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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Genetic loci determining total immunoglobulin E levels from birth through adulthood

To the Editor,

Total immunoglobulin E (IgE) is an intermediate phenotype and a potential therapeutic target for allergic diseases. Recent genome-wide association studies (GWASs) have discovered single-nucleotide polymorphisms (SNPs) to be associated with IgE levels, including genes residing within the major histocompatibility complex (MHC) region across several populations.¹⁻⁵ However, limited susceptible genes from GWASs for IgE levels have been reported in Asian populations, while only Yatagai et al⁵ revealed SNPs within the human leukocyte antigen (*HLA*)-C region are associated with IgE levels in 1180 Japanese adults. Here, we aimed to investigate potential functional influence of susceptible genes associated with IgE levels by performing integrative analyses.

We conducted a GWAS of total IgE levels in a Taiwanese population (415 subjects recruited from the Prediction of Allergies in Taiwanese CHildren (PATCH) schoolchildren cohort⁶) and performed replication analyses in three independent cohorts: a Taiwanese birth cohort (431 newborns from the PATCH birth cohort,⁷ which is different to the children enrolled in the PATCH schoolchildren cohort), a Taiwanese schoolchildren cohort (another independent 838 PATCH schoolchildren), and a Caucasian adult cohort (1120 adults from the Chicago Food Allergy Study⁸). Characteristics of the discovery and replication samples are presented in Table S1. The study design and analysis pipeline are shown in Figure S1. Detailed methods are provided in the Supporting information.

Nine independent SNPs associated with IgE levels were identified at a suggestive significance level $P < 10^{-5}$ in the discovery sample (Table 1; Figure 1A) and only rs660895 was replicated in three independent samples ($P = 2.69 \times 10^{-3}$ for independent schoolchildren; $P = 1.90 \times 10^{-2}$ for birth cohort; $P = 3 \times 10^{-3}$ for adult cohort; $p_{\text{meta-analysis}} = 3.24 \times 10^{-10}$ for all study cohorts). The regional association plot for rs660895 in the *HLA-DRB1/DQA1/DQB1* gene region at 6p21.32 is presented in Figure 1B. We did not genotype those associated SNPs downstream *HLA-DQB1* for replication

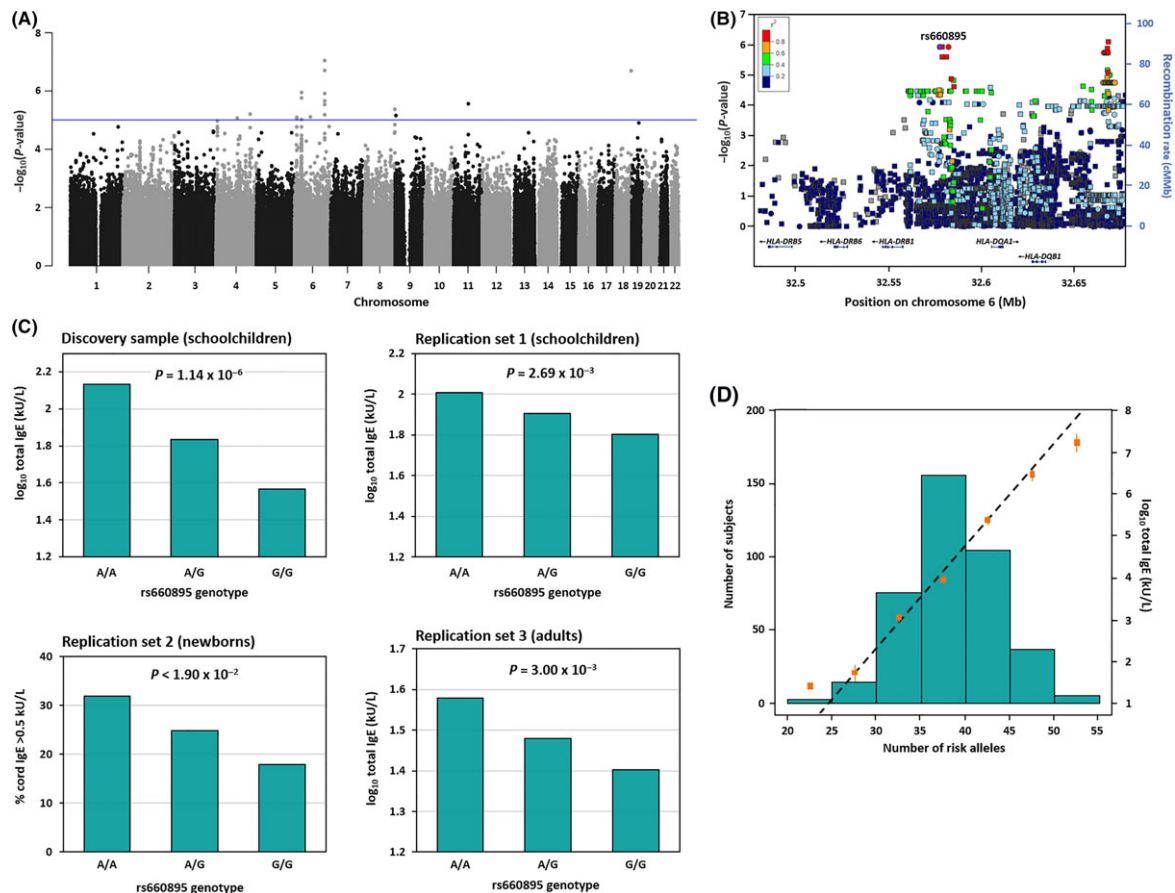
because they are in strong linkage disequilibrium (LD) with SNP rs660895 ($r^2 > 0.8$). Lower IgE levels were found in subjects carrying one or two copies of the rs660895-G alleles in all study samples of schoolchildren, newborns, and adults (Figure 1C). No SNP at a genome-wide significance level was identified in the discovery cohort and only one of nine potentially associated SNPs was replicated in three independent cohorts, which might reflect the low power due to limited sample size.

