Gene expression in African Americans and Latinos reveals ancestry-specific patterns of genetic architecture

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ABSTRACT

We explored the role of genetic ancestry in shaping the genetic architecture of whole blood gene expression using whole genome and RNA sequencing data from 2,733 African American and Hispanic/Latino children. We find that heritability of gene expression significantly increases with greater proportion of genome-wide African ancestry and decreases with higher levels of Indigenous American ancestry. Fine-mapping of expression quantitative trait loci (eQTLs) in individuals with predominantly African or Indigenous American ancestry revealed ancestry-specific eQTLs in over 30% of heritable genes. We leveraged our data to train genetically derived transcriptome prediction models, which identified significantly more associated genes when applied to 28 traits from a multi-ancestry population. Our findings underscore the importance of increasing representation from ancestrally diverse populations in genomic studies to enable new discoveries and ensure their equitable translation.

INTRODUCTION

Gene expression has been extensively studied as a trait affected by genetic variation in humans¹. Expression quantitative trait loci (eQTLs) have been identified in most genes^{2–4} and extensive analyses across multiple tissues have demonstrated both tissue-specific and shared eQTLs². Genome-wide association studies (GWAS) tend to identify loci that are enriched for eQTLs⁵. Colocalization of eQTLs with GWAS has become an important element of identifying causal genes and investigating the biology underlying genetic susceptibility to disease⁶. More recently, transcriptome-wide association studies (TWAS) have been developed to systematically leverage eQTL data by imputing transcriptomic profiles in external datasets, which has led to the discovery of trait-associated genes that were often missed by GWAS^{7,8}.

GWAS have identified thousands of loci for hundreds of diseases and disease-related phenotypes in human populations⁹. However, non-European ancestry populations are significantly under-represented in GWAS^{10,11} and in studies of gene expression and eQTLs. We and others have shown that gene expression prediction models trained in predominantly European ancestry reference datasets, such as the Genotype-Tissue Expression (GTEx) project², have substantially lower accuracy to predict gene expression levels when applied to populations of non-European ancestry^{3,12,13}. The importance of having ancestry-matched training datasets for prediction accuracy is also reflected by the limited cross-population portability of other multi-SNP prediction models, such as polygenic risk scores (PRS)^{14,15}. Therefore, the limited diversity in genetic association studies and reference datasets is a major obstacle for applying existing integrative genomic studies to non-European populations.

To address this gap, we leveraged whole genome and RNA sequencing data from 2,733 African American and Latino children from the Genes-environments and Admixture in Latino Americans (GALA II) study and the Study of African Americans, Asthma, Genes, and Environments (SAGE) to characterize the genetic architecture of whole blood eQTLs. The diversity within the GALA II/SAGE population enabled us to evaluate how genetic ancestry relates to the heritability of gene expression, and systematically quantify the prevalence of ancestry-specific eQTLs. Lastly, we

developed a powerful set of TWAS models to facilitate genetic association analyses in multi-ancestry populations.

RESULTS

Demographic characteristics of GALA II and SAGE participants

We analyzed data from a total of 2,733 participants from the GALA II and SAGE asthma case-control studies, including 757 self-identified African Americans (AA), 893 Puerto Ricans (PR), 784 Mexican Americans (MX), and 299 other Latinos (LA) who did not self-identify as Mexican American or Puerto Rican (Table 1, Table S1). Participants identified all four grandparents as Latino for GALA II or African American for SAGE. The median age of the participants varied from 13.2 (PR) to 16.0 (AA) years old. About 50% of the participants were female and 45% (MX) to 62% (PR) had physician-diagnosed asthma. For each participant we estimated genome-wide genetic ancestry (global ancestry) proportions, visualized in Figure 1. Median global African ancestry was highest in AA (82.6%), followed by PR (19.7%), and lowest in MX (3.5%).

Variability of gene expression explained by common genetic variation increases with African ancestry

We compared the heritability (h^2) and genetic variance (V_G) of whole blood gene expression across self-identified race/ethnicity groups (AA, PR, MX) and populations defined based on genetic ancestry. V_G corresponds to the observed variance in transcriptome levels attributed to common genetic variation (minor allele frequency [MAF] ≥ 0.01) within the *cis*-region (see Methods). Heritability (h^2) was calculated as the ratio of V_G to total phenotypic variance. There was an overarching trend whereby increasing proportion of African ancestry was positively associated with h^2 and V_G (Figure 2a-b, Table S2). *Cis*-heritability was significantly higher in AA ($h^2=0.158$) compared to PR ($h^2=0.135$; Wilcoxon rank sum test: $p=2.2 \times 10^{-50}$) and MX ($h^2=0.123$; $p=3.3 \times 10^{-134}$), as well as PR compared to MX ($p=2.2 \times 10^{-25}$).

In examining genetic ancestry, we considered both global ancestry and local ancestry. Global ancestry is defined as the genome-wide average ancestry for each individual and local ancestry is

defined as the number of alleles (0, 1 or 2) derived from distinct ancestral populations at a given locus. The same pattern was observed in the distribution of h^2 and V_G when we assigned participants to high global African ancestry (AFR_{high} , $AFR > 50\%$) and low global African ancestry (AFR_{low} , $AFR < 10\%$) groups (Table S1-2, Figure S2c-d). *Cis*-heritability and V_G were significantly higher in AFR_{high} compared to AFR_{low} groups (Wilcoxon rank sum test: $p=9.6 \times 10^{-126}$ for h^2 ; V_G : $p=7.6 \times 10^{-106}$ for V_G). The opposite was observed for Indigenous American (IAM) ancestry, with higher h^2 and V_G in IAM_{low} ($<10\%$ IAM ancestry) compared to IAM_{high} ($\geq 50\%$ IAM ancestry) groups ($p=3.1 \times 10^{-103}$ for h^2 ; $p=4.5 \times 10^{-159}$ for V_G).

Next, we refined the observed relationship with heritability by examining ancestry at the locus level. For each gene, individuals with homozygous local African ancestry (AFR/AFR) were compared to those with heterozygous local African and European ancestry (AFR/EUR) at the transcription start site. Consistent with global ancestry, h^2 and V_G were significantly higher in AFR/AFR homozygotes compared to AFR/EUR and lower in IAM/IAM compared to IAM/EUR heterozygotes (Figure 2e-h). Within the pooled GALA/SAGE II population, V_G was significantly higher in AFR/AFR compared to AFR/EUR group (Wilcoxon rank sum test: $p=2.00 \times 10^{-7}$; Table S3). Conversely, the V_G in IAM/IAM was significantly lower compared to EUR/EUR ($p=1.76 \times 10^{-7}$ for Pooled;) or IAM/EUR ($p=1.62 \times 10^{-8}$ for Pooled; Table S3). All associations remained statistically significant in analyses stratified by self-reported race/ethnicity (Table S3; $p=5.70 \times 10^{-5}$ for AFR/AFR vs. AFR/EUR in AA; $p=2.98 \times 10^{-4}$ for IAM/IAM vs. EUR/EUR in MX; $p=7.67 \times 10^{-11}$ for IAM/IAM vs. IAM/EUR in MX).

