

ORIGINAL ARTICLE

Asthma and Rhinitis

Novel eosinophilic gene expression networks associated with IgE in two distinct asthma populations

Yamini V. Virkud^{1,2}  | Rachel S. Kelly²  | Damien C. Croteau-Chonka² | Juan C. Celedón³  | Amber Dahlin² | Lydiana Avila⁴ | Benjamin A. Raby² | Scott T. Weiss² | Jessica A. Lasky-Su²

¹Division of Allergy and Immunology, Department of Pediatrics, Harvard Medical School, Massachusetts General Hospital for Children, Boston, Massachusetts

²Channing Division of Network Medicine, Department of Medicine, Harvard Medical School, Brigham and Women's Hospital, Boston, Massachusetts

³Division of Pediatric Pulmonary Medicine, Allergy and Immunology, Children's Hospital of Pittsburgh of UPMC, University of Pittsburgh, Pittsburgh, Pennsylvania

⁴Department of Pediatrics, Hospital Nacional de Niños, San Jose, Costa Rica

Correspondence

Yamini Virkud, Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.
Email: yvirkud@mgh.harvard.edu

Funding information

This manuscript is supported by an R01 grants from the National Heart, Lung, and Blood Institute of the National Institutes of Health to Brigham and Women's Hospital, NHLBI 1R01HL123915-01 JALS, the NIH grant R37 HL066289 (STW). D.C.C.-C. was supported by a grant from the National Heart, Lung, and Blood Institute of the US National Institutes of Health (K01 HL127265). Y.V.V. was supported by a grant from the National Institute of Allergy and Infectious Diseases of the US National Institutes of Health (K23AI130408).

Summary

Background: Asthma represents a significant public health burden; however, novel biological therapies targeting immunoglobulin E (IgE)-mediated pathways have widened clinical treatment options for the disease.

Objective: In this study, we sought to identify gene transcripts and gene networks involved in the determination of serum IgE levels in people with asthma that can help inform the development of novel therapeutic agents.

Methods: We analysed gene expression data from a cross-sectional study of 326 Costa Rican children with asthma, aged 6 to 12 years, from the Genetics of Asthma in Costa Rica Study and 610 young adults with asthma, aged 16 to 25 years, from the Childhood Asthma Management Program trial. We utilized differential gene expression analysis and performed weighted gene coexpression network analysis on 25 060 genes, to identify gene transcripts and network modules associated with total IgE, adjusting for age and gender. We used pathway enrichment analyses to identify key biological pathways underlying significant modules. We compared findings that replicated between both populations.

Results: We identified 31 transcripts associated with total IgE that replicated between the two study cohorts. These results were notable for increased eosinophil-related transcripts (including *IL5RA*, *CLC*, *SMPD3*, *CCL23* and *CEBPE*). Pathway enrichment identified the regulation of T cell tolerance as important in the determination of total IgE levels, supporting a key role for *IDO1*.

Conclusions and Clinical Relevance: These results provide robust evidence that biologically meaningful gene expression profiles (relating to eosinophilic and regulatory T cell pathways in particular) associated with total IgE levels can be identified in individuals diagnosed with asthma during childhood. These profiles and their constituent genes may represent novel therapeutic targets.

KEYWORDS

allergic asthma, computational biology, eosinophils, gene expression, network medicine

1 | INTRODUCTION

Asthma, allergies and related disorders have doubled in prevalence during recent decades and are among the most common chronic diseases in industrialized countries, with asthma affecting 26 million US children and adults and allergies affecting ~42.5% of the population.¹⁻⁴ A large proportion of asthma cases are characterized by an increase in serum immunoglobulin E (IgE) levels,⁵ and a dose-response relationship between IgE levels and disease severity has been demonstrated.⁶ Total IgE is an important therapeutic target, and existing biological therapies (including omalizumab and recently approved reslizumab and mepolizumab) target IL-4, IL-5, IL-13 and IgE-specific pathways.⁷ However, therapeutic trials of these biological agents only demonstrate benefits among subjects with more severe asthma subtypes.⁷ These findings provide further evidence that asthma comprises a diverse range of immunological and clinical phenotypes, which require an equally diverse variety of personalized therapies.

Given the high heritability of IgE (estimated to be up to 0.8 in cord blood),⁸ a better understanding of the causal genetic factors underlying IgE levels among individuals with asthma may lead to novel IgE-focused treatments for a subset of cases. While many of the genetic and genomic studies of total IgE levels have been performed in genetically heterogeneous populations,⁹⁻¹² we hypothesized that investigating a more homogeneous population, specifically a semipopulation isolate (Costa Rican children with asthma), will enable the improved identification of novel biological targets. A genetic study of this population previously identified an association between polymorphisms in *IL13* and serum IgE, eosinophil count and asthma exacerbations.¹³

The aim of this study was to use a network genomics approach to identify novel differentially expressed genes and gene networks associated with serum total IgE in two populations with asthma: the “Genetics of Asthma in Costa Rica Study” (GACRS) and “The Childhood Asthma Management Program” (CAMP) cohort. By comparing the semi-isolated GACRS population, which is characterized by a high asthma prevalence (24% in children), a more homogenous genetic background and a highly allergic population, to the heterogeneous CAMP population, we aimed to improve the robustness and generalizability of our findings.¹⁴ These approaches using gene coexpression networks have been useful in identifying gene signatures associated with severe subtypes of asthma, but have not previously been employed in association with IgE levels in asthma.^{15,16} Elucidating the biology underlying serum total IgE, particularly as it pertains to asthma severity, is vital to develop improved strategies for the prevention, management and treatment of asthma and allergic conditions.

2 | METHODS

2.1 | GACRS population and study design

Participants in the GACRS are members of a Hispanic population semi-isolated from the Central Valley of Costa Rica, which has one of the highest rates of asthma world-wide.¹⁷ From February 2001 to

August 2008, questionnaires were sent to the parents of 16 912 children (aged 6-14 years) enrolled in 140 Costa Rican schools; 9180 (54.3%) questionnaires were returned. Children were eligible for the study if they had asthma (physician-diagnosed asthma and ≥ 2 respiratory symptoms or asthma attacks in the prior year) and a high probability of having ≥ 6 great grandparents born in the Central Valley of Costa Rica. A total of 1189 children with asthma and their parents (ie 383 parent-child trios) were enrolled. A total of 383 children provided blood samples, on which whole-blood RNA gene expression profiling was performed, using the Illumina HumanHT-12v4 microarray. All participating children completed a protocol including a slightly modified and translated version of the questionnaire used in the Collaborative Study on the Genetics of Asthma.¹⁸ Serum total IgE level was determined using the UniCAP 250 system (Pharmacia & Upjohn, Kalamazoo, MI), with samples measured in duplicate, conducted at the same time as gene expression profiling. Written parental consent and child's assent were obtained. The study was approved by the Institutional Review Boards of the Hospital Nacional de Niños (San Jose, Costa Rica) and Brigham and Women's Hospital (Boston, MA, USA) (IRB Protocol Number 2000P001130).

2.2 | CAMP population and study design

CAMP is a multi-centre, randomized, double-masked, clinical trial designed to determine the long-term (~16.5 years of follow-up) effects of three inhaled treatments for mild-to-moderate asthma in children aged 5 to 12 at baseline: placebo, nedocromil or budesonide.¹⁹ Asthma was defined based on having both a clinical history (with the presence of symptoms or the use of an inhaled bronchodilator at least twice weekly or the use of daily medication for asthma) and airway responsiveness to methacholine (as measured by 20% decline in forced expiratory volume (FEV1) at doses ≤ 12.5 mg/mL of methacholine). A follow-up study to the primary trial extracted blood samples from 620 CAMP subjects at early adulthood (aged 16-25, a mean 11.6 years (range 10-13.5 years) after enrolment) for gene expression profiling using the same Illumina HumanHT chip and concurrently measured serum total IgE (two individuals for whom IgE was measured >18 months from gene expression profiling were removed from the analysis). Of these, 610 subjects had complete phenotype data for downstream analysis.

2.3 | Gene expression profiling

In both populations, whole-blood gene expression was assayed with 47 009 probes on the Illumina HumanHT-12 v4 Expression Bead-Chip (Illumina, Inc., San Diego, CA, USA) that passed stringent and commonly used quality control metrics.²⁰ Expression data were log₂-transformed and quantile-normalized using the R package “lumi” (version 2.22) and Bioconductor.²¹ Principal components of gene expression were generated both for GACRS and for CAMP. Prior to downstream statistical analyses, a standard non-specific variance filter was applied to the expression data using the R package

“genefilter.” Probes not annotated with a valid Entrez gene identifier or Human Genome Organization gene symbol and probes with interquartile ranges (IQR) of expression variance below the 50th percentile were excluded,²² ensuring that the most informative probes were used for analysis. The data were then collapsed to assign a single probe to each gene based on the largest IQR of expression variance. This resulted in a total of 25 059 and 24 971 gene probes for the GACRS and CAMP analyses, respectively. All 24 971 genes in the CAMP data set were also represented in the larger GACRS data set.

2.4 | Statistical analyses

All analyses were first conducted in the GACRS and then conducted independently in the CAMP population for validation of the significant findings using a parallel analysis design and identical methods. As in previous study designs,^{9,23} we aimed to compare findings that replicated in both a relative genetic isolate (GACRS) and an outbred population (CAMP).¹⁹

2.4.1 | Differential gene expression analysis

Generalized linear regression models (GLM)²⁴ were constructed to identify differentially expressed genes associated with log-transformed total serum IgE levels. We adjusted for age (due to the age differences in our two cohorts), sex (due to prior genetic associations with IgE²⁴) and the first two principal components of gene expression to account for any batch effects (all concurrent with serum total IgE measurements). The Benjamini-Hochberg procedure was used to control the false discovery rate (FDR) with q -value set at 0.05.²⁵

2.4.2 | Gene network analysis

Gene expression modules have previously been shown to be enriched for biological functions. We hypothesized that using weighted gene coexpression network analysis (WGCNA),²⁶ we would be able to refine the results of the regression analysis to identify networks of highly significant genes that are expressed together in groups or “modules” of coexpressed genes. These modules can then be related to phenotypic and baseline traits. WGCNA was used to generate a network describing correlation patterns between the genes. Using hierarchical clustering based on topological overlap and applying a soft thresholding power of 5, chosen to achieve a scale-free topology fitting index >0.9 (explaining over 90% of the variance), the interconnectedness between all possible gene pairs was quantified and they were assigned to gene coexpression modules. A dynamic clustering approach was utilized with a deep split of 0.8, and a minimum module size of 30 was specified, to further refine the degree of detail each module contained without influencing module structure, based on recommended defaults.²⁶ We then removed all uncorrelated transcripts that were assigned to the grey module, a module typically reserved for transcripts that do not map to a discrete module. Each module was summarized by an eigenvalue based on its first principal component, and the association between

the module eigenvalues and log₁₀-transformed serum total IgE, after adjustment for age at gene expression profiling and gender, was computed using multivariable linear regression.