We performed secondary analysis with IgE-related phenotypes, specifically, atopy, allergic rhinitis, asthma, eczema, and either one of allergic rhinitis, asthma, or eczema using 1235 PATCH schoolchildren. Rs660895 was associated with atopy ($P = 1.81 \times 10^{-3}$), allergic rhinitis ($P = 1.20 \times 10^{-4}$), and either one of allergic rhinitis, asthma or, eczema ($P = 2.31 \times 10^{-3}$) (Table 1). Seven of the 9 IgE-associated SNPs identified in the discovery analysis were associated with atopy, indicating their potential broader relevance to atopy (Table 1).

To evaluate potential functional influence, we mined two eQTL databases in four specific tissues (blood, skin, lung, and lymphoblastoid cells). The minor allele of rs660895-G was associated with lower expression of *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* genes in all four tissues, suggesting potential regulatory function of rs660895. We performed SNP-methylation associations using 34 945 CpG sites on chromosome 6 in 218 whole blood samples from the Chicago Food Allergy Study. We found that methylation levels at 27 of the 53 probes in the *HLA-DRB1/DQA1/DQB1* region were associated with genotypes at SNP rs660895 in the Chicago Food Allergy Study after correcting for multiple testing (Table S2). Three peanut-allergy-related differentially methylated probes (DMPs),⁸ cg15982117 ($p_{\text{FDR}} = 1.77 \times 10^{-2}$), cg17039645 ($p_{\text{FDR}} = 7.23 \times 10^{-4}$), and cg18111114 ($p_{\text{FDR}} = 1.20 \times 10^{-6}$) were associated with SNP rs660895, further supporting potential functional influence of SNP rs660895 not only on total IgE but also on allergic diseases (Table S2). Among the three peanut-allergy-related DMPs, cg15982117 and cg18111114 locate in the *HLA* gene region. In

TABLE 1 Genome-wide association results of total IgE levels in discovery and replication in PATCH schoolchildren^a and secondary analysis for association with IgE-related phenotypes in the combined PATCH schoolchildren samples (n = 1235)

Band	SNP	Minor allele	Discovery (n = 397)		Replication (n = 838)		Combined schoolchildren (n = 1235)	
			β (95% CI)	P-value ^b	β (95% CI)	P-value	Atopy OR ^c (95% CI)	P-value
4q23	rs34879386	T	0.51 (0.29, 0.73)	8.52×10^{-6}	-0.02 (-0.18, 0.14)	8.30×10^{-1}	1.55 (1.03, 2.34)	3.42×10^{-2}
4q32.1	rs2348622	A	0.60 (0.35, 0.86)	6.28×10^{-6}	0.09 (-0.10, 0.27)	3.65×10^{-1}	2.63 (1.54, 4.51)	4.20×10^{-4}
6p21.32	rs660895	G	-0.27 (-0.37, -0.16)	1.14×10^{-6}	-0.12 (-0.19, -0.04)	2.69×10^{-3}	0.74 (0.61, 0.89)	1.81×10^{-3}
6q13	rs16885806	C	0.28 (0.16, 0.40)	5.72×10^{-6}	-0.001 (-0.09, 0.09)	9.81×10^{-1}	1.41 (1.13, 1.78)	2.98×10^{-3}
6q24.1	rs9376674	A	0.37 (0.24, 0.51)	9.12×10^{-8}	-0.07 (-0.17, 0.02)	1.35×10^{-1}	1.17 (0.92, 1.49)	1.95×10^{-1}
8q24.3	rs55869541	C	-0.24 (-0.35, -0.14)	4.34×10^{-6}	-0.05 (-0.13, 0.02)	1.74×10^{-1}	0.89 (0.74, 1.07)	2.05×10^{-1}
9p24.2	rs497374	T	0.59 (0.34, 0.85)	7.06×10^{-6}	0.16 (-0.02, 0.33)	7.84×10^{-2}	1.61 (1.01, 2.57)	4.50×10^{-2}
11q13.3	rs11263535	G	-0.30 (-0.43, -0.18)	2.78×10^{-6}	-0.02 (-0.12, 0.07)	6.39×10^{-1}	0.73 (0.58, 0.91)	6.11×10^{-3}
18q23	rs7236372	T	-0.29 (-0.40, -0.18)	2.03×10^{-7}	0.02 (-0.06, 0.10)	5.54×10^{-1}	0.77 (0.63, 0.93)	7.66×10^{-3}

