

An epigenome-wide association study of total serum immunoglobulin E concentration

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Immunoglobulin E (IgE) is a central mediator of allergic (atopic) inflammation. Therapies directed against IgE can alleviate hay fever¹ and allergic asthma^{1,2}. Genetic association studies have not yet identified novel therapeutic targets or pathways underlying IgE regulation^{3–6}. We therefore surveyed epigenetic associations between serum IgE concentrations and methylation at loci concentrated in CpG islands genome wide in 95 nuclear pedigrees, using DNA from peripheral blood leukocytes. We validated positive results in additional families and in subjects from the general population. Here we show replicated associations—with a meta-analysis false discovery rate less than 10^{-4} —between IgE and low methylation at 36 loci. Genes annotated to these loci encode known eosinophil products, and also implicate phospholipid inflammatory mediators, specific transcription factors and mitochondrial proteins. We confirmed that methylation at these loci differed significantly in isolated eosinophils from subjects with and without asthma and high IgE levels. The top three loci accounted for 13% of IgE variation in the primary subject panel, explaining the tenfold higher variance found compared with that derived from large single-nucleotide polymorphism genome-wide association studies^{3,4}. This study identifies novel therapeutic targets and biomarkers for patient stratification for allergic diseases.

Asthma, atopic dermatitis (eczema) and hay fever are IgE-related diseases that are increasing in prevalence and are a major source of disability. Systematic knowledge of IgE production is limited, beyond the regulation of IgE creation in B cells by interleukin (IL)-4 released from T_H2 cells and eosinophils⁷. Genome-wide association studies show polymorphisms in *STAT6*, *FCER1A*, the *IL4/RAD50* locus and the major histocompatibility complex (MHC) to be associated with high IgE concentrations^{3–6}, but these single-nucleotide polymorphisms (SNPs) combined account for only 1–2% of the variation in serum IgE⁴.

CpG methylation is associated with gene silencing and the patterning of gene expression that determines cellular types and functions⁸,

and islands of CpG (CGIs) sequences are positioned near the promoters of 40% of human genes⁹. *IL4* expression has been related to upstream epigenetic variation in DNA methylation in T cells¹⁰, encouraging us to search genome wide for other CGIs associated with IgE serum concentrations.

We used Illumina HumanMethylation27 arrays to target individual CpG sites (loci) within proximal promoter regions of 14,475 genes. The panel is enriched for genomic regions regulating expression, but does not cover all functionally important CpG sites. We excluded from downstream analyses any loci with SNPs overlapping the Illumina probe sequence, and established that direct bisulphite pyrosequencing correlates robustly with the array used in this study (Extended Data Fig. 1) and elsewhere¹¹.

We investigated nuclear families from the Medical Research Council Asthma (MRCA) panel, in which we have previously carried out genome-wide SNP association studies for IgE levels and asthma¹². The panel contained 355 subjects (183 male) with a mean age in children of 12.2 years (ranging from 2 to 39 years) and in adults of 42 years (27 to 61 years) (Table 1). One-hundred and thirteen children had doctor-diagnosed asthma. We found replication cohorts in 149 Caucasian subjects selected equally from the top and bottom deciles of IgE distribution in 1,614 unselected volunteers for the Poblagaeth Asthma Prifysgol Abertawe (PAPA) study (students and staff from Swansea University)¹³; and in 160 subjects in an asthmatic family panel from the Saguenay–Lac-Saint-Jean region (SLSJ) of Québec¹⁴, with a mean age in children of 16 years (ranging from 5 to 50 years; 40 with doctor-diagnosed asthma) and adults of 44 years (31 to 79 years) (Table 1).

We fitted models with log_e-normalized IgE (ln(IgE)) as a dependent variable and methylation status for each Illumina probe as a predictor, with age, sex, parental status, interactions and batch identifiers as covariates. We identified 34 loci with a false discovery rate (FDR) < 0.01 (Fig. 1 and Supplementary Table 1) in 32 different CGIs in the MRCA

Table 1 | Subject characteristics

	MRCA (discovery)	PAPA (1st replication)	SLSJ (2nd replication)
Number	355	149	160
Age (mean (range))	28 (2–61)	21 (18–30)	29 (5–79)
N (%) female	17 (48.5%)	72 (48.3%)	80 (50.0%)
N (%) asthmatic	175 (49.3%)	34 (22.8%)	69 (43.1%)
N (%) smoker	45 (12.7%)	33 (22.1%)	28 (17.5%)
Eosinophil count (mean ± s.e.m.) per μl	406 ± 383	246 ± 214	242 ± 205
Geometric mean serum IgE (range) (IU l ⁻¹)†	320 (1–4,999)	663 (0–18,800)	412 (2–7,653)

s.e.m., standard error of the mean.

*Normal range, <350 cells per μl.

†Normal range, <100 IU l⁻¹.

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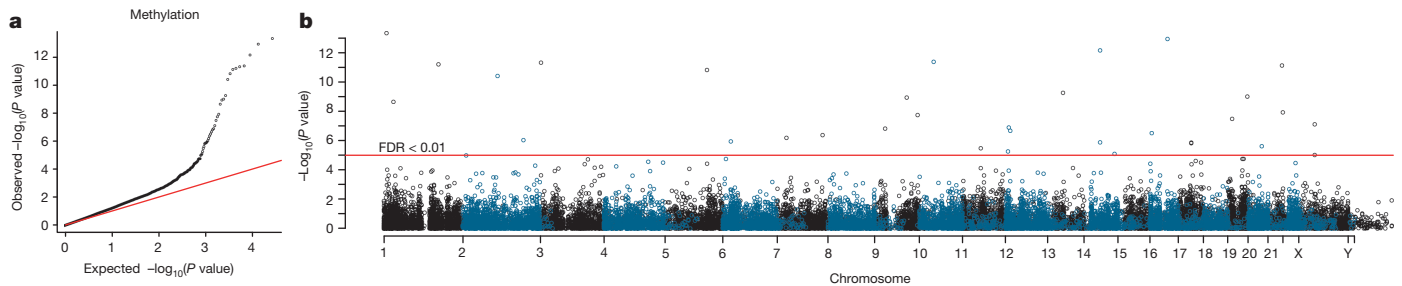


Figure 1 | Manhattan plot of the results of the genome-wide methylation association study. **a**, **b**, The results of genome-wide association testing to CGIs are shown for 27,000 loci in 355 subjects from the MRCA panel of families. **a**, The QQ plot showing observed versus expected $-\log_{10}(P \text{ values})$ for

association at all loci. **b**, Manhattan plot showing chromosomal locations of $-\log_{10}(P \text{ values})$ for association at each locus. The red line illustrates the threshold for an $FDR < 0.01$.

panel. After replication in the PAPA and SLSJ panels, a meta-analysis combining the results identified 36 loci with $FDR < 10^{-4}$ and 62 loci with an $FDR < 0.005$ (Table 2 and Supplementary Table 1). All loci showed associations with the same anti-correlated direction in the three data sets (Table 2). A radioallergosorbent test (RAST) index, quantifying IgE against common allergens¹⁵, showed similar but non-independent associations, suggesting common regulation of total and specific IgE. Testing of models with asthma as the dependent variable showed only *LPCAT2* (also known as *AYTL1*) and *ZNF22* to be associated with asthma independently of IgE levels ($P = 7.7 \times 10^{-5}$ and $P = 1.8 \times 10^{-4}$).