2.4.3 | Pathway enrichment analysis

Pathway enrichment analysis using Gene Ontology (GO) terms was performed using an online tool from the GO Consortium (<http://geneontology.org/page/go-enrichment-analysis>), relying on the PANTHER Overrepresentation Test (release 20171205)²⁷ with an $\alpha < 0.01$, fold enrichment >5 and containing at least 2 genes transcripts from our study included in the GO category. We identified key biological pathways among (a) the differentially expressed genes identified using the multivariate linear regression models and (b) the gene transcripts of WGCNA-generated modules associated with serum IgE.

2.4.4 | Overrepresentation analysis

We conducted gene set overrepresentation analysis using replicated gene transcripts common to both the GACRS and CAMP population, in comparison with the Molecular Signatures Database version 6.1 (<http://software.broadinstitute.org/gsea/msigdb/>).⁽²⁹⁾ We specifically used hypergeometric tests with FDR to correct for multiple hypothesis testing and compared our data set with the C7 database, which comprises 4872 immunological gene sets that were curated based on published human and murine studies of the immune system from the Gene Expression Omnibus.²⁹

3 | RESULTS

3.1 | Study populations

The baseline characteristics of the GACRS and CAMP study populations are described in Table 1. The GACRS comprises a Hispanic/Latino population, while CAMP was predominantly white European (68%) and only 10% Hispanic/Latino. The proportion of males and females in the two cohorts were similar, with a higher percentage of males in both. The age of asthma onset was young in both cohorts: 2.4 years in the GACRS and 3.0 years in CAMP. Gene expression profiling and the measurement of serum IgE levels were performed on blood samples taken in childhood (mean: 9.1 years) in the GACRS and in early adulthood in CAMP (mean: 20.4 years). Importantly, there was no significant difference in serum IgE levels between the two cohorts, despite the age difference.

3.2 | Differential gene expression analysis

A total of 81 gene transcripts were significantly associated with serum IgE levels in the GACRS cohort after adjustment for age at expression profiling, gender and the first two principal components, and controlling for the FDR. Sixty-nine of these gene transcripts (85%) were positively associated with serum total IgE, and 12 (15%)

TABLE 1 Demographics of GACRS population and CAMP populations

	GACRS	CAMP	P-Value
Number of participants	326	610	
Sex, n (%)			
Female	140 (43%)	226 (37%)	0.10
Male	186 (57%)	384 (63%)	
Race/ethnicity			
Hispanic/Latino	326 (100%)	59 (10%)	na
American Indian or Alaskan Native	0 (0%)	4 (1%)	
Black or African American	0 (0%)	94 (15%)	
East/South-east Asian	0 (0%)	4 (1%)	
European	0 (0%)	417 (68%)	
Multiple races	0 (0%)	32 (5%)	
Age at gene expression profiling, mean (SD)	9.1 (1.8)	20.4 (2.2)	<0.001
Age of asthma onset (y), mean (SD)	2.4 (2.2)	3.0 (2.4)	<0.001
log ₁₀ IgE (kU/L), mean (SD)	2.5 (0.7)	2.5 (0.6)	0.74
Atopic dermatitis	38 (25%)	174 (29%)	0.44
Allergic rhinitis	300 (92%)	310 (51%)	<0.001
Inhaled corticosteroid use	173 (53%)	125 (23%)	<0.001

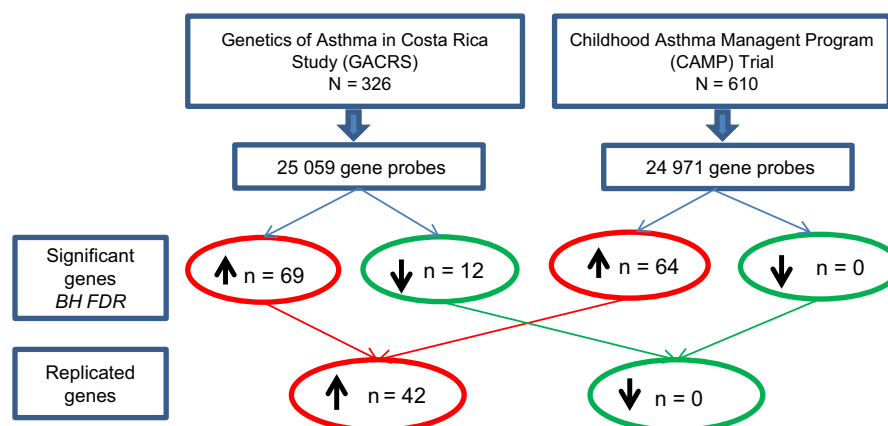
were negatively associated (Figure 1). Differential gene expression analysis showed no association of these genes with atopic dermatitis or allergic rhinitis. Of these 81 gene transcripts, 42 (54%) were replicated in CAMP with a concordant direction of effect; all 42 were overexpressed with increasing IgE levels (Figure 2, Table S1). We then conducted a sensitivity analysis, adjusting for inhaled corticosteroid use in both populations, with 36 (86%) transcripts still significant (Table S1). Of note, all of the transcripts identified in CAMP remained significantly associated with IgE after adjusting for original treatment group from the CAMP randomized trial. The 42 transcripts replicated in CAMP and GACRS were nominally enriched ($P < 0.01$, fold enrichment > 5, number of genes > 1) for 32 biological processes

(Table S2). Although none of these 32 processes was robust to the most stringent correction for multiple testing, Bonferroni correction, a number had biologically plausible functional annotations, particularly those related to immune responses, such as “regulation of T cell tolerance induction.”

3.3 | Gene network analysis

The gene expression network analysis was first performed in the GACRS. The samples were clustered, resulting in the exclusion of three samples identified as outliers. From the 25 059 transcripts, hierarchical clustering based on the topological overlap then designated the 10 073 transcripts into 32 modules (43%) (Figure S1A). Five modules, as summarized by their eigengenes, were associated with serum IgE level ($\alpha < 0.05$) (Figure S1A). Only one module, containing 103 gene transcripts, was robust to correction for multiple testing ($P = 4.8 \times 10^{-11}$). This module was also associated with the more stringent subtype of asthma ($P = 0.002$), defined by physician diagnosis and the presence of airway hyper-reactivity on methacholine challenge, but was not associated with allergic rhinitis or atopic dermatitis. The module was also not associated with inhaled corticosteroid use, but had a nominal association with oral steroid use in the last year ($P = 0.04$). Pathway enrichment analysis of these 103 transcripts identified 52 GO-defined biological processes (Table S3), including 12 of the 32 processes identified in the differential gene expression analysis and additional processes relating to immune function.

WGCNA was then applied to the CAMP data set using the same network construction approach; one sample was excluded as an outlier. Within the remaining 611 samples, the gene transcripts were clustered into 40 modules including 15 120 transcripts (61%). Fourteen modules were associated with serum IgE levels after adjustment for age and gender ($P < 0.05$), and one module was robust to correction for multiple testing ($P = 6.8 \times 10^{-14}$) (Figure S1B). This module was also associated with inhaled corticosteroid use ($P = 0.0003$). This module contained 38 transcripts that were enriched for 25 biological processes (Table S4).

**FIGURE 1** Number of genes significantly associated with serum total IgE in the GACRS and CAMP populations by differential gene expression. BH FDR, Benjamini-Hochberg adjusted false discovery rate

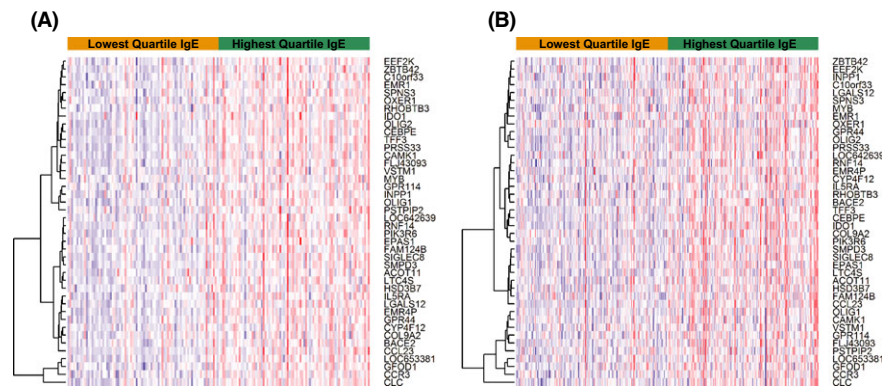


FIGURE 2 Heat map comparing differential gene expression of the 42 replicated genes by IgE (top vs bottom quartile) in the (A) GACRS and (B) CAMP populations. All individuals were included in the differential gene expression analysis; however, the top quartile IgE (green) and the bottom quartile IgE (yellow) were displayed for comparison purposes

To determine any genomic and biological similarities between the significant GACRS and CAMP modules, the overlap in terms of gene transcripts and enriched biological processes was computed. Of the 103 transcripts in the significant GACRS module and the 38 transcripts in the significant CAMP module, all 38 were common to both (Table S5). Of the 52 and 25 enriched GO-defined biological processes from the GACRS and CAMP modules, respectively, 14 were common to both (Table S5). Finally, the similarities between the differential gene expression analysis and the WGCNA analyses were compared (Figure 3). Thirty-one transcripts (Table 2), and twelve enriched biological processes, including a number related to immune response, were common to both sets of analyses in both cohorts (Table 3) and further confirmed the similarities in results between the two independent populations. Based on the literature, eight of the thirty-one gene transcripts have been previously linked to IgE, while the remainder represent novel associations (Table 2).