^aSubjects included in the discovery stage and replication were recruited from the two independent PATCH schoolchildren cohorts.^bBold values indicated either *p*-value less than 10^{-5} in the discovery stage or *p*-value less than 10^{-2} in the replication stage and secondary analysis.^cAdjusted covariates included age, sex, and serum cotinine levels.**FIGURE 1** A, Manhattan plot for genome-wide association of 2 258 185 SNPs with total IgE levels. Note: The blue line shows the threshold ($P = 10^{-5}$) for selection of genomic regions for further replication analysis. B, Regional association plot at the 6p21.32 region for total IgE levels. Note: Circle dots are genotyped SNPs and square dots are imputed SNPs. C, Association of subjects carrying different genotypes of rs660895 with total IgE levels in the discovery PATCH schoolchildren cohort (top-left), replication PATCH schoolchildren cohort (top-right), replication PATCH birth cohort (bottom-left), and the Chicago Food Allergy Study (bottom-right). D, The combined genetic effects of 40 SNPs on total IgE levels in the PATCH schoolchildren cohort. Note: Subjects are binned by total number of alleles associated with increased total IgE levels with adjustment of age, sex, and serum cotinine levels (x-axis); the right y-axis presents total IgE levels; and the number of subjects in each bin is provided on the left y-axis. The regression line representing the correlation between total IgE levels and number of risk alleles is shown in black dash line.

Allergic rhinitis		Asthma		Eczema		Asthma/Allergic rhinitis/Eczema	
OR ^c (95% CI)	P-value	OR ^c (95% CI)	P-value	OR ^c (95% CI)	P-value	OR ^c (95% CI)	P-value
1.15 (0.77, 1.72)	4.85×10^{-1}	1.00 (0.54, 1.89)	9.82×10^{-1}	0.47 (0.25, 0.90)	2.30×10^{-2}	1.08 (0.73, 1.59)	6.91×10^{-1}
1.19 (0.75, 1.89)	4.55×10^{-1}	0.66 (0.28, 1.56)	3.40×10^{-1}	1.26 (0.74, 2.15)	3.97×10^{-1}	1.21 (0.77, 1.89)	4.18×10^{-1}
0.67 (0.55, 0.82)	1.20×10^{-4}	0.96 (0.71, 1.31)	8.17×10^{-1}	0.95 (0.75, 1.20)	6.63×10^{-1}	0.74 (0.61, 0.89)	2.31×10^{-3}
1.13 (0.90, 1.42)	2.89×10^{-1}	1.22 (0.87, 1.72)	2.46×10^{-1}	1.18 (0.89, 1.54)	2.45×10^{-1}	1.19 (0.95, 1.48)	1.24×10^{-1}
1.17 (0.92, 1.49)	2.06×10^{-1}	0.93 (0.63, 1.37)	7.15×10^{-1}	1.15 (0.86, 1.54)	3.43×10^{-1}	1.14 (0.90, 1.45)	2.64×10^{-1}
0.84 (0.69, 1.01)	7.47×10^{-1}	0.98 (0.73, 1.31)	8.66×10^{-1}	0.89 (0.71, 1.13)	3.43×10^{-1}	0.85 (0.70, 1.02)	7.47×10^{-1}
0.99 (0.63, 1.57)	9.88×10^{-1}	0.83 (0.39, 1.77)	6.32×10^{-1}	1.47 (0.89, 2.42)	1.33×10^{-1}	0.99 (0.64, 1.54)	9.83×10^{-1}
1.11 (0.88, 1.40)	3.88×10^{-1}	1.00 (0.70, 1.44)	9.69×10^{-1}	1.06 (0.79, 1.39)	6.97×10^{-1}	1.12 (0.89, 1.41)	3.13×10^{-1}
0.97 (0.79, 1.19)	7.82×10^{-1}	0.86 (0.63, 1.18)	3.59×10^{-1}	1.13 (0.88, 1.44)	3.37×10^{-1}	1.04 (0.86, 1.27)	6.78×10^{-1}

addition, a previous epigenome-wide association study revealed that DMPs in the *HLA-DQB1* gene region were likely responsible for IgE-mediated food allergy and also might be relevant to the potential pathway named "intestinal immune network for IgA production."⁹ By combing our and others' findings, we hypothesized that the identified SNP rs660895 may impact DNA methylation level of *HLA-DRB1/DQA1/DQB1* gene regions, which subsequently alters gene expression and results in potential functional implication. These hypotheses, however, await further validation by performing genotyping, DNA methylation profiling, and gene expression measurement in a single cohort.

