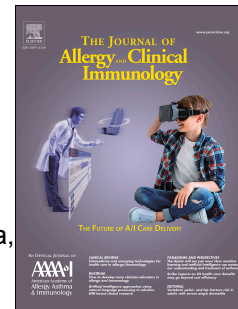


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Multi-ethnic genome-wide and HLA association study of total serum IgE

Michelle Daya, PhD, Corey Cox, MS, Nathalie Acevedo, MD PhD, Meher P. Boorgula, MS, Monica Campbell, MS, Sameer Chavan, MS, Michael H. Cho, MD, Gloria L. David, PhD MHSc, Priyadarshini Kachroo, PhD, Jessica Lasky-Su, PhD, Xingnan Li, PhD, Caitlin P. McHugh, PhD, Dandi Qiao, PhD, Nicholas Rafaels, MS, Lisa A. Beck, MD, Eugene R. Bleeker, MD, Luis Caraballo, MD PhD, Adrienne L. Cupples, PhD, Camila A. Figueiredo, PhD, Richard L. Gallo, MD PhD, Jon Hanifin, MD, Nadia N. Hansel, MD, Tissa R. Hata, MD, Craig P. Hersh, MD, Jennifer Knight-Madden, MBBS PhD, Donald Y.M. Leung, MD PhD, Emma Guttman-Yassky, MD PhD, Deborah A. Meyers, PhD, George O'Connor, PhD, Carole Ober, PhD, Peck Y. Ong, MD, Victor E. Ortega, MD PhD, Amy S. Paller, MD, Nirupama Putcha, MD, Robert M. Reed, MD, Lynda C. Schneider, MD, Edwin K. Silverman, MD PhD, Mark K. Slifka, PhD, Jonathan M. Spergel, MD, Ramachandran S. Vasan, MD, Karine A. Viaud-Martinez, Harold Watson, MD, Scott T. Weiss, MD PhD, NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium, Ingo Ruczinski, PhD, Terri H. Beaty, PhD, Rasika A. Mathias, ScD, Kathleen C. Barnes, PhD



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1 **Title: Multi-ethnic genome-wide and HLA association study of total serum IgE**

2 *Authors:*

3 Michelle Daya PhD¹

4 Corey Cox MS¹

5 Nathalie Acevedo MD PhD²

6 Meher P. Boorgula MS¹

7 Monica Campbell MS¹

8 Sameer Chavan MS¹

9 Michael H. Cho MD³

10 Gloria L. David PhD MHSc⁴

11 Priyadarshini Kachroo PhD⁵

12 Jessica Lasky-Su PhD⁵

13 Xingnan Li PhD⁶

14 Caitlin P. McHugh PhD⁷

15 Dandi Qiao PhD⁵

16 Nicholas Rafaels MS¹

17 Lisa A. Beck MD⁸

18 Eugene R. Bleeker MD⁶

19 Luis Caraballo MD PhD²

20 Adrienne L. Cupples PhD⁹

21 Camila A. Figueiredo PhD¹⁰

22 Richard L. Gallo MD PhD¹¹

23 Jon Hanifin MD¹²

- 24 Nadia N. Hansel MD¹³
- 25 Tissa R. Hata MD¹¹
- 26 Craig P. Hersh MD⁵
- 27 Jennifer Knight-Madden MBBS PhD¹⁴
- 28 Donald Y.M. Leung MD PhD¹⁵
- 29 Emma Guttman-Yassky MD PhD¹⁶
- 30 Deborah A. Meyers PhD⁶
- 31 George O'Connor PhD¹⁷
- 32 Carole Ober PhD¹⁸
- 33 Peck Y. Ong MD¹⁹
- 34 Victor E. Ortega MD PhD²⁰
- 35 Amy S. Paller MD²¹
- 36 Nirupama Putcha MD¹³
- 37 Robert M. Reed MD²²
- 38 Lynda C. Schneider MD²³
- 39 Edwin K. Silverman MD PhD⁵
- 40 Mark K. Slifka PhD¹²
- 41 Jonathan M. Spergel MD²⁴
- 42 Ramachandran S. Vasan MD²⁵
- 43 Karine A. Viaud-Martinez²⁶
- 44 Harold Watson MD²⁷
- 45 Scott T. Weiss MD PhD⁵
- 46 NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium

47 Ingo Ruczinski PhD²⁸

48 Terri H. Beaty PhD²⁹

49 Rasika A. Mathias ScD²⁹

50 Kathleen C. Barnes PhD¹

51 *Affiliations:*

52 1. Department of Medicine, University of Colorado, Aurora, CO

53 2. Institute for Immunological Research, University of Cartagena, Cartagena, Colombia

54 3. Channing Division of Network Medicine and Division of Pulmonary and Critical Care