3.4 | Overrepresentation analysis

To further clarify the immunological relevance of the 31 validated transcripts, we compared our gene set to a database of immunological signatures, yielding three gene sets that were enriched (Table S6). These three gene sets encompassed gene transcripts up-regulated in neutrophils (*GPR44*, *CCR3*, *IL5RA*, *CLC*, *SIGLEC8*; *FDR* q value < 0.001), bone marrow-derived macrophages (*EMR1*, *CCL23*, *OLIG2*, *OLIG1*; $q < 0.011$) and eosinophils (*SMPD3*, *CCL23*, *IDO1*, *CEBPE*; $q < 0.011$).

4 | DISCUSSION

Novel biological therapies targeting total IgE-mediated pathways have widened clinical treatment options for asthma. A better understanding of the biology underlying total IgE levels should help refine and improve these therapeutic approaches. We used differential gene expression approaches and network analysis, followed by a variety of pathway enrichment techniques, to identify novel gene transcripts coregulated with determinants of serum IgE levels in individuals with asthma. The combination of statistical approaches allowed us to identify the most biologically relevant transcripts working in combination to influence total IgE. Crucially, we had access to two independent populations and achieved a high level of replication, with 52% of transcripts replicated using GLM and 37% using WGCNA. Combining the results of these two methods yielded ultimately 31 biologically relevant and informative transcripts that were associated with serum total IgE. It is also notable that the key gene coexpression network module associated with IgE was also associated with the more stringent definition of asthma based on physician diagnosis and methacholine challenge, suggesting that these 31 replicated gene transcripts reflect not simply an IgE-related gene signature but also an allergic asthma signature. This network module was associated with the use of inhaled corticosteroid in the CAMP cohort, though not in the GACRS cohort. While it is difficult to determine whether this is due to confounding with severity of asthma, we reported that

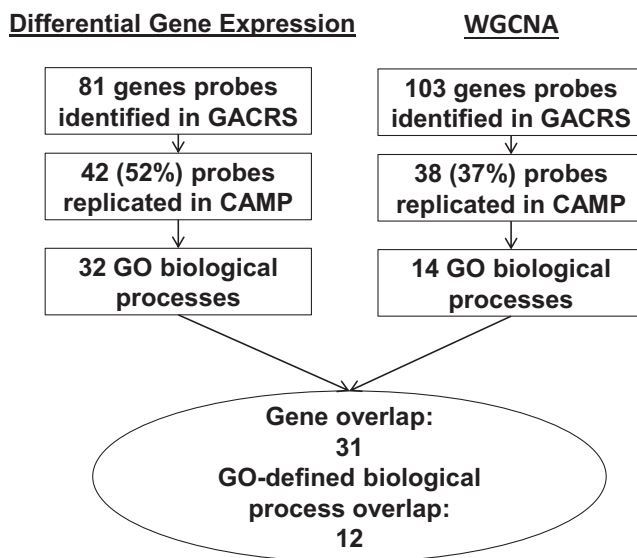


FIGURE 3 Comparison of the total IgE gene expression profiles generated using differential gene expression and network approaches

TABLE 2 Replicated gene transcripts (n = 31) associated with total IgE using two different statistical approaches in two distinct cohorts with asthma: GACRS and CAMP

Gene symbol	Gene name	Chr	GACRS P-value	CAMP P-value	Sig. adjusted for ICS	Linked with eosinophils		
						Esnaul ³⁶	Nakajima ³⁷	MSigDB ²⁸
PRSS33	Protease, serine, 33	16	2.8×10^{-9}	2.4×10^{-10}	■	●		
OLIG2	Oligodendrocyte lineage transcription factor 2	21	1.7×10^{-8}	2.3×10^{-12}	■	●		
TFF3	Trefoil factor 3 (intestinal)	21	2.4×10^{-8}	6.7×10^{-13}	■		●	
CLC ^a	Charcot-Leyden crystal protein	19	3.8×10^{-8}	4.0×10^{-9}	■	●	●	
CCL23	Chemokine (C-C motif) ligand 23; macrophage inflammatory protein 1-alpha (MIP-1-alpha)	17	1.5×10^{-7}	3.0×10^{-10}	■		●	●
EMR4P	Adhesion G protein-coupled receptor E4, pseudogene	19	1.5×10^{-7}	7.1×10^{-6}	■	●		
OLIG1	Oligodendrocyte transcription factor 1	21	1.5×10^{-7}	7.8×10^{-7}	■	●		
GPR44 ^a	Prostaglandin D2 receptor 2, G protein-coupled receptor 44, (aka CRTH2)	11	2.0×10^{-7}	2.1×10^{-8}	■	●	●	
FLJ43093	Unknown	6	2.5×10^{-7}	2.0×10^{-8}	■			
CEBPE	CCAAT/enhancer binding protein (C/EBP), epsilon	14	2.7×10^{-7}	2.6×10^{-13}	■			●
PYROXD2	Pyridine nucleotide-disulphide oxidoreductase domain 2 (PYROXD2)	10	3.3×10^{-7}	3.8×10^{-10}	■	●		
SORD2	Sorbitol dehydrogenase	15	1.5×10^{-6}	4.9×10^{-5}	■			
SIGLEC8 ^a	Sialic acid binding Ig-like lectin 8	19	1.6×10^{-6}	5.0×10^{-9}	■	●		
PIK3R6 ^a	Phosphoinositide-3-kinase, regulatory unit 6	17	2.9×10^{-6}	2.0×10^{-8}	■	●		
LGALS12	Galectin 12	11	6.1×10^{-6}	3.7×10^{-4}	■	●		
GFOD1	Glucose-fructose oxidoreductase domain containing 1	6	8.7×10^{-6}	1.8×10^{-3}	■			
CCR3 ^a	Chemokine (C-C motif) receptor 3	3	1.8×10^{-5}	9.2×10^{-5}	■	●	●	
IL5RA ^a	Interleukin 5 receptor, alpha	3	3.5×10^{-5}	0.020	■	●	●	
SMPD3	Sphingomyelin phosphodiesterase 3, neutral membrane	16	5.6×10^{-5}	2.2×10^{-6}	■			●
INPP1	Inositol polyphosphate-1-phosphatase	2	1.5×10^{-4}	6.4×10^{-5}	■			
EMR1	Adhesion G protein-coupled receptor E1 (alias ADGRE1)	19	2.5×10^{-4}	5.5×10^{-3}	■		●	
ACOT11	Acyl-CoA thioesterase 11	1	2.6×10^{-4}	2.1×10^{-4}	■			
CAMK1	Calcium/calmodulin-dependent protein kinase I	3	3.0×10^{-4}	6.8×10^{-5}	■	●	●	
VSTM1	V-set & transmembrane domain containing 1	19	4.6×10^{-4}	1.9×10^{-6}	■	●		
RNF14	Ring finger protein 14	5	4.6×10^{-4}	3.1×10^{-5}	■			
SPNS3	Spinster homolog 3 (Drosophila)	17	6.2×10^{-4}	6.6×10^{-9}	■	●		
ZBTB42	Zinc finger and BTB domain containing 42	14	7.5×10^{-4}	2.7×10^{-8}	■			
IDO1 ^a	Indoleamine 2,3-dioxygenase 1 (aka INDO)	8	1.1×10^{-3}	2.4×10^{-10}	■		●	●
LOC642639	Unknown	9	1.2×10^{-3}	1.7×10^{-3}	■			
LTC4S ^a	Leukotriene C4 synthase	5	2.5×10^{-3}	6.9×10^{-6}	■	●		
CYP4F12	Cytochrome P450, family 4, subfamily F, polypeptide 12	19	4.2×10^{-3}	2.0×10^{-5}	■	●		

CAMP, Childhood Asthma Management Program; GACRS, Genetics of Asthma in Costa Rica Study; MSigDB, Molecular Signature Database.

●: known association with eosinophils.

■: Significant in both cohorts after adjustment for recent inhaled corticosteroid (ICS) use.

^aKnown association with IgE.

all of transcripts reported in Table 2 remained significantly associated with IgE levels by differential gene expression even after adjusting for the use of inhaled corticosteroids.

Within the pathway enrichment analyses of the total IgE-associated transcripts, we identified the immune relevant GO category, "regulation of tolerance induction," which was highlighted due to

TABLE 3 Gene ontology defined biological processes associated with total IgE using two different statistical approaches in two different cohorts: GACRS and CAMP cohorts

GO biological process	# Genes in GO group	Differential gene expression		WGCNA			
		GACRS and CAMP		GACRS		CAMP	
		Fold enrichment	Raw P value	Fold enrichment	Raw P value	Fold enrichment	Raw P value
Regulation of leucocyte-mediated immunity (GO:0002703): <i>CLC</i> , <i>PIK3R6</i>	167	9.7	0.0038	6.3	0.0014	10.5	0.0030
Regulation of leucocyte proliferation (GO:0070663): <i>IDO1</i> , <i>CLC</i>	219	7.4	0.0080	5.8	0.0007	8.0	0.0064
Regulation of lymphocyte-mediated immunity (GO:0002706): <i>CLC</i> , <i>PIK3R6</i>	126	12.9	0.0017	6.7	0.0034	13.9	0.0014
Regulation of lymphocyte proliferation (GO:0050670): <i>IDO1</i> , <i>CLC</i>	210	7.7	0.0071	6.0	0.0006	8.4	0.0057
Regulation of mononuclear cell proliferation (GO:0032944): <i>IDO1</i> , <i>CLC</i>	211	7.7	0.0072	6.0	0.0006	8.3	0.0058
Regulation of T-cell tolerance induction (GO:0002664): <i>IDO1</i> , <i>CLC</i>	12	89.9	0.0003	35.1	0.0019	97.4	0.0003
Regulation of tolerance induction (GO:0002643): <i>IDO1</i> , <i>CLC</i>	18	60.0	0.0006	23.4	0.0040	64.9	0.0005
Neuron fate commitment (GO:0048663): <i>OLIG1</i> , <i>OLIG2</i>	70	15.4	0.0079	12.0	0.0004	16.7	0.0067
Oligodendrocyte differentiation (GO:0048709): <i>OLIG1</i> , <i>OLIG2</i>	65	16.6	0.0068	13.0	0.0003	27.0	0.0002
Positive regulation of glial cell differentiation (GO:0045687): <i>OLIG2</i> , <i>CLC</i>	33	32.7	0.0019	19.1	0.0007	35.4	0.0016
Positive regulation of gliogenesis (GO:0014015): <i>OLIG2</i> , <i>CLC</i>	51	31.7	0.0001	16.5	0.0001	22.9	0.0037
Regulation of glial cell differentiation (GO:0045685): <i>OLIG2</i> , <i>CLC</i>	59	18.3	0.0057	14.3	0.0002	19.8	0.0049