As a parallel approach for evaluating the role of genetic ancestry in the context of whole blood gene expression, we tabulated the number of heritable genes for which global and/or local ancestry was significantly associated ($FDR < 0.05$) with transcript levels. The proportion of global AFR ancestry was associated with the expression of 326 (2.4%) and 589 (4.5%) of heritable genes in AA and PR, respectively (Table S4). Associations with local, but not global AFR ancestry were more common (8.9% in AA; 10.9% in PR). Few genes were associated with both global and local AFR ancestry (1.5% in AA and 2.5% in PR). Among genes associated with both global and local AFR

ancestry in AA, global AFR ancestry explained a median of 1.8% of variation in gene expression, while local AA accounted for 3.8% of variation (Figure S1). Local IAM ancestry was significantly associated with the expression of 9.8% of genes in MX, compared to 2.8% for global. The variation in gene expression accounted for by local IAM ancestry was also higher (3.5%) than global (1.8%) in genes associated with both global and local IAM ancestry.

Identification of ancestry-specific eQTLs

We next sought to understand patterns of eQTLs in the GALA and SAGE datasets and identify potential novel population-specific eQTLs. We identified 19,568 genes with at least one eQTL (eGenes) in the pooled samples and 17,351, 16,980, and 15,975 eGenes in African American, Puerto Rican, and Mexican American samples, respectively (Table S5, Figure S2).

To characterize ancestry-related differences in the genetic determinants of gene expression, we built a framework to identify eQTLs with potential ancestry-specific effects on gene expression levels. We then classified them based on their ancestry-specific properties, including allele frequency and effect size (Figure 3a). We termed these eQTLs ancestry-specific eQTLs (anc-eQTLs). To identify anc-eQTLs, we compared the lead eQTL signals of *cis*-heritable genes (Table S6) between high and low ancestry groups (AFR_{high} vs AFR_{low}; IAM_{high} vs IAM_{low}). We identified the lead eQTL signal of each gene in each ancestry group (AFR_{high}, AFR_{low}, IAM_{high} and IAM_{low}) by fine-mapping 95% credible set of causal variants using CAVIAR¹⁶.

We divided anc-eQTLs into three tiers of ancestry-specificity in multiple steps, first based on overlap in the 95% credible sets for each gene, followed by analyses accounting for differences in eQTL effect size, allele frequency, and linkage disequilibrium (LD) between ancestry groups (see Methods). Over 30% (n=2,961) and 8% (n=679) of heritable genes had at least one AFR_{high}-eQTL and IAM_{high}-eQTL, respectively. We define the three tiers of ancestry-associated eQTLs below.

Tier 1 anc-eQTLs had non-overlapping 95% credible sets because they were only common (MAF ≥ 0.01) in high ancestry groups and were thus considered to be the most ancestry specific. Over 28% (n=2,695) of genes contained at least one Tier 1 AFR_{high} lead eQTL, while 7% (n=562) of genes

contained a Tier 1 IAM_{high} eQTL (Figure 3a, left branch; Table S7). Tier 2 anc-eQTLs also had non-overlapping 95% credible sets for each gene but were commonly segregating ($MAF \geq 0.01$) in both high and low ancestry groups. This class of anc-eQTLs was identified using PESCA¹⁷, which accounted for population-specific LD patterns. We identified 109 genes (1.1%) with eQTLs having a posterior probability (PP) > 80% of being specific to AFR_{high} and 33 genes (0.4%) matching the same criteria for IAM_{high}. Lastly, we found that over 50% of genes (5,058 for AFR and 5,355 for IAM) contained eQTLs that were shared between high and low ancestry groups. Among these shared signals, only a small proportion of eQTLs exhibited effect size heterogeneity, as assessed using Cochran's Q test. We refer to these as Tier 3 anc-eQTLs (2.0% for AFR; 1.0% for IAM) and note this tier has the lowest level of ancestry-specificity.

Next, we explored whether the ancestry-specific eQTLs were previously associated with diseases/traits based on the NHGRI-EBI GWAS catalog¹⁸. We found 70 unique anc-eQTLs in 61 genes associated with 84 diseases/traits reported in 65 publications (Table S8, supplementary file 1). Representative examples of disease-associated anc-eQTLs are shown in (Figure 3b-d). A Tier 1 AFR_{high}-specific eQTL of the *ABAT* gene (rs1641047) was associated with systemic sclerosis (SSc) in a study that compared European and African American SSc patients¹⁹. It is common in African Americans in SAGE ($MAF = 0.04$) but rare in Mexican Americans in GALA II ($MAF = 2 \times 10^{-3}$, Figure 3b) and Europeans ($MAF = 2 \times 10^{-4}$, $n=15,666$). A Tier 2 IAM_{high}-specific eQTL of *SPSB2* gene (rs11064437) was previously reported as a risk variant for colorectal cancer in East Asian populations²⁰, likely due to the fact that it is present at higher frequencies in this population (East Asian $MAF = 0.26$). In our dataset, the lead *SPSB2* eQTL in the IAM_{high} group (rs11064437) was independent from that of the IAM_{low} group (rs6489739), as indicated by low LD ($r^2=0.16$, Figure 3d). A Tier 3 AFR_{high}-specific eQTL of *KCNK17* gene (rs34246110) was associated with type 2 diabetes in two independent studies performed in Japanese and multi-ancestry (European, African American, Hispanic and Asian) populations^{21,22}. The detection of this association in multiple populations was consistent with the characteristics of Tier 3 anc-eQTLs in that they share lead eQTL signals with other populations but likely exhibit heterogeneous effect sizes.

Gene expression prediction models from admixed populations increase power for gene discovery

We generated gene expression imputation models from GALA II and SAGE following the PrediXcan approach⁷. We used the pooled population (n=2,733) to generate models with significant prediction (see Methods) for 11,830 heritable genes with mean cross-validation (CV) $R^2=0.157$ (Table S9 and Figure S3). We also generated population-specific models for African Americans (10,090 genes, CV $R^2=0.180$), Puerto Ricans (9,611 genes, CV $R^2=0.163$), and Mexican Americans (9,084 genes, CV $R^2=0.167$). In sensitivity analyses adjusting for local ancestry, we did not observe gains in predictive performance (Table S10). For genes with local ancestry estimates, adjusting for local ancestry did not result in improved model performance (AA: CV $R^2=0.177$; PR: CV $R^2=0.154$; MX: CV $R^2=0.159$).