To account for SNPs with moderate effect size, we used multi-marker modeling to determine a minimum set of 40 SNPs with $P < 1.05 \times 10^{-4}$ that best predict IgE levels. Among genes corresponding to the 40 SNPs, we discovered additional related genes, including *AP1AR*, *HLA-C*, and *SYK*. Figure 1D shows that subjects carrying increasing number of risk alleles among the 40 SNPs had increasing IgE levels.

Furthermore, we performed gene-based analysis and identified 87 IgE-associated genes. The results from gene ontology analysis suggested that 14 out of the 87 genes (*ADGRG6*, *CHST8*, *CORO2A*, *CSF2RB*, *FGF4*, *HCG27*, *HLA-DQA1*, *HLA-DQA2*, *HLA-DQB1*, *HLA-DRB1*, *IL27*, *PMS1*, *ROS1*, and *TPMT*) were potentially relevant to immunological diseases (P -values ranging from 4.93×10^{-2} to 9.77×10^{-8}). The network plot shows that the 14 genes either directly or indirectly interact with other genes in the networks linking to MHC or immunoglobulin (Figure S2). Nonetheless, replication analyses have not been performed yet for the results from the multi-marker analyses or the gene-based analyses. It would be of interest to further validate the results observed in this study.

It is known that IgE levels vary across different age groups, as well as different ethnic groups. Additionally, recent GWASs suggested that *HLA-DQ* and/or *HLA-DR* genes in the 6p21.32 region may harbor genetic variants associated with IgE levels and related

allergic diseases.^{2-4,10,11} But to our knowledge, no studies have simultaneously investigated whether susceptible genetic variants in this region have consistent impact on IgE levels across different age groups, specifically from birth to adulthood.

In summary, our results discovered that rs660895 in the *HLA-DRB1/DQA1/DQB1* gene region residing at 6p21.32 was associated with IgE levels and replicated in our two independent Taiwanese pediatric cohorts (birth and schoolchildren cohorts), as well as in Caucasian adults in the Chicago Food Allergy Study. Rs660895 identified in this study was in various degrees of LD (r^2 ranging from 10^{-4} to 0.23) with alleles reported by previous GWASs.^{2,10,11} We also found that rs660895 was associated with atopy, allergic rhinitis, and either one of allergic rhinitis, asthma, or eczema, suggesting that rs660895 may broadly affect risk for allergic diseases. In terms of functional relevance, our results indicated that the minor allele of rs660895-G was associated with reduced expressions of *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQB1* genes in four examined tissues; rs660895 was associated with three peanut-allergy-related DMPs, lending further support to the potential functional impact of rs660895, or alleles in LD with it. As such, it is likely rs660895 may play a functional role in not only regulating IgE levels, but also triggering other allergy-related diseases. Multi-marker modeling further identified a set of 40 SNPs that were jointly associated with IgE levels, showing that subjects carrying more risk alleles at those associated SNPs tended to have elevated IgE levels. The results from gene-based, gene ontology, and network analyses provide suggestive evidence that the identified 14 genes were related to immune response in function. Together, further validation and functional studies through different integrative approaches are required to better understand the role of *HLA-DR* and/or *HLA-DQ* genes in IgE regulation.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Allergen-specific IgE over time in women before, during and after pregnancy

To the Editor,

A strong association has been found between IgE reactivity to allergens and asthma, eczema and rhinitis but these diseases also exist in non sensitized individuals.¹ During pregnancy, IgG, IgM and IgA have been found to decrease between first and third trimester and to increase postpartum but the decrease in IgG was mainly due to hemodilution.² For IgE, studies report a decrease in total IgE concentration in plasma from pregnancy to after delivery in allergic mothers³; however, this decrease was not observed for allergen-specific IgE (asIgE) to common inhalant allergens.³ In order to tolerate the semiallogeneic fetus, a regulated homeostasis between the Th1/Th2/Th17 (with additional Th9 and Treg) cell subsets and their dynamic and complex inter-relationship is essential during pregnancy.^{4,5} To our knowledge, no study has included measures of asIgE before conception to study the trajectory of asIgE in sensitized women. Our aim was to investigate changes of asIgE before, during and after pregnancy.