the up-regulation of Charcot-Leyden crystal galectin (*CLC*) and indoleamine-pyrrole 2,3-dioxygenase (*IDO1*) in association with increased total IgE levels. Both genes are expressed in eosinophils, and their activity is stimulated following binding of IgE. *CLC* levels have been positively associated with eosinophilic airway inflammation and aspirin-induced asthma^{30,31} and have also been associated with expansion of T regulatory cells.³² *IDO1* encodes an enzyme involved in tryptophan catabolism and suppresses helper and effector T cells while activating regulatory T cells.³³ These findings are notable given that regulatory T cell activity is thought to be deficient in allergic asthma,³⁴ yet we found a positive association of increasing IgE with increased *IDO1* and *CLC*. We hypothesize that this represents a regulatory mechanism induced to curb the allergic immune response in the setting of asthma. This hypothesis is supported by findings describing a spectrum of increased *IDO1* activity that is higher among atopic individuals compared to healthy controls and highest among asymptomatic atopic subjects compared to symptomatic ones, suggesting that *IDO1* may be involved in inducing a state of clinical unresponsiveness in allergic individuals.³⁵ Further study is needed to clarify the roles of *IDO1* and serum IgE in asthma.

Overrepresentation analysis suggested the presence of various immunological signatures such as enrichment of neutrophil,

macrophage and eosinophil-associated genes. In particular, comparison with a gene set of eosinophils compared to Th1 cells showed that 4 of our 31 transcripts overlapped, including *SMPD3*, *CCL23*, *IDO1* and *CEBPE*. These findings support the involvement of eosinophil-associated genes in total IgE regulation. At least 20 of these 31 validated transcripts were previously found to be up-regulated in eosinophils.^{36,37} Esnault et al³⁶ compared gene expression in sputum eosinophils from individuals with mild asthma before and after allergen challenge and found 365 up-regulated transcripts. Sixteen of our 31 transcripts overlapped with that set, providing further validation for the genes as being strongly associated with the asthmatic response to allergen (Table 2). Esnault et al also conducted experiments comparing the changes in allergen-induced gene expression in bronchoalveolar lavage cells, before and after treatment with mepolizumab, to block IL-5 pathways. Of the gene transcripts that were up-regulated after allergen exposure but down-regulated after mepolizumab treatment, 10 of our transcripts overlapped, suggesting involvement in IL-5-related pathways of inflammation in asthma (*CYP4F12*, *EMR4P*, *IL5RA*, *LGALS12*, *LTC4S*, *PIK3R6*, *PRSS33*, *SIGLEC8*, *SPNS3*, *VSTM1*). In another gene expression analysis of eosinophils compared with mast cells, neutrophils and mononuclear cells, Nakajima et al³⁷ identified 30 eosinophil-specific genes; 9 of our 31

transcripts overlapped with that set, further supporting the association between eosinophilic inflammation and total IgE levels. While we found high levels of replication between our populations and with other microarray data, we also compared our findings with GWAS studies of IgE and were unable to find similarities.^{9,38} However, given the limited overlap between IgE-associated GWAS findings and those of asthma,³⁸ it is not surprising that our replicated transcripts associated with IgE in asthmatics did not coincide with these prior association signals.

The most notable aspect of this analysis is the high degree of replication of the identified transcripts, using two distinct statistical methods in two different patient populations, as well as in comparison with gene sets described in the literature. Some of these genes, such as *IL5RA* and *GPR44* (*CRTH2*, receptor for prostaglandin D₂), represent known therapeutic targets, with IL-5 receptor antagonists showing some efficacy in uncontrolled eosinophilic asthma.³⁹ *CCR3* (eotaxin-3), a chemokine receptor, has been associated with allergic asthma, although *CCR3*-targeted therapies appear to be less efficacious in humans than in mouse models.^{40,41} In addition, the leukotriene pathway has been a therapeutic target for asthma for decades, although specific inhibitors for *LTC4S* (leukotriene C₄ synthase) have only recently been developed and evaluated in mouse models.^{42,43} The identification of these known targets using our analytical approach corroborates the possibility that the other transcripts in this set may be equally useful as therapeutic targets.

Other genes from our eosinophil-related gene sets have known associations with allergic asthma and may also have therapeutic or diagnostic potential, including *CCL23*, *CLC*, *CEBPE*, *CAMK1*, *IDO1*, *SIGLEC8*, *SMPD3* and *SPNS3*.⁴⁴⁻⁵¹ Potential therapeutic targets include cell surface receptors, such as *Siglec8*, an inhibitory receptor on eosinophils with polymorphisms associated with asthma.⁴⁵⁻⁴⁷ *CCL23* is an eosinophilic chemokine that has been identified as a possible biomarker for anti-IL13-targeted therapies.^{48,49} Several of these are enzymes expressed within eosinophils, such as *CLC*, *IDO1*, *SMPD3* and *SPNS3*, and the latter two are both involved in the sphingolipid pathways, known to mediate airway hyperresponsiveness and mast cell activation.^{50,51} Another gene *CAMK1* has been implicated as an asthma control gene,²⁰ but has not been identified previously with IgE or an allergy-related phenotype among individuals diagnosed with asthma. Of the calmodulin-dependent kinases, *CAMK2* and *CAMK4* have been implicated in bronchoconstriction and smooth muscle proliferation,^{52,53} suggesting that *CAMK1* may also play a role in asthma pathogenesis.

We also report the expression of a number of novel genes, including *EMR1*, *EMR4P* and *PIK3R6*, which were significant in our data and have been shown to be up-regulated in eosinophilic asthma in other studies. These genes have no known mechanistic association with total IgE or asthma but have been implicated in allergic processes such as allergic rhinosinusitis and anaphylaxis.^{54,55} *EMR1* and *EMR4P* encode G protein-coupled receptors that may be particularly useful as therapeutic targets. While these genes do not have known roles in IgE or asthma, their replication in both of our

cohorts, as well as other published gene sets, implicates them as potentially novel biomarkers or therapeutic targets.

Our study design and analysis does have some limitations. The cross-sectional nature of the expression and phenotype data limit the ability to make true causal inferences. The use of whole-blood gene expression profiles may not be as physiologically relevant as lung tissue-specific profiling; however, the use of less invasive sampling methods allows for greater sample sizes and more robust replication. While it could prove useful to split the whole-blood expression data into specific cell populations, these techniques are in development and still face significant limitations, including concerns about accuracy and ability to detect expression profiles of cell subsets.^{56,57} Recent studies support the utility of whole-blood transcriptomics in the setting of asthma.^{58,59} We cannot exclude the possibility that these transcripts play a role in atopic disease in general; however, the WGCNA analysis showing an association with a stricter definition of asthma in the GACRS population, and a lack of association with either atopic dermatitis or allergic rhinitis, supports a more prominent role in allergic asthma. Also, GO-based enrichment analyses are subject to annotation bias and can often yield high-level information about biological processes that are ubiquitous to all cellular function; thus, we attempted to improve the biological relevance of this analysis by placing more stringent criteria on fold enrichment and the number of genes required.

This analysis benefits from the GACRS population being a highly homogeneous relative genetic isolate with consistent and controllable environmental exposures, detailed clinical information and minimal population stratification. We have abundant data demonstrating that the genetic findings in the GACRS cohort are generalizable to CAMP. There are over 15 studies of validated susceptibility loci replicated in GACRS and CAMP with similar effect size and direction of effect, whether identified by GWAS,⁶⁰⁻⁶⁶ by linkage and positional cloning⁶⁷⁻⁶⁹ or by candidate gene testing.^{23,64,70-73} However, we acknowledge that there are ethnic, genetic and environmental differences between the GACRS population and the North American CAMP population, which may have limited our ability to find additional shared gene probes. Focusing on only replicated gene transcripts may have missed important gene probes relevant only in a single population and could have limited the detection of novel biological targets. In addition, the gene expression profiling was performed on childhood samples from GACRS and adulthood samples from CAMP. Given the striking overlap in our findings over both ethnicity and age, the generalizability of these results is a strength rather than a limitation.

In conclusion, we present robust and reproducible evidence for consistent gene expression profiles of serum total IgE in individuals with asthma. Indeed, we found similar profiles with clear biological relevance, regardless of the statistical approach used: multivariable regression or network-based approaches. Pathway enrichment identified an important role for regulation of T cell tolerance. In addition to gene transcripts with known or suspected relationships with IgE-related pathways, we identified a number of novel transcripts. Many of the genes encoding these transcripts have been reported in other

studies of asthma, suggesting that they may represent novel therapeutic targets or biomarkers. Further functional validation of these transcripts and their roles in influencing total IgE and asthma will be essential to developing novel strategies for improved primary prevention, management and treatment of asthma.

ACKNOWLEDGEMENTS

We thank the participants and families who have so generously participated in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Yamini Virkud - data analysis, data interpretation, writing the manuscript, figures and literature search. Rachel Kelly - data analysis, data interpretation, writing the manuscript, figures and literature search. Damien C. Croteau-Chonka - data analysis and data interpretation. Juan Celedon - study design and data collection. Amber Dahlin - data interpretation, literature search and manuscript revision. Lydiana Avila - study implementation and data collection. Benjamin A. Raby - study design, data collection and manuscript revision. Scott T. Weiss - study design, data collection and manuscript revision. Jessica A. Lasky-Su - data analysis, data interpretation and manuscript revision.