Our TWAS models were successfully validated in the Study of Asthma Phenotypes and Pharmacogenomic Interactions by Race-Ethnicity (SAPPHIRE)²³, an independent adult population of African Americans (Figure S4). As expected, both pooled and AA-specific TWAS models, which contained the highest number of ancestry-matched individuals, had significantly highest gene expression prediction accuracy in SAPPHIRE compared to other models (correlation $R^2=0.026$ for AA compared to 0.023 for PR [Wilcoxon rank sum test: $p=2.39 \times 10^{-5}$], 0.020 for MX [$p=1.19 \times 10^{-17}$ and 0.015 for GTEx v8 [$p=5.43 \times 10^{-85}$] models).

To evaluate the potential of TWAS models built from GALA II and SAGE pooled samples (hereafter referred to as GALA/SAGE models) to improve gene discovery in admixed populations, we applied our models to GWAS summary statistics for 28 traits from the multi-ancestry Population Architecture using Genomics and Epidemiology (PAGE) study²⁴ and conducted parallel analyses using TWAS models based on GTEx v8^{2,7} and the Multi-Ethnic Study of Atherosclerosis (MESA)³. GTEx v8 whole blood models are based on 670 subjects of predominantly European ancestry (85%) with median age between 50-60 years old². MESA models impute monocyte gene expression³ based on a sample of African American and Hispanic/Latino individuals (MESA_{AFHI}:

n=585). As such, MESA more closely resembles the ancestry composition of our population as well as subjects included in the PAGE study.

The total number of genes examined was 39% to 82% higher in GALA/SAGE compared to GTEx (n=7,249) and MESA_{AFHI} (n=5,555), with an intersection of 3,146 genes (Figure 4a, left). Restricting to genes shared across all three models, CV R^2 was significantly higher in GALA/SAGE compared to GTEx ($p=5.1 \times 10^{-159}$) and MESA_{AFHI} ($p=9.9 \times 10^{-65}$), which is expected based on the substantially larger sample size of GALA/SAGE (Figure 4a). More importantly, we observed the same trend with an intersection of 3,003 genes using GALA/SAGE TWAS models built in African Americans only (n=757), which was a population of comparable sample size (Figure 4b). Association results across 28 PAGE traits demonstrate that TWAS using GALA/SAGE (pooled) models identified a larger number of significant gene-trait pairs (n=380, FDR<0.05), followed by MESA_{AFHI} (n=303), and GTEx (n=268), with only 30 genes (35 gene-trait pairs) significant in all three analyses (Figure 4c).

GALA/SAGE models yielded a larger number of associated genes than MESA in 80% of analyses (binomial test: $p=0.012$) and 79% compared to GTEx ($p=0.019$). Of the 330 genes with FDR<0.05 in GALA/SAGE, 143 (43%) were not present in GTEx and 199 (60%) were not present in MESA_{AFHI}. For genes that were significant in at least one TWAS, z-scores in GALA/SAGE were highly correlated with GTEx (Figure 4c; $r=0.74$, $p=3.5 \times 10^{-64}$) and MESA_{AFHI} (Figure 4d; $r=0.55$, $p=8.5 \times 10^{-27}$), suggesting that most genes have concordant effects even if they fail to achieve statistical significance in both analyses (Figure 4c). Despite the higher correlation with GTEx TWAS z-scores, we observed a higher proportion of gene-trait pairs significant (FDR<0.05) in GALA/SAGE that were not even nominally associated ($p<0.05$) in GTEx (33%), compared to 18% in MESA_{AFHI}.

The high-density lipoprotein (HDL) cholesterol trait exhibited one of the largest differences in TWAS associations, with over 60% more significant genes identified using GALA/SAGE models (n=29) than GTEx predictions (n=11; Figure 4c). Models for several associated genes, including *CETP* which has been consistently implicated in HDL cholesterol transport, were not available in GTEx. The top-ranking HDL cholesterol-associated gene, *CD36* (z-score= -10.52, $p=6.9 \times 10^{-26}$) had Tier 1 AFR_{high}-specific eQTLs (rs3211938) that were not present at an appreciable frequency in

populations with low African ancestry (MAF in European = 1.3×10^{-4}). The difference in MAF may explain why *CD36* was not detected using GTEx (z-score=0.057, $p=0.95$), even though all 43 variants used in GTEx prediction model were available in PAGE summary statistics. In addition to HDL cholesterol levels, *CD36* expression was also associated with levels of C-reactive protein (z-score= 5.30, $p=1.1 \times 10^{-7}$).

In addition to multi-ancestry analyses, we also demonstrate the utility of ancestry-specific gene expression prediction models for the discovery of trait-gene association using TWAS. We conducted a TWAS of neutrophil counts in African ancestry subjects using population-specific summary statistics from Chen et al.²⁵ and ancestry-matched GALA/SAGE models, which identified 139 genes. Eleven of the 139 genes identified were outside of the Duffy locus on 1q23 with FDR<0.05 (Figure 5b). Novel findings included *CREB5* ($p=1.5 \times 10^{-14}$), *DARS* ($p=2.9 \times 10^{-8}$), *CD36* ($p=1.1 \times 10^{-5}$), *PPT2* ($p=1.3 \times 10^{-5}$), *SSH2* ($p=4.7 \times 10^{-5}$), *TOMM5* ($p=2.9 \times 10^{-4}$), and *ARF6* ($p=3.4 \times 10^{-4}$).

DISCUSSION

Our comprehensive analysis in a uniquely large and diverse multi-racial/ethnic population revealed new insight into the role of genetic ancestry in shaping the genetic architecture of whole blood gene expression that may have broad implications for other complex traits. We found that *cis*-heritability of gene expression increased with higher proportions of African ancestry. We also demonstrated that in populations of mixed ancestry, *cis*-heritability was highest with increasing local African ancestry. The consistency of this pattern in analyses considering global and local ancestry, as well as in pooled and stratified analyses within populations (African Americans or Puerto Ricans), suggests this is unlikely to be attributed to confounding by social or environmental factors. Parallel analyses examining Indigenous American Ancestry revealed an inverse relationship – with genetic variance and *cis*-heritability of gene expression decreasing in individuals with higher levels Indigenous American ancestry compared to European ancestry.