55 Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard

56 Medical School, Boston, MA remhc@channing.harvard.edu

57 4. Rho, Inc., Durham, NC

58 5. Channing Division of Network Medicine, Department of Medicine, Brigham and

59 Women's Hospital and Harvard Medical School, Boston, MA

60 6. Division of Genetics, Genomics and Precision Medicine, Department of Medicine,

61 University of Arizona, Tucson, AZ

62 7. Department of Biostatistics, University of Washington, Seattle, WA

63 8. University of Rochester Medical Center, Rochester, NY

64 9. Department of Biostatistics, Boston University School of Public Health, Boston, MA

65 10. Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Brazil

66 11. University of California San Diego, San Diego, CA

67 12. Oregon Health & Science University, Portland, OR

68 13. Pulmonary and Critical Care Medicine, School of Medicine, Johns Hopkins

69 University, Baltimore, MD

14. Caribbean Institute for Health Research, The University of the West Indies Kingston, Jamaica
15. Division of Pediatric Allergy & Immunology, National Jewish Health, Denver, CO
16. Icahn School of Medicine at Mount Sinai, New York, NY
17. Department of Medicine, Boston University School of Medicine, Boston, MA
18. Department of Human Genetics, University of Chicago, Chicago, IL
19. Division of Clinical Immunology and Allergy, Children's Hospital Los Angeles and University of Southern California, Los Angeles, CA
20. Department of Internal Medicine, Center for Precision Medicine, Wake Forest School of Medicine, Winston-Salem, NC
21. Northwestern University and Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL
22. University of Maryland School of Medicine, Baltimore, MD
23. Boston Children's Hospital and Harvard Medical School
24. Philadelphia, Department of Pediatrics, Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA
25. Departments of medicine and Epidemiology, Boston University Schools of Medicine and Public Health, Boston, MA
26. Illumina Laboratory Services, Illumina, Inc., 5200 Illumina Way, San Diego CA 92122
27. Faculty of Medical Sciences, The University of the West Indies, Queen Elizabeth Hospital, Bridgetown, St. Michael, BB11000, Barbados

28. Department of Biostatistics, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

29. Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD

Address correspondence to: Kathleen C. Barnes,
University of Colorado Anschutz Medical Campus
13001 E. 17th Place, Room 5330A, Aurora, CO 80045 | Mail Stop F563
E: Kathleen.Barnes@cuanschutz.edu | P: 303-724-9734

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Abstract

Background: Total serum IgE (tIgE) is an important intermediate phenotype of allergic disease. Whole genome genetic association studies across ancestries may identify important determinants of IgE.

Objective: By leveraging data from the NHLBI Trans-Omics for Precision Medicine (TOPMed) program, the Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA) and the Atopic Dermatitis Research Network (ADRN), we aim to increase understanding of genetic variants affecting tIgE production across the ancestry and allergic disease spectrum (N=21,901).

Methods: We performed genome-wide association within strata of study, disease, and ancestry groups, and combined results via a meta-regression approach that models heterogeneity attributable to ancestry. We also tested for association between HLA alleles called from whole genome sequence data and tIgE, assessing replication of associations in HLA alleles called from genotype array data. For details, please see the Methods section in this article's Online Repository at www.jacionline.org.

Results: We identified six loci at genome-wide significance ($P < 5 \times 10^{-9}$), including four loci previously reported as genome-wide significant for tIgE, as well as new regions in chr11q13.5 and chr15q22.2, also identified in prior GWAS of atopic dermatitis and asthma. In the HLA allele association study, HLA-A*02:01 was associated with decreased tIgE (discovery $P = 2 \times 10^{-4}$, replication $P = 5 \times 10^{-4}$, discovery+replication $P = 4 \times 10^{-7}$) and HLA-DQB1*03:02 was strongly associated with decreased tIgE in Hispanic/Latino ancestry populations (Hispanic/Latino discovery+replication $P = 8 \times 10^{-8}$).

Conclusion: We performed the largest GWAS and HLA association study of tIgE focused on ancestrally diverse populations and found several known tIgE and allergic disease loci that are relevant in non-European ancestry populations.

Key messages:

- This study is the largest genome-wide and HLA association study of tIgE performed to date in populations of diverse ancestry
- We show that known tIgE and allergic disease loci, discovered primarily in European ancestry groups, are relevant to non-European ancestry groups
- We also identified specific HLA alleles associated with tIgE with evidence of replication

Capsule Summary: Known tIgE and allergic disease loci are relevant in non-European ancestry populations. HLA-A*02:01 and HLA-DQB1*03:02 are associated with decreased levels of tIgE.