The variable methylation site upstream of *IL4* has a well-studied effect on IL-4 production^{16,17} and IgE regulation, with methylation anti-correlated with expression in the same direction as in our study. We looked for SNP associations at this locus by imputation with the 1000

Genomes project phase 1 SNPs and indels in all three panels, analysing the 20,746 variants within 1 megabase upstream or downstream of the *IL4* 5' untranslated region. We found no significant SNP associations with IgE after accounting for multiple testing.

We carried out Mendelian randomization to test for a causal effect of IL-4 methylation on IgE¹⁸, choosing the SNP showing the strongest association to methylation at the IL-4 CpG probe (cg26787239) as the instrumental variable. The first stage *F*-test statistics for the MRCA and SLSJ panels ($F = 16.4$ and 26.2) indicated effects strong enough to ensure the validity of the method. In the MRCA panel, the association between the instrument SNP (*rs12311504*) and IgE before adjusting for IL-4 methylation was $P = 0.03$, and $P = 0.53$ after adjustment, indicating that methylation mediated most of the SNP effect. The meta-analysis *P* for a causal effect was 6.8×10^{-4} , suggesting that the locus

Table 2 | Meta-analysis of association of total serum IgE concentration in three subject panels

Probe	Symbol	Function	MRCA (<i>P</i> value)	SLSJ (<i>P</i> value)	PAPA (<i>P</i> value)	Meta-analysis (<i>P</i> value)
cg01998785	<i>LPCAT2</i>	Lysophospholipid metabolism	1.2×10^{-13}	8×10^{-3}	9.6×10^{-6}	1.2×10^{-18}
cg10159529	<i>IL5RA</i>	Cytokine signalling	5.1×10^{-12}	2.1×10^{-4}	7.2×10^{-5}	2.2×10^{-18}
cg01614759	<i>ZNF22</i>	Transcription factor	4.4×10^{-12}	3.4×10^{-3}	2.8×10^{-6}	2.8×10^{-18}
cg15996947	<i>L2HGDH</i>	Mitochondrial oxidoreductase	7.4×10^{-13}	1.0×10^{-2}	3.7×10^{-5}	2.8×10^{-17}
cg26787239	<i>IL4</i>	Cytokine signalling	1.6×10^{-11}	1.3×10^{-3}	4.8×10^{-4}	3.2×10^{-16}
cg18783781	<i>SLC25A33</i>	Mitochondrial transport: dendritic-cell endocytosis	5.0×10^{-14}	3.4×10^{-2}	6.2×10^{-3}	4.4×10^{-15}
cg13221796	<i>RB1</i>	Transcription factor	5.7×10^{-10}	6.0×10^{-2}	4.9×10^{-6}	2.5×10^{-14}
cg01770400	<i>SERPINC1</i>	Anti-thrombin	6.6×10^{-12}	7.0×10^{-3}	1.3×10^{-2}	5.3×10^{-14}
cg02643667	<i>TFF1</i>	Mucus-stabilizing secreted protein	7.9×10^{-12}	1.3×10^{-1}	2.6×10^{-3}	7.6×10^{-13}
cg21627181	<i>SLC17A4</i>	Sodium/phosphate co-transporter	1.1×10^{-6}	1.4×10^{-4}	3.8×10^{-4}	1.1×10^{-12}
cg20189937	<i>L2HGDH</i>	Mitochondrial oxidoreductase	1.3×10^{-6}	2.4×10^{-3}	3.2×10^{-5}	2.6×10^{-12}
cg26457013	<i>TMEM86B</i>	Lysoplasmalogenase: phospholipid metabolism	1.0×10^{-9}	9.5×10^{-2}	9.0×10^{-4}	7.1×10^{-12}
cg20503329	<i>COL15A1</i>	Cell shape, motility, adhesion	1.2×10^{-9}	3.0×10^{-1}	8.8×10^{-5}	9.5×10^{-12}
cg03693099	<i>CEL</i>	Secreted carboxyl ester lipase	1.8×10^{-8}	1.1×10^{-1}	8.3×10^{-5}	1.3×10^{-11}
cg00079056	<i>SPINK4</i>	Serine peptidase inhibitor	1.5×10^{-7}	6.4×10^{-2}	3.1×10^{-5}	1.8×10^{-11}
cg09676390	<i>ADARB1</i>	Pre-mRNA editing of the glutamate receptor	1.2×10^{-8}	7.0×10^{-2}	8.0×10^{-4}	3.1×10^{-11}
cg15998761	<i>MFS06</i>	MHC receptor homologue	9.3×10^{-7}	1.3×10^{-2}	1.7×10^{-4}	4.5×10^{-11}
cg25494227	<i>TMEM52B</i>	Transmembrane protein	1.3×10^{-7}	2.0×10^{-1}	1.2×10^{-5}	5.1×10^{-11}
cg11398517	<i>FAM112A</i>	Unknown	2.4×10^{-6}	7.4×10^{-3}	3.3×10^{-4}	1.0×10^{-10}
cg06690548	<i>SLC7A11</i>	Cystine/glutamate antiporter: dendritic-cell differentiation	2.7×10^{-5}	3.3×10^{-4}	1.1×10^{-3}	1.8×10^{-10}
cg17784922	<i>KEL</i>	Metallo-endopeptidase	4.2×10^{-7}	7.9×10^{-3}	4.4×10^{-3}	2.1×10^{-10}
cg16050349	<i>PIK3CB</i>	Catalytic subunit for PI3K-β: activation of neutrophils	4.0×10^{-5}	1.7×10^{-3}	2.3×10^{-4}	3.2×10^{-10}
cg25636075	<i>TMEM41A</i>	Transmembrane protein	2.5×10^{-4}	5.1×10^{-5}	7.7×10^{-4}	3.9×10^{-10}
cg08404225	<i>IL5RA</i>	Cytokine signalling	2.3×10^{-4}	3.3×10^{-3}	8.4×10^{-6}	4.1×10^{-10}
cg09447105	<i>PDE6H</i>	Inhibitory subunit of cGMP phosphodiesterase	2.2×10^{-7}	1.3×10^{-7}	4.0×10^{-4}	5.3×10^{-10}
cg05215575	<i>SEPT12</i>	Cell shape, motility, adhesion	3.1×10^{-7}	2.1×10^{-1}	2.7×10^{-4}	1.2×10^{-9}
cg26136776	<i>KLF1</i>	Erythroid-specific transcription factor	3.3×10^{-8}	4.3×10^{-1}	6.6×10^{-4}	1.5×10^{-9}
cg17749520	<i>ITGA2B</i>	Platelet fibronectin receptor: role in coagulation	1.4×10^{-6}	2.5×10^{-2}	3.5×10^{-3}	1.8×10^{-9}
cg24459209	<i>PRG3</i>	Eosinophil major basic protein homologue	3.3×10^{-6}	2.9×10^{-2}	1.1×10^{-3}	1.8×10^{-9}
cg00002426	<i>SLMAP</i>	Sarcolemma-associated protein	7.9×10^{-5}	8.3×10^{-3}	1.6×10^{-4}	2.4×10^{-9}
cg15357945	<i>PRG2</i>	Eosinophil granule major basic protein	2.2×10^{-3}	2.8×10^{-5}	5.8×10^{-4}	3.1×10^{-9}
cg17582777	<i>EFNA3</i>	Receptor protein-tyrosine kinase	1.1×10^{-4}	3.1×10^{-2}	8.2×10^{-5}	8.6×10^{-9}
cg19881895	<i>SLC43A3</i>	Transmembrane protein	7.5×10^{-5}	2.8×10^{-3}	6.7×10^{-3}	1.6×10^{-8}
cg18254848	<i>CLC</i>	Lysophospholipid metabolism	1.8×10^{-5}	4.4×10^{-2}	4.6×10^{-3}	4.5×10^{-8}
cg21631409	<i>ALDH3B2</i>	Enzyme or kinase	2.3×10^{-4}	1.7×10^{-2}	1.2×10^{-3}	6.8×10^{-8}
cg00536175	<i>GATA1</i>	Eosinophil-specific transcription factor	7.9×10^{-8}	4.0×10^{-1}	5.1×10^{-2}	1.4×10^{-7}