These observations are consistent with the overall pattern of heterozygosity of these populations. African populations consistently show the highest heterozygosity since all other populations

passed through a bottleneck during their migration out of Africa^{26,27}. Indigenous American populations have passed through additional bottlenecks which possibly date to, or prior to, the settling of the Americas^{28,29}. The finding that heritability tracks with heterozygosity across human populations may be expected from fundamental assumptions that there are more segregating causal variants in populations with higher heterozygosity³⁰. However, to our knowledge, this has not yet been systematically demonstrated for whole blood gene expression, particularly in a sufficiently large racially/ethnically diverse sample size.

A second major finding of our work is that over 30% of heritable genes have ancestry-specific eQTLs and these are predominantly comprised of Tier 1 variants that were rare ($MAF < 0.01$) or not polymorphic in another population. Tier 2 ancestry eQTLs are a less striking, albeit interesting class of variants which were present at a sufficient frequency ($MAF > 0.01$) in both high and low ancestry groups but did not map to the same eQTL signal. Tier 2 eQTLs could arise due to differences in environmental effects on gene expression, multiple causal variants at the same locus that are in different degrees of LD with each other, or *trans*-effects, which are contributed by genetic variation outside of the *cis*-region. Additional research is needed to elucidate the underlying reasons for more subtle ancestry-driven differences in eQTL effects.

Given genetic architecture of complex traits is, to variable degree, mirrored or driven by the genetics of gene expression³¹, our work has several important implications. Observations regarding heritability suggest genetic prediction of complex traits in populations with at least 50% global African ancestry should be at least as accurate, if not more effective, than in populations with low proportion of African ancestry. However, for most complex traits the accuracy of polygenic prediction models in populations of predominantly African ancestry significantly lags behind other populations¹⁵, particularly those of European ancestry. Although there are caveats associated with using heritability to gauge the future potential of genetic risk prediction models³², our results imply that current deficits in predictive performance in admixed populations are likely to be a consequence of insufficient sample sizes and systematic underrepresentation. The substantial prevalence of ancestry-specific eQTLs implies that analytic approaches alone will yield

limited improvements in the generalizability of genetic prediction models, including European-ancestry derived polygenic risk scores (PRS). For instance, fine-mapping approaches that rely on accounting for differential LD tagging to identify causal variants will recover some deficits in predictive performance, but will not compensate for missing genetic predictors. Our results reinforce the conclusion that developing truly generalizable genetic prediction models requires capturing the full spectrum of genetic variance in non-European populations. As such, access to sufficiently large and racially/ethnically and ancestrally diverse populations is the main rate limiting step.

Previous work by Gay *et al.* assessed the amount of variance in gene expression explained by global and/or local ancestry³³. It was reported that local ancestry explains at least 7% of the variance in residual expression for 1% of expressed genes in an analysis of 117 admixed individuals from GTEx. Using sample sizes ranging from 757 (AA) to 893 (PR) in GALA II/SAGE, we found that local ancestry was a significant predictor of transcript levels for at least 10% of heritable genes, explaining between 2.1% (PR) and 5.1% (AA) of residual variance. Consistent with Gay *et al.*, we observed that local ancestry explains a larger proportion of variance in gene expression corrected for global ancestry. However, contrary to their findings, we found that, on average, local ancestry also explains more variance in residual gene expression than global ancestry.

The importance of comprehensively accounting for genetic determinants of trait variation in multi-ethnic populations is illustrated in our TWAS results for 28 traits from the PAGE study. TWAS models trained in the racially/ethnically and ancestrally diverse GALA II/SAGE study identified significantly more trait-associated genes than GTEx and MESA respectively. This is consistent with the findings of Geoffroy *et al.*¹³ using GTEx and MESA models, as well as other observations^{12,13} that ancestry-matched models improve power for gene discovery in admixed populations. Over 40% of significantly associated TWAS genes detected using GALA/SAGE models were not available in GTEx, which underscores how biologically meaningful associations may be overlooked in studies that exclusively rely on European ancestry-based predictions. The top two HDL cholesterol-associated genes, *CETP* in 16q13 and *CD36* in 7q21, with established effects on lipid

metabolism^{34–37}, were not detected in TWAS using GTEx due differences in eQTLs. The finding for *CD36* is compelling since this gene was associated with multiple phenotypes and contains Tier 1 anc-eQTLs that are specific to individuals with high African ancestry, consistent with earlier findings that evolutionary pressures have elevated genetic divergence at this locus^{38,39}. *CD36* encodes a transmembrane protein that binds many ligands, including collagen, thrombospondin, and long-chain fatty acids, and serves as a negative regulator of angiogenesis⁴⁰. Beyond lipid metabolism, its most extensively studied function involves mediating the adherence of erythrocytes infected with *Plasmodium falciparum*, the parasite responsible for severe malaria^{41,42}.

An even more striking example of ancestry-specific genetic architecture involves the Duffy antigen receptor gene (*ACKR1*) on 1q23, which is responsible for persistently lower white blood cell and neutrophil counts in African ancestry individuals^{43,44}. Common African-derived alleles at this locus confer a selective advantage against *Plasmodium vivax* malaria and are extremely rare in European populations. Expression of *ACKR1* could not be imputed using GTEx or MESA, but this causal gene was captured by the pooled and AA-specific GALA/SAGE TWAS models. Furthermore, we identified 11 genes associated with neutrophil counts outside of the Duffy locus, including associations not reported in the source GWAS by Chen *et al*²⁵. We replicated the *PSMD3* signal in 17q21⁴⁵, which was previously identified in African Americans, as well as *CREB5*, *SSH2*, and *PPT2*, which were discovered in European ancestry populations⁴⁶. We also identified several novel genes that have not previously been linked to hematologic traits: *DARS1* in 2q31.1 modulates reactivity to mosquito antigens⁴⁷, while *TOMM5* has been implicated in lipoprotein phospholipase A2 activity⁴⁸.

Taken together, our TWAS findings suggest that models trained in racially/ethnically and ancestrally diverse populations improve power for identifying trait-associated genes in shared biological pathways. When applied to admixed populations, GALA II/SAGE imputation models benefit from having more similar allele frequency profiles to the target datasets, such as PAGE, as well as more accurate modeling of LD. However, we note that adjustment for local ancestry

did not improve the predictive performance of our TWAS models. Previous analyses of GTEx data indicated that local ancestry adjustment increases power for discovery in *cis*-eQTL mapping^{33,49}, however this has not been assessed explicitly within the TWAS framework. It is possible that the slight decrease in CV R^2 occurs due to overadjustment, since local ancestry may serve as a proxy for information already captured by genetic variants in the *cis*-region.

