text{ value})$) versus the corresponding expected statistic. The line corresponds to the null distribution.

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KORA S3/F3 500 K analysis. No single SNPs reached genome-wide significance, but the scan pointed to the gene encoding the alpha chain of the high affinity receptor for IgE (*FCER1A*) on chromosome 1 (Figure 1A). Particularly the quantile-quantile-plot of the P values illustrates observed significant associations beyond those expected by chance (Figure 1B).

Replication and Fine-Mapping

For replication in the independent population-based KORA S4 cohort ($N = 3,890$), we used the following inclusion criteria: (i) $P < 10^{-4}$ in the genome wide analysis (39 SNPs, 35 expected); (ii) $P < 10^{-3}$ with at least one neighboring SNPs (± 100 kb) with $P < 10^{-3}$ (45 SNPs). The specific results for all SNPs in the GWAS and KORA S4 are given in supplementary table S3. Six SNPs were significantly associated with total IgE levels in KORA S4 with P values ranging from 2.47×10^{-4} to 3.23×10^{-9} (given a Bonferroni-corrected significance level of 5.10×10^{-4}). The strongest associations were observed for rs2427837 ($P = 3.23 \times 10^{-9}$), which is located in the 5' region of *FCER1A*, and rs12368672 ($P = 2.03 \times 10^{-6}$), which is located in the 5' region of *STAT6*. In addition, all 4 *RAD50* SNPs which had been selected in the GWAS could be replicated.

Effect estimates of the SNPs in *FCER1A* and *STAT6* were only slightly lower compared to those in the KORA S3/F3 500 K

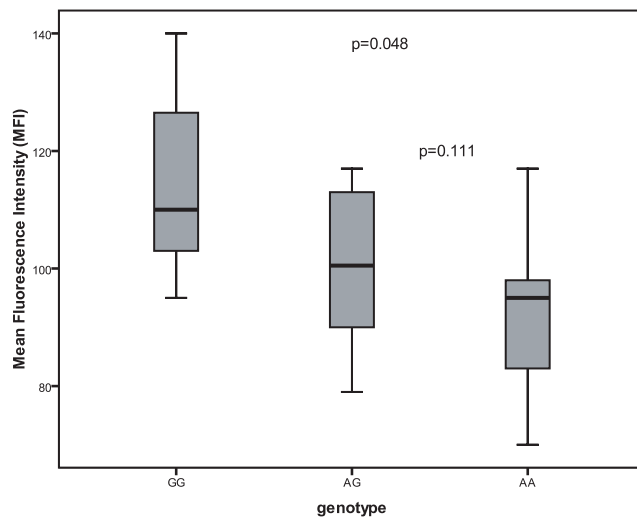


Figure 2. Expression of the FCER1 alpha chain on IgE-stripped basophils. PBMCs were isolated from individuals displaying high sIgE levels and FCER1 alpha chain expression was measured after stripping IgE from its receptor by lactic acid buffer incubation by FACS. Results are expressed as mean fluorescence intensity for FCER1A in the basophile gate. Significance was calculated using the Student's t-test. doi:10.1371/journal.pgen.1000166.g002

sample whereas clearly lower effects were observed for the SNPs in *RAD50*. The rare allele “G” of the top ranked SNP rs2427837 in *FCER1A* had an estimated effect per copy of -0.212 based on the logarithm of total IgE. This translates into an estimated decrease of 19.1% in total serum IgE level for the heterozygote genotype and 34.6% for the rare homozygote genotype, which was significantly associated with an increased FCER1A expression on IgE-stripped basophils (Figure 2).

The estimated effect of the *STAT6* SNP rs12368672 was 0.156 resulting in an increase of total IgE of 16.9% and 36.6% for the heterozygote and rare homozygote genotype, respectively. The most significant SNP in the *RAD50* gene (rs2706347) had an effect estimate of 0.143 ($P = 2.26 \times 10^{-4}$) with an associated increase in total IgE of 15.4% and 33.1%. Altogether the variance of total IgE level explained by genotypes of the three replicated regions was about 1.9%.

To fine-map the regions of strong association in greater detail, we selected additional SNPs covering the *FCER1A* and *RAD50* gene region based on HapMap data from individuals of European ancestry. In addition, two previously described promoter SNPs of *FCER1A* (rs2251746, rs2427827) [15,16], as well as 2 SNPs in the *RAD50* hypersensitive site 7 (RHS7) in intron 24 (rs2240032, rs2214370) [17] were included. In total, 14 SNPs were genotyped in KORA S4. We found the strongest association in the proximal promoter region of the *FCER1A* gene, at rs2251746, which was in strong LD ($r^2 = 0.96$) with rs2427837 (Table 1 and Figure 3). The contribution of the two alleles of rs2251746 in homozygotes and heterozygotes is given in Figure S1. Their effect is observed across the full range of IgE values. The strongest observed association of SNP rs2251746 and the distribution of the SNPs in the region are shown in Figure 3A. None of the *RAD50* SNPs in the fine-mapping showed distinctly stronger association with total IgE (Figure 3B). We additionally sequenced all *FCER1A* exons with adjacent intronic sequences in 48 male and 48 female samples selected equally from the extremes of the serum IgE distribution in 3,890 individuals from the KORA S4 cohort. We identified two new mutations, each present in one individual only, and concurrently

confirmed three SNPs already annotated in public databases (dbSNP) with validated minor allele frequencies in Europeans. None of the novel mutations were predicted to have functional consequences (for details see Text S1 and Tables S5 and S6). Haplotype analysis for the *FCER1A* gene showed lower total IgE levels with effect estimates ranging from -0.18 to -0.32 for a haplotype described by the rare “G” allele of rs2427837 and the rare “C” allele of rs2251746 (haplotype frequency 26.4%) in comparison to all other common haplotypes carrying both major alleles (Table S7).

For further replication of the KORA S4 results in the population-based children cohorts GINI ($n = 1,839$), LISA ($n = 1,042$) and ISAAC ($n = 2,998$) the top 6 SNPs: rs2251746, rs2427837, rs2040704, rs2706347, rs3798135, rs7737470 and rs12368672 were tested for association with total serum IgE levels. In GINI, all SNPs except rs12368672 yielded significant P values ranging from 0.029 to 8.14×10^{-6} . After correction for multiple testing SNP rs2706347 is slightly above the significance level. In LISA, the two *FCER1A* polymorphisms rs2251746 and rs2427837 were strongly associated ($P = 4.18 \times 10^{-5}$ and 6.58×10^{-5}), while the *RAD50* SNPs showed consistent trends, but no statistical significance. In ISAAC, the effect estimates of the two *FCER1A* SNPs were distinctly smaller than in the other replication samples but in the same direction and significantly associated with P values of 2.11×10^{-4} for rs2251746 and of 4.27×10^{-4} for rs2427837. The *RAD50* SNPs showed effect estimates in concordance with the other replication samples but were only borderline significant. Additional analysis of markers in the *RAD50-IL13* region in a subset of 526 children from the ISAAC replication cohort (for details see Table S9) indicated presence of one linkage disequilibrium (LD) block, which encompasses the entire *RAD50* gene and extends into the promoter region of the *IL13* gene, whereas rs20541 showed low levels of LD with *RAD50* variants ($r^2 < 0.3$) (Figure S2).

In the combined analysis of all replication samples both selected *FCER1A* SNPs ($P = 1.85 \times 10^{-20}$ and 7.08×10^{-19} for rs2251746 and rs2427837, respectively) and *RAD50* SNPs ($P = 6.28 \times 10^{-7}$ – 4.46×10^{-8}) were significantly associated with IgE levels. Effect estimates were consistent throughout all replication cohorts.

Association Analysis with Dichotomous Traits

In a *post hoc* analysis of the KORA S4 and ISAAC replication cohorts, *FCER1A* polymorphisms rs2251746 and rs2427837 showed association with allergic sensitization ($P = 7.78 \times 10^{-4}$ and 1.95×10^{-3} in KORA, $P = 0.025$ and 0.032 in ISAAC), while there were no significant associations for the dichotomous traits asthma, rhinitis and atopic eczema (AE). However, the number of cases for these traits was relatively low. We therefore additionally typed a cohort of 562 parent-offspring trios for AE from Germany and a population of 638 asthma cases and 633 controls from UK. In these cohorts we observed weak associations of *RAD50* variants with eczema ($P = 0.007$ – 0.01) and with asthma ($P = 0.017$ – 0.002 , Table S8).

Discussion

In this large-scale population-based GWAS with follow-up investigations in 9,769 individuals from 4 independent population-based study samples we show that functional variants of the gene encoding the alpha chain of the high affinity receptor for IgE (*FCER1A*) are of major importance for the regulation of IgE levels.

The high affinity receptor for IgE represents the central receptor of IgE-induced type I hypersensitivity reactions such as the liberation of vasoactive mediators including serotonin and

Table 1. Association between total IgE and selected SNPs in the GWAS sample and in the four replication samples.

		GWAS KORA S3/F3			Replication KORA S4			Replication GINI			Replication LISA			Replication ISAAC			Combined		
		n = 1,530			n = 3,890			n = 1,839			n = 1,042			n = 2,998			n = 9,769		
Gene	SNP	Est.	P value	Est. %	Est.	P value	Est. %	Est.	P value	Est. %	Est.	P value	Est. %	Est.	P value	Est. %	Est.	P value	Est. %
FCER1A	rs2511211				−0.206	9.28E-08	−18.59												
FCER1A	rs10489854				0.153	2.85E-02	16.52												
FCER1A	rs2494262				0.122	1.67E-04	12.99												
FCER1A	rs2427837	−0.235	6.19E-05	−20.94	−0.212	3.23E-09	−19.12	−0.219	2.51E-05	−19.64	−0.280	6.58E-05	−24.56	−0.145	4.27E-04	−13.53	−0.202	7.08E-19	−18.27
FCER1A	rs12565775				0.119	2.45E-02	12.56												
FCER1A	rs2427824				0.082	2.52E-02	8.49												
FCER1A	rs3845625				0.085	5.09E-02	8.82												
FCER1A	rs2427827				0.120	2.45E-04	12.72												
FCER1A	rs2251746				−0.227	6.07E-10	−20.29	−0.236	8.14E-06	−20.99	−0.290	4.18E-05	−25.17	−0.153	2.11E-04	−14.16	−0.213	1.85E-20	−19.21
RAD50	rs2069812				−0.052	1.42E-01	−4.98												
RAD50	rs2706347	0.236	4.05E-05	26.62	0.143	2.26E-04	15.43	0.122	2.91E-02	13.02	0.118	1.01E-01	12.56	0.095	2.70E-02	9.96	0.120	6.28E-07	12.80
RAD50	rs6884762				0.034	7.22E-01	3.46												
RAD50	rs17772565				−0.096	2.27E-01	−9.17												
RAD50	rs17772583				−0.058	1.24E-01	−5.62												
RAD50	rs3798135	0.227	6.58E-05	25.48	0.142	2.32E-04	15.20	0.173	2.00E-03	18.91	0.107	1.37E-01	11.26	0.101	1.75E-02	10.64	0.129	6.69E-08	13.82
RAD50	rs2040704	0.221	9.25E-05	24.73	0.140	2.47E-04	14.97	0.158	4.40E-03	17.14	0.111	1.21E-01	11.73	0.112	8.22E-03	11.83	0.130	4.46E-08	13.90
RAD50	rs7737470	0.231	4.81E-05	25.99	0.142	2.27E-04	15.28	0.163	3.70E-03	17.70	0.100	1.64E-01	10.55	0.087	4.13E-02	9.12	0.123	3.35E-07	13.07
RAD50	rs2240032				0.137	4.01E-04	14.67												
RAD50	rs2214370				0.136	5.95E-01	14.54												
STAT6	rs12368672	0.167	8.52E-04	18.18	0.156	2.03E-06	16.93	0.016	7.34E-01	1.65	0.075	2.44E-01	7.78			0.108	1.52E-05	11.44	