Loci with an FDR for the meta-analysis $< 10^{-4}$ are shown; a full list of significant associations can be found in Supplementary Table 1. MRCA contained 355 subjects, PAPA 149 and SLSJ 160. Markers are identified through their Illumina identification numbers and the associated gene symbol is derived from the Illumina annotation updated through PubMed. Note that two probes from *IL5RA* and from *L2HGDH* are associated to IgE concentrations.

represents a functionally validated epigenetic association with a complex phenotype.

Several loci were annotated to genes that encode proteins characteristic of eosinophils (Table 2 and Supplementary Table 1). *IL5RA* encodes a receptor that selectively stimulates eosinophil production and activation¹⁹; *CCR3* encodes the eosinophil eotaxin receptor; *IL1RL1* encodes the receptor for the eosinophil-activating cytokine IL-33; *PGR2* (also known as *GPR142*) encodes eosinophil granule major basic protein (*PRG2*); *PGR3* (also known as *GPR139*) is a *PGR2* homologue; and *GATA1* is an eosinophil transcription factor. We therefore tested whether methylation at our associated loci marked activation in eosinophils purified from peripheral blood, studying eight asthmatics with high serum IgE levels (>110 international units (IU) l^{-1}), eight asthmatics with low serum IgE (<110 IU l^{-1}) levels, and eight controls (mean age of all subjects 31 years (range 6–56 years), eight females and two current smokers). Asthmatics in both groups were on a maintenance regime of inhaled β -agonists, augmented with inhaled glucocorticoids during exacerbations.

We observed the lowest levels of methylation in the subjects with asthma and high IgE, and that methylation in asthmatics with low IgE was intermediate to controls (Fig. 2) ($P < 0.05$; Supplementary Table 1), supporting our initial results. Partitioning the data into high or low IgE groups led to similar conclusions. The range of variation for the principal loci was narrower in asthmatics with high IgE (Fig. 2) than in the other two groups, suggesting the enrichment of a distinctive eosinophil subset in atopic asthma. This is consistent with the recognized mixture of eosinophil populations in human blood and eosinophil activation in atopic disease²⁰. Comparison of methylation levels in the MRCA panel

for our IgE-associated loci ($P < 0.001$) with an independent study of eosinophils isolated from normal subjects²¹ confirmed correlations with methylation status ($R = 0.64$).

Lineage commitment to particular cell types is accompanied by specific methylation changes⁸, and it has been suggested that DNA from mixtures of cells (such as peripheral blood leukocytes (PBLs)) will not support epigenome-wide association studies of complex diseases²¹. We extracted DNA from unfractionated populations of PBLs so that our methylation patterns reflect the numbers and the activity of different cells in each specimen. We further explored whether our associations to IgE reflected carriage in particular cell types by fitting regression models that included differential white cell counts. We identified partial associations with eosinophil numbers for all IgE-associated loci (Supplementary Table 2 and Fig. 3), consistent with independent effects on IgE from the numbers of eosinophils and the activity of the loci within eosinophils.

It is well recognized that the regulation of IgE production against particular antigens may reside in T cells and B cells as well as in eosinophils²². However, our regression models found that the top IgE associations were not accounted for by concomitant correlation with lymphocyte counts (Supplementary Table 2). We further examined the distribution of IgE-associated CpG loci in leukocyte subsets we isolated and in subsets from published data²¹. The results showed robustly in both data sets that low levels of methylation at the IgE-associated loci were confined to eosinophils (Extended Data Fig. 2).

Surrogate CpG markers that identify lymphocyte subsets can be used as an alternative to white cell counts in association models²³. We also applied these methods to our data (Extended Data Table 1). This analysis

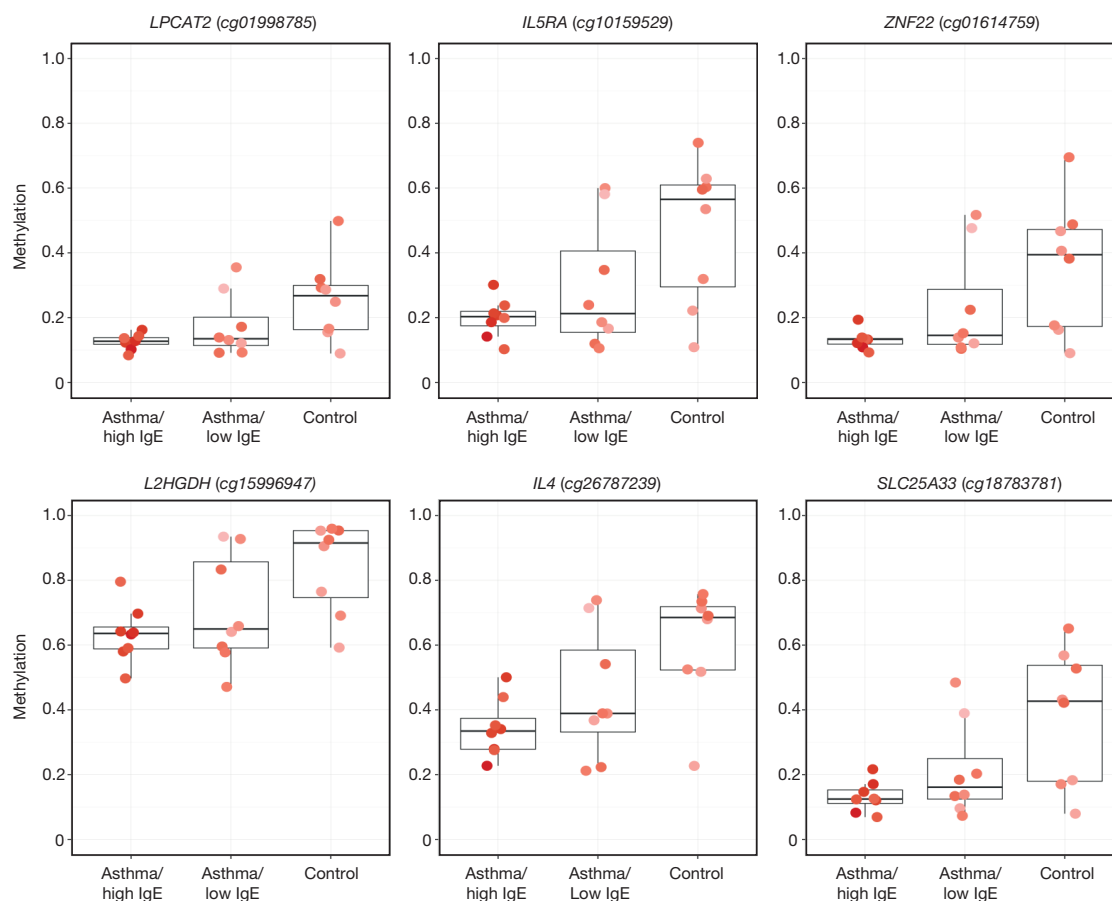


Figure 2 | Boxplots of methylation at selected CpG loci in isolated eosinophils from subjects with and without asthma and high total serum IgE concentrations (>110 IU l^{-1}). Subjects were recruited from the SLSJ population. Methylation (β) is shown on a scale of 0–1 for eight subjects in each

group. Boxplots show means and interquartile ranges. The intensity of the data point colour is proportional to total serum IgE. All loci exhibited reduced variability and levels of methylation in the subjects with asthma and high IgE ($P < 0.05$).