ORCID

Yamini V. Virkud  <http://orcid.org/0000-0001-7328-7215>

Rachel S. Kelly  <http://orcid.org/0000-0003-3023-1822>

Juan C. Celedón  <http://orcid.org/0000-0002-6139-5320>

REFERENCES

- Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy*. 2004;59(5):469-478.
- Devereux G. The increase in allergic disease: environment and susceptibility. Proceedings of a symposium held at the Royal Society of Edinburgh, 4th June 2002. *Clin Exp Allergy*. 2003;33(3):394-406.
- U.S. Department of Health and Human Services, National Institutes of Health. Global Initiative for Asthma Management and Prevention. NHLBI/WHO Workshop Report. 1995;95-3659.
- Akinbami LJ, Moorman JE, Bailey C, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. *NCHS Data Brief*. 2012;94:1-8.
- Peng Z. Vaccines targeting IgE in the treatment of asthma and allergy. *Hum Vaccin*. 2009;5(5):302-309.
- Naqvi M, Choudhry S, Tsai HJ, et al. Association between IgE levels and asthma severity among African American, Mexican, and Puerto Rican patients with asthma. *J Allergy Clin Immunol*. 2007;120(1):137-143.
- Bice JB, Leechawengwongs E, Montanaro A. Biologic targeted therapy in allergic asthma. *Ann Allergy Asthma Immunol*. 2014;112(2):108-115.
- Husby S, Holm NV, Christensen K, Skov R, Morling N, Petersen PH. Cord blood immunoglobulin E in like-sexed monozygotic and dizygotic twins. *Clin Genet*. 1996;50(5):332-338.
- Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med*. 2010;363(13):1211-1221.
- Sleiman PM, Flory J, Imielinski M, et al. Variants of DENND1B associated with asthma in children. *N Engl J Med*. 2010;362(1):36-44.
- Granada M, Wilk JB, Tuzova M, et al. A genome-wide association study of plasma total IgE concentrations in the Framingham Heart Study. *J Allergy Clin Immunol*. 2012;129(3):840-845. e21.
- Lasky-Su J, Himes BE, Raby BA, et al. HLA-DQ strikes again: genome-wide association study further confirms HLA-DQ in the diagnosis of asthma among adults. *Clin Exp Allergy*. 2012;42(12):1724-1733.
- Hunninghake GM, Soto-Quiros ME, Avila L, et al. Polymorphisms in IL13, total IgE, eosinophilia, and asthma exacerbations in childhood. *J Allergy Clin Immunol*. 2007;120(1):84-90.
- Asher MI, Montefort S, Bjorksten B, et al. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and Three repeat multicountry cross-sectional surveys. *Lancet*. 2006;368(9537):733-743.
- Bigler J, Boedigheimer M, Schofield JP, et al. A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts. *Am J Respir Crit Care Med*. 2017;195(10):1311-1320.
- Modena BD, Bleecker ER, Busse WW, et al. Gene expression correlated to severe asthma characteristics reveals heterogeneous mechanisms of severe disease. *Am J Respir Crit Care Med*. 2017;195:1449-1463.
- Pearce N, Ait-Khaled N, Beasley R, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax*. 2007;62(9):758-766.
- Blumenthal MN, Banks-Schlegel S, Bleecker ER, Marsh DG, Ober C. Collaborative studies on the genetics of asthma—National Heart, Lung and Blood Institute. *Clin Exp Allergy*. 1995;25:29-32.
- Childhood Asthma Management Program Research Group. The Childhood Asthma Management Program (CAMP): design, rationale, and methods. *Childhood Asthma Management Program Research Group. Control Clin Trials*. 1999;20(1):91-120.
- Croteau-Chonka DC, Qiu W, Martinez FD, et al. Gene expression profiling in blood provides reproducible molecular insights into asthma control. *Am J Respir Crit Care Med*. 2017;195(2):179-188.
- Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*. 2004;5(10):R80.
- Bourgon R, Gentleman R, Huber W. Independent filtering increases detection power for high-throughput experiments. *Proc Natl Acad Sci USA*. 2010;107(21):9546-9551.
- Hunninghake GM, Soto-Quiros ME, Avila L, et al. TSLP polymorphisms are associated with asthma in a sex-specific fashion. *Allergy*. 2010;65(12):1566-1575.
- Raby BA, Soto-Quiros ME, Avila L, et al. Sex-specific linkage to total serum immunoglobulin E in families of children with asthma in Costa Rica. *Hum Mol Genet*. 2007;16(3):243-253.
- Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc*. 1995;57(1):289-300.
- Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*. 2008 Dec; 29(9):559-571.
- Starownik R, Michalak J, Bar K, Plaza P, Muc K, Rechberger T. An uncommon case of inflammatory infiltration of the urinary bladder in the long-term process of the purulent inflammation of the cervix and vaginal fornix, complicated with vesicovaginal fistula of unknown etiology. *Cent European J Urol*. 2013;66(1):101-103.

28. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005;102(43):15545-15550.
29. Godec J, Tan Y, Liberzon A, et al. Compendium of Immune Signatures Identifies Conserved and Species-Specific Biology in Response to Inflammation. *Immunity*. 2016;44(1):194-206.
30. Chua JC, Douglass JA, Gillman A, O'Hehir RE, Meeusen EN. Galectin-10, a potential biomarker of eosinophilic airway inflammation. *PLoS One*. 2012;7(8):e42549.
31. Devouassoux G, Pachot A, Laforest L, et al. Galectin-10 mRNA is overexpressed in peripheral blood of aspirin-induced asthma. *Allergy*. 2008;63(1):125-131.
32. Rabinovich GA, Toscano MA. Turning 'sweet' on immunity: galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol*. 2009;9(5):338-352.
33. Baban B, Chandler PR, Sharma MD, et al. IDO activates regulatory T cells and blocks their conversion into Th17-like T cells. *J Immunol*. 2009;183(4):2475-2483.
34. Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity*. 2009;31(3):438-449.
35. von Bubnoff D, Fimmers R, Bogdanow M, Matz H, Koch S, Bieber T. Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure. *Clin Exp Allergy*. 2004;34(7):1056-1063.
36. Esnault S, Kelly EA, Sorkness RL, Evans MD, Busse WW, Jarjour NN. Airway factor XIII associates with type 2 inflammation and airway obstruction in asthmatic patients. *J Allergy Clin Immunol*. 2016;137(3):767-773. e6.
37. Nakajima T, Matsumoto K, Suto H, et al. Gene expression screening of human mast cells and eosinophils using high-density oligonucleotide probe arrays: abundant expression of major basic protein in mast cells. *Blood*. 2001;98(4):1127-1134.
38. Potaczek DP, Kabesch M. Current concepts of IgE regulation and impact of genetic determinants. *Clin Exp Allergy*. 2012;42(6):852-871.
39. Molino NA, Gossage D, Kolbeck R, Parker JM, Geba GP. Molecular and clinical rationale for therapeutic targeting of interleukin-5 and its receptor. *Clin Exp Allergy*. 2012;42(5):712-737.
40. Provost V, Larose MC, Langlois A, Rola-Pleszczynski M, Flamand N, Laviolette M. CCL26/eotaxin-3 is more effective to induce the migration of eosinophils of asthmatics than CCL11/eotaxin-1 and CCL24/eotaxin-2. *J Leukoc Biol*. 2013;94(2):213-222.
41. Ahmadi Z, Hassanshahi G, Khorramdelazad H, Zainodini N, Koochakzadeh L. An overlook to the characteristics and roles played by eotaxin network in the pathophysiology of food allergies: allergic asthma and atopic dermatitis. *Inflammation*. 2016;39(3):1253-1267.
42. Ago H, Okimoto N, Kanaoka Y, et al. A leukotriene C4 synthase inhibitor with the backbone of 5-(5-methylene-4-oxo-4,5-dihydrothiazol-2-ylamino) isophthalic acid. *J Biochem*. 2013;153(5):421-429.
43. Niegowski D, Kleinschmidt T, Ahmad S, et al. Structure and inhibition of mouse leukotriene C4 synthase. *PLoS One*. 2014;9(5):e96763.
44. Gombart AF, Kwok SH, Anderson KL, Yamaguchi Y, Torbett BE, Koeffler HP. Regulation of neutrophil and eosinophil secondary granule gene expression by transcription factors C/EBP epsilon and PU.1. *Blood*. 2003;101(8):3265-3273.
45. Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood*. 2003;101(12):5014-5020.
46. Angata T. Associations of genetic polymorphisms of Siglecs with human diseases. *Glycobiology*. 2014;24(9):785-793.
47. Gao PS, Shimizu K, Grant AV, et al. Polymorphisms in the sialic acid-binding immunoglobulin-like lectin-8 (Siglec-8) gene are associated with susceptibility to asthma. *Eur J Hum Genet*. 2010;18(6):713-719.
48. Youn BS, Zhang SM, Broxmeyer HE, et al. Characterization of CKbeta8 and CKbeta8-1: two alternatively spliced forms of human beta-chemokine, chemoattractants for neutrophils, monocytes, and lymphocytes, and potent agonists at CC chemokine receptor 1. *Blood*. 1998;91(9):3118-3126.
49. Syed F, Huang CC, Li K, et al. Identification of interleukin-13 related biomarkers using peripheral blood mononuclear cells. *Biomarkers*. 2007;12(4):414-423.
50. Khavandgar Z, Murshed M. Sphingolipid metabolism and its role in the skeletal tissues. *Cell Mol Life Sci*. 2015;72(5):959-969.
51. Ono JG, Worgall TS, Worgall S. Airway reactivity and sphingolipids-implications for childhood asthma. *Mol Cell Pediatr*. 2015;2(1):13.
52. Girodet PO, Ozier A, Bara I, Tunon de Lara JM, Marthan R, Berger P. Airway remodeling in asthma: new mechanisms and potential for pharmacological intervention. *Pharmacol Ther*. 2011;130(3):325-337.
53. Sanders PN, Koval OM, Jaffer OA, et al. CaMKII is essential for the proasthmatic effects of oxidation. *Sci Transl Med*. 2013;5(195):195ra97.
54. Legrand F, Tomasevic N, Simakova O, et al. The eosinophil surface receptor epidermal growth factor-like module containing mucin-like hormone receptor 1 (EMR1): a novel therapeutic target for eosinophilic disorders. *J Allergy Clin Immunol*. 2014;133(5):1439-1447. e1-8.
55. Biethahn K, Orinska Z, Vigorito E, et al. miRNA-155 controls mast cell activation by regulating the PI3Kgamma pathway and anaphylaxis in a mouse model. *Allergy*. 2014;69(6):752-762.
56. Wang L, Oh WK, Zhu J. Disease-specific classification using deconvoluted whole blood gene expression. *Sci Rep*. 2016;6:32976.
57. Shen-Orr SS, Gaujoux R. Computational deconvolution: extracting cell type-specific information from heterogeneous samples. *Curr Opin Immunol*. 2013;25(5):571-578.
58. Vijverberg SJ, Hilvering B, Raaijmakers JA, Lammers JW, Maitland-van der Zee AH, Koenderman L. Clinical utility of asthma biomarkers: from bench to bedside. *Biologics*. 2013;7:199-210.
59. Croteau-Chonka DC, Qiu W, Martinez FD, et al. Gene expression profiling in blood provides reproducible molecular insights into asthma control. *Am J Respir Crit Care Med*. 2017;195(2):179-188.
60. Verlaan DJ, Berlivet S, Hunninghake GM, et al. Allele-specific chromatin remodeling in the ZBP2/GSDMB/ORMDL3 locus associated with the risk of asthma and autoimmune disease. *Am J Hum Genet*. 2009;85(3):377-393.
61. Torgerson DG, Ampleford EJ, Chiu GY, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet*. 2011;43(9):887-892.
62. Forno E, Lasky-Su J, Himes B, et al. Genome-wide association study of the age of onset of childhood asthma. *J Allergy Clin Immunol*. 2012;130(1):83-90. e4.
63. Lasky-Su J, Lange N, Brehm JM, et al. Genome-wide association analysis of circulating vitamin D levels in children with asthma. *Hum Genet*. 2012;131(9):1495-1505.
64. Lasky-Su J, Lyon HN, Emilsson V, et al. On the replication of genetic associations: timing can be everything!. *Am J Hum Genet*. 2008;82(4):849-858.
65. Himes BE, Lasky-Su J, Wu AC, et al. Asthma-susceptibility variants identified using probands in case-control and family-based analyses. *BMC Med Genet*. 2010;11:122.
66. Du R, Litonjua AA, Tantisira KG, et al. Genome-wide association study reveals class I MHC-restricted T cell-associated molecule gene (CRTAM) variants interact with vitamin D levels to affect asthma exacerbations. *J Allergy Clin Immunol*. 2012;129(2):368-373. e1-5.
67. Hersh CP, Raby BA, Soto-Quiros ME, et al. Comprehensive testing of positionally cloned asthma genes in two populations. *Am J Respir Crit Care Med*. 2007;176(9):849-857.
68. Murphy A, Tantisira KG, Soto-Quiros ME, et al. PRKCA: a positional candidate gene for body mass index and asthma. *Am J Hum Genet*. 2009;85(1):87-96.
69. Hunninghake GM, Lasky-Su J, Soto-Quiros ME, et al. Sex-stratified linkage analysis identifies a female-specific locus for IgE to