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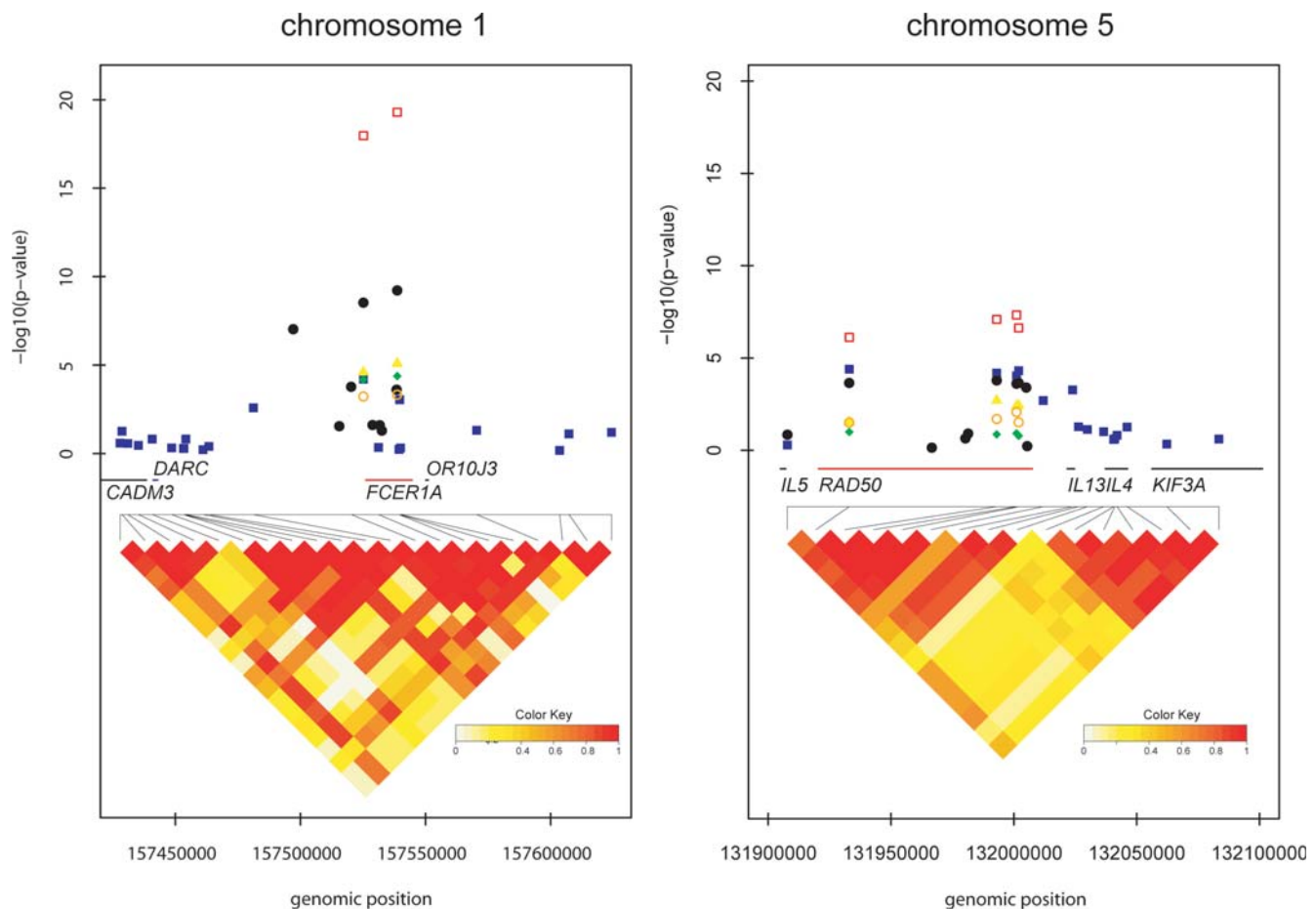


Figure 3. P value and pairwise linkage disequilibrium diagram of the region on chromosome 1q23, area of *FCER1A* (panel A), and chromosome 5q31, area of *RAD50* (panel B). Pairwise LD, measured as D' , was calculated from KORA S3/F3 500 K. Shading represents the magnitude of pairwise LD with a white to red gradient reflecting lower to higher D' values. Gene regions are indicated by colored bars. P value diagram: The x-axis represents the genomic position. The y-axis shows $-\log_{10}(P \text{ values})$ of KORA S3/F3 500 K (blue), KORA S4 (black), GINI (yellow), LISA (green), ISAAC (orange), combined replication samples (red). doi:10.1371/journal.pgen.1000166.g003

histamine, but also for the induction of profound immune responses through the activation of NF κ B and downstream genes [18]. It is usually expressed as a $\alpha\beta\gamma_2$ complex on mast cells and basophils, but additionally as a $\alpha\gamma_2$ complex on antigen-presenting cells (APCs) as shown for dendritic cells and monocytes [18]. Interestingly, in APCs, IgE-recognition of allergens also leads to facilitated allergen uptake via FCER1 and thereby contributes to a preferential activation of Th2-subsets of T-cells. Its expression is substantially influenced by the binding of IgE to either form of the receptor as bound IgE apparently protects the receptor from degradation and thus enhances surface expression without *de novo* protein synthesis. Of note, binding of IgE in the two different complexes only uses the alpha subunit of the receptor lacking contact sites with the beta or gamma subunits. Consequently, the expression level of the alpha subunit is crucial for IgE levels on immune cells [18].

Previous studies suggested linkage of atopy to the gene encoding the β chain of the high-affinity IgE receptor (*FCER1B*) [19]. *FCER1B* plays a critical role in regulating the cellular response to IgE and antigen through its capacity to amplify FCER1 signalling and regulate cell-surface expression [18], and there have been several studies which reported an association of *FCER1B* variants and atopy-related traits but conflicting results for total IgE [20,21,22,23,24,25,26,27,28]. In a more recent study, no associ-

ation between *FCER1B* tagSNPs and IgE levels was observed [22]. The 500 k random SNP array contained only one SNP within as well as 31 SNPs within a 100-kb region around this gene, which were not significantly associated with total IgE. However, we cannot rule out that we missed relevant variants in this gene.

In the present study we identified *FCER1A* as susceptibility locus in a genome-wide association scan and replicated association of the *FCER1A* polymorphism rs2427837 with serum IgE levels in a total of 9,769 individuals from 4 independent population-based cohorts with a combined P value of 7.08×10^{-19} . This SNP is in complete LD with the *FCER1A* polymorphism rs2251746, for which we observed a combined P value of 1.85×10^{-20} .

Besides the continuous cycling of the IgE receptor subunits from intracellular storage pools to the surface, there is also a substantial expression of the alpha subunit after stimulation with IL-4 which requires *de novo* protein synthesis [18]. This induction is stimulated by the transcription factor GATA-1, which has a binding site in the putative promoter region of the *FCER1A* gene. Notably, in a previous study with Japanese individuals it could be shown that the minor allele of the polymorphism rs2251746 is associated with higher FCER1A expression through enhanced GATA-1 binding [15]. In line with this we observed an increased cell surface expression of FCER1A on IgE-stripped basophils from individuals homozygous for the "G" allele at rs2427837 (Figure 2). Analysis of

the correlation of FCER1A expression with IgE levels in 320 KORA samples where whole genome blood expression profiles were available revealed no significant effect. However, FCER1A expression showed a significant dependency on IL-4 ($P=0.0087$) and GATA-1 expression ($P=1.4\times 10^{-4}$), confirming the known stimulation pathway. Interestingly, we found a highly significant dependency of FCER1A expression on GATA-2 transcript levels ($p=7.8\times 10^{-27}$). While whole blood expression levels could easily obscure the situation in basophils, this finding might indicate a novel regulatory mechanisms of FCER1A expression via GATA-2 [18].

The large (>50 kb) *RAD50* gene, which encodes an ubiquitously expressed DNA repair protein, is located within the Th2-cytokine locus on chromosome 5q31, which has been linked with total IgE [29]. It contains multiple conserved non-coding sequences with presumed regulatory function [30]. Remarkably, evidence has been provided for the presence of a locus control region (LCR) within a 25 kb segment of the 3' region of this gene, which plays an important role in the regulation of Th2 cytokine gene transcription [31]. The core of this LCR is constituted by four *RAD50* hypersensitive sites (RHS) in intron 21 (RHS4-6) and 24 (RHS7) [17,32,33]. The finding of an association between *RAD50* variants and IgE levels is new and biologically compelling. However, it has to be considered that so far *RAD50* has not emerged as candidate, but that several known candidate genes for atopy-related traits map to this region with strong linkage disequilibrium, especially *IL13*, which is one of the strongest and widely replicated candidate genes [10,11]. Notably, two functional *IL13* polymorphisms, *IL13*-1112CT (rs1800925) in the promoter region and *IL13*+2044GA (*IL13* Arg130Gln, rs20541) in Exon 4, have been shown to be associated with a range of atopy-related disorders. *IL13*+2044GA (rs20541) did not pass our selection criteria, and *IL13*-1112CT (rs1800925) is not contained in the Affymetrix 500 K Array Set. Additional analysis of markers in this region including these two SNPs showed one LD block encompassing the entire *RAD50* gene and extending into the *IL13* promoter region, whereas rs20541 showed low levels of LD with *RAD50* SNPs (Figure S2). Thus, we cannot reliably differentiate the specific source of the signal between *RAD50* and *IL13* in our data. Functional studies are needed to assess whether *RAD50* is a true causal gene and to identify the causal genetic variants modulating IgE levels in this region.

The identification and positive replication of the *STAT6* locus, which is located in one of the most frequently identified genomic regions linked to atopy-related phenotypes [34], serves as positive control for the experiment. Our results confirm previous candidate studies which showed that genetic variants in the gene encoding *STAT6*, a key regulatory element of the TH2 immune response, contribute to the regulation of total serum IgE [35,36].

Other previously reported candidate genes for total IgE showed no or only weak signals in our genome-wide scan (Tables S10 and S11). However, it has to be considered that there are only very few genes that have been associated in the first place to IgE such as *STAT6*, whereas most reported candidate genes for total IgE were investigated in asthma or eczema cohorts [10,11]. In addition, there have been queries with regard to replication for many of the genes reported. Thus, our data obtained in a population-based and ethnically homogeneous sample (South German Caucasians) are not readily comparable with previous candidate gene studies. Furthermore some previously implicated variants were covered insufficiently by the 500 k random SNP array (Table S10).

In summary, in this first GWAS on total IgE *FCER1A* was identified and replicated as new susceptibility locus at which common genetic variation influences serum IgE levels. In addition, our data suggest that variants within the *RAD50* gene might

represent additional factors within cytokine gene cluster on chromosome 5q31, emphasizing the need for further investigations in this intriguing region.

Methods

Subjects and Study Design

A detailed description of the GWAS population and the replication samples is given in Text S1 and Table S1. In all studies informed consent has been given, and all studies have been approved by the local ethical committees. The participants were of European origin.

KORA S3/F3 500 K and Replication Sample KORA S4

The study population for the GWAS (KORA S3/F3 500 K) and the first replication cohort were recruited from the KORA S3 and S4 surveys. Both are independent population-based samples from the general population living in the region of Augsburg, Southern Germany, and were examined in 1994/95 (KORA S3) and 1999/2001 (KORA S4). The standardized examinations applied in both surveys have been described in detail elsewhere [37]. In the KORA S3 study 4,856 subjects (participation rate 75%), and in KORA S4 in total 4,261 subjects have been examined (participation rate 67%). 3,006 subjects participated in a follow-up examination of S3 in 2004/05 (KORA F3). For KORA S3/F3 500 K we selected 1,644 subjects of these participants in the age range 25 to 69 years including 1,530 individuals with total IgE level available. From KORA S4, DNA samples from 3,890 individuals with total IgE level were available. Total and specific IgE antibodies to aeroallergens ($S\times I$) were measured using RAST FEIA CAP system (Pharmacia, Freiburg, Germany). Specific sensitization was defined as specific IgE levels ≥ 0.35 KU/l (CAP class ≥ 1).

GINI and LISA Replication Samples

GINI (German Infant Nutritional Intervention Program) and LISA (Influences of lifestyle-related factors on the immune system and the development of allergies in childhood study) are two ongoing population-based birth cohorts conducted in Germany. A detailed description of screening and recruitment has been provided elsewhere [38]. Briefly, the GINI birth cohort comprises 5,991 newborns, who were recruited between January 1996 and June 1998 in 16 maternity wards in Wesel and Munich, Germany. Children with a positive medical history of atopic disease were invited to a randomized clinical trial with hydrolyzed formulae [39]. The LISA birth cohort study includes 3,097 neonates who were recruited between December 1997 and January 1999 in Munich, Leipzig and Wesel, Germany. Blood samples were collected from 1,962 (51%) and 1,193(50%) children from the GINI and LISA study, respectively, at age 6. Total IgE was determined by standardized methods with CAP-RAST FEIA (Pharmacia Diagnostics, Freiburg, Germany).

ISAAC Replication Sample

Between 1995 and 1996, a cross sectional study was performed in Munich and in Dresden, Germany as part of the International Study of Asthma and Allergy in Childhood phase II (ISAAC II) to assess the prevalence of asthma and allergies in all schoolchildren attending 4th class in both cities (age 9 to 11 years) [40]. Serum measurements for total and specific IgE were performed according to standardized procedures as previously described [40]. Allergic sensitization was defined as positive prick test reaction to at least one out of six common aeroallergens. Within the study population

of 5,629 children, all children of German origin with DNA and total IgE level available were included in this analysis ($n = 2,998$).

KORA S3/F3 500 K Genotyping and Quality Control

Genotyping for KORA S3/F3 500 K was performed using Affymetrix Gene Chip Human Mapping 500 K Array Set consisting of two chips (Sty I and Nsp I). Genomic DNA was hybridized in accordance with the manufacturer's standard recommendations. Genotypes were determined using BRLMM clustering algorithm. We performed filtering of both conspicuous individuals and single nucleotide polymorphisms (SNPs) to ensure robustness of association analysis. Details on quality criteria are described in Text S1 and Table S2.

SNP Selection for Replication and Fine-Mapping

The power of the replication was estimated for a difference in log total IgE per allele of 0.2 and a nominal significance level of 0.05. The power to detect a true association was above 85% in KORA S4, GINI and ISAAC; whereas in LISA it was about 55%. No single SNPs in the GWAS reached genome-wide significance using a Bonferroni threshold of 1.4×10^{-7} . To fine map the replicated loci in KORA S4 we selected tagging SNPs and used the pairwise tagging algorithm ($r^2 > 0.8$) implemented in HAPLOVIEW 3.3 (HapMap data release #22, March 2007, on NCBI B36 assembly, dbSNP b126) and additionally selected putative functional SNPs in *FCER1A* and *RAD50*.