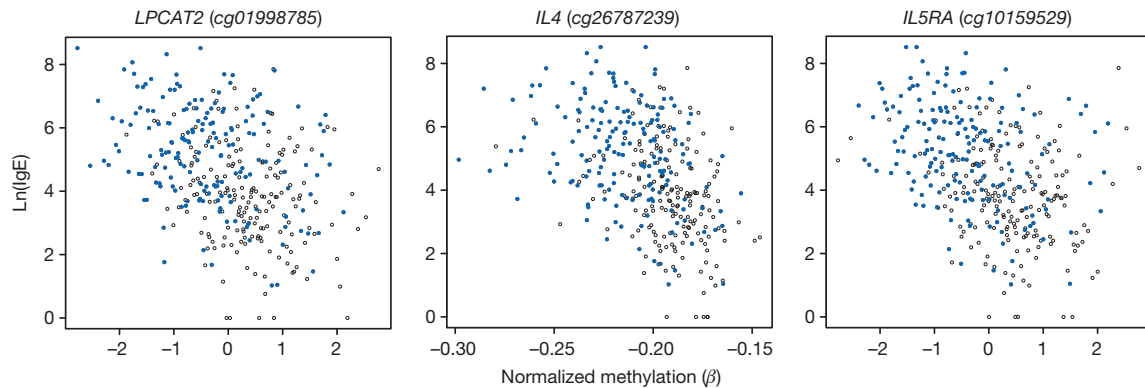


Figure 3 | Association of selected CpG loci to total serum IgE concentrations in the MRCA panel, partitioned by eosinophil counts. Methylation values for 355 individuals normalized around a mean of 0 on the

abscissa (x) with $\ln(\text{IgE})$ levels on the ordinate. Blue dots indicate subjects with eosinophil counts greater than the median for the MRCA panel.

provided further evidence that T-cell subsets do not have strong effects on these loci.

The variance (standard deviation) in our IgE-associated CpG loci was on average 4.4-fold larger in isolated eosinophils than in PBLs from the MRCA data set, indicating an attenuation of effect size in PBLs that would mask associations rather than magnify them. The power to detect cell-specific associations from PBLs depends on the proportion of each cell type, the effect size in specific cells, and the sample size. We estimated that we had 90% power to detect loci accounting for 10% of variance in IgE in the MRCA panel and >99% power in the combined panels (Extended Data Fig. 3). Although our ability to detect associations was enhanced by eosinophil counts that were above the normal range for many of our subjects (Table 1), the power estimates and the large observed effect size encourage epigenome-wide association studies of other diseases in which PBLs may be important.

We investigated the variance attributable to different loci in the MRCA panel through a stepwise regression that included all significant CGI associations together with differential white cell counts, age, sex and parental status. Using a cut-off of corrected $P < 0.1$ for inclusion, we found that *SLC25A33*, *LPCAT2* and *L2HGDH* predicted the serum IgE concentration independently of each other and of eosinophil counts.

In the MRCA panel, the top three CpGs independently explained 13.5% of IgE variation and the eosinophil counts explained a further 8.8%. In the SLSJ panel the top three CpGs explained 8.3% IgE variation and eosinophil counts a further 15.5%. (We were not able to estimate variances meaningfully in the PAPA data set, because the samples were selected by extreme IgE values). The regression models therefore matched the results from isolated eosinophils, leading us to the conclusion that the methylation status of eosinophils and their numbers were both related to IgE levels.

Methylation levels were highly correlated between loci, and similar estimations of variance were obtained with forced entry of other significantly associated markers, so the results do not imply that *SLC25A33*, *LPCAT2* and *L2HGDH* are the most important loci. DNA methylation is not meiotically heritable and the variance in IgE attributable to these loci does not impact on the problem of missing heritability.

Overall, the most significant association was to *cg01998785*, within a CGI adjacent to *LPCAT2*. *LPCAT2* encodes lyso-platelet-activating factor (PAF) acetyltransferase, which is essential to induced formation of PAF, a potent pro-inflammatory lipid mediator²⁴. It is of interest that hypocoactive variants of plasmatic PAF-acetylhydrolase are associated with atopy and asthma²⁵. Other significant associations annotated to genes involved in phospholipid metabolism included lysoplasmalogenase (*TMEM86B*), *CEL* (carboxyl ester lipase) and *CLC*.

GATA1 is a known eosinophil transcription factor and subsequent investigations will determine if the other associated transcription factors *ZNF22*, *RB1* and *KLF1* regulate eosinophil activation. Other associations may encode proteins released from eosinophil granules, including

PRG2, *PRG3*, *SERPINC1* (antithrombin), *TFF1* (which may protect the mucosa), *CEL* and the polyvalent serine protease inhibitor *SPINK4*. Genes encoding mitochondrial proteins (*L2HGDH* and *SLC25A3*) are consistent with mitochondrial suppression of apoptosis in activated eosinophils²⁶.

Although the relationship between methylation and gene expression at these loci requires further investigation in isolated cells, our results support the recognition that eosinophils are an important source of cytokines and other pro-inflammatory molecules at the site of allergic inflammation⁷. Eosinophils are required locally for the maintenance of bone-marrow plasma cells²⁷, allowing direct regulation of IgE production in specialized environments. Clinically, the presence of eosinophilia in the peripheral blood or airways identifies a subgroup of refractory asthmatic individuals in whom therapies directed at eosinophils may be effective²⁸. The measurement of methylation at these loci may further identify patients responsive to therapies directed at eosinophils or individual gene products.

Cigarette smoking may increase serum IgE, and we found anti-correlated associations to current cigarette smoking with *F2RL3* ($P = 8.6 \times 10^{-17}$) and *GPR15* ($P = 4.6 \times 10^{-9}$). The SLSJ data set confirmed these associations ($P = 2.5 \times 10^{-6}$ and $P = 6.6 \times 10^{-7}$), in keeping with previous studies^{29,30}. Adjusting for smoking had minimal impact on the top hits for IgE and neither locus affected IgE in our subjects. *F2RL3* and *GPR15* may represent therapeutic targets to counter the effects of tobacco smoke and their methylation status may prospectively predict consequences of smoking.

Our epigenome-wide association study has discovered reproducible CGI associations accounting for a variation in the total serum IgE that is tenfold higher than that derived from large SNP genome-wide association studies⁴. In contrast to SNP studies, association to methylation levels captures responses to environmental factors and the loci should not be assumed to cause disease. Nevertheless, our findings suggest the presence of novel therapeutically tractable pathways underlying IgE production.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 29 January; accepted 27 November 2014.

Published online 18 February 2015.

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Supplementary Information is available in the online version of the paper.