- cockroach in Costa Ricans. *Am J Respir Crit Care Med*. 2008;177(8):830-836.
70. Sharma S, Litonjua AA, Tantisira KG, et al. Clinical predictors and outcomes of consistent bronchodilator response in the childhood asthma management program. *J Allergy Clin Immunol*. 2008;122(5):921-928. e4.
71. Sharma S, Raby BA, Hunninghake GM, et al. Variants in TGFB1, dust mite exposure, and disease severity in children with asthma. *Am J Respir Crit Care Med*. 2009;179(5):356-362.
72. Bunyavanich S, Melen E, Wilk JB, et al. Thymic stromal lymphopoietin (TSLP) is associated with allergic rhinitis in children with asthma. *Clin Mol Allergy*. 2011;9:1.
73. Sharma S, Murphy AJ, Soto-Quiros ME, et al. Association of VEGF polymorphisms with childhood asthma, lung function and airway responsiveness. *Eur Respir J*. 2009;33(6):1287-1294.

SUPPORTING INFORMATION

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

How to cite this article: Virkud YV, Kelly RS, Croteau-Chonka DC, et al. Novel eosinophilic gene expression networks associated with IgE in two distinct asthma populations. *Clin Exp Allergy*. 2018;48:1654–1664. <https://doi.org/10.1111/cea.13249>

Supplementary Tables and Figures

Supplementary Table 1: Gene probes significantly associated with total IgE in the GACRS and CAMP using differential gene expression. . ■ Significant in both cohorts after adjustment for recent inhaled corticosteroid (ICS) use.

Probe IDs	Gene Symbols	Chromosome	Adjusted P-Value		Significant after ICS adjustment	
			GACRS	CAMP	GACRS	CAMP
2470364	PRSS33	16	2.79E-09	2.44E-10	■	■
1780239	OLIG2	21	1.65E-08	2.27E-12	■	■
7570484	TFF3	21	2.39E-08	6.74E-13	■	■
2030142	CLC	19	3.77E-08	4.03E-09	■	■
5360048	CCL23	17	1.49E-07	2.96E-10	■	■
2030403	OLIG1	21	1.49E-07	7.83E-07	■	■
7320324	EMR4P	19	1.49E-07	7.10E-06	■	■
940519	GPR44	11	1.97E-07	2.14E-08	■	■
6450504	FLJ43093	6	2.45E-07	2.02E-08	■	■
2940767	CEBPE	14	2.67E-07	2.62E-13	■	■
1030278	C10orf33	10	3.28E-07	3.85E-10	■	■
5720470	LOC653381	15	1.51E-06	4.92E-05	■	■
780369	SIGLEC8	19	1.55E-06	5.00E-09	■	■
510463	PIK3R6	17	2.92E-06	2.02E-08	■	■
4180338	LGALS12	11	6.06E-06	3.72E-04	■	■
5720059	GFOD1	6	8.71E-06	1.81E-03	■	■
2190671	CCR3	3	1.77E-05	9.16E-05	■	■
1240446	CAT	11	2.08E-05	-	■	
6200253	THBS4	5	2.27E-05	-	■	
1850068	C13orf27	13	2.32E-05	-	■	
5870474	RHOBTB3	5	2.72E-05	2.83E-03	■	
830519	IL5RA	3	3.51E-05	2.01E-02	■	■
4490324	SMPD3	16	5.63E-05	2.24E-06	■	■
7550022	STXBP5	6	6.70E-05	-	■	
6660315	INPP1	2	1.53E-04	6.42E-05	■	■
4480497	SIGLEC10	19	1.57E-04	-	■	
130187	PAPSS1	4	1.79E-04	-	■	
2510356	EMR1	19	2.46E-04	5.50E-03	■	■
2710400	DSC2	18	2.49E-04	-	■	
1050253	ACOT11	1	2.56E-04	2.14E-04	■	■
4610338	CAMK1	3	3.04E-04	6.83E-05	■	■
4290368	PSTPIP2	18	3.22E-04	1.87E-03	■	■
1300546	VSTM1	19	4.64E-04	1.87E-06	■	■
4200047	RNF14	5	4.65E-04	3.10E-05	■	■

1740022	SPNS3	17	6.16E-04	6.60E-09	■	■
4810181	ZBTB42	14	7.51E-04	2.69E-08	■	■
520053	OXER1	2	9.28E-04	2.64E-03	■	
1430541	LOC647308	14	9.38E-04	-	■	
2230678	ACACB	12	1.01E-03	-	■	
5050093	KBTBD11	8	1.01E-03	-	■	
5570711	IDO1	8	1.07E-03	2.44E-10	■	■
60576	LOC642639	9	1.18E-03	1.70E-03	■	■
1660703	MGC72080	7	2.31E-03	-	■	
5390661	VLDLR	9	2.31E-03	-	■	
1230286	LTC4S	5	2.54E-03	6.86E-06	■	■
7610131	EPAS1	2	3.24E-03	2.36E-02	■	
1230487	SRGAP3	3	3.32E-03	-	■	■
2680202	HYAL3	3	3.40E-03	-	■	
5820373	ALDH6A1	14	4.07E-03	-	■	
6560239	CYP4F12	19	4.23E-03	2.00E-05	■	■
7320441	C7orf68	7	4.55E-03	-	■	
2640735	BHLHB2	3	5.76E-03	-	■	
1300242	ZNF440	19	6.61E-03	-	■	
4640398	C6orf97	6	7.94E-03	-	■	
1240201	BACE2	21	8.20E-03	2.31E-02	■	
4050768	CHST13	3	8.20E-03	-	■	
5290148	GPT2	16	8.81E-03	-	■	
150309	SLC24A3	20	1.17E-02	-	■	
6380040	COL9A2	1	1.19E-02	1.35E-03	■	■
2640731	HTR3A	11	1.19E-02	-	■	
290189	FAM124B	2	1.32E-02	1.16E-02	■	■
2480068	CD63	12	1.47E-02	-	■	
4150017	PAQR7	1	1.53E-02	-	■	
6650196	E2F5	8	1.54E-02	-	■	
4120224	GPR18	13	1.54E-02	-	■	
2570661	TRERF1	6	1.73E-02	-	■	
5260142	C20orf79	20	1.79E-02	-	■	
3440689	ACYP1	14	2.20E-02	-	■	
7560180	SUSD1	9	2.24E-02	-	■	
2760500	CD38	4	2.34E-02	-	■	
2350278	PNOC	8	2.58E-02	-	■	
3420300	EEF2K	16	2.66E-02	1.21E-04	■	■
5220612	ECHDC3	10	2.76E-02	-	■	
1500608	HSD3B7	16	3.15E-02	1.29E-02	■	
4640768	VPREB3	22	3.62E-02	-	■	
780725	ADAM8	10	3.80E-02	-	■	
2070520	CDCA7	2	3.80E-02	-	■	
7380670	MYB	6	4.04E-02	3.10E-05		■