Interestingly, the correlation of TWAS summary statistics was higher with GTEx than MESA_{AFHI}, which may partly reflect the lack of neutrophils present in monocyte gene expression in MESA_{AFHI} compared to whole blood in GALA/SAGE and GTEx. However, the scarcity of discordant TWAS findings (null in one analysis or significant associations with opposite directions of effect) or missing genes between GALA II/SAGE and MESA, indicate that similarity in ancestry may partly compensate for differences in the cell type analyzed. Furthermore, GALA II/SAGE and GTEx conducted whole-genome sequencing, whereas MESA TWAS models are based on imputed genotype data, although we this effect should be limited as we filter to common variants. While our study population is comprised of pediatric studies, TWAS analyses of medically relevant traits and chronic conditions in PAGE identified more genes than adult-derived prediction models. This implies that the power gained from ancestry-matched prediction and large sample size of the training set may outweigh substantial differences in age between participants in our cohort and participants in PAGE studies that range across adulthood.

In evaluating the contributions of our work, a few limitations should be acknowledged. Although our analyses were limited to a single tissue, we note that whole blood is one of the most clinically-informative and commonly-collected samples, and for over 60% of genes whole blood transcriptomes can significantly capture expression levels in other tissues⁵⁰. Thus, our observations regarding the genetic architecture of whole blood eQTLs in non-European ancestry populations are likely generalizable to other tissues. Our approach for ancestry-specific eQTLs may result in an underestimation of the number of these loci. We assumed that each gene had one causal eQTL locus and focused all comparisons on the corresponding 95% credible set. This assumption is likely violated for genes with multiple independent eQTLs, which would limit our

ability to assess the ancestry-specificity of all signals. In general, we believe this is a conservative assumption that would lead us to potentially miss some ancestry-specific eQTLs. Detection of Tier 2 anc-eQTLs by the PESCA method relies on having regions that are approximately LD independent in both populations to estimate the proportion of causal variants. This estimate may be biased if there is residual LD between regions, which is particularly a challenge in admixed populations with longer-range LD. Lastly, our comparison of TWAS models may be slightly biased against GTEx since we did not apply MASHR models, which predict a larger number of genes using fine-mapped eQTLs⁵¹. We chose to compare with elastic net GTEx models instead because GALA/SAGE TWAS models were developed using the same analytic pipeline.

Despite these limitations, our study leveraged a uniquely large and diverse sample of 2,733 African American and Latino youths to advance our understanding of the interplay between genetic ancestry and genetic determinants of gene expression. Our systematic approach for evaluating the ancestry-specificity of whole blood eQTLs revealed that most of this signal is due to Tier 1 anc-eQTLs, which are only polymorphic in predominantly AFR and IAM ancestry populations. These ancestry-specific eQTLs are present in a large fraction of genes, which highlights the need for larger genomic studies in globally representative populations to detect functional genetic variants and evaluate their role in disease susceptibility. We also developed genetic prediction models of whole blood transcriptomes and demonstrated that they covered more genes and, in TWAS based on multi-ethnic populations, performed better than similar models built in European populations. In summary, the results of our study have implications not only for the genetic prediction of gene expression, but for other complex traits, and ultimately for the equitable translation of precision medicine efforts to all populations worldwide.

METHODS

Study population

This study examined African American, Puerto Rican and Mexican American children between 8-21 years of age with or without physician-diagnosed asthma from the Genes-environments and Admixture in Latino Americans II (GALA II) study and the Study of African Americans, Asthma, Genes & Environments (SAGE). The inclusion and exclusion criteria are previously described in detail^{52,53}. Briefly, participants were eligible if they were 8-21 years of age and identified all four grandparents as Latino for GALA II or African American for SAGE. Study exclusion criteria included the following: 1) any smoking within one year of the recruitment date; 2) 10 or more pack-years of smoking; 3) pregnancy in the third trimester; 4) history of lung diseases other than asthma (for cases) or chronic illness (for cases and controls).

The local institutional review board from the University of California San Francisco Human Research Protection Program approved the studies (IRB# 10-02877 for SAGE and 10-00889 for GALA II). All subjects and their legal guardians provided written informed consent.

Whole genome sequencing data and processing

Genomic DNA samples extracted from whole blood were sequenced as part of the Trans-Omics for Precision Medicine (TOPMed) whole genome sequencing (WGS) program⁵⁴ and the Centers for Common Disease Genomes of the Genome Sequencing Program. WGS was performed at the New York Genome Center and Northwest Genomics Center on a HiSeq X system (Illumina, San Diego, CA) using a paired-end read length of 150 base pairs (bp), with a minimum of 30x mean genome coverage. DNA sample handling, quality control, library construction, clustering, and sequencing, read processing and sequence data quality control are previously described in detail⁵⁴. All samples were jointly genotyped by the TOPMed Informatics Research Center. Variant calls were obtained from TOPMed data freeze 8 VCF files generated based on the GRCh38 assembly. Variants with a minimum read depth of 10 (DP10) were used for analysis unless otherwise stated.

RNA sequencing data generation and processing

Total RNA was isolated from PAXgene tube using MagMax™ for Stabilized Blood Tubes RNA Isolation Kit (Applied Biosystem, P/N 4452306). Globin depletion was performed using GLOBINclear™ Human (Thermo Fisher Scientific, cat. no. AM1980). RNA integrity and yield were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

Total RNA was quantified using the Quant-iT™ RiboGreen® RNA Assay Kit and normalized to 5ng/ul. An aliquot of 300ng for each sample was transferred into library preparation which was an automated variant of the Illumina TruSeq™ Stranded mRNA Sample Preparation Kit. This method preserves strand orientation of the RNA transcript. It uses oligo dT beads to select mRNA from the total RNA sample. It is followed by heat fragmentation and cDNA synthesis from the RNA template. The resultant cDNA then goes through library preparation (end repair, base ‘A’ addition, adapter ligation, and enrichment) using Broad-designed indexed adapters substituted in for multiplexing. After enrichment the libraries were quantified with qPCR using the KAPA Library Quantification Kit for Illumina Sequencing Platforms and then pooled equimolarly. The entire process is in 96-well format and all pipetting is done by either Agilent Bravo or Hamilton Starlet.

Pooled libraries were normalized to 2nM and denatured using 0.1 N NaOH prior to sequencing. Flowcell cluster amplification and sequencing were performed according to the manufacturer’s protocols using the HiSeq 4000. Each run was a 101bp paired-end with an eight-base index barcode read. Each sample was targeted to 50M reads. Data was analyzed using the Broad Picard Pipeline which includes de-multiplexing and data aggregation.