In the prospective longitudinal cohort study Born into Life, we followed 106 women during 2010-2013 living in Stockholm before, during and after pregnancy.⁶ In total, 413 blood samples were collected at baseline (before conception, $n = 102$), at gestational week 10-14 ($n = 64$) and 26-28 ($n = 93$), at admission to hospital ($n = 74$), and 3 days after delivery ($n = 80$). Plasma from these blood samples at all time points were analyzed for IgE reactivity against a mixture of 11 inhalant allergens, Phadiatop[®] and 6 food allergens, fx5[®] (Thermo Fisher Scientific, Uppsala, Sweden). If the test screened positive for Phadiatop[®] ≥ 0.35 kU_A/L, asIgE antibodies to the most common single airborne allergens, cat, dog, horse-epithelium, birch and timothy were analyzed. We did not include house dust mite allergens, since they are not abundant in Sweden. If screening was positive for fx5[®] ≥ 0.35 kU_A/L, the sample was subsequently analyzed for asIgE antibodies to the single allergens extract of egg, milk, cod, wheat and soy. To investigate change over time in each specific allergen (as well as all

airborne allergens taken together), a mixed tobit model was applied. Correction for multiple testing was made by the Bonferroni method. Since very few women ($n = 5$) tested positive for fx5[®], we only present descriptive statistics for these food allergens. Details on study population, data-sources, variables and statistical analyses are provided in this article's online supporting information. The Regional Ethics Review Board in Stockholm, Sweden, granted ethical approval. Written informed consent was obtained from the study participants.

In total, $n = 38$ women screened positive for Phadiatop[®], $n = 5$ women screened positive for fx5[®], $n = 4$ women screened positive for both Phadiatop[®] and fx5[®] and $n = 67$ women were negative for both Phadiatop[®] and fx5[®]. See Table S1 for baseline descriptive in this article's online supporting information.

Figure 1A and Table S2 in this article's online supporting information display the geometric mean for IgE airborne allergens concentration over time with 95% Confidence Interval (CI). The highest geometric mean concentration of asIgE for all time points was found for birch. The geometric mean concentration of IgE to birch was 0.57 (95% CI 0.39-0.83) kU_A/L. At baseline, before conception, the IgE concentration to birch was 0.41 (0.19-0.88) kU_A/L and 3 days after delivery 0.72 (0.31-1.70) kU_A/L. The geometric mean concentration of IgE to cat was 0.21 (0.16-0.28) kU_A/L, at baseline 0.21 (0.11-0.40) kU_A/L and 0.24 (0.09-0.63) kU_A/L week 10-14 during pregnancy. Figure 1B shows the specific food allergens over time with 95% CI where IgE to soy displays the highest concentration although low in numbers (max $n = 5$) for all food allergens, Table S2.

Table 1 shows results from the multivariate mixed tobit model. Levels of IgE to birch increased significantly over time, with $\beta = 1.006$ (95% CI: 1.002-1.010), which means that by 1 week progress in pregnancy the IgE geometric mean concentration increased by 0.6%. This remained significant after correction for multiple testing using Bonferroni, with similar results for those with at least one test above 0.10 kU_A/L. The largest regression coefficient was found when all airborne allergens were taken together in one analysis, $\beta = 1.007$, and the smallest was seen for timothy, $\beta = 1.003$ however nonsignificant.

Supporting Information

Genetic Loci Determining Total Immunoglobulin E Levels from Birth through Adulthood

DETAILED METHODS

Study subjects

Prediction of Allergies in Taiwanese Children (PATCH) schoolchildren cohort. PATCH schoolchildren cohort is a prospective population-based cohort study launched in 2007 to investigate the epidemiology and predictive factors of asthma and allergies in schoolchildren. Detailed descriptions of the PATCH schoolchildren cohort has been reported previously^{E1}. Of the 1,315 subjects who had total immunoglobulin E (IgE) measurement, 1,253 subjects had blood samples with sufficient DNA for genotyping were included in this study (415 in the discovery cohort (median (interquartile range (IQR)) of age: 10.35 years (8.32-12.20)) and 838 in the replication cohort (median (IQR) of age: 9.99 years (8.23-11.68))).

PATCH birth cohort. Four hundred and thirty one newborns who had cord blood IgE data and had blood samples with sufficient DNA for genotyping were included as an independent replication cohort. Detailed descriptions has been described previously^{E2}.

Chicago Food Allergy Study. Further replication was performed in 1,120 Caucasian adults from the Chicago Food Allergy Study (median (IQR) of age: 38.18 years (34.86-41.54)) with total IgE, genotype and DNA methylation data^{E3}.

Measurement of total IgE levels

Serum total IgE levels and cord blood IgE levels were measured in duplicates by ImmunoCAP (Phadia, Uppsala, Sweden).

Definition of IgE-related phenotypes

Atopy was defined as a positive Phadiatop Infant test result (≥ 0.35 PAU/L). Subjects with ≥ 0.35 PAU/L were defined as having atopy and with < 0.35 PAU/L as having non-atopy. Physician diagnosis of allergic rhinitis, asthma, and eczema were obtained using a modified International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire reported by parents of the study schoolchildren.

Genome-wide association and replication analysis

Genotyping and quality control: In the discovery stage, genome-wide genotyping was performed using the Illumina HumanOmni2.5-4v1 array (Illumina, San Diego, CA, USA) for 415 PATCH schoolchildren. Quality assurance (QA) and quality control (QC) assessments were performed based on the protocol described in PLINK^{E4}. A total of 2,258,185 SNPs from 397 unrelated schoolchildren were included for subsequent analyses.