Acknowledgements This work was supported by the Freemasons' Grand Charity. The study was also funded by the Wellcome Trust under grants WT 077959 and WT096964, the UK Medical Research Council, a grant to G.M.L. from Génome Québec, le Ministère de l'Enseignement supérieur, de la Recherche, de la Science et de la Technologie (MESRST), Québec and McGill University, and the National Institutes of Health R01s HL101251-01 and P01-ES18181. M.F.M. and W.O.C.M.C. are Joint Wellcome Trust Senior Investigators, W.O.C.M.C. is a National Institute for Health Research Senior Investigator and C.L. is the Chairholder of the Canada Research Chair on Genetic Determinants in Asthma. We thank A.-M. Madore and V. T. Vaillancourt for the eosinophil isolation and M. Laviolette and N. Flamand for their advice on this technique.

Author Contributions S.A.G.W.-O., W.O.C.M.C., G.M.L. and M.F.M. planned the initial study. S.A.G.W.-O., A.B. and K.C.C.W. performed measurements of methylation status. L.L. and W.O.C.M.C. led statistical analyses of the data with S.A.G.W.-O. and G.M.L.: most analyses were carried out by L.L. G.M.L. and T.M.P. led discussions on replication strategy, methylation assays and cell-specific methylation, with contributions from M.F.M., D.A.S. and I.V.Y. E.G. validated Illumina probes with bisulphite sequencing. C.L. led studies of SLSJ families with T.J.H., and G.A.D. and J.M.H. led studies of the PAPA subjects. C.L. led studies of isolated eosinophils. M.H., L.R. and S.B. recruited subjects and studied lymphocyte subsets. W.O.C.M.C. wrote the first draft of the paper. All authors contributed to the interpretation of the results and the writing of the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to W.O.C.M.C. (w.cookson@imperial.ac.uk).

METHODS

Phenotyping. Ethical approval for the study was obtained from the NHS Multicentre Research Ethics Committee for the MRCA subjects; from the Swansea Joint Scientific Research Committee and Swansea Research Ethics Committee for the Swansea (PAPA) subjects; and from le Centre de Santé et des Services Sociaux de Chicoutimi for the SLSJ families. Written informed consent was obtained from all subjects or in the case of children, from their parents. Asthma was doctor defined. Following a standard respiratory questionnaire, all subjects submitted to venipuncture. Differential white cell counts were measured by automated counter. Total serum IgE and specific serum IgE to whole HDM (*Dermatophagoides pteronyssinus*) and Timothy grass pollen (*Phleum pratense*) were measured using the Immucap FEIA (Pharmacia AB). The levels of specific IgE were converted to RAST units according to Pharmacia recommendations. A combined RAST index was calculated for each individual as the sum of the RAST scores to HDM and Timothy grass¹⁵.

Detection of methylation status. DNA was extracted after red cell lysis and centrifugation to recover leukocyte nuclear pellets. DNA samples were bisulphite converted using the Zymo EZ DNA Methylation kit (Zymo Research) with an input of 1,000 ng. The assay was carried out as per the Illumina Infinium Methylation instructions, using the HumanMethylation27 BeadChips (Illumina). These interrogate 27,578 of CpG sites for the extent of DNA methylation. Data were visualized using the BeadStudio software, and samples that failed quality control were repeated. Raw methylation data were exported from the GenomeStudio software. For the Illumina HumanMethylation27 BeadChip data, quantile normalization of intensity was applied to all methylated and unmethylated probes for all samples together. The methylation β values were recalculated as the ratio of methylated probe signal/ (total signal + 100). The Touleimat and Tost³¹ analysis pipeline was used for the HumanMethylation450 BeadChip. Individual data points with detection $P > 0.01$ or number of beads < 3 were treated as missing data, as were samples with more than 20% missing probes. The lumi package³² was used for background and colour bias correction. BeadChip ID and position on chip were included as categorical covariates to account for potential batch effects. Quantile normalization across samples was applied to probes within each functional category (CpG island, shelf, shore, and so on) separately to correct the shift of methylation β values between Infinium I and Infinium II probes on the HumanMethylation450 BeadChip. Probe overlaps with any frequent SNP (minimum allele frequency (MAF) $> 5\%$ in 1000 Genomes Project phase 1 EUR population) in the probe sequence or in position +1 or +2 of the query site (depending on Infinium I or Infinium II status) were removed. The use of meta-analysis to combine 27K and 450K data together with this implementation of the Tost pipeline ensured our analysis was not confounded by probe differences.

Isolation of human eosinophils. Isolation of human eosinophils was as described³³. Briefly, platelet-rich plasma was removed from 200 ml using centrifugation, followed by Dextran-mediated sedimentation to remove erythrocytes and removal of mononuclear cells using a lymphocyte separation medium. Hypotonic lysis with sterile water removed remaining erythrocytes and other granulocytes were removed using negative selection with anti-CD16 MicroBeads. DNA was extracted using the QIAamp DNA Blood Mini Kit. Methylation was assessed using Illumina 450K arrays, with analysis restricted to significantly associated probes from the meta-analysis.

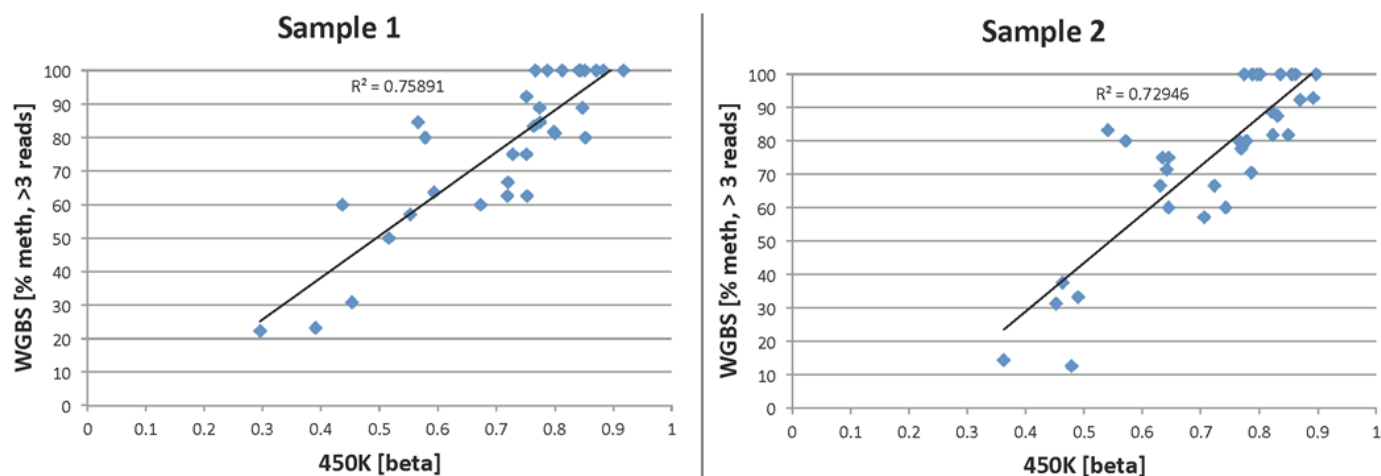
Statistical analyses. To investigate the association with the total serum IgE concentration we tested for association with log-normalized IgE ($\ln(\text{IgE})$) as response with methylation (β) at each locus as predictor while including sex, age, parent indicator, age $\times$ sex and age $\times$ parent interactions in the model, together with batch indicators captured by Illumina chip ID and position of chip (such as

operators, sample wells, plates, runs, and reagents). We applied inverse normal transformation to methylation measures to remove the effect of outliers. We used the R function `lme()` in the nlme package to implement a linear mixed model, assuming a compound symmetry variance-covariance structure to account for correlation of phenotypes among family members. The R code for the discovery stage of association in the MRCA panel was:

```
index = list.na(methylation)
fam = familyID[index]
par = parent[index]
methylation = methylation[index]
methylation = qnorm(rank(methylation)/(length(methylation)+1), mean = 0, sd = 1)
lnige = LNIGE[index]
age = AGE[index]
sex = SEX[index]
lm2 = lme(lnige ~ sex + age + methylation + par + sex*age + age*par, random = ~1 | fam)
```