RNA-seq reads were further processed using the TOPMed RNA-seq pipeline for Year 3 and Phase 5 RNA-seq data (supplementary file 2 obtained from https://topmed.nhlbi.nih.gov/sites/default/files/TOPMed_RNAseq_pipeline_COREyr3.pdf) . Count-level data were generated using GRCh38 human reference genome and GENCODE 30 for transcript annotation.

Count-level quality control (QC) and normalization were performed following the Genotype-Tissue Expression (GTEx) project v8 protocol (https://github.com/hakyimlab/PredictDB_Pipeline_

GTEX_v7). Sample-level QC included removal of RNA samples with RIN < 6, genetically related samples (equal or more related than third degree relative), and sex-discordant samples based on reported sex and their *XIST* and *RPS4Y1* gene expression profiles. Count distribution outliers were detected as follows: (i) Raw counts were normalized using the trimmed mean of M values (TMM) method in edgeR⁵⁵ as described in GTEx v8 protocol. (ii) The log2 transformed normalized counts at the 25th percentile of every sample were identified (count_{q25}). (iii) The 25th percentile (Q25) of count_{q25} was calculated. (iv) Samples were removed if their count_{q25} was lower than -4 as defined by visual inspection (Figure S6).

Estimation of hidden confounding factors

To account for hidden confounding factors such as batch effects, technical and biological variation in the sample preparation, and sequencing and/or data processing procedures, latent factors were estimated using the Probabilistic Estimation of Expression Residuals (PEER) method⁵⁶. Optimization was performed according to approach adopted by GTEx with the goal to maximized eQTL discovery⁵⁷. A total of 50 (for AA, PR, MX, pooled samples) and 60 (for AFR_{high}, AFR_{low}, IAM_{high}, IAM_{low}) PEER factors were selected for downstream analyses (Figure S7).

Estimation of global and local genetic ancestry

Genetic principal components (PCs), global and local ancestry, and kinship estimation on genetic relatedness were computed using biallelic single nucleotide polymorphisms (SNPs) with a PASS flag from TOPMed freeze 8 DP10 data as described previously^{58,59}. Briefly, genotype data from European (CEU), African (YRI) and Indigenous American (IAM) ancestral populations were used as the reference panels for global and local ancestry estimation assuming three ancestral populations. Global ancestry was estimated by ADMIXTURE⁶⁰ in supervised while local ancestry was estimated by RFMIX version 2 with default settings⁶¹. Throughout this study, local ancestry of a gene was defined as the number of ancestral alleles (0, 1, or 2) at the transcription start site.

Comparative analyses were performed based on two different sample grouping strategies, by self-identified race/ethnicity or by global ancestry. Self-identified race/ethnicity included four

groups – African Americans (AA), Puerto Ricans (PR), Mexican Americans (MX), and the pooling of AA, PR, MX and other Latinos (pooled). For groups defined by global ancestry, samples were grouped into high ($> 50\%$, AFR_{high} or IAM_{high}) or low ($< 10\%$, AFR_{low} or IAM_{low}) global African or Indigenous American ancestry (Figure S5). The sample size for each group is shown in Table S1.

Cis-heritability of gene expression

The genetic region of *cis*-gene regulation was defined by 1MB region flanking each side of the transcription start site (*cis*-region). *Cis*-heritability (h^2) of gene expression was estimated using unconstrained GREML⁶² analysis (--reml-no-constrain), and estimation was restricted to common autosomal variants ($MAF \geq 0.01$). Inverse-normalized gene expression was regressed on PEER factors, and the residuals were used as the phenotype for GREML analysis. Sex and asthma case-control status was used as categorical covariates, while age at blood draw and the first 5 genetic PCs were used as quantitative covariates. *Cis*-heritability was estimated separately for each self-identified race/ethnicity group (AA, PR, MX and pooled) and groupings based on global (AFR_{high} , AFR_{low} , IAM_{high} and IAM_{low}) and local ancestry (described below). Differences in the distribution of h^2 and genetic variance (V_G) between groups were tested using two-sided Wilcoxon tests. These analyses included all genes and were not restricted to *cis*-heritable genes. For analyses based on local ancestry, we also tested whether the proportion of genes with higher V_G in the AFR/AFR group was higher than that in AFR/EUR group using a two-sided binomial test. Parallel analyses were also conducted for Indigenous American ancestry (IAM/IAM vs. EUR/EUR and IAM/IAM vs. IAM/EUR).

Association of global and local ancestry with gene expression

Methods from Gay *et al* (2020)³³ was modified to identify genes associated with global and local ancestry (Figure S1). In step 1, inversed normalized gene expression was regressed on age, sex and asthma status (model 0). In step 2, the residuals from model 0 were regressed on global ancestry (model 1). In step 3, the residuals from model 1 were regressed on local ancestry (model 2) to identify genes that are associated with local ancestry. False discovery rate of 0.05 (R package qvalue) was applied to step 2 and 3 separately to identify genes that were significantly associated

with global and/or local ancestry. Step 1 to step 3 were run separately for African and Indigenous American ancestry. For heritable genes that were associated with global and/or local ancestry, a joint model of regressing global and local ancestry from residuals from model 0 was also examined to assess the percentage of variance of gene expression explained by global and/or local ancestry.

Identification of eGenes, cis-eQTLs and ancestry-specific cis-eQTLs

Raw gene counts were processed and eQTLs were identified using FastQTL⁶³ according to the GTEx v8 pipeline (<https://github.com/broadinstitute/gtex-pipeline>). Age, sex, asthma status, first 5 genetic PCs, and PEER factors were used as covariates for FastQTL analysis. *Cis*-eQTLs were defined as genetic variants that have nominal p values less than the nominal p value threshold estimated from the permutation step of FastQTL. eGenes are defined as genes with at least one eQTL.

Ancestry-specific eQTL (anc-eQTL) mapping was performed in participants stratified by high and low global African and Indigenous American ancestry (see “Grouping samples by self-identified race/ethnicity or global ancestry”). We developed a framework to identify anc-eQTLs by focusing on the lead eQTL signal for each gene and compared these between high and low global ancestry groups (AFR_{high} vs AFR_{low}; IAM_{high} vs IAM_{low}) (Figure 3a).

Anc-eQTLs were classified into three tiers as described below based on differential effect size and allele frequency, while accounting for linkage disequilibrium (LD) between eQTLs (Figure 3a). For every protein coding and heritable (GCTA h^2 LRT $p < 0.05$) eGene (Table S6), the lead eQTL signal was identified using CAVIAR¹⁶ assuming one causal locus ($c=1$). The 95% credible set of the high and low global ancestry group were compared to determine if there was any overlap of the 95% credible set (overlapping or non-overlapping lead eQTL signals).