Key words: total serum IgE, human leukocyte antigen, genome-wide association study, atopic dermatitis, asthma, multi-ethnic

Abbreviations: total serum IgE (tIgE), genome-wide association study (GWAS), human leukocyte antigen (HLA), whole genome sequence (WGS), Trans-Omics for Precision Medicine (TOPMed), Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA), Atopic Dermatitis Research Network (ADRN), atopic dermatitis (AD), Framingham Heart Study (FHS), epidermal differentiation complex (EDC), T helper type 2 (TH2), Genotype-Tissue Expression (GTEx), major histocompatibility complex (MHC), Genetic susceptibility to Asthma and pollution in Peru (GASP), linkage disequilibrium (LD)

Introduction

Total serum IgE (tIgE) is an important marker of atopy, estimated to affect between 27-36% of individuals living in developed countries^{1, 2}. Total IgE is highly elevated in individuals suffering from atopic diseases such as asthma and is considered a risk factor for this disease. While several tIgE genome-wide association studies (GWAS) have been reported³⁻⁷, these have mostly been limited to European ancestry populations. The largest GWAS to date included 14,745 European ancestry samples⁵, and the only multi-ethnic GWAS to date included 4,292 samples⁴. Collectively, these GWAS have identified four genome-wide significant loci, with the human leukocyte antigen (HLA) region being the most consistently reported. In this study, we aimed to increase our understanding of genetic factors affecting tIgE across the ancestry and allergic disease spectrum.

Results and Discussion

We combined existing tIgE and genome-wide data from three large NIH-funded initiatives, including whole genome sequence (WGS) data from the NHLBI Trans-Omics for Precision Medicine (TOPMed) program⁸ and GWAS array data from the Consortium on Asthma among African-ancestry Populations in the Americas (CAAPA)⁹ and the Atopic Dermatitis Research Network (ADRN)¹⁰, and conducted a GWAS of tIgE in >20,000 subjects from 16 studies (23% African, 18% Hispanic/Latino, 59% European ancestry, Figure 1A). As expected, mean tIgE is higher in asthmatics and participants with atopic dermatitis (AD) compared to control groups and population-based studies, and different by ancestry group (Figure 1B, Table E1). Due to this heterogeneity of tIgE

distribution, we defined GWAS strata by study, disease group, ancestry group and genotyping platform (WGS data vs. GWAS array data imputed using the TOPMed reference panel¹¹). We performed GWAS separately for each stratum, and combined results via a meta-regression approach that models allelic effects along genetic axes of variation representative of ancestry as well as residual heterogeneity reflecting non-genetic differences between stratum (more detailed Methods in this article's Online Repository at www.jacionline.org, Figure E3)⁸. This approach does not penalize effects that are ancestry-specific and quantifies heterogeneity attributable to aggregate genetic ancestry.

Six loci reached genome-wide significance ($P < 5 \times 10^{-9}$ as recently recommended by Lin¹², Figure 2, Table E2). All four loci previously reported as genome-wide significant by tlgE GWAS^{3, 5, 7} were identified, as well as two loci not previously reported in tlgE GWAS but reported by prior GWAS of AD and asthma: chr11q13.5 (*EMSY*+*LRRC32*), reported in early GWAS of AD¹³ and eosinophilic esophagitis¹⁴, and chr15q22.2 (*RORA2*+*ANXA2*+*VPS13C*), reported in an early GWAS of asthma⁵. Both these regions also reached genome-wide significance in a recent GWAS of asthma in the UK Biobank¹⁵. We contrast these findings with relatively weak associations observed for the epidermal differentiation complex (EDC) region and the chr17q12-21 locus, the strongest AD and asthma GWAS known genetic associations (pink dots in Figure 2). *EMSY* affects transcription of interferon genes¹⁶ and may shift the immune response to T helper Type 1, thereby reducing the T helper type 2 (TH2) immune response, while *RORA2* regulates transcription of genes modifying TH2 cell responses¹⁷. Therefore, the chr11q13.5 (*EMSY*) and chr15q22.2 (*RORA2*) associations

likely reflect changes in the TH2 immune response rather than other biological pathways conferring risk for developing allergic disease.

P-values for heterogeneity attributable to genetic ancestry were used to assess evidence for effects that may differ by ancestry. For 3 of the 6 genome-wide significant loci (chr1q23.2, chr11q13.5, chr12q13.3) we observed no such evidence ($P_{\text{anc}} > 0.05$ in Table E2), and only marginal evidence of heterogeneity attributable to ancestry for chr15q22.2 (minimum $P_{\text{anc}} = 0.03$, Table E2). Effect sizes and direction of effect are generally consistent by ancestry for the lead variants in these loci (Figure E5). Heterogeneity attributable to ancestry at the chr5q31.1 locus is likely due to lack of association in the Hispanic/Latino ancestry group and a weaker effect in the African ancestry group (Figure E5). For the chr6p21.32 HLA locus, all three ancestries contributed to the association signal, but with varying effect sizes by ancestry group ($P_{\text{anc}} < 0.05$ in Table E2) and gene locus (Figures E5, E7). However, we qualify that genetic ancestry is only captured in aggregate at the analysis stratum level, the ancestry spectrum in the Hispanic/Latino group is particularly diverse (Figure E3), and our study could not account for unmeasured environmental and socio-economic factors. Therefore, we caution that loci with association signals that are heterogeneous by ancestry should not be interpreted as biological differences between ancestry groups.