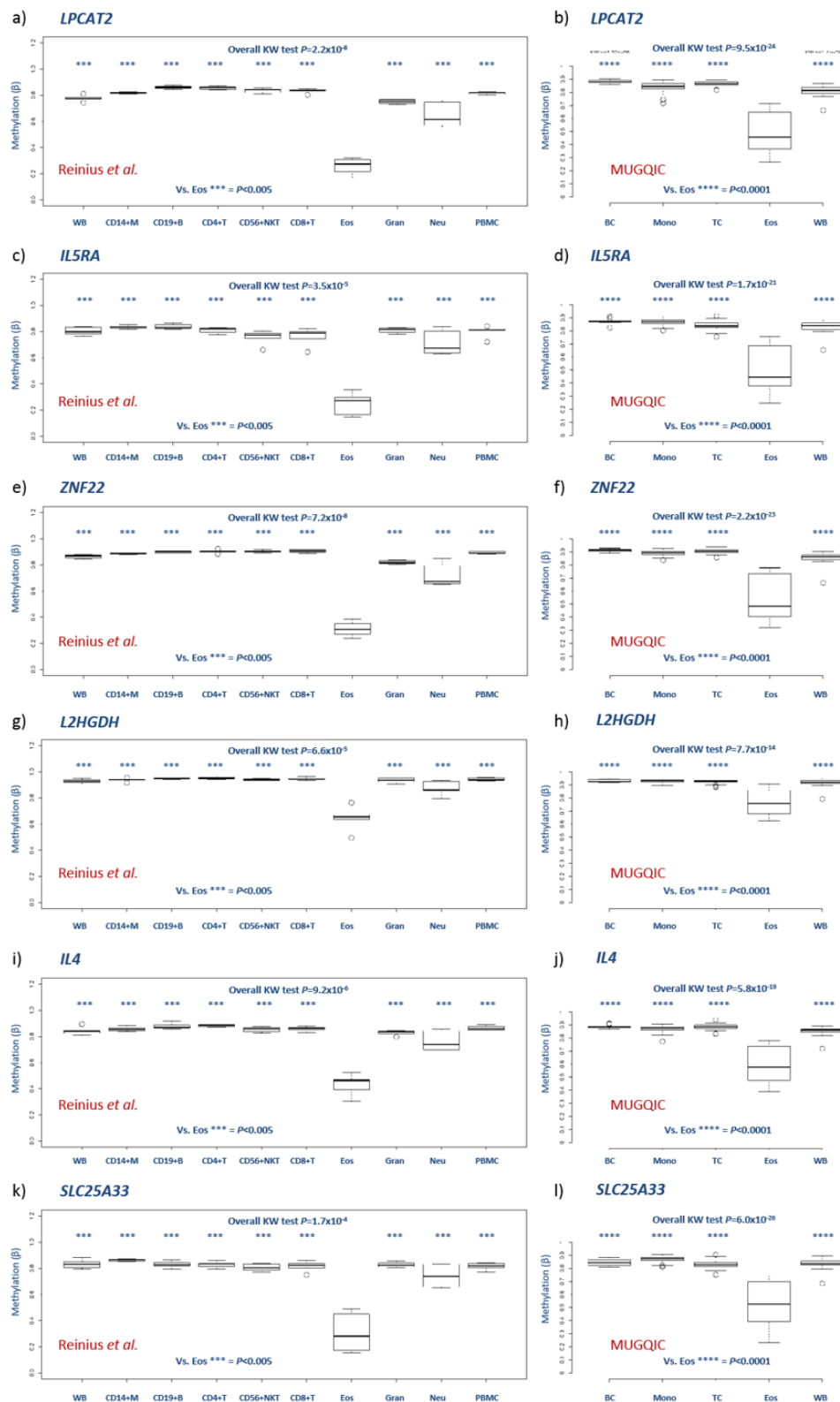
The residual methylation values after removal of the effects of chip ID and position for the genome-wide significant loci in the MRCA, PAPA and SLSJ panels are provided in Supplementary Tables 3–5, together with phenotypic and covariate parameters. We calculated FDRs and applied Bonferroni corrections to adjust for multiple comparisons to 27,578 probes. The same analyses were carried out in the PAPA and SLSJ subjects before meta-analysis of the three data sets. We use a weighted z-score method for meta-analysis based on P value and effect direction from individual studies with weights proportion to the square root of the sample size of the individual study³⁴. SNPs and indels from the 1000 Genomes Project phase 1 release (14 March 2012 haplotypes) were imputed using MINIMAC³⁵. SNPs or indels with imputation quality score $R^2 < 0.3$ were removed from downstream analysis. We carried out Mendelian randomization to assess the causal effect of IL-4 methylation on IgE level through a two-stage least squares instrumental variable regression³⁶ implemented in the ivreg2.r program (<http://diffuseprior.wordpress.com/2012/05/03/an-ivreg2-function-for-r/>). We tested association trends in isolated eosinophils by exact regression (Cytel Studio 9) with asthma/high IgE coded as 2, asthma/low IgE coded as 1, and controls as 0. Covariates for age, sex and batch were included in the model and to test the hypothesis that low levels of methylation were associated with high IgE, P values were one-sided. Differences in methylation between peripheral blood leukocyte subsets were assessed with Kruskal–Wallis tests, using two-sided P values.