Thirty-eight SNPs had suggestive significance ($p < 10^{-5}$) in the discovery stage and 9 with linkage disequilibrium (LD) $r^2 < 0.5$ (default option in PLINK) were selected from the 38 SNPs for replication genotyping.

Imputation: We performed imputation in the discovery sample using the IMPUTE2 software^{E5}, based on all populations in the 1000 Genomes Project as a reference panel. We applied the post-imputation filtering by excluding variants with imputation info scores < 0.9 . As a result, there were 6,073,890 genotyped and imputed SNPs on the autosomal chromosomes for subsequent data analyses in the discovery stage.

Association analysis: We performed genome-wide associations of SNPs with serum total IgE using linear regression models implemented in PLINK. We used an additive genetic model, adjusting for age, sex, serum cotinine levels and physician-diagnosed asthma. We also used linear regression to assess association in the replication analyses including the same covariates. LocusZoom was used to generate regional association plots^{E6}.

Meta-analysis: We performed meta-analysis for the discovery and three replication cohorts using the z-statistics method implemented in the META program^{E7}.

Secondary analysis: In addition to serum total IgE, we performed logistic regression models and tested the association of each replicated SNP with IgE-related phenotypes in the 1,235 PATCH schoolchildren, including atopy (yes/no), allergic rhinitis (yes/no), asthma (yes/no), eczema (yes/no), and either one of allergic rhinitis, asthma or eczema (yes/no). In these analysis, we adjusted for age, sex and serum cotinine levels.

Expression quantitative trait locus (eQTL) analysis

We further queried whether SNP rs660895 has been reported as an expression quantitative trait locus (eQTL) in two eQTL databases, Genotype-Tissue Expression browser (GTEx, <https://www.gtexportal.org/home/>) and HaploReg (<http://archive.broadinstitute.org/mammals/haploreg/haploreg.php>). Because serum

total IgE levels are highly associated with allergic disorders, we searched gene expression in four relevant tissue types: blood, skin, lung and lymphoblastoid cells.

SNP-methylation association tests

Using available DNA methylation data measured by the Infinium HumanMethylation450 BeadChips obtained from the Chicago Food Allergy Study, we tested associations between methylation QTL (meQTL) on chromosome 6 and SNP rs660895 identified from GWAS using 218 whole blood samples in the Chicago Food Allergy Study. Details of the data handling has been reported previously^{E3}. Linear regression models with adjustment of age, sex and estimated cell composition were employed using the 'limma' package in R/ Bioconductor^{E8}, with ComBat-transformed M-values at each CpG site.

Multi-marker modeling

It has been noted that some underlying associated SNPs may not reach genome-wide levels of significance in single-SNP analyses due to their small effect size, but these truly associated, small-effect SNPs may jointly affect total IgE levels. We therefore conducted multi-SNP modeling using the empirical Bayesian elastic net (EBEN) implemented in the EBEN R package^{E9}. Due to the high number of SNPs and relatively small sample size, a pre-screening step is typically recommended to enhance the computational feasibility and stabilize the numerical performance when fitting a penalized regression on GWAS SNPs^{E10}. First, from a total of 6,073,890 genotyped and imputed SNPs, we performed LD pruning to remove strongly correlated SNPs (i.e. $r^2 > 0.5$) using PLINK and resulted in 428,105 SNPs. We then applied the adaptive false negative control (AFNC) procedure on the marginal GWAS p-values^{E11} using the AFNC R package and selected the top 211 SNPs (corresponding to $p\text{-value} < 1.05 \times 10^{-4}$). AFNC is a signal inclusion procedure that is based on controlling false negative rate and aims to include a large proportion of associated SNPs with high confidence while not incurring too many unnecessary null SNPs. Finally, we performed the EBEN analysis on the AFNC selected SNPs, and identified a set of 40 SNPs for estimating polygenic risk effect on total IgE levels, adjusting for covariates, such as age, gender, serum cotinine levels and physician-diagnosed asthma.

Gene ontology and network analysis

In addition to the single-SNP association analyses, we also performed gene-based association analysis using the extended Simes procedure (GATES)^{E12}

implemented in the KGG software package. SNPs that were within 50 kb upstream or downstream to a gene were assigned to the gene for the analysis. Given the gene-level p -values, we applied AFNC to select a set of promising genes for the gene ontology and network analyses so to evaluate potential biological and functional relevance of the identified signals. The later analyses were carried out to evaluate potential biological functional relevance for the identified gene set through the Ingenuity Knowledge Base implemented in a bioinformatics application, the Ingenuity Pathway Analysis (IPA) (QIAGEN Inc., <http://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis>). Fisher's exact test was used to determine significant signals from the gene ontology and network analyses. Statistical significance was declared using a p -value less than 0.05.