SNP Genotyping and Quality Control in the Replication Samples

In all replication samples genotyping of SNPs was realized with the iPLEX (Sequenom San Diego, CA, USA) method by means of matrix assisted laser desorption ionisation-time of flight mass spectrometry method (MALDI-TOF MS, Mass Array, Sequenom, San Diego, CA, USA) according to the manufacturers instructions. In KORA S4 for 7 of 84 replicated SNPs a deviation from Hardy-Weinberg-Equilibrium was observed (P value < 0.01). In LISA, GINI and ISAAC all replicated SNPs were in HWE. Details on genotyping are described in Text S1 and Table S4.

Mutational Analysis by Cycle Sequencing

FCER1A exons were amplified with intronic primers (Tables S5 and S6) and were directly sequenced using a BigDye Cycle sequencing kit (Applied Biosystems). Genomic DNA (~ 30 ng) was subjected to PCR amplification carried out in a 15 μ l volume containing 1 $\times$ PCR Master Mix (Promega), 0.25 μ M of each forward and reverse primer under the following cycle conditions: initial step at 95°C for 5 min, for 30 cycles at 95°C for 30 s, 58°C (exon 1 62°C) for 30 s, and 72°C for 30 s; and final extension at 72°C for 5 min.

Statistical Analysis of Genetic Effects

In the KORA S3/F3 500 K sample possible population substructures were analyzed (Text S1). Additive genetic models assuming a trend per copy of the minor allele were used to specify the dependency of logarithmic values of total IgE levels on genotype categories. The result is a multiplicative model on the original scale of total IgE with effects interpreted in percental changes. All models were adjusted for gender and in the adult cohorts we adjusted additionally for age. We used a linear regression algorithm implemented in the statistical analysis system R (<http://www.r-project.org/>) and SAS (Version 9.1.). To select significant SNPs in the genome-wide screening and the replications we used conservative Bonferroni thresholds which corresponded to a nominal level of

0.05. Haplotype reconstruction and haplotype association analysis was performed in the KORA S4 replication sample using the R-library *HaploStats* that allows including all common haplotypes in the linear regression and incorporating age and gender as covariates. The most common haplotype served as reference. Details on haplotype analysis are described in Text S1.

Gene Expression Analysis

Peripheral blood (2.5 ml) was drawn from individuals participating in the KORA study under fasting conditions. The blood samples were collected between 10–12am directly in PAXgene (TM) Blood RNA tubes (PreAnalytiX). The RNA extraction was performed using the PAXgene Blood RNA Kit (Qiagen). RNA and cRNA quality control was carried out using the Bioanalyzer (Agilent) and quantification was done using Ribogreen (Invitrogen). 300–500 ng of RNA was reverse transcribed into cRNA and biotin-UTP labeled using the Illumina TotalPrep RNA Amplification Kit (Ambion). 1,500 ng of cRNA was hybridized to the Illumina Human-6 v2 Expression BeadChip. Washing steps were carried out in accordance with the Illumina technical note # 11226030 Rev. B. The raw data were exported from the Illumina "Beadstudio" Software to R. The data were converted into logarithmic scores and normalized using the LOWESS method [41]. The association between *FCER1A* gene expression (independent variable) and IgE level (dependent variable) was computed using the linear regression model adjusted for gender.

Supporting Information

Figure S1 Box plot comparing the total IgE levels for the genotypes at rs2251746. The x axis represents the three genotype groups: TT (major homozygote), CT (heterozygote) and CC (minor homozygote). The y axis is the total IgE level on a logarithmic scale. Plot was created in R using the box plot function from the graphics package.

Found at: doi:10.1371/journal.pgen.1000166.s001 (0.38 MB TIF)

Figure S2 Patterns of pairwise LD between the SNPs at the RAD50-IL13 locus.

Found at: doi:10.1371/journal.pgen.1000166.s002 (0.03 MB TIF)

Table S1 Description of study populations.

Found at: doi:10.1371/journal.pgen.1000166.s003 (0.05 MB DOC)

Table S2 KORA S3/F3 500K SNP exclusion. Detailed breakdown of SNPs that were monomorphic or did not pass quality control and therefore did not enter analysis.

Found at: doi:10.1371/journal.pgen.1000166.s004 (0.04 MB DOC)

Table S3 Details on the association analysis of SNPs selected for replication (additive model).

Found at: doi:10.1371/journal.pgen.1000166.s005 (0.25 MB DOC)

Table S4 Genotyping details on replication and fine-mapping stages.

Found at: doi:10.1371/journal.pgen.1000166.s006 (0.15 MB DOC)

Table S5 Primers used to amplify the exons of *FCER1A*.

Found at: doi:10.1371/journal.pgen.1000166.s007 (0.04 MB DOC)

Table S6 Mutational analysis of *FCER1A* exons.

Found at: doi:10.1371/journal.pgen.1000166.s008 (0.04 MB DOC)

Table S7 Associations between *FCER1* haplotypes and IgE levels in KORA S4. Results correspond to the single SNP analyses where presence of A (rs2427837) and C (rs2251746) alleles at respective positions were strongly associated.

Found at: doi:10.1371/journal.pgen.1000166.s009 (0.05 MB DOC)

Table S8 Association analysis of *FCER1* and *RAD50* variants with AE in 562 German AE trios and with asthma in 638 UK asthma cases and 633 controls.

Found at: doi:10.1371/journal.pgen.1000166.s010 (0.06 MB DOC)

Table S9 Extended SNP analysis in the *RAD50*-*IL13* region in a subset of 526 children from the ISAAC replication cohort and association with total IgE levels.

Found at: doi:10.1371/journal.pgen.1000166.s011 (0.05 MB DOC)

Table S10 Genes that have been associated with total IgE ordered by their chromosomal position.

Found at: doi:10.1371/journal.pgen.1000166.s012 (0.16 MB DOC)

Table S11 Affymetrix SNPs in selected candidate genes for total IgE, which yielded a nominal p-value <0.05 in the GWAS. Genes are ordered by their chromosomal position.

Found at: doi:10.1371/journal.pgen.1000166.s013 (0.14 MB DOC)

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Text S1 Supplementary information.

Found at: doi:10.1371/journal.pgen.1000166.s014 (0.10 MB DOC)

Acknowledgments

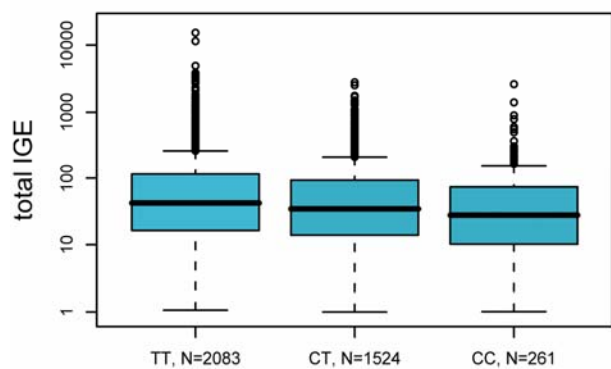
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Author Contributions

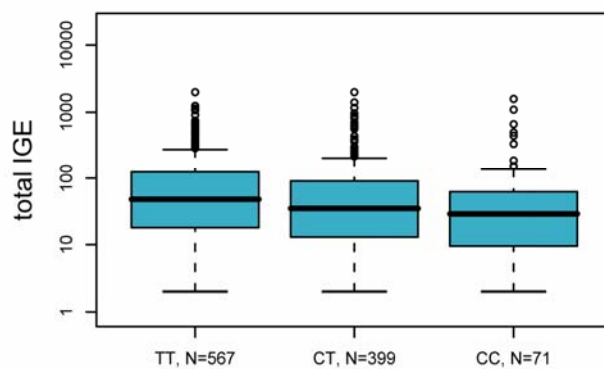
Conceived and designed the experiments: S Weidinger, N Klopp, T Meitinger, HE Wichmann, T Illing. Performed the experiments: E Rodriguez, M Mempel, N Klopp, H Prokisch, D Mehta. Analyzed the data: S Weidinger, C Gieger, H Baurecht, H Gohlke, S Wagenpfeil, M Depner, L Liang, T Illing. Contributed reagents/materials/analysis tools: S Weidinger, H Gohlke, M Ollert, J Ring, H Behrendt, J Heinrich, N Novak, T Bieber, U Krämer, D Berdel, A von Berg, CP Bauer, O Herbarth, S Koletzko, T Meitinger, E von Mutius, MF Moffatt, W Cookson, M Kabesch, HE Wichmann. Wrote the paper: S Weidinger, C Gieger, MF Moffatt, M Kabesch, T Illing.

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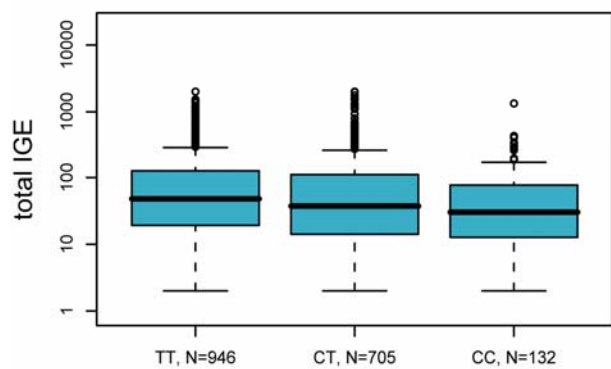
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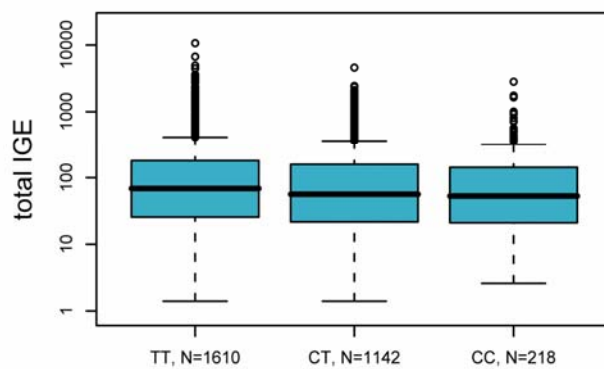
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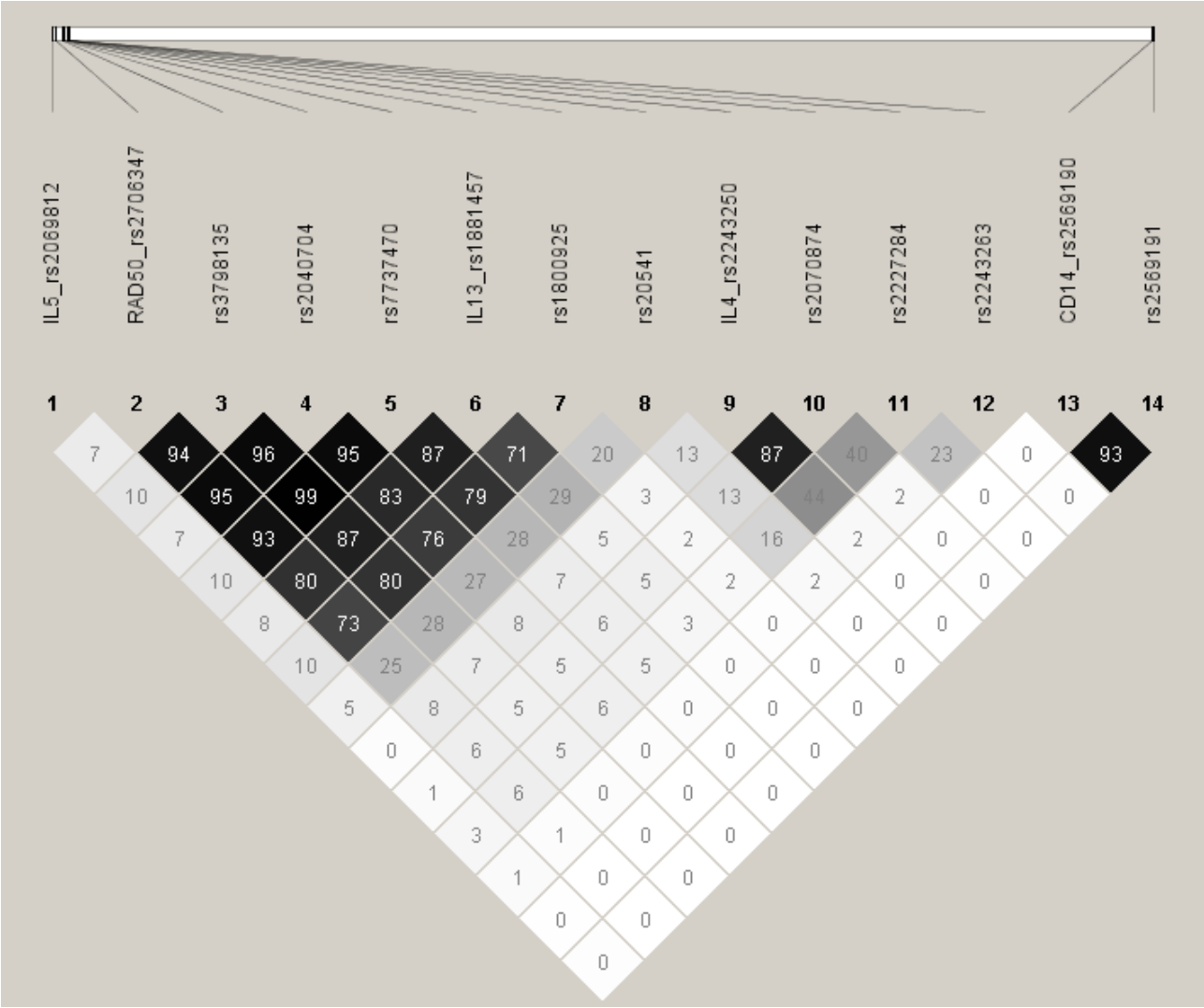