In addition to genetic heterogeneity between study strata, the wide array of geographical locations and clinical settings participants were recruited from also represents heterogeneity in environmental exposures. The meta-regression approach we used to combine association results also explicitly models residual heterogeneity⁸, thereby - at least in part - accounting for heterogeneity in environmental exposures,

reducing the risk of over and under estimation of p-values. None of the lead variants in our study showed evidence of residual heterogeneity ($P_{het} > 0.05$ in Table E2, Figure E6), and only one of the credible set genome-wide significant variants showed evidence of residual heterogeneity (rs3024971 in the chr12q1.3 STAT6 locus, $P_{het}=0.03$, Table E2), which may be due to environmental exposure difference and/or disease group. However, several genome-wide significant variants not included in the credible set did show evidence of residual heterogeneity ($P_{het} \leq 0.05$ in Table E3), particularly variants in the HLA locus, perhaps reflecting varying exposure to environmental allergens.

We performed co-localization analysis using gene expression data available through GTEx (multiple tissues) and eQTLGen (blood) to test for causal variants in common between tlgE and gene expression¹⁸. This analysis showed expression of *FCER1A*, *IL13* and *STAT6* may be causally related to genetic control of tlgE production (posterior probability of one common causal variant [$PPH4$] > 0.99 , Figure 3). Although the role of the protein products of these genes in the TH2 immune response is well known (*i.e.*, *FCER1A* is an IgE receptor; *IL13* is a TH2 cytokine, *STAT6* mediates TH2 inflammation), it has previously been speculated the chr1q23.2 association may be related to *DARC*^{3, 19} and chr5q31.1 to *RAD50*⁷; our results support the notion that *FCER1A* and *IL13* are the more likely candidates.

The role of the HLA locus in the TH2 immune response has been well established, but the relative importance of particular HLA genes is still a subject of debate. In our study, we observed the strongest association in the vicinity of the *HLA-DRB+DQA+DQB* gene region (an effect that appears relevant in all 3 ancestry groups)

but note statistical signal peaks are also observed close to the *HLA-A* and *HLA-B+C* gene regions (Figure 4, Figure E7). We observed low levels of linkage disequilibrium (LD) between the *HLA-A*, *HLA-B-C* and *HLA-DRB-DQA-DQB* lead variants (Figure 4). Thus, these are likely independent association signals, and our finding suggests both major histocompatibility complex (MHC) class II and class I genes play a role in tlgE production.

To further investigate the role of specific HLA alleles, we performed HLA allele calling from WGS data using HLA-LA²⁰ and from GWAS array data using HIBAG²¹ followed by association analysis (see Online Repository Methods). We used the TOPMed WGS data sets for discovery and GWAS array data sets for replication. In this analysis, carriers of the *HLA-A*02:01* allele had lower levels of tlgE (discovery $P = 2 \times 10^{-4}$, replication $P = 5 \times 10^{-4}$, discovery+replication $P = 4 \times 10^{-7}$) (Table 1, Figures E13-E14). The HLA A2 serotype has previously been reported as associated with tlgE in subjects with ragweed sensitivity²². An association with *HLA-DQA1*03:01* was also replicated, but we note a consistent lower allele frequency in GWAS array data sets compared to WGS data (Figure E12), which indicates potential bias in this allele call. Due to heterogeneity by ancestry at the HLA locus in our GWAS, we also performed an exploratory analysis combining discovery and replication data sets by ancestry group to identify any additional ancestry related associations. This revealed a strong association between *HLA-DQB1*03:02* and tlgE in individuals with Hispanic/Latino ancestry ($P = 7 \times 10^{-8}$, Table 1), with some evidence of replication in individuals of European ancestry ($P = 0.03$, Table 1, Figure E12). *HLA-DQB1*03:02* was recently reported by the Genetic susceptibility to Asthma and pollution in Peru (GASP) study (one of the constituent

studies in our analysis) as associated with tIgE ($P = 2 \times 10^{-4}$)²³, and this association was replicated in the other Hispanic/Latino ancestry populations in our study (Hispanic/Latino meta-analysis excluding GASP $P = 8 \times 10^{-5}$).