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Table S1. Characteristics of the study subjects

	Discovery	Replication		
	Schoolchildren (<i>n</i> =415)	Schoolchildren (<i>n</i> =838)	Newborns (<i>n</i> =431)	Adults (<i>n</i> =1,120)
Age (y), mean $\pm$ SD	10.51 $\pm$ 2.85	10.12 $\pm$ 2.44	0	38.58 $\pm$ 5.52
Female sex, <i>n</i> (%)	210 (50.60%)	433 (51.67%)	194 (45.01%)	541 (48.3%)
Serum IgE (kU/L), mean $\pm$ SD ^a	325.43 $\pm$ 590.62	272.59 $\pm$ 493.27	NA	93.79 $\pm$ 259.55
Cord blood IgE (kU/L), mean $\pm$ SD ^b	NA	NA	0.96 $\pm$ 4.76	NA
Atopy ^c	237 (57.1%)	481 (57.4%)	NA	NA

NOTE

IgE, immunoglobulin E; SD: standard deviation; NA, not applicable.

^a The suggestive cutoff value of serum total IgE for schoolchildren is 77.7 kU/L^{E13} and for adults is 100 kU/L^{E14}.

^b 130 of 431 newborns (30.2%) had cord blood IgE > 0.5 kU/L.

^c Atopy was defined as a positive Phadiatop Infant test result ($\geq$ 0.35 PAU/L).

Table S2. Methylation probes locating in the *HLA-DRB1/DQA1/DQB1* gene region are associated with SNP rs660895 in the Chicago Food Allergy Cohort.

Probe	Chromosome	Nearest gene	Gene location	Position	SNP rs660895	
					β	$p_{\text{FDR}}^{\dagger}$
cg10765922	6p21.32	<i>HLA-DRB1</i>	Gene body	32552022	15.04	4.97×10^{-8}
cg24147543	6p21.32	<i>HLA-DRB1</i>	Gene body	32554481	4.14	2.51×10^{-3}
cg24760581	6p21.32	<i>HLA-DRB1</i>	TSS1500	32557970	33.23	4.35×10^{-16}
cg00598125	6p21.32	<i>HLA-DRB1</i>	Gene body	32555411	5.42	7.28×10^{-4}
cg26036029	6p21.32	<i>HLA-DRB1</i>	Gene body	32552443	7.71	7.59×10^{-5}
cg00211215	6p21.32	<i>HLA-DRB1</i>	Gene body	32552246	36.42	1.61×10^{-17}
cg24242384	6p21.32	<i>HLA-DRB1</i>	Gene body	32551954	15.01	4.97×10^{-8}
cg17480035	6p21.32	<i>HLA-DRB1</i>	Gene body	32552670	44.17	6.29×10^{-21}
cg19575208	6p21.32	<i>HLA-DRB1</i>	Gene body	32551888	2.78	9.17×10^{-3}
cg07984380	6p21.32	<i>HLA-DRB1</i>	Gene body	32547019	2.34	1.42×10^{-2}
cg15982117	6p21.32	<i>HLA-DRB1</i>	Gene body	32552106	2.04	1.77×10^{-2}
cg16514085	6p21.32	<i>HLA-DRB1</i>	Gene body	32552152	1.35	3.52×10^{-2}
cg13778567	6p21.32	<i>HLA-DQA1</i>	Gene body	32609783	63.96	5.24×10^{-30}
cg02919082	6p21.32	<i>HLA-DQA1</i>	Gene body	32605694	6.96	1.57×10^{-4}
cg24143305	6p21.32	<i>HLA-DQA1</i>	TSS1500	32604865	5.24	8.43×10^{-4}
cg12192813	6p21.32	<i>HLA-DQB1</i>	Gene body	32632108	27.56	1.67×10^{-13}
cg11986643	6p21.32	<i>HLA-DQB1</i>	1 st exon	32634316	18.78	1.31×10^{-9}
cg25597745	6p21.32	<i>HLA-DQB1</i>	Gene body	32634245	15.03	4.97×10^{-8}
cg10982913	6p21.32	<i>HLA-DQB1</i>	Gene body	32632731	10.18	6.18×10^{-6}
cg14323910	6p21.32	<i>HLA-DQB1</i>	Gene body	32628305	7.66	7.77×10^{-6}
cg05724777	6p21.32	<i>HLA-DQB1</i>	1 st exon	32634362	6.38	2.79×10^{-4}
cg13353717	6p21.32	<i>HLA-DQB1</i>	1 st exon	32634276	5.40	7.3×10^{-4}
cg15752756	6p21.32	<i>HLA-DQB1</i>	TSS200	32634362	4.69	1.50×10^{-3}
cg23336481	6p21.32	<i>HLA-DQB1</i>	1 st exon	32634344	4.36	2.01×10^{-3}
cg03403720	6p21.32	<i>HLA-DQB1</i>	Gene body	32632970	2.79	9.17×10^{-3}
cg22984282	6p21.32	<i>HLA-DQB1</i>	Gene body	32632568	2.55	1.16×10^{-2}
cg04696840	6p21.32	<i>HLA-DQB1</i>	Gene body	32633354	2.17	1.61×10^{-2}
cg17039645*	6p21.32	<i>C6orf10</i>	Gene body	32294503	5.45	7.23×10^{-4}
cg18111114*	6p21.32	<i>HLA-DRB5</i>	Tss1500	32498493	11.84	1.20×10^{-6}
cg15982117*	6p21.32	<i>HLA-DRB1</i>	Gene body	32552106	4.23	1.77×10^{-2}

NOTE:

* Methylation probe is reported to be associated with peanut allergy in Hong et al. (*Nat Commun* 2015;6:6304)^{E3}.