rs2251746, GINI



rs2251746, ISAAC





	GWAS	Replication samples			
	KORA S3/F3 500K	S4	LISA	GINI	ISAAC
Number total	1530	3890	1042	1839	2998
Number males	758 (49.5%)	1927 (49.5%)	565 (54.2%)	933 (50.7%)	1510 (50.4%)
Number females	772 (50.5%)	1963 (50.5%)	477 (45.8%)	906 (49.3%)	1488 (49.6%)
Mean age (range)	52.3 (25-69)	49.1 (25-74)	6.0	6.0	9.6 (8-12)
Geometric mean total IgE in kU/L (95% CI)					
Total	42.41 (39.56 - 45.47)	38.88 (37.17 - 40.68)	39.97 (36.63 -- 43.62)	43.92 (41.18-46.85)	68.92 (65.50-72.52)
Men	54.10 (49.01 - 59.72)	49.01 (46.04 - 52.18)	49.53 (43.82 - 55.98)	50.17 (45.77 - 54.99)	77.56 (72.08-83.46)
Women	33.40 (30.36 - 36.74)	30.98 (29.08 - 33.00)	31.01 (27.49-34.98)	38.30 (35.01 - 41.90)	61.14 (56.99- 65.58)

Reason for exclusion	Number of SNPs excluded
Monomorphic	17415
HWE (deviation from HWE (P-value < 10^{-6}))	4527
MAF (minor allele frequency < 0.03)	64850
Genotyping efficiency (GE < 0.95)	35904
HWE + MAF	346
HWE + GE	8405
MAF + GE	4972
HWE + MAF + GE	44
Total	136463

				GWAS (KORA S3/F3 500K) (n=1530)				Replication KORA S4 (n=3890)			
rs number	Position*	Gene Structure*	Gene*	MAF	Estimate	P value	Genotyping efficiency	MAF	Estimate	P value	Genotyping efficiency
rs2427837	chr1:157525169	5' region	FCER1A	26.57%	-0.235	6.19E-05	95.68%	27.42%	-0.212	3.23E-09	99.24%
rs12368672	chr12:55798737	5' region	STAT6	38.25%	0.167	8.52E-04	99.39%	38.00%	0.156	2.03E-06	99.53%
rs2706347	chr5:131933016	intronic	RAD50	23.70%	0.236	4.05E-05	99.09%	22.14%	0.143	0.000225629	98.60%
rs7737470	chr5:132001962	intronic	RAD50	24.22%	0.231	4.81E-05	99.94%	22.56%	0.142	0.000226596	99.26%
rs3798135	chr5:131993008	intronic	RAD50	24.38%	0.227	6.58E-05	99.82%	22.67%	0.142	0.000231899	99.61%
rs2040704	chr5:132001076	intronic	RAD50	24.59%	0.221	9.25E-05	99.70%	23.06%	0.140	0.000246599	99.11%
rs17500878	chr4:38379324			28.68%	0.195	0.000259962	99.70%	28.09%	0.102	0.004851719	98.06%
rs4345891	chr10:1704379	intronic	ADARB2	25.51%	-0.213	0.000174564	99.21%	27.65%	-0.094	0.009460605	99.61%
rs7735200	chr5:178335013			16.07%	-0.279	3.09E-05	99.94%	16.09%	0.096	0.026525431	99.80%
rs17411897	chr15:48070212	intronic	ATP8B4	3.90%	0.500	8.48E-05	99.76%	3.62%	-0.187	0.031437837	99.56%
rs10863711	chr1:204812348	intronic	RASSF5	43.24%	0.182	0.00024168	99.88%	42.06%	-0.064	0.048615797	98.92%
rs10738507	chr9:18304001			47.20%	0.178	0.000306891	96.72%	46.73%	-0.062	0.054093381	99.53%
rs2833584	chr21:32258059	intronic	HUNK	10.77%	0.351	7.96E-06	96.29%	10.41%	0.101	0.057010423	99.43%
rs2801020	chr10:42277336			18.73%	-0.243	0.000126407	99.70%	19.30%	-0.058	0.150731057	99.83%
rs4867943	chr5:169558250			31.17%	0.193	0.000432635	98.36%	31.51%	0.052	0.154633138	90.78%
rs2520094	chr9:135909806	intronic	BRD3	30.79%	0.198	0.000351965	95.13%	30.27%	0.047	0.175791773	99.80%
rs10771978	chr12:32717095			27.10%	-0.214	0.000165691	100.00%	26.88%	0.047	0.18740227	96.95%
rs2248775	chr1:69469896			27.28%	0.185	0.00068095	100.00%	27.08%	0.046	0.204922228	99.51%
rs4943387	chr13:36004633			28.03%	-0.198	0.000325101	99.51%	27.54%	0.044	0.232691386	99.58%
rs829612	chr2:30484591			43.93%	0.179	0.000275827	99.64%	44.13%	-0.038	0.239047104	99.53%
rs2508756	chr11:75921549	intronic	C11orf30	44.34%	-0.189	0.000137891	99.88%	43.44%	-0.037	0.257370461	99.21%
rs3768513	chr1:157278841	intronic	IFI16	14.46%	0.282	6.53E-05	99.51%	13.75%	-0.051	0.282437895	99.26%
rs2273758	chr1:114082240	intronic	PHTF1	39.62%	0.168	0.000681559	98.48%	38.94%	-0.035	0.293351985	99.53%
rs1956849	chr14:98034246			33.08%	-0.200	0.000117626	98.30%	34.67%	0.034	0.304748531	98.18%
rs2277615	chr17:40290220	intronic	EFTUD2	32.36%	0.220	4.67E-05	99.33%	33.51%	-0.035	0.308459254	99.68%
rs17628255	chr17:40304977	intronic	EFTUD2	12.05%	-0.268	0.000375127	98.18%	12.65%	0.027	0.565723563	90.90%
rs849538	chr2:206296221	intronic	NRP2	48.84%	0.206	3.03E-05	99.70%	48.38%	0.033	0.309237792	99.53%
rs849540	chr2:206294712	intronic	NRP2	49.60%	0.208	2.19E-05	100.00%	49.44%	0.031	0.328662965	99.46%
rs4380643	chr5:132273842	intronic	AFF4	30.21%	0.261	1.57E-06	99.27%	29.96%	0.035	0.312424707	99.88%
rs10479013	chr5:132272685	intronic	AFF4	20.42%	0.253	6.95E-05	95.01%	19.98%	0.032	0.421161712	99.46%
rs6702619	chr1:99818834			49.03%	0.176	0.00034666	100.00%	49.46%	-0.032	0.31344827	99.43%
rs8019638	chr14:50900116			9.45%	0.361	3.67E-05	95.92%	10.60%	-0.047	0.336200308	98.94%

rs1326099	chr13:58786055			36.53%	-0.201	9.73E-05	97.51%	37.36%	-0.032	0.337439528	99.73%
rs1410269	chr13:58733622			37.41%	-0.194	0.000226089	96.41%	38.73%	-0.031	0.351171139	99.70%
rs2037010	chr3:165730724			37.58%	-0.187	0.00019371	99.88%	38.66%	0.031	0.353456645	98.11%
rs787633	chr10:95109238	intronic	FER1L3	28.86%	0.186	0.000645271	99.39%	27.03%	0.033	0.372385463	99.53%
rs17123958	chr14:51011874			3.18%	0.558	6.77E-05	99.57%	3.89%	-0.073	0.382256705	99.14%
SNP_A-1782550#	chr4:83698942	intronic	FLJ12993	10.01%	0.297	0.000359165	98.78%	10.67%	0.044	0.401044371	99.83%
rs2922309	chr2:177188300			15.10%	-0.258	0.000221428	99.70%	12.81%	0.040	0.407423846	99.61%
rs481689	chr5:84812541			20.85%	0.251	4.70E-05	99.76%	19.58%	0.034	0.408848387	99.66%
rs7328732	chr13:27714278	intronic	PAN3	29.75%	0.186	0.00051414	99.88%	29.47%	-0.027	0.438666196	99.24%
rs2001011	chr7:141616055			26.65%	-0.192	0.000533399	99.27%	25.94%	-0.028	0.446796897	98.33%
rs6464482	chr7:141609452			26.52%	-0.188	0.000702127	99.76%	25.90%	-0.028	0.448583256	94.86%
rs990157	chr13:53279249			34.89%	-0.181	0.000456037	99.64%	33.94%	-0.027	0.456490645	90.88%
rs6452647	chr5:84576005			21.13%	0.240	5.18E-05	99.88%	28.84%	0.021	0.461247955	99.66%
rs10793491	chr10:43598732			23.77%	0.239	2.79E-05	98.54%	25.27%	0.027	0.465259604	98.99%
rs4392995	chr9:27826571			3.13%	0.571	5.94E-05	100.00%	2.57%	-0.064	0.539416773	97.07%
rs6651173	chr8:133236822	intronic	KCNQ3	45.65%	0.196	6.42E-05	100.00%	47.05%	0.020	0.542785448	99.48%
rs7526120	chr1:106451947			6.92%	-0.364	0.00014804	99.82%	6.86%	-0.040	0.549785326	90.88%
rs16868519	chr5:16425052			10.65%	-0.327	2.88E-05	99.64%	10.55%	0.030	0.555169543	99.43%
rs3907223	chr5:16434517			10.69%	-0.319	4.39E-05	99.88%	10.81%	0.030	0.557570801	99.80%
rs9474972	chr6:54694596			41.36%	0.179	0.00038587	99.94%	42.33%	0.019	0.5638028	99.51%
rs10114741	chr9:27792613			3.13%	0.560	8.67E-05	99.21%	2.68%	-0.055	0.581560391	98.82%
rs951260	chr10:43520976			23.54%	0.237	3.25E-05	99.88%	25.18%	0.020	0.584192725	99.58%
rs12516809	chr5:16461984			10.51%	-0.339	1.90E-05	98.36%	10.68%	0.028	0.587300136	99.34%
rs7980576	chr12:32983423			43.52%	0.172	0.000479738	99.94%	43.79%	-0.017	0.587542252	99.04%
rs17519439	chr13:26541135	exonic	USP12	8.16%	-0.394	1.75E-05	97.26%	7.88%	-0.031	0.60362714	99.11%
rs460175	chr9:97517939			25.23%	-0.216	0.000102464	99.57%	24.07%	-0.019	0.60602295	98.33%
rs9287421	chr2:133030315	intronic	GPR39	16.88%	0.252	0.000151598	99.64%	17.17%	-0.022	0.611104018	99.04%
rs10492336	chr12:113069963			34.40%	-0.183	0.000315143	98.84%	34.38%	-0.016	0.643737179	99.48%
rs7861951	chr9:27808130			3.11%	0.563	8.32E-05	99.70%	2.74%	-0.044	0.655983548	99.61%
rs1230658	chr1:114019974	intronic	MAGI3	23.72%	0.222	9.70E-05	100.00%	24.84%	-0.016	0.663736739	99.46%
rs1217200	chr1:113962639	intronic	MAGI3	24.27%	0.234	4.72E-05	95.50%	24.58%	-0.015	0.68833338	94.56%
rs12515725	chr5:168523878	intronic	SLIT3	43.48%	0.207	3.61E-05	99.76%	43.62%	0.013	0.690861139	99.70%
rs157936	chr7:130236163			28.66%	0.229	2.91E-05	96.90%	27.56%	0.014	0.700046468	94.86%
rs7574303	chr2:128357845	intronic	AMMECR1L	12.16%	-0.303	4.60E-05	98.54%	11.53%	0.019	0.704428723	99.26%
rs11139093	chr9:83057977			29.47%	0.200	0.000521885	97.63%	30.61%	-0.013	0.716678564	99.70%

rs2931116	chr5:84639708			20.87%	0.236	9.75E-05	99.51%	20.51%	0.014	0.734486799	99.53%
rs1602452	chr4:130781241			8.80%	-0.308	0.00042454	99.51%	8.58%	0.019	0.751626083	90.97%
rs7215172	chr17:40030029			50.00%	0.184	0.000181668	99.88%	49.56%	0.011	0.753000567	91.20%
rs10090180	chr8:29475483			12.45%	0.294	0.000129093	95.50%	12.15%	0.015	0.759514839	99.68%
rs12341214	chr9:27811861			3.16%	0.569	6.45E-05	99.15%	2.69%	-0.030	0.762393969	99.34%
rs1591000	chr6:78808445			34.52%	-0.209	6.26E-05	99.64%	36.25%	-0.010	0.765918278	99.53%
rs731955	chr13:98668641	intronic	PHGDHL1	37.75%	-0.193	0.000286722	95.07%	37.98%	-0.010	0.769839524	99.78%
rs4840947	chr8:8269553			29.62%	-0.199	0.000240051	98.78%	32.05%	0.007	0.826590489	99.73%
rs1953289	chr13:81015510			9.14%	0.288	0.000590014	99.51%	7.99%	0.013	0.829028779	97.84%
rs4293602	chr2:48586448	intronic	CCDC128	27.18%	0.209	0.000188381	99.94%	28.16%	-0.007	0.844508489	99.29%
rs10510391	chr3:8500794			10.93%	-0.267	0.00057424	99.94%	10.10%	-0.008	0.876882235	99.68%
rs6897714	chr5:84626651			21.21%	0.237	9.70E-05	98.36%	20.44%	0.004	0.927041041	98.84%
rs6748436	chr2:30453138			22.44%	0.213	0.000328849	98.11%	23.34%	-0.002	0.950180315	98.77%
rs7229385	chr18:67508569			44.74%	0.198	5.35E-05	99.94%	43.59%	-0.002	0.96217847	99.73%
rs2821195	chr9:11679891			35.60%	0.199	0.000114315	99.70%	36.93%	-0.002	0.963397047	99.70%
rs7541801	chr1:101813107			43.95%	-0.188	0.000183747	100.00%	43.66%	-0.001	0.98458379	98.48%
rs4450260	chr13:108001372			38.70%	-0.198	0.000117232	99.57%	40.55%	0.000	0.993558965	96.83%