It is worth noting that, while tIgE is an important marker for atopic disease, it is a poor measure of symptoms with less clinical utility compared to measures of allergen-specific IgE. GWAS and especially HLA-association studies of allergen-specific IgE will likely yield better mechanistic insights into the genetic risk for atopic disease compared to tIgE. For example, a previous study reported a much stronger overlap in genetic association between asthma and allergen specific IgE compared to the overlap between asthma and tIgE²⁴. While 5 of the 6 genome-wide significant loci in our study have been reported as genome-wide significant in allergic disease GWAS of asthma, atopic dermatitis, allergic rhinitis or eosinophilic esophagitis²⁵, the FCER1A locus is a notable exception. This suggests that tIgE production is partly regulated by mechanisms other than the production of the more clinically relevant allergen-specific IgE. However, the limited availability of large publicly available genetic data sets with comparable between-study allergen-specific IgE measures curtail opportunities to conduct robust genetic association studies of allergen-specific IgE.

Our study only investigated the association between individual HLA genes and tIgE. Due to long-range LD across the HLA region, multivariate haplotype association analysis of HLA alleles may provide more statistical power to detect HLA associations. However, such analysis require non-missing allele calls across all genes, which would reduce the available HLA discovery sample by ~10% and the HLA replication sample by ~50%. In addition, HLA haplotypes likely have even larger frequency differences

between ancestry groups compared to individual HLA alleles, complicating cross-ancestry analysis. For these reasons, we opted to not perform multivariate HLA association analysis in our study.

In summary, we performed the largest GWAS and HLA association study for tlgE focused on ancestrally diverse populations. We conclude two of the four tlgE GWAS loci reported previously showed no evidence of heterogeneity by ancestry and that these loci are likely relevant in other ancestry groups. We also demonstrated chr11q13.5 and chr15q22.2, recently reported in a large European ancestry asthma GWAS¹⁵, are associated with tlgE production and may therefore play a role in allergic disease in non-European ancestry populations. Due to differing patterns of LD across population groups, multi-ethnic studies are particularly useful for identifying causal variants, and the lack of ancestry heterogeneity observed for credible variants in genome-wide significant loci (Table E2) suggest that these variants are plausible causal variants. Our results support the notion that both MHC class I and II genes can affect risk for allergic disease, and we identified replicated associations between tlgE and specific HLA alleles A*02:01 and DQB1*03:02.

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Figure 1: Clinical characteristics. 1A) Breakdown of ancestry, disease group and genotyping platform across studies. 1B) Barplot of the total serum IgE IU/ml geometric mean by study, stratified by allergic/non-allergic group, colored by ancestry.

Figure 2: tlgE GWAS Manhattan plot. Red dots denote loci previously reported by the tlgE GWAS, light blue dots denote loci not previously reported by tlgE GWAS, and pink dots denote the chr1q21 EDC and chr17q12-21 regions. Diamonds denote lead SNPs from previous GWAS. The red line denotes genome-wide significance ($P=5 \times 10^{-9}$), the blue line denotes suggestive significance ($P=10^{-6}$).

Figure 3: Locus zoom plots of tlgE and gene expression regions with evidence for co-localization. 3A) FCER1A (ENSG00000179639.10) in Adipose Visceral Omentum (PPH4=0.9986), 3B) IL13 (ENSG00000169194) in Testis (PPH4=0.9961), 3C) STAT6 (ENSG00000166888) in Cultured Fibroblasts (PPH4=0.9984).

Figure 4: Associations in the HLA locus. 4A) Locus zoom plot. Lead variants in each peak are colored red. The red horizontal line denotes the 5×10^{-9} genome-wide significance threshold, the blue line denotes suggestive significance at 10^{-6} . 4B) Linkage disequilibrium (r^2) between lead variants in African (ASW), European (CEU) and Hispanic/Latino (MXL) 1000 Genomes populations.