For genes with non-overlapping lead eQTL signals (Figure 3a, left branch), eQTLs from the high group were categorized into Tier 1 anc-eQTLs if they were common variants (MAF ≥ 0.01) in the high group but rare or absent in the low group. If the eQTLs were common variants in both the high and low groups, their specificity was further assessed using PESCA¹⁷ for differential effect

size while accounting for LD between eQTLs. LD pruning on eQTLs with $r^2 > 0.95$ was performed prior to PESCA analysis. All eQTL pairs with $r^2 > 0.95$ were identified in both the high and low groups and only those pairs common to both groups were removed. In the PESCA analysis, each eQTL obtained a posterior probability whether it was specific to the high group (PP_{high}), low group (PP_{low}) or shared between the two groups (PP_{shared}). All eQTLs in the credible set of the high group were required to obtain the highest posterior probability in the high group ($PP_{\text{high}} > PP_{\text{low}}$ and $PP_{\text{high}} > PP_{\text{shared}}$) and pass the $PP_{\text{high}} > 0.8$ cutoff to be considered Tier 2 ancestry-specific eQTLs.

For genes with overlapping lead eQTL signals (Figure 3a, right branch), Cochran's Q statistics were used to test for effect size heterogeneity between high and low ancestry groups. An eQTL with significant heterogeneity in effect size was defined as having a p value of Cochran's Q smaller than the bonferroni threshold ($0.05/n_{\text{Gene}}$, where n_{Gene} was the number of genes tested and was 5,058 for AFR and 5,355 for IAM ancestry, see Table S7 row C). Since we assume the 95% credible set corresponds to a single lead eQTL signal, all eQTLs in the credible set of the high group were required to have a significant heterogeneous effect size to be qualified into Tier 3 anc-eQTLs.

Development of gene prediction models and transcriptome-wide association analyses

Gene prediction models for *cis*-gene expression were generated using common variants and elastic net modeling implemented in the PredictDB v7 pipeline (https://github.com/hakyimlab/PredictDB_Pipeline_GTEEx_v7). Models were filtered by nested cross validation (CV) prediction performance and heritability p value ($\rho_{\text{avg}} > 0.1$, $z_{\text{score_pval}} < 0.05$ and GCTA h^2 LRT p value < 0.05). Sensitivity analysis was performed by generating gene prediction models by including additional number of ancestral alleles as covariates for local ancestry adjustments. For AA, number of African ancestral allele count was used while European and Indigenous American ancestral alleles were used as covariates.

Out-of-sample validation of the gene expression prediction models were done using the African American asthma cohort, Study of Asthma Phenotypes and Pharmacogenomic Interactions by

Race-Ethnicity (SAPPHIRE)²³. Predicted gene expression from SAPPHIRE genotypes was generated using the predict function from MetaXcan. Genotypes of SAPPHIRE samples were generated by whole genome sequencing through the TOPMed program and were processed the same way as GALA II and SAGE. RNA-seq data from SAPPHIRE were generated as previously described⁶⁴ and were normalized using TMM in edgeR. Predicted and normalized gene expression data were compared to generate correlation R^2 .

To assess the performance of the resulting GALA/SAGE models we conducted transcriptome-wide association studies (TWAS) of 28 traits using GWAS summary statistics from the Population Architecture using Genomics and Epidemiology (PAGE) Consortium study by Wojcik et al²⁴. Analyses were performed using S-PrediXcan with whole blood gene prediction models from GALA II and SAGE (GALA/SAGE models), GTEx v8, and monocyte gene expression models from the Multi-Ethnic Study of Atherosclerosis (MESA) study³.

Data availability

TOPMed WGS and RNA-seq data from GALA II and SAGE are available on dbGaP under accession number phs000920.v4.p2 and phs000921.v4.p1, respectively. TOPMed WGS data from SAPPHIRE are available under the dbGaP accession number phs001467.v1.p1. Models for performing transcriptome wide association studies developed from GALA II and SAGE are freely available upon request from the corresponding authors. Due to use restrictions and prohibition of public posting of genomic summary results from GALA II and SAGE, please email the corresponding authors to set up data transfer.

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AUTHOR CONTRIBUTIONS

ACYM, LK, KLK, SSO, MAS, CRG, NZ, EGB, EZ contributed to the conception or design of the work.

ACYM, LK, DH, CE, SH, JRE, NG, SG, SX, HG, AOO, JRS, MAL, LKW, LNB, CRG, NZ, EGB, EZ contributed to the acquisition, analysis, or interpretation of data. ACYM, LK, JRE, KLK, AOO, SSO, LNB, CRG, NZ, EGB, EZ have drafted the work or substantively revised it. All authors approved the submission of this manuscript.

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Figure 1. Study overview.

Figure 1. Study overview. This study included TOPMed whole genome sequencing and whole transcriptome data generated from whole blood samples of SAGE African American and GALA II Latino individuals (n=2,733). Global ancestry proportions for all individuals are shown in the top panel. **Middle row:** we studied the genetic architecture of gene expression including *cis*-heritability and genetic component of gene expression variation (see Figure 2 for details), and association of gene expression with global and/or local ancestry (see Figure S1 for details). We also identified eQTLs, eGenes and ancestry-specific eQTLs (see Figure 3 for details). **Bottom:** we generated gene prediction models (left panel) and applied them in TWAS based on GWAS summary statistics generated from the PAGE study. We also compared TWAS results generated from GTEx v8 whole blood and MESA monocyte-derived models.

Figure 2. *Cis*-heritability of gene expression and variance explained by genetic component.

Figure 2. *Cis*-heritability (h^2) of gene expression and genetic component of gene expression variance (V_G). **a, b** | Estimated h^2 and V_G of whole blood gene expression in self-identified race/ethnicity groups. **c, d** | Estimated h^2 and V_G in groups defined by genetic ancestry, comparing subjects with > 50% global ancestry (high) to < 10% of the same ancestry (low). **e, f** | Estimated h^2 and V_G in groups defined by local ancestry at gene's transcriptional start site (TSS), comparing subjects with 100% (AFR/AFR) to 50% (AFR/EUR) local African ancestry. **g, h** | Similar to e and f but for 100% (IAM/IAM) to 50% (IAM/EUR) local Indigenous ancestry at the TSS. Two-sided p-values are based on the Wilcoxon rank sum test.

Figure 3. Identification and examples of ancestry-specific eQTLs.