Affymetrix 500k probe set ID, rs number not available

* HapMap data release #22, April 2007, on NCBI B36 assembly, dbSNP b126

rs number	2nd primer 5'-3'	1st primer 5'-3'	Extension primer 5'-3'
rs10090180	ACGTTGGATGTATGTCAGTCTCCTTGGTCC	ACGTTGGATGTTGCTTGGCCTGAGAAGATG	TCACAGAGCTCCTTTGG
rs10114741	ACGTTGGATGCTGAGATATCCTGTTACGCC	ACGTTGGATGACTGAGGCGTTTTCTTACCC	CTAGATCCTGTTACGCCCTGTAGAG
rs10479013	ACGTTGGATGGGAGTGTGAAACTCCATCTC	ACGTTGGATGCAGCCGCTCAAGCATATCTT	TGTTTTAACAATGAATGAAGTAAC
rs10489854	ACGTTGGATGCAAGGAGGTCAAGAATGCCC	ACGTTGGATGATACCCAGACTATGACTGAC	GGCAAGAATGCCCTTCACC
rs10492336	ACGTTGGATGTCAGTTCTAACCATTATGGC	ACGTTGGATGCAGCCTAACCTTTCAGAATT	GCAATGTTTATTATGAGATAGCCC
rs10510391	ACGTTGGATGCAGGCATGGGCAATATTATG	ACGTTGGATGTTAGCTGACCACCTGTTCTG	GATCAACTTCTAAAAAAGGCATTGTA
rs10738507	ACGTTGGATGTACGTTGTCCTTGGAGAAAC	ACGTTGGATGCAACTTAGTAGCAAAACACC	TTTCAAGTCCTTTGCCCATTTTTTTTA
rs10771978	ACGTTGGATGCTGCCTCGTGTATTTATTCC	ACGTTGGATGCCAGCAAAATATACTGCCTG	GTGATGAAGTACTTGTAGGCATA
rs10793491	ACGTTGGATGTCTTGGGCTAGGAGACAATG	ACGTTGGATGAGTCTTAGGTCTGAGCTGTG	GATCCTTTCTATGAAACCTCCA
rs10863711	ACGTTGGATGTCTTCTGTAAAGCTGTGCCC	ACGTTGGATGAGTTGTGTCCTAGGCCACAG	CTGTGCCCATGTACTC
rs11139093	ACGTTGGATGATGACAAGGTAAGTACAGAC	ACGTTGGATGCACATGTTTGGATGTTGCAG	GAGAGTGTTCATAACTTTCA
rs1217200	ACGTTGGATGTAATTGTTGGTGTTCACCTC	ACGTTGGATGTTGGGCGACAAGAGTGAAAC	TTTGGTGTTCACCTCATATCA
rs1230658	ACGTTGGATGCCTGTCTGATTTCCAAACCC	ACGTTGGATGTACAGCTCGTCAAGAACTGC	ACCCATGTTCTTGTCCAC
rs12341214	ACGTTGGATGCTTGAAGACAGGGTGGGAGA	ACGTTGGATGTGCCCAGCACTGAATGAATG	AAGGGTGGGAGATGAAGA
rs12368672	ACGTTGGATGATCTCCTTGCTTGAAGCCTG	ACGTTGGATGAAGGAGGAATGTTCTCAAGG	GGCTTGTCTGGAACCT
rs12515725	ACGTTGGATGAGCCAATGGGAGGGAAAATG	ACGTTGGATGTAGAATGGGACCTTGGGAAG	CCGGCTAATTCAATTCC
rs12516809	ACGTTGGATGACAGTCTCCTTTCTCTGAGC	ACGTTGGATGGTAAAGTAGTTAACTTTCTTC	GGTTCTTCTGAAAGAAATGTATTAAA
rs12565775	ACGTTGGATGACTTGGTTGATTTCTCCCCC	ACGTTGGATGGCTGTAATAACTGGGTAGAC	CCCACAATTCTAGTACTTCCCTTAAAT
rs1326099	ACGTTGGATGAGCTAATGGCCTCCATTTCC	ACGTTGGATGGATGCAGGCAGTGGAAAGTT	AGAAGGTGCCACATGG
rs1410269	ACGTTGGATGTGCCATATTGTCTATGCAGC	ACGTTGGATGCCTTAGCTGGATCCTATCTG	TCTCATTTTTTAGCATCATCAT
rs157936	ACGTTGGATGCTCAAGAGGTCAAAGTAGGC	ACGTTGGATGCTGGCACACAGTAGGTGTTT	TTGGTCATCCATTTATTCAGCAAA
rs1591000	ACGTTGGATGGGAAAACCAATTATCCCC	ACGTTGGATGGGGATGTAGAGTGTAAGG	TGGCCAGTCTGCAAAAT
rs1602452	ACGTTGGATGAGATGTCCATTTCTGCCTC	ACGTTGGATGGGAAGAAAGATGAAGAAAGC	GCCTCAGATTTGGGGAC
rs16868519	ACGTTGGATGTTGCCCATGATTCTATCATC	ACGTTGGATGGGTTTCACTCTGAAATGTTG	TTCTATCATCTTGGTAATATAACTG
rs17123958	ACGTTGGATGGCTAAGAACTCTCTGAAG	ACGTTGGATGGGAAGCACTATGATGTCAGG	TGGGTGAAATCAGCTC
rs17411897	ACGTTGGATGAAAAATGGAAGTCACTGCCC	ACGTTGGATGTAGGAAGAGATGAAGCCAGC	ACTCAGTGCCCAATAGTCTA
rs17500878	ACGTTGGATGCTGCAGACAATCCAGCAAAA	ACGTTGGATGTCTTTTTGGAAGCAGGCAGG	AACTCCAGCAAAAATCTACAAACTC
rs17519439	ACGTTGGATGACCCCCAAGGAATTCAAAGC	ACGTTGGATGGCTCCTCTTTTAAACAGCC	GGTGCAATTAATAAAGAGAAAAATG
rs17628255	ACGTTGGATGTGCAAGGTCACCATGAGTTC	ACGTTGGATGTCCCTCACTGTTAGTGCTTC	GGTTGATGTCTGCCTCCATAC
rs17772565	ACGTTGGATGCAGTCTAGTGCCTTAGCATC	ACGTTGGATGTTATTCCTGCCTTGACTGCC	GGGGTGCCTTAGCATCAACAAG
rs17772583	ACGTTGGATGTTGTGTATTACAGAACCCTC	ACGTTGGATGCCAGAGTGCTTTACTTAGCG	GATTCCAGAACCCTCATAGCATG
rs1953289	ACGTTGGATGATAGCTCAATACCCAAATGC	ACGTTGGATGTTATGCATTGGGGATTAGAC	CAATGCTATAAATTCACACATTTTCTT
rs1956849	ACGTTGGATGCTGTGCCTCTGAAAACAGC	ACGTTGGATGGCAAGACATGCTGAAAGCAC	TGAAAACAGCAAGTCC

rs2001011	ACGTTGGATGGGGTTGAGTGAAGCAGGTGT	ACGTTGGATGACCTACCCTGACTTTGATGC	TGATTGAAGCAGGTGTTGATAG
rs2037010	ACGTTGGATGACACATGCACACATACGCAC	ACGTTGGATGGAGTTTTGGCTGCTTCTAGG	CCCCACACACATTTTCATAACTTTT
rs2040704	ACGTTGGATGAAAGAGTTTCCTCTGGATGG	ACGTTGGATGCTGTCATAGTGTAGATAGGG	TTCCTCTGGATGGTTTTTCTTAT
rs2069812	ACGTTGGATGCAAGATGATGTCCAGACTCC	ACGTTGGATGCAATCAGAAAAGCACAGCTTG	GTCCAGACTCCTGGATCT
rs2214370	ACGTTGGATGGAAGTTCTCACTAAAGCAGG	ACGTTGGATGATCCCAGGCCAAGGAGTAAG	TGTATAAATATTTTATTGAGTTTTCTT
rs2240032	ACGTTGGATGCTGCTGAGCTCTCCTTCTG	ACGTTGGATGGGTCAAAGGAGGTGTTTCAG	ACCCGCTGCCAGAAAGGGACT
rs2248775	ACGTTGGATGTTACTTCCAGAGGACAAGGC	ACGTTGGATGTTACAGCCGTGTTGGTTTC	GCTTTCCTGATAAGACAATGAT
rs2251746	ACGTTGGATGAGGCACAGCTGATGGGTAA	ACGTTGGATGCTGGAGAGATCTAAGGCTTC	GATGGGTTAACCAGATATGA
rs2273758	ACGTTGGATGAGCAGCAAAAAGAACTGTC	ACGTTGGATGCTTTGTTTGGCTGTTACTAC	TAACTGTCATTGCTTATTCC
rs2277615	ACGTTGGATGTTACTCTTGCTCAGGAATC	ACGTTGGATGCAACAGCAGGTTAAAAGAGC	GGGCTTTTTGGTGATG
rs2427824	ACGTTGGATGTGTTGTTTCAGAGACAGGTGG	ACGTTGGATGAGGGTGTCTGTGTTTGGC	GGGCGAGACAGGTGGACAAAA
rs2427827	ACGTTGGATGCACCTGGCATATGTTTGGTA	ACGTTGGATGCAACTTAGAAAAGTGGGATGC	CCCGCTGCTGCTGTTTTATTCTGC
rs2427837	ACGTTGGATGCTCTCTGTGTTACTACCTGG	ACGTTGGATGCAGGTGCCCTCAAGGATTAT	AAGACTTTCTCCCCATGATG
rs2494262	ACGTTGGATGAGCCTTCAGGTTCTACCTTC	ACGTTGGATGCCAAGTCTCCTGAGATCATC	CTACATTCTTACCTCTTAGTAGCATG
rs2508756	ACGTTGGATGTGGATTTATTCCCCACCCTC	ACGTTGGATGGACATTGCTTTGAGTAAGTC	CCCACCCTCACCTTGA
rs2511211	ACGTTGGATGCAGTGTCAAAGTTGTATACAG	ACGTTGGATGCTCAACTGATCTAACCAGG	GGGAGACAGAATAGCTTTCTGTAAGTAC
rs2520094	ACGTTGGATGTTGAAGCCCTTTCCTCTCTG	ACGTTGGATGCAAGCTGGATGCTCAATGTC	CAAGTGGGAGGAAGTGAGG
rs2706347	ACGTTGGATGCAGAAGACATCAGAACTAGG	ACGTTGGATGAGAATTTTTGTGTCCTTGC	AGGGAAAATGAAACATTTTATGATGA
rs2801020	ACGTTGGATGTATCTGCTTTCAGCTCCAGG	ACGTTGGATGCCCCATTTTAAGACATCAGG	CTCCAGGTTGTAGCTATT
rs2821195	ACGTTGGATGCCACACACATCCTGTTTGTC	ACGTTGGATGATATGTAGGCTTGGAGATAG	TGTCATTCTTATTACCA
rs2833584	ACGTTGGATGTTCTGTGGATTCACAAGGGC	ACGTTGGATGTCTATTAATTCTTCGGCTC	ATGCTGTTTTCAACTCAAG
rs2922309	ACGTTGGATGAGTAATTCACTGGATGGCCC	ACGTTGGATGTTCTCAGTTTAGCACAGGAC	TCCCTCTCTCACAAAC
rs2931116	ACGTTGGATGTGTTTGTGCTGTAGGGATGG	ACGTTGGATGGTGTTATAAAGTTGTCTGGTC	GGATGGAGAAAACAGGCATATT
rs3768513	ACGTTGGATGCTGATGTATGGTGAGAGAGC	ACGTTGGATGTCCCCCAGTATTACTATACC	AAGGAGAAAGGATCGCAAAG
rs3798135	ACGTTGGATGAAAGCACTCCTGTACCTCTC	ACGTTGGATGGAAAAGTGCCTCAGTCACAG	CTCCACCAAGCTCCCTCATAG
rs3845625	ACGTTGGATGGAAAAGGAAGAGGATAGTGG	ACGTTGGATGTATACCAGCTGACAAATGAC	ATAGTGGAATATCTGTTTACTTT
rs3907223	ACGTTGGATGGCATGGATAAGGGATTCTTTC	ACGTTGGATGCTGAAACCAGGTCAGTGAAG	TATCATGGATTTTAATACAGCAA
rs4293602	ACGTTGGATGATCTCCATGGCAAGTCACTC	ACGTTGGATGCTCTGGTTCTGTTTTGATGG	GCAATTACAAAACAGGC
rs4345891	ACGTTGGATGTGGACAGCCTGCCATCGTTT	ACGTTGGATGGCTGCAAAGGAGTTGAAAG	GCCATCGTTTTCTCT
rs4380643	ACGTTGGATGCACATGGAACAGAGGGATTG	ACGTTGGATGAGGATCATATTGGGGAGCTG	AATTGTTTTTGTGTTTTAAATGGGA
rs4392995	ACGTTGGATGGCAGTGTTTCTCCCTTGGTC	ACGTTGGATGCCATCATCTGCACAGAGATA	CCCTTGTTTCTCCCTTGGTCTGTGATA
rs4450260	ACGTTGGATGTGAAGGAAAGCAAGGAGGAC	ACGTTGGATGCCCTTGTGCCTTTTTATAG	CCACAATGTTGTTTCGGTATC
rs460175	ACGTTGGATGCTGTCTGGAAATCAAGTTGC	ACGTTGGATGAACACAGTAAACTCAGATG	TCACGTTGCATTTTTCTTCCCCATG
rs481689	ACGTTGGATGGGGTGCTTGTGTTGTAGTATC	ACGTTGGATGGGTAAACCATTGGTTGTTG	AGTATCTGCTGATATCTCTGG