Allele	All		African		European		Hispanic/Latino	
	Beta [95% CI]	P	Beta [95% CI]	P	Beta [95% CI]	P	Beta [95% CI]	P
Discovery								
A*02:01	-0.06 [-0.08,-0.03]	2×10⁻⁴	-0.06 [-0.15,0.03]	2×10 ⁻¹	-0.05 [-0.09,-0.02]	6×10⁻⁴		
DQA1*03:01	-0.07 [-0.10,-0.03]	4×10⁻⁵	-0.02 [-0.10,0.06]	6×10 ⁻¹	-0.06 [-0.10,-0.02]	2×10⁻³	-0.16 [-0.25,-0.07]	3×10⁻⁴
DQA1*05:01	0.06 [0.03,0.09]	7×10⁻⁵	0.04 [-0.02,0.11]	2×10 ⁻¹	0.06 [0.02,0.09]	2×10⁻³	0.12 [0.03,0.20]	1×10⁻²
DQB1*03:01	0.06 [0.03,0.09]	3×10⁻⁵	0.05 [-0.01,0.11]	8×10 ⁻²	0.06 [0.03,0.09]	5×10⁻⁴	0.07 [-0.02,0.16]	1×10 ⁻¹
DQB1*03:03	-0.08 [-0.13,-0.04]	2×10⁻⁴	-0.18 [-0.34,-0.02]	2×10⁻²	-0.07 [-0.12,-0.02]	3×10⁻³	-0.19 [-0.43,0.06]	1×10 ⁻¹
DRB1*04:04	-0.13 [-0.19,-0.07]	1×10⁻⁵	0.00 [-0.30,0.31]	1.00	-0.15 [-0.21,-0.09]	3×10⁻⁶	-0.01 [-0.23,0.21]	9×10 ⁻¹
DRB1*11:01	0.08 [0.04,0.12]	2×10⁻⁴	0.10 [0.02,0.19]	2×10⁻²	0.07 [0.02,0.12]	6×10⁻³	0.10 [-0.06,0.26]	2×10 ⁻¹
Replication								
A*02:01	-0.05 [-0.08,-0.02]	5×10⁻⁴	-0.08 [-0.15,-0.00]	4×10⁻²	-0.06 [-0.09,-0.02]	4×10⁻³	-0.03 [-0.07,0.02]	3×10 ⁻¹
DQA1*03:01	-0.08 [-0.12,-0.05]	4×10⁻⁶	-0.11 [-0.24,0.02]	1×10 ⁻¹	-0.06 [-0.11,-0.01]	2×10⁻²	-0.10 [-0.16,-0.05]	1×10⁻⁴
DQA1*05:01	0.03 [-0.01,0.06]	2×10 ⁻¹	-0.03 [-0.11,0.05]	5×10 ⁻¹	0.02 [-0.02,0.07]	3×10 ⁻¹	0.07 [-0.00,0.14]	7×10 ⁻²
DQB1*03:01	0.02 [-0.01,0.04]	2×10 ⁻¹	0.06 [-0.01,0.13]	1×10 ⁻¹	0.03 [-0.01,0.07]	2×10 ⁻¹	-0.01 [-0.05,0.04]	7×10 ⁻¹
DQB1*03:03	-0.04 [-0.08,0.01]	2×10 ⁻¹	-0.10 [-0.30,0.10]	3×10 ⁻¹	-0.03 [-0.10,0.03]	3×10 ⁻¹	-0.03 [-0.10,0.05]	5×10 ⁻¹
DRB1*04:04	-0.01 [-0.10,0.08]	8×10 ⁻¹	-0.10 [-0.76,0.55]	8×10 ⁻¹	0.04 [-0.07,0.15]	5×10 ⁻¹	-0.13 [-0.29,0.04]	1×10 ⁻¹
DRB1*11:01	0.03 [-0.01,0.07]	2×10 ⁻¹	0.03 [-0.06,0.12]	5×10 ⁻¹	0.05 [-0.01,0.11]	8×10 ⁻²	-0.00 [-0.07,0.07]	9×10 ⁻¹
Ancestry								
A*02:01			-0.07 [-0.13,-0.01]	2×10⁻²	-0.05 [-0.08,-0.03]	8×10⁻⁶	-0.03 [-0.07,0.02]	3×10 ⁻¹
A*29:02			0.06 [-0.05,0.16]	3×10 ⁻¹	0.09 [0.04,0.14]	2×10⁻⁴	0.02 [-0.06,0.10]	6×10 ⁻¹
B*40:01			-0.11 [-0.26,0.03]	1×10 ⁻¹	-0.09 [-0.14,-0.05]	3×10⁻⁵	-0.03 [-0.23,0.18]	8×10 ⁻¹
C*03:04			-0.03 [-0.10,0.04]	3×10 ⁻¹	-0.07 [-0.11,-0.04]	1×10⁻⁴	-0.01 [-0.07,0.05]	8×10 ⁻¹
DPB1*03:01			-0.09 [-0.18,-0.01]	3×10⁻²	-0.06 [-0.09,-0.03]	2×10⁻⁴	-0.02 [-0.09,0.06]	6×10 ⁻¹
DQA1*03:01			-0.04 [-0.11,0.02]	2×10 ⁻¹	-0.06 [-0.09,-0.03]	1×10⁻⁴	-0.12 [-0.16,-0.07]	3×10⁻⁷
DRB1*04:04			-0.01 [-0.29,0.26]	9×10 ⁻¹	-0.10 [-0.16,-0.05]	2×10⁻⁴	-0.08 [-0.21,0.05]	2×10 ⁻¹
DQB1*03:02			-0.04 [-0.12,0.04]	3×10 ⁻¹	-0.03 [-0.07,-0.00]	3×10⁻²	-0.12 [-0.16,-0.08]	8×10⁻⁸

Table 1: Significant HLA allele associations. Associations in the discovery data set with $P < 0.05/155$ (the number of alleles tested), replicated associations with $P < 0.05$, and associations in the combined discovery+replication data set with $P < 0.05/155$ in any of the ancestry groups, are included in this table. P-values < 0.05 are in bold font.