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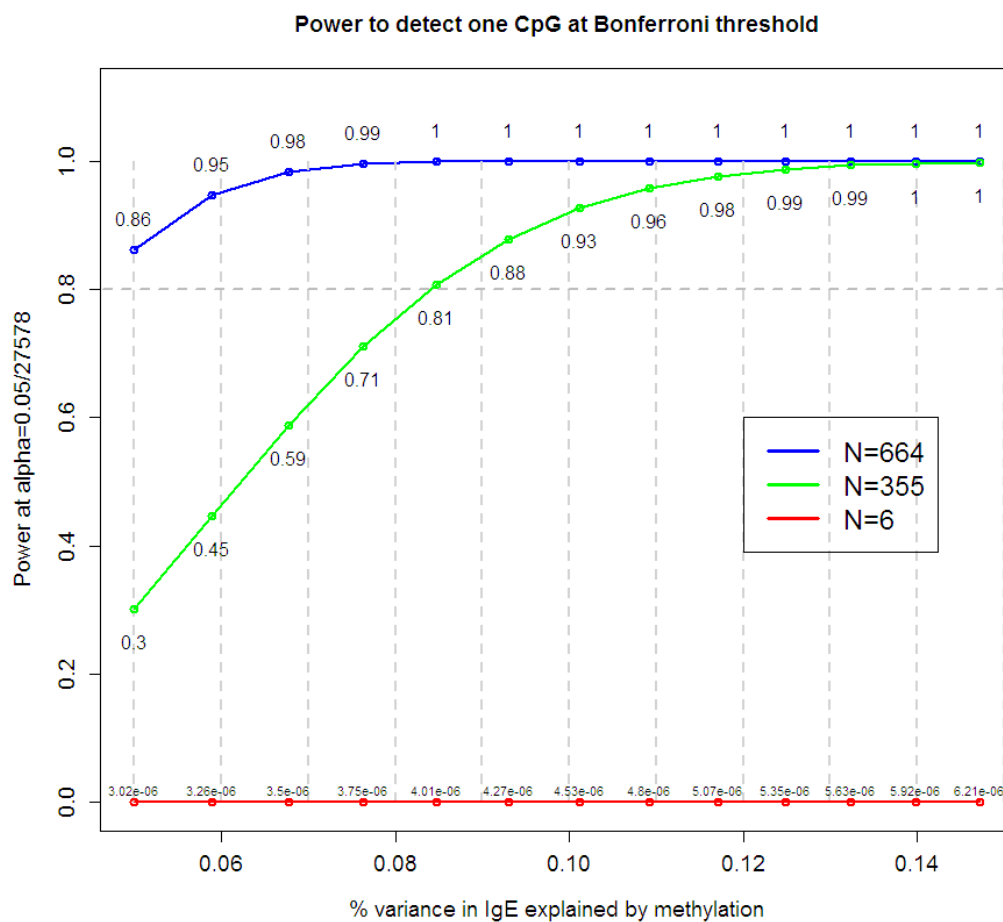
Extended Data Figure 1 | Concordance in methylation status at IgE-associated loci when comparing whole-genome bisulphite sequencing with the Illumina platform. These results were produced by us (E.G. and T.M.P.) at the Genome Québec Innovation Centre. The figures show a comparison between IgE-associated CpG probes using Illumina 450K (*x*-axis) and whole-genome bisulphite sequencing (WGBS) (*y*-axis) platforms for two

samples (left and right panels) with 20-fold sequence coverage. The results show a high R^2 between platforms (0.76 and 0.73). The median of the correlation coefficients for our IgE-associated loci across 30 different samples (using WGBS at various depths) was $R^2 = 0.76$. This can be compared with the global assessment of all overlapping 450K sites, which is $R^2 = 0.81$.



Extended Data Figure 2 | Distribution of methylation status at IgE-associated loci in isolated leukocyte subsets. a–l, The figure shows the distribution of methylation in PBL subsets at the most strongly IgE-associated loci. CpG methylation was measured by the Illumina Infinium 450K platform. Boxplots show means and interquartile ranges. a, c, e, g, i, k, Results from publically available data derived from six healthy controls²¹. Lower levels of methylation with wider variation are observed in eosinophils when compared to whole blood (WB) and subsets comprising CD14⁺ monocytes (CD14+M); CD19⁺ B cells (CD19+B); CD4⁺ T cells (CD4+T); CD56⁺ natural killer cells

(CD56+NK); CD8⁺ T cells (CD8+T); granulocytes (Gran); neutrophils (Neu) and peripheral blood mononuclear cells (PBMCs). b, d, f, h, j, l, Results from cells isolated and analysed by us at the McGill University Genome Québec Innovation Centre (MUGQIC). Eosinophils (Eos) (from 24 subjects in the SLSJ panel) also show lower levels of methylation with wider variation compared to whole blood (22 SLSJ subjects), and to subsets including B cells (BC; 9 control subjects), monocytes (Mono; 76 control subjects) and T cells (TC; 74 control subjects). KW Test, Kruskal–Wallis one-way analysis of variance.



Extended Data Figure 3 | Power estimations to detect eosinophil-specific effects in DNA from PBL. The figure shows that our original MRCA data set (green line) and our combined data set (blue line) are well powered to detect signals of the magnitude observed in our three groups of subjects. The red line shows the power of a sample size of six, as described previously²¹, to detect

differences in CpG methylation in unfractionated PBLs. The mean variance (as standard deviation (s.d.)) for the IgE-associated loci was 0.036 in PBLs from our primary MRCA panel and 0.023 in the whole blood normal samples from ref. 21, demonstrating that our results were consistent with the previous experiment.

Extended Data Table 1 | Comparison of surrogate variable analyses with direct white cell counts in association models