rs4840947	ACGTTGGATGTTTAAATCCATCCCTGGCCC	ACGTTGGATGAGTCCCCGAATGAATGAGAG	CCTGGCCCATAGTGAG
rs4867943	ACGTTGGATGTTCTCAACAGGCAAGTCAGG	ACGTTGGATGGCCGATCAATGGCAAATACG	AGGTCAGGAGCTGCTAAGATAAA
rs4943387	ACGTTGGATGACAGCATAGAACAGTGCTGG	ACGTTGGATGCATTTGTCCATCTATCCCTG	GTGCTGGCATAGGGTT
rs6452647	ACGTTGGATGGCAACTACTTGGCTACTGTG	ACGTTGGATGCCATTCTGTAAAGAGGCAC	ATCTATCTCTTACTCCAGGCAAT
rs6464482	ACGTTGGATGTCACAGGTAGAGATAGAGGC	ACGTTGGATGGCTTTCCCCCACCTTTTTTC	ATAGGCTGTAAATAATAAGGTCTTAC
rs6651173	ACGTTGGATGGTGTGCAAAGCCAGGATTC	ACGTTGGATGTTTCCAGGTTTTCTTGGCCC	ACTCCTAAACTAGCTCTAC
rs6702619	ACGTTGGATGGAAAGCACTTCCTCCAACCTG	ACGTTGGATGTGCTTAAGCAGGACTGAGAG	CTCCTGTGGATCAGTTT
rs6748436	ACGTTGGATGGGAAAACATTTGCATTGCAG	ACGTTGGATGTAGTCAAGCAGATGAGACAG	GCAGTTCAGTTCTATATTCTAATTTAT
rs6884762	ACGTTGGATGGCAGAAAAGTTGAGAAGCAAG	ACGTTGGATGTCTCCACTGTAAAGTTACTG	TTTACATAGTCTCAACATTTCT
rs6897714	ACGTTGGATGTGAGCCCAAGCTTGATACTG	ACGTTGGATGGGTTGCAGCAAAGTATTATTC	ATGAAGCACTGGGATGG
rs7215172	ACGTTGGATGTCAGATCCTTCATGCAGGTG	ACGTTGGATGGCAGCAAATTTCCGTGACAG	CAGGTGACGCCACATT
rs7229385	ACGTTGGATGCTATACACCAGGCTTCATGC	ACGTTGGATGCTAGTTGCTGCATCCTCTGG	AATTCAGATTACTTGGGCTTCCA
rs731955	ACGTTGGATGACTTTTCTCTGGCATGCAGG	ACGTTGGATGGGAGGAAAGAGTGAGCTGTA	TGTTTCAGGACTAGGATTT
rs7328732	ACGTTGGATGGCAAACATGAAACAACCCAC	ACGTTGGATGAGAATGTCCTTCATCCCAGC	CCCCAACAACCCACTAGACACTA
rs7526120	ACGTTGGATGGGCAGAATCACTCTTGAGAC	ACGTTGGATGGTCAGGCACCTAAAGTTCTC	CCTTAGATATGTAGAGCCA
rs7541801	ACGTTGGATGTAGGAAAGCATGCTGGGTAG	ACGTTGGATGGATAGAGATGAACAGAGGAC	CCTCTGCTCATAGTTTTCTGAGA
rs7574303	ACGTTGGATGTGCAGTTCCTGATCAAAGTC	ACGTTGGATGGGACAAAATGTTCCAAAAGTC	TATTGGTTCTCTTTTCTATAGTTTCA
rs7735200	ACGTTGGATGAATCATGCACCAAGCTGCAC	ACGTTGGATGTGACCACTACAACCTGCTCTG	CCTGCACTCTTGAAACATCTTCT
rs7737470	ACGTTGGATGAATGGTGGGAAGCTCTTTGC	ACGTTGGATGGTTGGTTAGCTTCAGGAGAG	CTCAGGCTCAGCTACTA
rs7861951	ACGTTGGATGAGAGTGGATTTAGAGTAGGG	ACGTTGGATGAAGTCACAATGCGTCTCCAG	AAGGGTTTAAGAAATAATCACCTCA
rs787633	ACGTTGGATGGGAAATGATTTTGGCAGCAT	ACGTTGGATGGGGAAGTCAGGGTTTGAATC	GGATTTTGGCAGCATAATCTAGTAGT
rs7980576	ACGTTGGATGGGGTTTCCAGTTATAGGCAG	ACGTTGGATGTGAGCAGTAAATCCAAAGGG	ATAGGCAGGGATAGAGA
rs8019638	ACGTTGGATGATTGTTGGGTTTGGTTTGGG	ACGTTGGATGACCCACAATCTTCCCCTCTG	CTTTGCTTTTTTCATTTTTTCCTG
rs829612	ACGTTGGATGACGCCTGCAAGATTTCCAC	ACGTTGGATGTGCCGTGGAAGGAGATGAG	CAAGATTTCCACTCTTCAC
rs849538	ACGTTGGATGGTGAAAGATTTGTTCCCTCTCC	ACGTTGGATGCTAACAGCCACCACTACTTC	AAGATCCATGTTGGTCTT
rs849540	ACGTTGGATGCAAGAGCTCAGGAGAGAAAC	ACGTTGGATGAAATGCAGCAGTGCATTGAC	GACAAAGGAGCCAGAAG
rs9287421	ACGTTGGATGTGTACGAATCAGAGAGACAG	ACGTTGGATGTATTTTGGGAGGAGAGCCAG	GAGACAGTATATGGACAGC
rs9474972	ACGTTGGATGCTGGTAATAAGTTGACCCAC	ACGTTGGATGGGACAGTGAAGGTAGAAGTG	CAACATATTAACAGCAATATCTAC
rs951260	ACGTTGGATGAATCAACCACAGGAACTCAG	ACGTTGGATGTCTGGCTCTTTCACCCAAAC	GCAGGAACTCAGGAATGG
rs990157	ACGTTGGATGTTGGCATCCATACTGCTGTG	ACGTTGGATGTGTGACATGTAAGGGGATTG	TGCTGTGAATAGCTGTAATG
SNP_A-1782550 [#]	ACGTTGGATGTAAAGCTACATGGCACCATC	ACGTTGGATGTGATGGCTTAGCTTGGGCTC	CACGGCACCATCAGCTCCA

[#] Affymetrix 500k probe set ID, rs number not available.

	Forward	Reverse
Exon 1	GCAGTAGCCTCCCTTAATGAC	CCTACTCTCAAGGAAGGGGC
Exon 2	AGAAGCAAAACCAGGCACAG	CAGCTGCTCCCCAAATTC
Exon 3	CCCACACCCAGATTCTAGTCC	AGGAAAGCAATGCCCAGAC
Exon 4	TGATTGTCAGAATATTGCTTCG	TCTGGTGTCTAACCCTTGG
Exon 5	GACACATGCTCTATGCGTGG	AGCTGCAACTGCTCAGGC
Exon 6	CAAAGCTTGGTCTTTCTCTTAGG	AGAACTTCATGGTCCTTGCC

SNP	Sequence variant	Position	Gene structure	MAF	Analyzed (n)
rs2251746	T/C	chr1:157538684	intronic	0.3	91
Mutation 1	A/G	chr1:157542690	intronic	0.005	79
rs41264475	C/A	chr1:157544313	exon 6	0.03	95
Mutation 2	A/G	chr1:157544428	exon 6 (3' UTR)	0.005	95
rs7549785	G/A	chr1:157544492	exon 6 (3' UTR)	0.17	95

No	rs2494262	rs2427837	rs12565775	rs2427824	rs3845625	rs2427827	rs2251746	freq	95% CI		est	95%CI		p-value
1	A	A	A	C	C	C	C	0.2637	0.2541	0.2732	-0.225	-0.312	-0.138	4.1×10^{-7}
2	A	G	A	C	C	C	T	0.1838	0.1754	0.1922	-0.045	-0.141	0.051	0.353
3	A	G	C	C	C	C	T	0.0508	0.0461	0.0556	0.090	-0.063	0.243	0.247
4	C	G	A	C	T	T	T	0.1627	0.1547	0.1707	0.008	-0.090	0.106	0.869
5	C	G	C	C	C	C	T	0.0474	0.0428	0.0520	0.010	-0.145	0.165	0.901
6	*	*	*	*	*	*	*	0.0239	0.0206	0.0272	0.041	-0.199	0.281	0.739
7	C	G	A	T	C	T	T	0.2676	0.2580	0.2773				

* haplotype frequency < 0.01.

SNP	Gene	Position	German families		UK case-control	
			Atopic eczema P-value	Total IgE P-value	Asthma P-value	Total IgE P-value
rs2511211	FCER1A	chr1:157497095	1.00	0.80	0.16	0.017
rs10489854	FCER1A	chr1:157515483	0.34	0.46	0.58	0.90
rs2494262	FCER1A	chr1:157520296	0.12	0.45	0.56	0.029
rs2427837	FCER1A	chr1:157525169	0.88	0.21	0.58	0.005
rs12565775	FCER1A	chr1:157528779	0.39	0.97	0.91	0.82
rs2427824	FCER1A	chr1:157531686	0.55	0.63	0.80	0.33
rs3845625	FCER1A	chr1:157532500	0.32	0.83	0.34	0.05
rs2427827	FCER1A	chr1:157538435	0.07	0.66	n.a.	n.a.
rs2251746	FCER1A	chr1:157538684	0.81	0.08	0.54	0.004
rs6884762	RAD50	chr5:131966629	0.19	0.68	0.47	0.043
rs17772565	RAD50	chr5:131980304	0.52	0.07	0.14	0.38
rs17772583	RAD50	chr5:131981409	0.00962	0.69	0.82	0.12
rs3798135	RAD50	chr5:131993008	0.00743	0.90	0.017	0.008
rs2240032	RAD50	chr5:132005026	0.00805	0.82	0.002	0.002

Gene	Chromosomal location [‡]	SNPs covered by the Affymetrix screening panel (gene +/- 100kb)	Significant variation reported [§]	Affymetrix SNP(s) in LD ($r^2 > 0.6$) with the reported variant	Estimate	P-value	Reference [#]
FLG	1q21	rs7345, rs10082235, rs3891075, rs7541193, rs6427005, rs6698058, rs9426902, rs4073768, rs6682411, rs11583896, rs16835600, rs4434872, rs4567311, rs4341393, rs4363451, rs9427232, rs12129468, rs4845576, rs4520447, rs4845578, rs11585416, rs12142130	R501X [§]	-	-	-	[8,14,15,16]
			2282del4 [§]	-	-	-	
CTLA4	2q33	rs10193976, rs10490573, rs11571292, rs11571293, rs11571304, rs11689629, rs12467195, rs16840016, rs17533594, rs17533727, rs1980422, rs231747, rs231790, rs231797, rs231814, rs3115966, rs3116487, rs3116497, rs3181106, rs3181107, rs3769683, rs4673259, rs4675367	rs231775	rs11571292	-0.0013	0.9793	[17,18]
			(AT) _n 3'UTR [§]	-	-	-	
			MH30/C [§]	-	-	-	
			CT60/A [§]	-	-	-	
			JO31/T [§]	-	-	-	
			JO30/A [§]	-	-	-	
			JO27_1/C [§]	-	-	-	
IRF1	5q31	rs274561, rs7731390, rs274554, rs274552, rs274547, rs6596075, rs17771891, rs4705943, rs4705944, rs2285673, rs11242111, rs6874639, rs11744116, rs4540166, rs4371745, rs10059611, rs10077785, rs2522057, rs10072700, rs2548999, rs2548997, rs739719, rs739718, rs2069812, rs2706347	rs17622656	rs2522057	-0.0851	0.0932	[19]
IL13	5q31	rs3798135, rs2040704, rs7737470, rs2158177, rs20541, rs2069757, rs2243218, rs2243248	rs20541	rs20541	0.2040	0.0005	[20,21,22,23, 24]
			rs1800925	-	-	-	