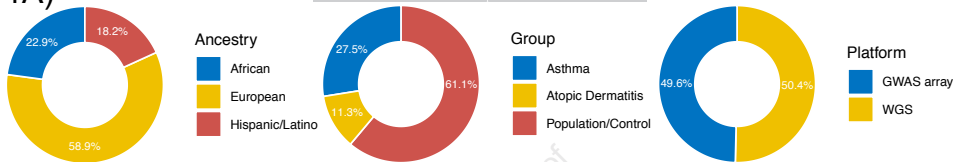
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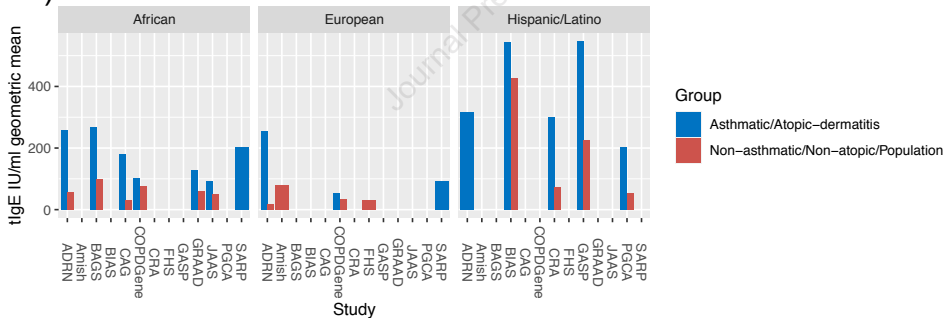
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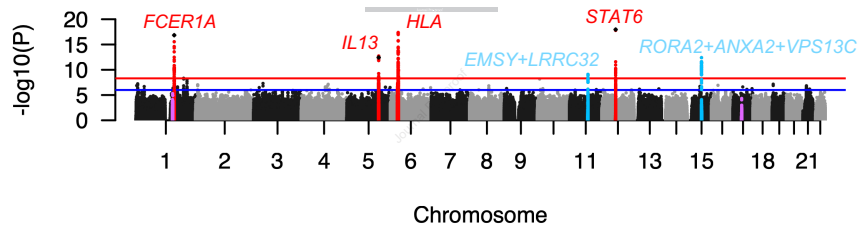
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1A)

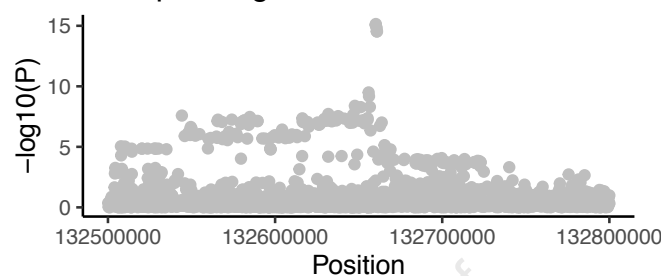
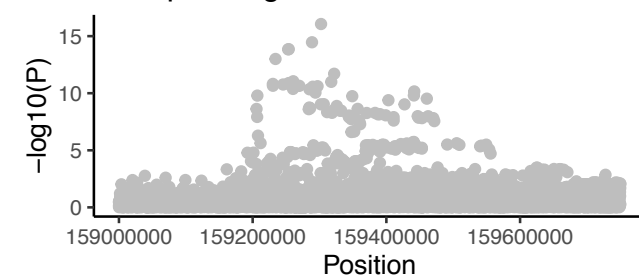


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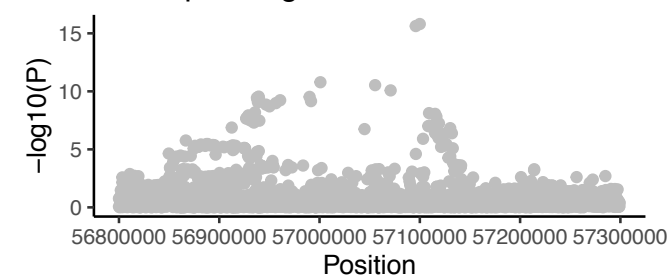