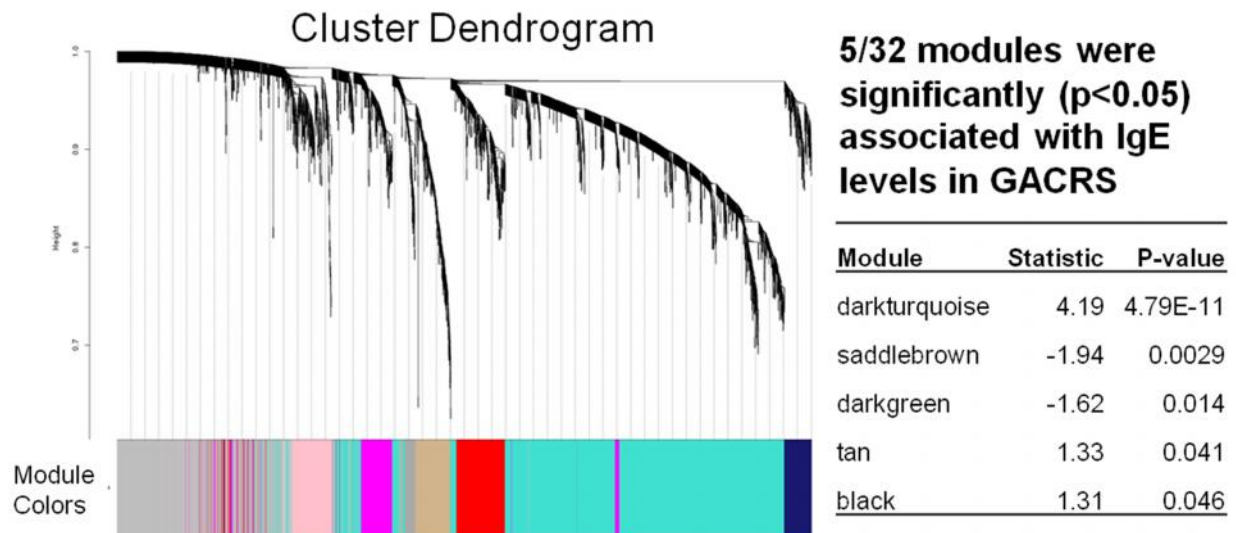
3440674	ASB2	14	4.24E-02	-	
7380273	GPR114	16	4.39E-02	1.75E-02	■
6380102	ATP8B3	19	4.70E-02	-	
3130543	RNASE3	14	-	4.15E-04	■
870138	ADORA3	1	-	8.04E-04	■
2640068	RNASE2	14	-	1.01E-03	■
1690446	PNPLA6	19	-	1.32E-03	■
5260070	HES4	1	-	1.39E-03	■
1400601	C20orf100	20	-	1.58E-03	■
2810253	ACSF2	17	-	1.70E-03	■
6280440	BIK	22	-	7.57E-03	■
1010736	TOX2	20	-	1.03E-02	■
1240025	C17orf38	17	-	1.29E-02	
10253	PPIL5	14	-	1.60E-02	
580475	OLFM2	19	-	1.70E-02	■
160437	BRI3BP	12	-	2.26E-02	
430446	KRT81	12	-	2.36E-02	
2360273	SLC16A14	2	-	2.49E-02	
5360735	FAM54B	1	-	2.70E-02	
4120750	OBFC1	10	-	3.09E-02	
2260747	HRK	12	-	3.09E-02	
6520215	ANXA1	9	-	3.09E-02	
4070424	C16orf93	16	-	3.61E-02	■
3130196	KCTD15	19	-	4.84E-02	
6020224	FBP1	9	-	4.87E-02	

Supplementary Table 2; Biological processes (raw $p < 0.01$, fold enrichment > 5 , genes > 1) enriched among the 42 replicated genes from the IgE-associated modules. *Of the 42 genes that were in the GACRS IgE-associated module and in the CAMP IgE-associated module; 39 could be mapped in GO.*

GO biological process	# Genes in GO Group	# Genes in this study	# Expected Genes	Fold Enrichment	Raw P- value
positive regulation of gliogenesis (GO:0014015)	51	3	0.09	31.74	0.0001
regulation of T cell tolerance induction (GO:0002664)	12	2	0.02	89.92	0.0003
regulation of tolerance induction (GO:0002643)	18	2	0.03	59.95	0.0006
regulation of gliogenesis (GO:0014013)	101	3	0.19	16.03	0.0009
positive regulation of neurogenesis (GO:0050769)	416	5	0.77	6.48	0.0010
regulation of lymphocyte mediated immunity (GO:0002706)	126	3	0.23	12.85	0.0017
leukotriene metabolic process (GO:0006691)	32	2	0.06	33.72	0.0018
positive regulation of nervous system development (GO:0051962)	477	5	0.88	5.66	0.0019
regulation of lymphocyte activation (GO:0051249)	478	5	0.89	5.64	0.0019
positive regulation of glial cell differentiation (GO:0045687)	33	2	0.06	32.7	0.0019
positive regulation of cell development (GO:0010720)	483	5	0.9	5.59	0.0020
monocarboxylic acid metabolic process (GO:0032787)	484	5	0.9	5.57	0.0020
positive regulation of dendritic spine development (GO:0060999)	40	2	0.07	26.98	0.0027
lymphocyte chemotaxis (GO:0048247)	43	2	0.08	25.09	0.0031
regulation of T cell activation (GO:0050863)	329	4	0.61	6.56	0.0033
regulation of leukocyte mediated immunity (GO:0002703)	167	3	0.31	9.69	0.0038
regulation of leukocyte cell-cell adhesion (GO:1903037)	344	4	0.64	6.27	0.0038
regulation of immune effector process (GO:0002697)	363	4	0.67	5.95	0.0046
lymphocyte migration (GO:0072676)	56	2	0.1	19.27	0.0052
regulation of glial cell differentiation (GO:0045685)	59	2	0.11	18.29	0.0057
oligodendrocyte differentiation (GO:0048709)	65	2	0.12	16.6	0.0068
regulation of lymphocyte proliferation (GO:0050670)	210	3	0.39	7.71	0.0071
regulation of mononuclear cell proliferation (GO:0032944)	211	3	0.39	7.67	0.0072
regulation of dendritic spine development (GO:0060998)	67	2	0.12	16.11	0.0072
positive regulation of dendrite development (GO:1900006)	69	2	0.13	15.64	0.0077

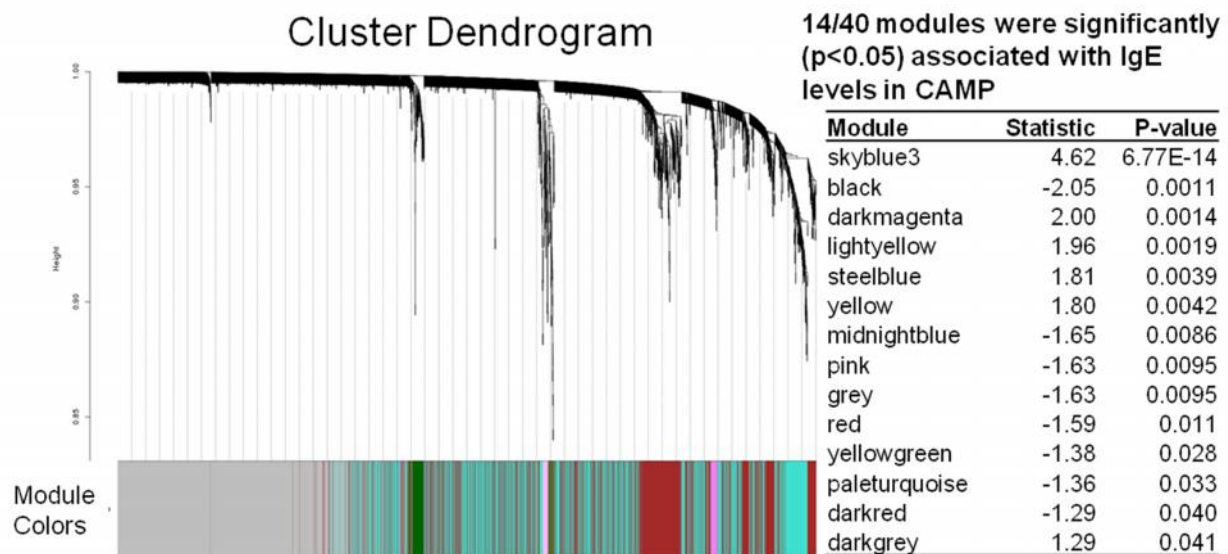
organic hydroxy compound metabolic process (GO:1901615)	421	4	0.78	5.13	0.0077
neuron fate commitment (GO:0048663)	70	2	0.13	15.42	0.0079
regulation of cell-cell adhesion (GO:0022407)	425	4	0.79	5.08	0.0079
regulation of leukocyte proliferation (GO:0070663)	219	3	0.41	7.39	0.0080
positive regulation of T cell differentiation (GO:0045582)	73	2	0.14	14.78	0.0085
chemokine-mediated signaling pathway (GO:0070098)	74	2	0.14	14.58	0.0087
cell fate commitment (GO:0045165)	227	3	0.42	7.13	0.0088

Supplementary Figure 1A: Cluster dendrogram detailing module assignment and the relationship between the resulting modules and serum total IgE in GACRS



The dark turquoise module, referred to as the GACRS module, was selected as the module of interest given its high level of significance.

Supplementary Figure 1B: Cluster dendrogram detailing module assignment and the relationship between the resulting modules and serum total IgE levels in CAMP



The sky blue 3 module, referred to as the CAMP module, was selected as the module of interest given its high level of significance.