IL4	5q31	rs2227284, rs2227282, rs2243290, rs2023822 rs11242127, rs194395, rs402959, rs30527, rs2069757, rs2243218, rs2243248	rs2243250	rs2243290 rs194395 rs402959	0.1354 0.1244 0.1323	0.0552 0.0993 0.0785	[25,26]
			rs2070874	rs2243290 rs194395 rs402959	0.1354 0.1244 0.1323	0.0552 0.0993 0.0785	
			rs2227284	rs2227284 rs2227282 rs11242127	0.0635 0.0658 0.0642	0.2594 0.2389 0.2474	
			rs2243266	rs2243290 rs194395 rs402959 rs30527	0,1354 0,1244 0,1323 0,1432	0,0552 0,0993 0,0785 0,0571	
			rs2243274	rs2243290 rs194395 rs402959 rs30527	0,1354 0,1244 0,1323 0,1432	0,0552 0,0993 0,0785 0,0571	
			rs2243288	rs2243290 rs194395 rs402959 rs30527	0,1354 0,1244 0,1323 0,1432	0,0552 0,0993 0,0785 0,0571	
			rs2243289	rs2243290 rs194395 rs402959 rs30527	0,1354 0,1244 0,1323 0,1432	0,0552 0,0993 0,0785 0,0571	
			rs2243290	rs2243290 rs194395 rs402959 rs30527	0,1354 0,1244 0,1323 0,1432	0,0552 0,0993 0,0785 0,0571	
			rs2243284	-	-	-	
CD14	5q31	rs1835959, rs250432, rs250430, rs250428, rs2282803, rs6550, rs2253378, rs2569169, rs2163786, rs2569192, rs5744441, rs3822356, rs17208187, rs753279, rs801399, rs2563335, rs801186, rs702396, rs801168, rs801167, rs17286731, rs801183	rs2569190	rs2163786 rs753279 rs801399 rs2563335 rs801168 rs801167 rs801183	-0.0111 0.0160 -0.0442 -0.0367 -0.0350 -0.0361 -0.0391	0.8236 0.7477 0.3718 0.4593 0.4815 0.4672 0.4298	[27,28]

SPINK5	5q32	rs1363530, rs17538716, rs7707803, rs10463395, rs1422998, rs1422997, rs6895394, rs17641748, rs10491342, rs7713918, rs7727019, rs17107665, rs1363720, rs1363719, rs953310, rs11958071, rs17718403, rs17718420, rs3756688, rs1423007, rs2287774, rs9325064, rs986494, rs1423001, rs12108690, rs4529181, rs2303066, rs2303069, rs1422987, rs3777142, rs988885, rs3815738, rs1862439, rs3777138, rs2052531, rs9325073, rs17704908, rs2112767, rs3756690, rs12187820, rs7717500, rs10068988, rs10067334, rs6881658, rs6895745, rs7703761, SNP_A-1807295, rs4270684, rs4452547, rs4259160, rs4499819, rs11745313, rs11168039, rs4705232, rs4705233, rs17096690, rs12517179, rs1432693, rs986673	rs2303067	rs10463395 rs1422998 rs1422997 rs6895394 rs7713918 rs3756688 rs1423007 rs9325064 rs986494 rs1423001 rs12108690 rs4529181 rs2303066 rs988885 rs1862439	0.0854 0.0857 0.1049 0.1206 0.1136 0.1038 0.0811 0.0923 0.0593 0.0908 0.0890 0.0799 0.0611 0.0664 0.0653	0.0867 0.0879 0.0416 0.0172 0.0239 0.0568 0.1119 0.0690 0.2597 0.0736 0.0796 0.1123 0.2067 0.1908 0.1982	[29]
ADRB2	5q31-32	rs17108682, rs13176278, rs9325115, rs2400642 rs1363543, rs6885118, rs1465405, rs6867058, rs6885216, rs919724, rs2082398, rs30309, rs30313, rs30319, rs30325, rs246503, rs246502, rs9285673, rs1347110, rs877741, rs877743, rs11168066, rs11959615, rs17778257, rs2400707, rs11957757, rs10066266, rs6897873, rs11168071, rs6580586, rs6884617, rs1432628, rs17640705, rs1560641, rs12520348, rs10042578, rs10036926, rs4705283, rs4705284, rs4705285, rs10875641, rs10075995, rs11746220, rs11744605,	rs1042714	rs11168066 rs11959615 rs2400707	-0.0733 -0.0775 -0.0607	0.1349 0.1356 0.2249	[30,31]

		rs17653203, rs17461431, rs4705291, rs4705292, rs11740851, rs12189287					
NOD1	7p15	rs38437, rs38449, rs38452, rs38466, rs38474, rs38488, rs2529439, rs11761080, rs12532057, rs16875290, rs2267710, rs975537, rs973002, rs929377, rs2240404, rs2190242, rs1003929, rs4723002, rs4723003, rs255121, rs255131, rs255152, rs255155, rs255156, rs255166, rs11771217, rs1971677, rs2075005, rs1061644, rs7810199	rs2907748	-	-	-	[32,33]
			rs2907749	-	-	-	
			rs2075822	-	-	-	
			rs2975632	-	-	-	
			ND1 +32656 ⁺ (rs6958571)	-	-	-	
			rs2736726	-	-	-	
			rs2075817	-	-	-	
			rs3020207	-	-	-	
			rs2075818	-	-	-	
			rs2235099	-	-	-	
			rs5743368	-	-	-	
GPRA	7p15-14	rs17198982, rs17788843, rs324978, rs324981, rs1419780, rs325464, rs17199659, rs17789420, rs17789642, rs17200135, rs17170015, rs17200455, rs10263313, rs11768189, rs17790961, rs4723388, rs2057819, rs2557768, rs1649218, rs1637670, rs1649238, rs1637682, rs10267957, rs328906, rs2023328, rs328928, rs2392303, rs329234, rs329235, rs329240, rs329251, rs329271, rs329277, rs329559, rs9648411, rs17158356	SNP546333 [§]	-	-	-	[34,35]
			rs740347	-	-	-	
IL18	11q22	rs10891323, rs578784, rs243908, rs544354, rs360729, rs1834481, rs360722, rs7106524, rs360718, rs10891337, rs11214127, rs4935984, rs11214136, rs2518345, rs3819331, rs2564873, rs2518360, rs17490906	rs360718	rs360718 rs10891337 rs11214127	-0.0441 -0.0137 -0.0149	0.4286 0.8105 0.7934	[36,37]
			rs360717	rs360718 rs10891337 rs11214127	-0.0441 -0.0137 -0.0149	0.4286 0.8105 0.7934	
			rs360721	-	-	-	
			rs1946519	-	-	-	
			rs187238	-	-	-	

STAT6	12q13	rs17119327, rs17119330, rs17119344, rs1552245, rs697222, rs17119386, rs2270737, rs7298475, rs17119416, rs12368672, rs4759277, rs1466535, rs7975818, rs1800176, rs4074308, rs1800168	rs3024974	rs17119327 rs17119330 rs17119344 rs1552245 rs697222 rs17119386 rs2270737 rs7298475 rs17119416	-0.0446 -0.0249 -0.0590 -0.0590 -0.1693 -0.0509 -0.0707 -0.0734 -0.0473	0.6214 0.7769 0.5134 0.5134 0.0288 0.5743 0.4350 0.4195 0.5911	[38,39,40]
			rs324011				
VDR	12q13	rs2072115, rs10747521, rs12818241, rs2544025, rs3782908, rs4760636, rs7418, rs2544029, rs2525051, rs7299689, rs7971594, rs7965281, rs10783215, rs2525046, rs7962898, rs11168268, rs2248098, rs2239182, rs2239180, rs12717991, rs2189480, rs2254210, rs11574046, rs2238136, rs4760658, rs4516035, rs11568820, rs7310552, rs7132324, rs10875700, rs7311856, rs4760671, rs7972027, rs11168314, rs11168316, rs10875704, rs10875705, rs10747527, rs10783221, rs11168325, rs11168326, rs1541408, rs2286024, rs2071358, rs1034762, rs7971880	rs2239185	-	-	-	[41,42]
			rs731236	rs2239182	-0.0331	0.5072	
			rs7975232	rs7965281 rs10783215 rs2525046 rs7962898 rs11168268 rs2248098	0.0182 0.0193 0.0303 0.0545 0.0364 0.0449	0.7130 0.6997 0.5426 0.2618 0.4657 0.3612	
				rs2239179	rs2239182	-0.0331	0.5072
				rs1540339	rs12717991	0.0544	0.2668
NOS1	12q24	rs7967489, rs7957302, rs957363, SNP_A-2052092, rs11068363, rs10850780, rs4238052, rs11068384, rs10774905, rs9658536, rs904658, rs9658498, rs3741476, rs10774909, rs2650163, rs12829185, rs816358, rs6490121, rs1607817, rs3825102, rs884847, rs7309163, rs532967, rs733334, rs7961147, rs478597, rs1483757, rs816293, rs471871, rs17509231, rs9658253, rs816292, rs12307921, rs492623, rs12826308,	rs2682826	rs10774909 rs12829185	0.0505 -0.0320	0.4174 0.6049	[43,44]

		rs1552229, rs1552230, rs1384873, rs10850820, rs7971155, rs11068469, rs10774915, SNP_A-1804024, rs708849, rs10850824, rs10774923, rs10850828, rs11068499, rs11068501, rs10774926, rs1093297, rs11068503, rs11068505, rs1068944, rs1580855, rs7298492, rs10850833					
PHF11	13q14	rs7338471, rs17072869, rs11617824, rs9535233, rs2057412, rs11619997, rs9591258, rs17072878, rs4942830, rs7992603, rs12429883, rs1980795, rs2031532, rs9568232, rs2981, rs3829366, rs6561534, rs9568239, rs3186024, rs7995684, rs1046028, rs3186013, rs7995006, rs3794376, rs1536192, rs7333969, rs3751383, rs17069328, rs9596148, rs9568258, rs9596150, rs9562900, rs11148150, rs11148151, rs882970, rs7981396, rs9526580, rs4941650, rs7327293, rs7331252, rs7332029, rs7335220	b5_2 ^{\$} b4_2 ^{\$} b5_3 ^{\$}	-	-	-	[45]
IL4RA-IL21R cluster	16p12-11	rs11641348, rs7186151, rs4547335, rs8059713, rs4395078, rs4547336, rs8057585, rs8044444, rs6498012, rs3024547, rs2239349, rs3024585, rs3024613, rs3024614, rs3024668, rs3024676, rs8832, rs16976728, rs4787426, rs7191188, rs6498015, rs6498016, rs2040788, rs6498017, rs722516, rs722517, rs1859308, rs7205704, rs8060368, rs2189521, rs11074858, rs7199138, rs8057551, rs8061992, rs3093315, rs3093317, rs3093341, rs3093366, rs3093367, rs3093378, rs12445873, rs12925626, rs232075, rs232081, rs232086, rs7198785, rs4787967	rs1805011	rs3024676	-0.0377	0.5731	[46,47,48]
			rs1805013	-	-	-	
			rs1801275	rs3024676	-0.0377	0.5731	
			rs1805012	rs3024676	-0.0377	0.5731	

CARD15	16q21	rs933566, rs8047222, rs745230, rs7199150, rs7186163, rs2066849, rs8050932, rs7202124, rs9933594, rs4785448, rs5743263, rs8057341, rs17221417, rs17312836, rs2066843, rs748855, rs1861758, rs1077861, rs3135499, rs8060598, rs3785142, rs4785450, rs17224078, rs1861760, rs4785460, rs6500336, rs9922324, rs16948899	rs2066845	-	-	-	[49,50]
			3020insC [§]	-	-	-	

[‡] location based on NCBI 36 entrez gene (<http://www.ncbi.nlm.nih.gov/sites/entrez>).

[§] reported variant associated with total IgE.

[#] references reporting association with total IgE (only positive association reports listed).

⁺ complex insertion–deletion polymorphism (indel), partially identified as rs6958571.

^{\$} no rs-number available.

Abbreviations: ADRB2 = beta-2-adrenergic receptor; CARD15 = nucleotide-binding oligomerization domain protein 2 (= NOD2); CD14 = monocyte differentiation antigen CD14; CTLA4 = cytoxic T lymphocyte-associated 4; FLG = filaggrin; GPRA = G protein-coupled receptor 154; IL4 = interleukin 4; IL4RA = interleukin 4 receptor, alpha; IL13 = interleukin 13; IL18 = interleukin 18; IL21R = interleukin 21 receptor; NOD1 = caspase recruitment domain-containing protein 4 (= CARD4); NOS1 = nitric oxide synthase 1; PHF11 = PHD finger protein 11; SPINK5 = serine protease inhibitor, Kazal-type, 5; STAT6 = signal transducer and activator of transcription 6; VDR = vitamin D receptor.