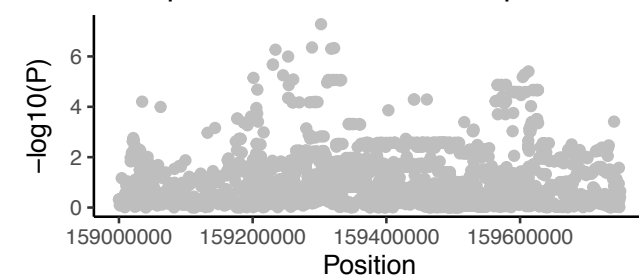
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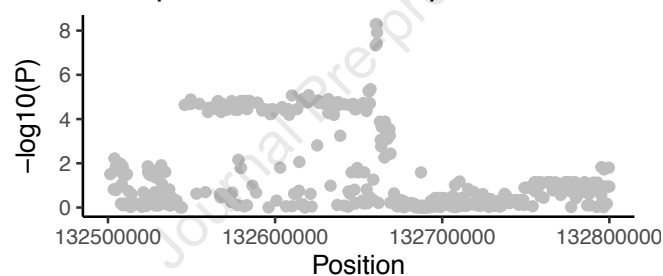
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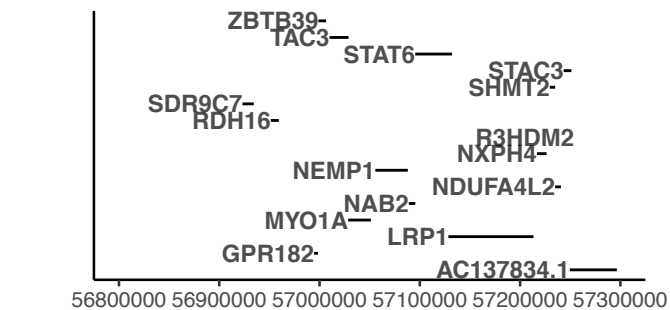
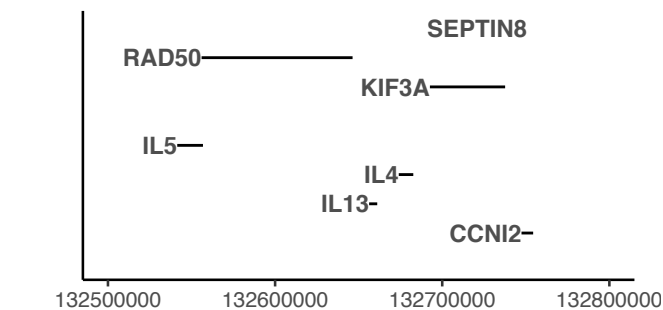
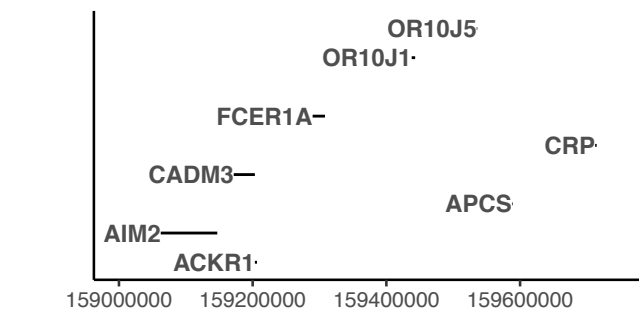
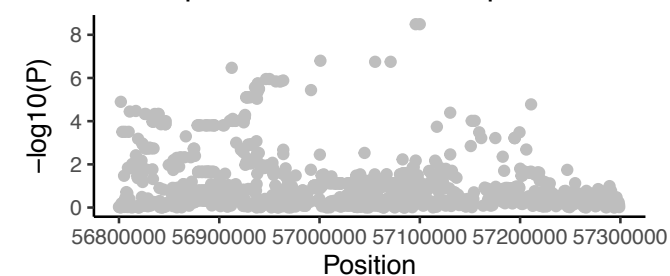
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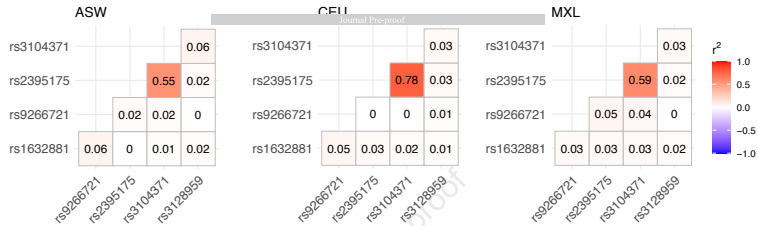
chr5q31.1 GTEx IL13 expression



chr12q13.3 GTEx STAT6 expression



4A)



4B)