[†] p_{FDR} is reported as p values after correcting for false discovery rate.

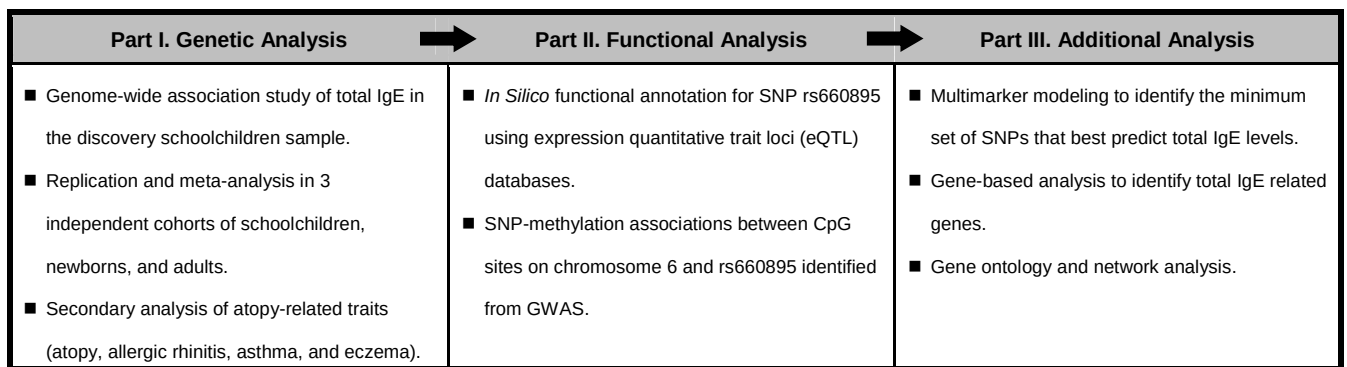


Figure S1. Study design and analysis process in the present study.

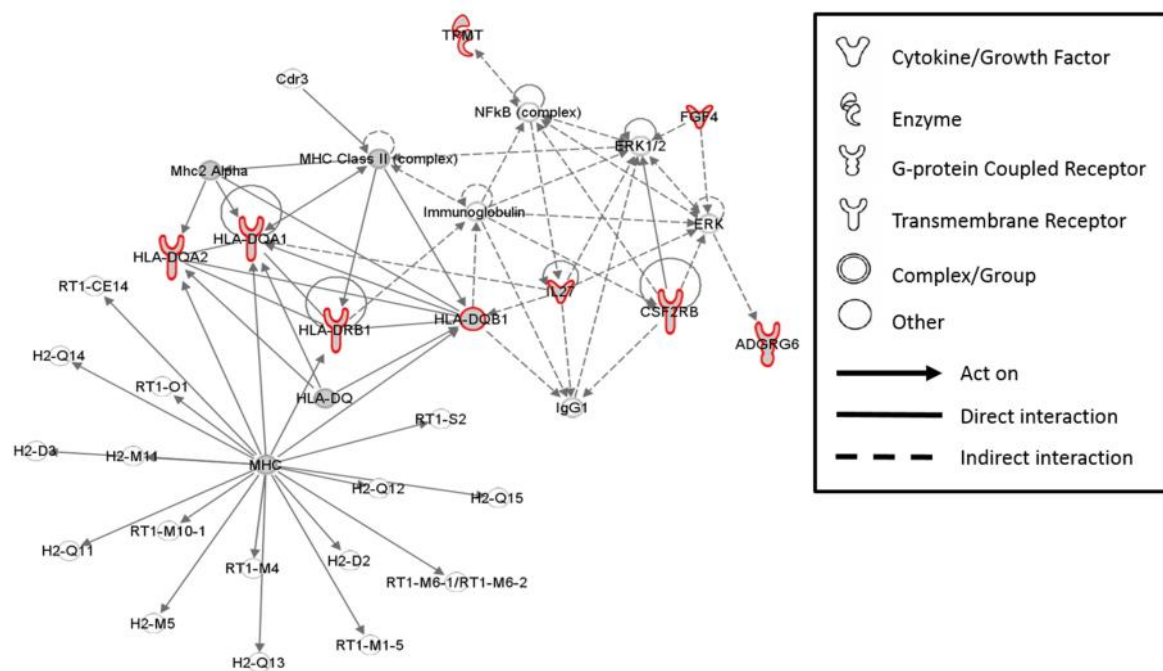


Figure S2. Network plot of the 14 identified genes involved in immunological diseases. **NOTE:** Nine out of the 14 identified genes are marked on the network plot.