We searched public databases for published candidate gene association studies for total IgE. We list only those for which association with total IgE has been reported in at least two independent white study populations of sufficient size (>200 subjects). In some of the cases we were not able to

derive rs numbers. LD calculations are based on HapMap (HapMap Data Release 23a/PhaseII Mar08, on NCBI B36 assembly, dbSNP b126; <http://www.hapmap.org/index.html.en>). References refer to positive reports only.

Gene	Chromosomal location	SNPs covered by the Affymetrix screening panel (+/- 100kb)	Position	Estimate	P-value
SELP	1q22-25	rs7474070	167782247	-0.3007	0.0047
		rs10910879	167784811	0.1516	0.0209
		rs12404218	167790915	-0.2945	0.0054
		rs742058	167823078	-0.2670	0.0416
		rs1157459	167835109	-0.2845	0.0301
		rs7882773	167835265	-0.1532	0.0100
		rs2281006	167842527	0.1279	0.0342
		rs17565766	167847788	0.1272	0.0421
		rs17565793	167847925	0.1232	0.0492
		rs7063044	167848418	0.1265	0.0447
		rs6608452	167848771	0.1276	0.0422
		rs12720462	167949349	-0.1742	0.0400
DPP10	2q14	rs11123288	115680236	-0.1278	0.0293
		rs7568150	116139130	-0.1107	0.0452
		rs10184097	116162717	-0.1086	0.0475
		rs4849421	116189249	-0.1164	0.0350
		rs1374271	116251152	0.1385	0.0123
		rs6735355	116264756	-0.1424	0.0179
		rs3768692	116318073	-0.1584	0.0407
		rs7420865	116330344	-0.1721	0.0265
		rs272041	116345018	0.1261	0.0182
IRF1	5q31	rs274561	131747127	0.1109	0.0269
		rs2706347	131933016	0.2357	4.1x10 ⁻⁵
IL13	5q31	rs3798135	131993008	0.2271	0.0001
		rs2040704	132001076	0.2210	0.0001
		rs7737470	132001962	0.2312	4.8x10 ⁻⁵
		rs2158177	132011957	0.1834	0.0020
		rs20541	132023863	0.2040	0.0005
SPINK5	5q32	rs7707803	147368427	-0.1640	0.0234
		rs1422997	147373857	0.1049	0.0416
		rs6895394	147379211	0.1206	0.0172
		rs7713918	147393844	0.1136	0.0239
GPRA	7p15-14	rs324978	34587572	-0.1084	0.0472
		rs17200455	34651236	-0.1840	0.0033
		rs10263313	34654233	-0.1808	0.0040
		rs4723388	34716060	0.1647	0.0142
		rs329271	34849117	0.1656	0.0136
FCER1B	11q13	rs1286289	59583253	-0.1195	0.0258
		rs1286170	59588506	-0.1192	0.0231
		rs501697	59589699	-0.1276	0.0182
		rs528823	59593673	-0.1253	0.0205
		rs558788	59608654	-0.1196	0.0269
		rs558678	59608689	-0.1271	0.0202
STAT6	12q13	rs697222	55721389	-0.1693	0.0288
		rs12368672	55798737	0.1673	0.0009
		rs4759277	55819957	0.1464	0.0041
		rs1466535	55820737	0.1475	0.0041
NOS1	12q24	rs10850820	116293199	0.0986	0.0452
		rs10774923	116343062	0.1076	0.0267
		rs11068501	116357854	0.1377	0.0449
		rs11068503	116364878	0.0993	0.0452
IL4RA-IL21R Cluster	16p12-11	rs7186151	27156882	0.1200	0.0222

		rs3024613	27271754	0.1031	0.0382
		rs16976728	27289213	0.1059	0.0410
		rs7205704	27308394	0.1550	0.0056
		rs3093378	27365559	0.1156	0.0269
		rs12445873	27381715	0.1685	0.0117
		rs12925626	27384049	0.1676	0.0121
		rs232075	27423252	0.1701	0.0112
		rs232081	27436681	0.1596	0.0204
		rs7198785	27465250	0.1953	0.0032
		rs4787967	27466060	0.1971	0.0031
CARD15	16q21	rs9922324	49423561	0.2370	0.0364
ADAM33	20p13	rs464831	3501750	0.1319	0.0172
		rs235544	3508655	0.1253	0.0357

Genome-wide scan on total serum IgE levels identifies

FCER1A as susceptibility locus

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Supplementary Methods

Subjects and study design

A description of the GWA study population and the replication samples is given in Table S1.

KORA S3/F3 500K genotyping and quality control

Genotyping for KORA S3/F3 500K was performed using Affymetrix 500K Array Set consisting of two chips (Sty I and Nsp I). Hybridisation of genomic DNA was done in accordance with the manufacturer's standard recommendations. Genotypes were determined using BRLMM clustering algorithm (http://www.affymetrix.com/support/technical/whitepapers/brlmm_whitepaper.pdf). The genotypes were determined in batches of at least 400 chips. For quality control purposes, we applied a positive control and a negative control DNA every 48 samples. The overall genotyping efficiency of the GWA was 98.26 %. Before statistical analysis, we performed filtering of both conspicuous chips and SNPs based on quality measures to ensure robustness of association analysis. On chip level only subjects with overall genotyping efficiencies of at least 93% for both chips and at most one discordant call for 50 SNPs being on both chips were included. In addition the called gender has to agree with the gender in the KORA study database. On SNP level from a total of 500,568 SNPs, we excluded for the purpose of this analysis all SNPs on chromosome X leaving 490,032 autosomal SNPs for the GWA screening step. From these 353,569 (72.15%) SNPs passed all quality control criteria, and were selected for the subsequent association analyses. Criteria leading to exclusion were genotyping efficiency < 95% and minor allele frequency (MAF) < 3%. An exact Fisher test has been used to detect deviations from Hardy Weinberg Equilibrium, and we excluded all SNPs with P-values below 10^{-6} . Details on the number of excluded SNPs per quality criterion are described in Table S2.

Detailed information on SNP selection for replication and finemapping

For replication in KORA S4 (population-based, n=3,890), we used the following inclusion criteria:

- (i) $P < 10^{-4}$ in stage 1 analysis
- (ii) $P < 10^{-3}$ with at least one neighboring SNP (± 100 kb) with $P < 10^{-3}$

Details on the SNPs selected for replication are shown in Table S3.

After we had identified the *FCER1A* and *RAD50* region through the genome-wide scan and replication, we additionally analysed 6 tagging SNPs in *FCER1A* as well as 4 tagging SNPs in *RAD50* using the pairwise tagging algorithm ($r^2 > 0.8$) implemented in HAPLOVIEW 3.3 (HapMap data release #22, March 2007, on NCBI B36 assembly, dbSNP b126. In addition, two previously described functional SNPs of *FCER1A* [1,2] and 2 SNPs in intron 24 of the *RAD50* hypersensitive site 7 (RHS7) [3] were genotyped.

SNP genotyping and quality control in the replication samples

Genotyping of SNPs was performed with the iPLEX™ (Sequenom San Diego, CA, USA) method by means of matrix assisted laser desorption ionisation-time of flight mass spectrometry method (MALDI-TOF MS, Mass Array™, Sequenom, San Diego, CA, USA) according to the manufacturers instructions.(www.sequenom.com). Shortly, 5 ng of genomic template DNA were amplified applying 5 units of HotStarTaq DNA Polymerase (Qiagen, Hilden, Germany). Thermocycling started with a single denaturation step for 15 min at 95°C, followed by 45 cycles of denaturation for 20 sec at 95°C, annealing for 30 sec at 56°C and extension at 72°C for 1 min, finally followed by 72°C for 10 min.

Subsequently PCR products were treated with shrimp alkaline phosphatase (SAP, Amersham, Freiburg, Germany) for 20 min at 37°C to remove excess dNTPs followed by 10 min at 85°C to inactivate SAP (Table S3). Base extension (iPLEX™ Sequenom, San Diego, CA) reactions in a final volume of 10 µl contained extension primers at a final concentration of 0.635 µM and 0.6 units ThermoSequenase (Amersham, Freiburg, Germany).

Base extension reaction conditions were 94°C for 2 min, followed by 40 cycles of 94°C for 5 sec, 52°C for 5 sec, and 72°C for 5 sec. Afterwards the base extension products were treated with SpectroCLEAN resin (Sequenom, San Diego, CA, USA). 20 nanoliters of the reaction solution were spotted onto a 384 format SpectroCHIP microarray (Sequenom, San Diego, CA, USA) presloaded with a matrix of 3-hydroxypicolinic acid (3-HPA). A modified Bruker Biflex MALDI-TOF MS was used for data acquisitions from the SpectroCHIP. Genotype calling was performed in real time with MassARRAY RT software version 3.0.0.4 (Sequenom, San Diego, CA, USA). For quality control 5% of the selected samples were genotyped in duplicate and in addition 4% of negative controls were used.

Primer sequences of the SNPs selected for replication and fine mapping are shown in Table S4.

Mutational analysis *FCER1A*

FCER1A exons were amplified with intronic primers and were directly sequenced using a BigDye Cycle sequencing kit (Applied Biosystems).

Details are provided in Tables S5 and S6.

Haplotype analysis

Haplotype reconstruction and association analysis from the unphased genotypes was performed on the KORA S4 replication sample using the SNP tagger implemented in HAPLOVIEW 3.3.2 [4] and the function *haplo.glm* implemented in the S-PLUS/R library *HaploStats* [5] (<http://mayoresearch.mayo.edu/mayo/research/biostat/splusfunctions.cfm>). The latter computes maximum likelihood estimates of the haplotype probabilities using an EM algorithm. All common haplotypes were included in the linear regression; the most common haplotype served as reference. Rare haplotypes (frequency <1%) were pooled and included in the model as one group. Each possible haplotype combination per individual entered the additive model as a weighted observation. The weights were calculated from the

posterior probabilities of the respective pair of haplotypes. Haplotype analysis was performed on all *FCER1A* and *RAD50* SNPs replicated in KORA S4. Results of the haplotype analysis, which was computed adjusted for gender and age, are reported in Table S7.

Analysis of population sub-structure

The association is very unlikely to be due to population stratification for two reasons: First, if the result would indeed be caused by population stratification, this stratification would have been the same in the KORA, LISA, GINI and ISAAC Study with the same substructures and the same extent of substructure in each of the three samples. Second a recent experimental assessment has found little population stratification to exist within and across Germany [6]. To test for possible population stratification in KORA S3/F3 500K an EIGENSOFT analysis was performed as described in [7]. A genomic control analysis led to a value of λ , the inflation factor, of 1.09.

Extended SNP analysis in the *RAD50-IL13* region in a subset of 526 children from the ISAAC replication cohort

Table S8 shows details of the additional SNPs typed in a subset of the ISAAC cohort and their association with total IgE. We report the p-values of a t-test in a dominant model. All tests are based on log-transformed IgE-values. Figure S2 shows the patterns of pairwise LD between the SNPs at the *RAD50-IL13* locus.

Additional association analysis of *FCER1A* and *RAD50* with AE and asthma

Associations of *FCER1A* and *RAD50* variants tagging the significant SNPs from the GWAS and replication studies on total IgE with the dichotomous traits AE and asthma were analysed in a cohort of 562 German parent-offspring trios for AE and a cohort of 638 asthma cases and 633 controls. German trios and UK asthma cases and controls were recruited and phenotyped as previously described [8,9]. In the family-setting we analysed association of

single SNPs with eczema using the classical transmission disequilibrium test (TDT), while association with log-transformed total IgE values was analysed with the quantitative version of the TDT (QTD). For association between asthma status and SNP genotype we fitted parametric model using the LAMP program. The program estimates disease allele frequency, a SNP allele frequency and three penetrances (disease prevalence is set to 5%) by assuming a multiplicative model and ignored linkage [10,11]. Association analysis of IgE was applied with MERLIN (-fastAssoc option). We fitted a simple regression model and used a variance component approach to account for the correlations between family members. The program estimates the additive effect of each SNP on the level of IgE and imputes missing genotype with expected genotype score using the Lander-Green algorithm [12,13].

Results are shown in Table S9.

Post-hoc analysis of previously reported candidate genes

In a secondary approach, we also examined the results of the GWAS with respect to previously reported candidate genes for total IgE (Table S10). To this end, we searched the public databases for candidate genes for total IgE identified in at least two independent cohorts of substantial size (>200 subjects).

We list all SNPs from within or around ($\pm 100\text{kb}$) these genes covered by the affymetrix 500k screening panel. In addition, we checked for affymetrix SNP(s) in LD ($r^2 > 0.6$) with reported variants and their association with total IgE in our GWAS. Occasionally it was not possible to pinpoint to one SNP as being more important among a number of SNPs with equally strong and potentially even independent effects ("multiple SNPs"). In addition, in some of the cases we were not able to derive rs numbers.

Table S11 shows all affymetrix SNPs from within or around ($\pm 100\text{kb}$) selected candidate genes which yielded a nominal p-value < 0.05 .

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