Figure 3. Identification of ancestry-specific eQTLs (anc-eQTL). **a** | Decision tree for the identification of anc-eQTLs. The lead eQTL signal of each heritable gene, represented by the 95% credible set, was identified by CAVIAR assuming 1 causal variant in each global ancestry high and low group separately (AFR_{high} , AFR_{low} , IAM_{high} and IAM_{low}). Sample size and global ancestry cutoff criteria used to classify individuals into each group are shown in the grey box at the top left corner. In genes with non-overlapping eQTL lead signal (left branch), Tier 1 anc-eQTLs were identified based on differences in allele frequency between groups ($MAF \geq 0.01$ in high and < 0.01 in low). Tier 2 anc-eQTLs were identified with the consideration of different effect size and LD pattern. To be considered Tier 2 ancestry-specific, all credible eQTLs in the high group were required to show the highest posterior probability to be causal specific to the high group (PP_{high}) and at least one of these eQTLs have to attain $PP_{high} > 0.8$. In genes with overlapping credible sets (right branch), Tier 3 anc-eQTLs were identified based on different effect size assessed by Cochran's Q. All credible eQTLs in the high group were required to have a Cochran's Q p-value smaller than the bonferroni threshold. Number of genes remaining for each ancestry (AFR and IAM) after each filtering step is indicated alongside each branch and in Table S6. The procedures are described in detail in the method section. **b** | An example of a Tier 1 AFR_{high} -specific eQTL (rs1641047) of the *ABAT* gene that was reported in GWAS catalog to be associated with systemic sclerosis. The minor allele, T allele, was associated with systemic sclerosis and was common ($MAF \geq 0.01$) in African Americans but rare in Puerto Ricans and Mexican Americans. **c** | An example of Tier 3 AFR_{high} -specific eQTLs (rs34247110 and rs3734618) of the *KCNK17* gene. Both eQTLs were shared between the 95% credible set (CAVIAR) of AFR_{high} and AFR_{low} groups. The eQTL, rs34247110, was reported in GWAS catalog by two different

studies to be associated with type 2 diabetes in European, African American, Hispanic and Asian populations. The detection of rs34247110 from multiple populations was consistent with the nature of overlapping lead eQTL signals for Tier 3 anc-eQTLs. Black and open white points and their whiskers indicate eQTL effect size and their 95% confidence interval (CI) in the AFR_{high} and AFR_{low} group, respectively. The two eQTLs show non-overlapping 95% CI of effect sizes between the AFR_{high} and AFR_{low} groups, supporting their identity as Tier 3 anc-eQTLs, which have significantly different effect size between the two groups (Cochran's Q $p < bonferroni$ threshold). Diamond and rectangle indicate effect size and their 95% confidence interval from meta-analysis using METASOFT. **d** | An example of a Tier 2 IAM_{high} -specific, colorectal cancer-associated eQTL (rs11064437) of the *SPSB2* gene. The top 2 panels on the left show non-overlapping 95% credible set (CAVIAR) between the IAM_{high} (black outline triangle) and IAM_{low} (grey outline triangle). The fill color of the triangle indicates LD of an eQTL with the eQTL marked by the vertical dotted line. The CAVIAR 95% credible eQTLs in IAM_{high} and IAM_{low} were in different LD blocks, as indicated by low LD represented by the fill of the triangle and LD plots on the right. LD plots showed eQTLs in the same order as the top two panels on the left. The bottom three panels on the left show the left eQTL had the highest posterior probability (PESCA) of being IAM -specific while the right eQTL had highest posterior probability of being shared between two groups. In the PESCA analysis, eQTLs were pruned by R^2 of 0.95 to remove eQTLs that have almost perfect LD with each other.

Figure 4. Comparison of transcriptome imputation model performance and transcriptome-wide association study (TWAS) results.

Figure 4. Comparison of transcriptome imputation model performance and transcriptome-wide association study (TWAS) results. **a,b** | Internal cross-validation R² values for each TWAS model restricting to the subset of overlapping genes. GALA/SAGE models were trained by pooled (a) or AA (b) samples. **c** | Summary of TWAS results across 28 traits from the PAGE GWAS meta-analysis. Venn diagrams depict the number of genes analyzed in each TWAS and the number significant (FDR<0.05) findings across all traits. **d,e** | Correlation in z-scores for the union of genes that were statistically significant in at least one TWAS model. Correlation between GALA/SAGE pooled models with GTEx (d) or MESA (e).

Figure 5. Transcriptome-wide association study (TWAS) results.

Figure 5. Transcriptome-wide association study (TWAS) results for **a** | HDL cholesterol using summary statistics from the PAGE multi-ancestry GWAS meta-analysis (N=33,063) and **b** | neutrophil counts from a GWAS meta-analysis of African ancestry individuals (N=13,476). Genes that achieved TWAS significance ($FDR < 0.05$) are indicated by circles with a black border and are labeled, except for chromosome 1 for neutrophil counts due to the large number of associations. Significantly associated genes for which expression levels could not be predicted using GTEx v8 elastic net models are indicated in red.

Table 1. Demographic characteristics of GALA II and SAGE participants included in this study.

	African American (N=757)	Puerto Rican (N=893)	Mexican (N=784)	Other Latino (N=299)	Pooled (N=2,733)
Age (years)	16.0 [6.6]	13.2 [4.8]	13.8 [6.5]	13.7 [5.7]	14.0 [6.3]
Sex					
Female	405 (53.5%)	451 (50.5%)	427 (54.5%)	158 (52.8%)	1,441 (52.7%)
Asthma status					
Case	433 (57.2%)	549 (61.5%)	351 (44.8%)	156 (52.2%)	1,489 (54.5%)
Genetic ancestry (%)					
African	82.6 [9.4]	19.7 [13.3]	3.5 [2.7]	8.3 [14.8]	17.5 [61.8]
Indigenous American	0.3 [0.9]	9.9 [3.6]	55.3 [23.2]	42.3 [43.2]	10.7 [45.2]
European	16.5 [9.5]	69.5 [13.6]	40.3 [21.9]	45.9 [20.8]	44.2 [43.8]
Recruitment center					
SF Bay Area	757 (100%)	0 (0%)	348 (44.4%)	109 (36.5%)	1,214 (44.4%)
Chicago	0 (0%)	31 (3.5%)	247 (31.5%)	52 (17.4%)	330 (12.1%)
Puerto Rico	0 (0%)	837 (93.7%)	0 (0%)	8 (2.7%)	845 (30.9%)
New York City	0 (0%)	22 (2.5%)	36 (4.6%)	86 (28.8%)	144 (5.3%)
Houston	0 (0%)	3 (0.3%)	153 (19.5%)	44 (14.7%)	200 (7.3%)

Summary statistics of quantitative variables are reported as median [interquartile range] while categorical variables were reported as count (percentage).