Supplementary Table 3: Biological processes enriched (raw $p < 0.01$, fold enrichment > 5 , genes > 1) among the 103 genes comprising the significant module in the GACRS Data. Of the 103 genes in the GACRS module; 100 could be mapped in GO

GO biological process	# Genes in GO Group	# Genes in this study	# Expected Genes	Fold Enrichment	Raw P-value
positive regulation of leukocyte activation (GO:0002696)	369	9	1.75	5.13	7.71E-05
positive regulation of cell activation (GO:0050867)	379	9	1.8	5	9.41E-05
positive regulation of gliogenesis (GO:0014015)	51	4	0.24	16.5	0.000134
regulation of gliogenesis (GO:0014013)	101	5	0.48	10.42	0.000149
regulation of glial cell differentiation (GO:0045685)	59	4	0.28	14.27	0.000227
oligodendrocyte differentiation (GO:0048709)	65	4	0.31	12.95	0.000322
regulation of forebrain neuron differentiation (GO:2000977)	4	2	0.02	100.1	0.000328
neuron fate commitment (GO:0048663)	70	4	0.33	12.02	0.000421
regulation of oligodendrocyte differentiation (GO:0048713)	31	3	0.15	20.36	0.000552
regulation of lymphocyte proliferation (GO:0050670)	210	6	1	6.01	0.000569
regulation of mononuclear cell proliferation (GO:0032944)	211	6	1	5.98	0.000583
positive regulation of glial cell differentiation (GO:0045687)	33	3	0.16	19.13	0.000655
regulation of leukocyte proliferation (GO:0070663)	219	6	1.04	5.76	0.000705
glial cell differentiation (GO:0010001)	148	5	0.7	7.11	0.000806
regulation of leukocyte mediated immunity (GO:0002703)	167	5	0.79	6.3	0.00136
positive regulation of astrocyte differentiation (GO:0048711)	10	2	0.05	42.08	0.00142
positive regulation of peptidyl-tyrosine phosphorylation (GO:0050731)	172	5	0.82	6.12	0.00154
ventral spinal cord development (GO:0021517)	46	3	0.22	13.72	0.00162
regulation of T cell tolerance induction (GO:0002664)	12	2	0.06	35.07	0.00194
gliogenesis (GO:0042063)	193	5	0.92	5.45	0.00251
production of molecular mediator involved in inflammatory response (GO:0002532)	15	2	0.07	28.06	0.00288
regulation of lymphocyte mediated immunity (GO:0002706)	126	4	0.6	6.68	0.00339
granulocyte differentiation (GO:0030851)	17	2	0.08	24.76	0.00359
negative regulation of hemopoiesis (GO:1903707)	128	4	0.61	6.58	0.00359
amine metabolic process (GO:0009308)	64	3	0.3	9.86	0.00395
regulation of tolerance induction (GO:0002643)	18	2	0.09	23.38	0.00398
negative regulation of wound healing (GO:0061045)	65	3	0.31	9.71	0.00412

somatic stem cell population maintenance (GO:0035019)	66	3	0.31	9.56	0.00429
regulation of wound healing (GO:0061041)	135	4	0.64	6.23	0.00431
protein ubiquitination involved in ubiquitin-dependent protein catabolic process (GO:0042787)	139	4	0.66	6.06	0.00476
aromatic amino acid family catabolic process (GO:0009074)	20	2	0.1	21.04	0.00481
positive regulation of dendrite development (GO:1900006)	69	3	0.33	9.15	0.00484
cell maturation (GO:0048469)	144	4	0.68	5.84	0.00538
positive regulation of myeloid leukocyte mediated immunity (GO:0002888)	23	2	0.11	18.3	0.00619
negative regulation of response to wounding (GO:1903035)	76	3	0.36	8.31	0.00626
positive regulation of vasculature development (GO:1904018)	151	4	0.72	5.57	0.00632
diencephalon development (GO:0021536)	78	3	0.37	8.09	0.00671
negative regulation of myeloid cell differentiation (GO:0045638)	79	3	0.38	7.99	0.00694
regulation of astrocyte differentiation (GO:0048710)	25	2	0.12	16.83	0.0072
regulation of response to wounding (GO:1903034)	158	4	0.75	5.33	0.00737
regulation of T cell proliferation (GO:0042129)	158	4	0.75	5.33	0.00737
regulation of glial cell proliferation (GO:0060251)	27	2	0.13	15.59	0.00827
regulation of transforming growth factor beta production (GO:0071634)	27	2	0.13	15.59	0.00827
positive regulation of lymphocyte differentiation (GO:0045621)	85	3	0.4	7.43	0.00843
regulation of blood coagulation (GO:0030193)	86	3	0.41	7.34	0.00869
regulation of hemostasis (GO:1900046)	86	3	0.41	7.34	0.00869
aromatic amino acid family metabolic process (GO:0009072)	28	2	0.13	15.03	0.00884
positive regulation of acute inflammatory response (GO:0002675)	28	2	0.13	15.03	0.00884
regulation of hematopoietic progenitor cell differentiation (GO:1901532)	87	3	0.41	7.26	0.00896
positive regulation of neutrophil chemotaxis (GO:0090023)	29	2	0.14	14.51	0.00942
B cell receptor signaling pathway (GO:0050853)	90	3	0.43	7.01	0.0098
embryonic placenta development (GO:0001892)	90	3	0.43	7.01	0.0098

Supplementary Table 4: Biological processes enriched (raw $p < 0.01$, fold enrichment > 5 , genes > 1) among the 38 genes comprising the significant module in the CAMP Data. Of the 38 genes in the module that were replicated in CAMP; 36 could be mapped in GO

GO biological process	# Genes in GO Group	# Genes in this study	# Expected Genes	Fold Enrichment	Raw P- value
oligodendrocyte differentiation (GO:0048709)	65	3	0.11	26.98	0.0002
regulation of T cell tolerance induction (GO:0002664)	12	2	0.02	97.42	0.0003
regulation of tolerance induction (GO:0002643)	18	2	0.03	64.94	0.0005
inflammatory response (GO:0006954)	465	5	0.8	6.28	0.0011
regulation of lymphocyte mediated immunity (GO:0002706)	126	3	0.22	13.92	0.0014
leukotriene metabolic process (GO:0006691)	32	2	0.05	36.53	0.0015
positive regulation of glial cell differentiation (GO:0045687)	33	2	0.06	35.42	0.0016
glial cell differentiation (GO:0010001)	148	3	0.25	11.85	0.0022
regulation of receptor activity (GO:0010469)	546	5	0.93	5.35	0.0023
regulation of cell activation (GO:0050865)	577	5	0.99	5.06	0.0029
regulation of leukocyte mediated immunity (GO:0002703)	167	3	0.29	10.5	0.0030
positive regulation of gliogenesis (GO:0014015)	51	2	0.09	22.92	0.0037
gliogenesis (GO:0042063)	193	3	0.33	9.09	0.0045
regulation of glial cell differentiation (GO:0045685)	59	2	0.1	19.81	0.0049
regulation of lymphocyte proliferation (GO:0050670)	210	3	0.36	8.35	0.0057
regulation of mononuclear cell proliferation (GO:0032944)	211	3	0.36	8.31	0.0058
regulation of cell-cell adhesion (GO:0022407)	425	4	0.73	5.5	0.0060
multicellular organismal response to stress (GO:0033555)	66	2	0.11	17.71	0.0060
regulation of leukocyte proliferation (GO:0070663)	219	3	0.37	8.01	0.0064
neuron fate commitment (GO:0048663)	70	2	0.12	16.7	0.0067
positive regulation of endothelial cell proliferation (GO:0001938)	73	2	0.12	16.01	0.0073
regulation of heart contraction (GO:0008016)	231	3	0.4	7.59	0.0074
regulation of angiogenesis (GO:0045765)	232	3	0.4	7.56	0.0074
chemokine-mediated signaling pathway (GO:0070098)	74	2	0.13	15.8	0.0075
regulation of vasculature development (GO:1901342)	256	3	0.44	6.85	0.0097

Supplementary Table 5: Replicated genes between the significant GACRS and CAMP modules R from WGCNA

Gene Symbol	Gene Name
ATB2	Ankyrin Repeat And BTB (POZ) Domain Containing 2
ACOT11	acyl-CoA thioesterase 11
ADORA3	Adenosine A3 Receptor
ATP8B3	ATPase, Aminophospholipid Transporter, Class I, Type 8B, Member 3
PYROXD2	Pyridine Nucleotide-Disulphide Oxidoreductase Domain 2 (previously c10orf33)
CACNG6	Calcium Channel, Voltage-Dependent, Gamma Subunit 6
CAMK1	calcium/calmodulin-dependent protein kinase I
CCL23	chemokine (C-C motif) ligand 23
CCR3	chemokine (C-C motif) receptor 3
CD9	CD9 Molecule
CEBPE	CCAAT/enhancer binding protein (C/EBP), epsilon
CLC	Charcot-Leyden crystal protein
CYP4F12	cytochrome P450, family 4, subfamily F, polypeptide 12
EMR1	Adhesion G Protein-Coupled Receptor E1 (alias ADGRE1)
EMR4P	Adhesion G Protein-Coupled Receptor E4, Pseudogene
FLJ43093	Unknown
GFOD1	Glucose-Fructose Oxidoreductase Domain Containing 1
GPR44	G protein-coupled receptor 44
IDO1	indoleamine 2,3-dioxygenase 1
IL5RA	Interleukin 5 Receptor, Alpha
INPP1	inositol polyphosphate-1-phosphatase
LGALS12	Galectin 12
LOC642639	Unknown
LOC653381	Sorbitol Dehydrogenase (alias: SORD2)
LTC4S	leukotriene C4 synthase
OLIG1	oligodendrocyte transcription factor 1
OLIG2	oligodendrocyte lineage transcription factor 2
PIK3R6	phosphoinositide-3-kinase, regulatory subunit 6
PRSS33	protease, serine, 33
RNASE2	Ribonuclease A Family Member 2
RNF14	ring finger protein 14
SIGLEC8	sialic acid binding Ig-like lectin 8
SMPD3	sphingomyelin phosphodiesterase 3, neutral membrane
SPNS3	spinster homolog 3 (Drosophila)
TFF3	trefoil factor 3 (intestinal)
THBS4	Thrombospondin 4
VSTM1	V-set and transmembrane domain containing 1
ZBTB42	zinc finger and BTB domain containing 42

Supplementary Table 6: Gene sets overlapping the gene set of 31 Common genes

Gene Set Description	# genes overlap	p-value	FDR (q-value)	Genes
Neutrophils versus Monocytes (GSE22886)	5	1.35×10^{-7}	6.55×10^{-4}	<i>GPR44, CCR3, IL5RA, CLC, SIGLEC8</i>
Bone Marrow derived macrophages with MLL4 knockout: control versus LPS stimulated (GSE30971)	4	6.57×10^{-6}	1.07×10^{-2}	<i>EMR1, CCL23, OLIG2, OLIG1</i>
Eosinophils versus Th1 cells (GSE3982)	4	6.57×10^{-6}	1.07×10^{-2}	<i>SMPD3, CCL23, IDO1, CEBPE</i>