a) Before adjusting cell counts					b) Adjusting for Houseman cell proportions							c) Adjusting for white cell counts						
Chr.	Position	Symbol	probe	pMethy	pMethy	pCD8T	pCD4T	pNK	pBCELL	pMON	pGRAN	pMethy	pEOS	pNEU	pLYM	pMON	pBAS	
1	9521654	SLC25A33	cg18783781	4.96E-14	5.58E-13	0.3625663	0.6489145	0.0830001	0.0735391	0.7715233	0.2564499	6.14E-06	0.00065	0.866661	0.678896	0.561016	0.551752	
16	54100210	LPCAT2	cg01998785	1.24E-13	3.81E-13	0.1560292	0.3633042	0.034881	0.0300831	0.7239839	0.1881862	2.36E-06	0.0003	0.294806	0.300248	0.445243	0.785135	
14	49849865	L2HGDH	cg15996947	7.39E-13	3.81E-11	0.3709841	0.6823007	0.125117	0.1583239	0.9387673	0.3768824	3.21E-05	0.000295	0.38062	0.519495	0.662394	0.890493	
10	44815441	ZNF22	cg01614759	4.42E-12	9.09E-11	0.3526504	0.6420391	0.1382946	0.0810812	0.654088	0.3845248	7.61E-05	0.000216	0.305013	0.42021	0.601202	0.937252	
3	3127530	IL5RA	cg10159529	5.06E-12	1.70E-10	0.8786628	0.9388517	0.2941882	0.2701973	0.9752202	0.5129874	0.000104	6.81E-05	0.877227	0.802458	0.551635	0.756105	
1	172153108	SERPINC1	cg01770400	6.59E-12	1.54E-11	0.1182883	0.3063038	0.0323251	0.0368702	0.574556	0.1896	6.36E-06	0.000109	0.236406	0.186757	0.667289	0.90807	
21	42659768	TFF1	cg02643667	7.87E-12	6.81E-11	0.234861	0.4646176	0.0330799	0.0706689	0.797703	0.1753888	9.07E-06	7.18E-06	0.384611	0.725574	0.672431	0.976409	
5	132036424	IL4	cg26787239	1.59E-11	2.13E-10	0.5058003	0.9922092	0.1723318	0.1996409	0.7364825	0.6544331	1.25E-05	1.66E-05	0.269226	0.259295	0.614387	0.77939	
2	110014086	LIMS3	cg18879041	4.06E-11	2.57E-09	0.3708111	0.7030526	0.1613161	0.2166898	0.9745824	0.474026	0.000865	0.000256	0.280498	0.382954	0.500203	0.963467	
13	47774920	RB1	cg13221796	5.66E-10	9.26E-11	0.1278218	0.3641314	0.0353766	0.0458363	0.7786051	0.2987393	2.84E-06	1.83E-05	0.029037	0.081617	0.516731	0.982861	
19	60432000	TMEM86B	cg26457013	1.02E-09	4.08E-09	0.3305229	0.5920938	0.036091	0.0792481	0.9078355	0.2711535	0.000691	1.35E-05	0.363948	0.317448	0.558421	0.740734	
9	100745613	COL15A1	cg20503329	1.19E-09	3.14E-08	0.4772819	0.7388277	0.1359031	0.1343368	0.9433195	0.4284566	0.001341	1.17E-05	0.443838	0.49736	0.401174	0.959364	
1	31166502	SDC3	cg07689731	2.33E-09	4.79E-09	0.5074834	0.7985815	0.0774502	0.0726484	0.7924958	0.4108301	0.002906	1.48E-05	0.415182	0.483806	0.437372	0.932638	
21	45317773	ADARB1	cg09676390	1.21E-08	1.32E-07	0.43369	0.6702876	0.0791369	0.1103603	0.9234331	0.3292778	0.000472	3.48E-07	0.432091	0.530921	0.462608	0.820656	
9	134926722	CEL	cg03693099	1.84E-08	1.01E-07	0.2926779	0.512945	0.0818301	0.1071869	0.9754856	0.3848947	0.000383	9.27E-06	0.099207	0.18224	0.575481	0.93957	
19	12859426	KLF1	cg26136776	3.34E-08	5.42E-07	0.2328607	0.4764967	0.0498737	0.087521	0.7661611	0.2312155	0.008494	4.25E-06	0.308908	0.378613	0.526765	0.764392	
X	48529968	GATA1	cg00536175	7.85E-08	5.70E-07	0.6056632	0.9159691	0.163687	0.1102612	0.8082841	0.5675393	0.002444	1.31E-06	0.230592	0.333849	0.423511	0.745501	
12	10222881	TMEM52B	cg25494227	1.29E-07	2.79E-07	0.4810187	0.6746518	0.0457622	0.0735827	0.9017706	0.3386288	8.31E-05	2.05E-08	0.349366	0.362883	0.456694	0.75634	
9	33229641	SPINK4	cg00079056	1.54E-07	1.23E-06	0.3893041	0.6595419	0.067607	0.090288	0.950103	0.3262354	0.015058	9.70E-07	0.327361	0.481372	0.49343	0.779557	
12	15017287	PDE6H	cg09447105	2.16E-07	1.80E-06	0.6038815	0.9752817	0.1325361	0.2619219	0.6725442	0.6818338	0.002327	6.73E-07	0.185319	0.308805	0.650588	0.72691	
16	4778723	FLJ25410	cg05215575	3.11E-07	7.40E-08	0.6354446	0.6693211	0.0382164	0.0635217	0.8705757	0.2084314	0.011151	9.08E-08	0.699883	0.891282	0.341595	0.856081	
7	142369547	KEL	cg17784922	4.24E-07	9.07E-07	0.6926023	0.9854571	0.0699431	0.1577229	0.8028496	0.5052827	0.001391	3.21E-08	0.505128	0.698535	0.367309	0.99912	
7	29199993	CHN2	cg21472642	6.51E-07	7.39E-07	0.5225896	0.8312874	0.0708089	0.0764603	0.9022296	0.4725488	8.45E-06	3.38E-09	0.104506	0.231601	0.595919	0.815002	
2	191009029	FLJ20160	cg15998761	9.33E-07	9.09E-06	0.4236314	0.824669	0.1393183	0.1145142	0.8527446	0.4643437	0.043732	5.71E-07	0.331558	0.449501	0.418024	0.785659	
6	25862169	SLC17A4	cg21627181	1.14E-06	1.42E-06	0.7663137	0.9149808	0.0591244	0.1414494	0.9511792	0.3972344	0.025202	5.30E-08	0.609342	0.865393	0.467443	0.746032	
14	49849874	L2HGDH	cg20189937	1.32E-06	1.16E-05	0.503935	0.9204654	0.0921306	0.1950088	0.8817908	0.5174749	0.007269	1.26E-07	0.297519	0.525586	0.480584	0.958405	
17	39822093	ITGA2B	cg17749520	1.37E-06	9.24E-06	0.3677214	0.6752543	0.046072	0.1157269	0.8717913	0.3274826	0.009625	3.11E-07	0.286277	0.406727	0.666483	0.903995	
17	39701014	SLC4A1	cg03580247	1.51E-06	1.76E-05	0.4966594	0.7030166	0.1566437	0.1201467	0.7706213	0.4809553	9.19E-05	7.97E-09	0.262889	0.282218	0.522557	0.944193	
20	41789039	FAM112A	cg11398517	2.42E-06	2.11E-05	0.431888	0.7316467	0.0506171	0.209415	0.8337169	0.4256838	0.006763	1.86E-07	0.220078	0.444385	0.592602	0.918166	
11	56904791	PRG3	cg24459209	3.33E-06	1.29E-05	0.5460024	0.9366639	0.0692143	0.1545826	0.8947187	0.4338765	0.094895	2.69E-07	0.444762	0.62114	0.500854	0.993962	
12	7792928	CLEC4C	cg22194129	5.46E-06	0.0001208	0.4977092	0.8378959	0.0710935	0.6019842	0.8886589	0.4447889	0.023532	2.38E-08	0.408481	0.759002	0.521453	0.818552	
14	95011802	C14orf49	cg16522484	8.04E-06	0.0001668	0.4308412	0.7282314	0.1486239	0.1112817	0.9482318	0.4206034	0.034072	1.01E-07	0.285777	0.449644	0.49125	0.837548	
X	48529554	GATA1	cg22543648	9.51E-06	7.12E-05	0.682124	0.9511741	0.1312401	0.1917101	0.6997158	0.4748147	0.02664	2.00E-08	0.585973	0.689124	0.425854	0.888172	
2	11727816	NTSR2	cg25657834	1.02E-05	2.04E-05	0.9354409	0.6331433	0.1373482	0.3261244	0.4920064	0.8015518	5.67E-05	4.84E-10	0.191813	0.526463	0.689339	0.954103	
19	48912054	IRGC	cg26251865	1.76E-05	9.19E-06	0.714389	0.9562959	0.0555475	0.1095196	0.6922884	0.4367507	0.011533	1.35E-08	0.495745	0.567965	0.468957	0.977824	
19	44919789	CLC	cg18254848	1.77E-05	2.33E-05	0.7122613	0.874791	0.0834931	0.1954486	0.6341788	0.5277066	0.161753	4.77E-08	0.462599	0.67872	0.424877	0.937408	
6	10635801	GCNT2	cg26385286	1.78E-05	3.88E-05	0.5747779	0.9758809	0.0654066	0.2046998	0.6574459	0.5308099	0.070861	4.31E-08	0.339931	0.593173	0.451377	0.902264	
3	150064527	CPA3	cg13424229	1.89E-05	2.29E-06	0.4329161	0.6962458	0.1048729	0.0674688	0.7084296	0.6214492	0.001236	4.96E-07	0.054043	0.094343	0.451245	0.955257	

a–c, The table shows the *P* values from regression models in the MRCA panel that predict $\ln(\text{IgE})$ at each locus before adjusting cell counts (a); after adjusting cell counts using Houseman surrogate variables (b); and after adjusting for cell subsets counted in our data (c). pMethyl measures the strength of association to IgE. CD8, CD4 and NK are T-cell subsets. All models are adjusted for sex, age, methylation, parent/child status, sex $\times$ age interaction, and age $\times$ parent interaction. BAS, basophils; EOS, eosinophils; GRAN, granulocytes; LYM, lymphocytes; MON, monocytes; NEU, neutrophil.