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*Hum Genet.* 2016 August ; 135(8): 869–880. doi:10.1007/s00439-016-1680-8.**Mapping Adipose and Muscle Tissue Expression Quantitative Trait Loci in African Americans to Identify Genes for Type 2 Diabetes and Obesity**

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## Abstract

Relative to European Americans, type 2 diabetes (T2D) is more prevalent in African Americans (AAs). Genetic variation may modulate transcript abundance in insulin-responsive tissues and contribute to risk; yet published studies identifying expression quantitative trait loci (eQTLs) in African ancestry populations are restricted to blood cells. This study aims to develop a map of genetically regulated transcripts expressed in tissues important for glucose homeostasis in AAs, critical for identifying the genetic etiology of T2D and related traits. Quantitative measures of adipose and muscle gene expression, and genotypic data were integrated in 260 non-diabetic AAs to identify expression regulatory variants. Their roles in genetic susceptibility to T2D, and related metabolic phenotypes were evaluated by mining GWAS datasets. eQTL analysis identified 1,971 and 2,078 *cis*-eGenes in adipose and muscle, respectively. *Cis*-eQTLs for 885 transcripts including top *cis*-eGenes *CHURC1*, *USMG5*, and *ERAP2*, were identified in both tissues. 62.1% of top *cis*-eSNPs were within  $\pm 50$ kb of transcription start sites and *cis*-eGenes were enriched for mitochondrial transcripts. Mining GWAS databases revealed association of *cis*-eSNPs for more

than 50 genes with T2D (e.g. *PIK3C2A*, *RBMS1*, *UFSP1*), gluco-metabolic phenotypes, (e.g. *INPP5E*, *SNX17*, *ERAP2*, *FN3KRP*), and obesity (e.g. *POMC*, *CPEB4*). Integration of GWAS meta-analysis data from AA cohorts revealed the most significant association for cis-eSNPs of *ATP5SL* and *MCCC1* genes, with T2D and BMI, respectively. This study developed the first comprehensive map of adipose and muscle tissue eQTLs in AAs (publically accessible at <https://mdsetaa.phs.wakehealth.edu>) and identified genetically-regulated transcripts for delineating genetic causes of T2D, and related metabolic phenotypes.

### Keywords

Expression Quantitative Trait (eQTL); Genotype; Transcript; Single nucleotide polymorphism (SNP); Adipose; Muscle; African American; Genomics; Diabetes; Obesity

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## INTRODUCTION

The importance of genetic factors in modulating the susceptibility to type 2 diabetes (T2D) is well established (Groop and Pociot, 2014). Relative to European Americans, T2D is twice as prevalent in African Americans (Cowie et al., 2010) and associated risk factors such as insulin resistance and obesity are more prevalent (Cowie et al., 1993). Whether genetic variation modulates molecular processes and contributes to the enhanced susceptibility to T2D in African Americans is unknown. Large-scale linkage, candidate-gene, and genome-wide association studies (GWAS), primarily in European and Asian populations, have identified approximately 88 loci associated with T2D and 83 loci associated with glucose homeostasis-related phenotypes (Mohlke and Boehnke, 2015). T2D-associated loci identified in GWAS reveal relatively weak effects, together explaining only a small fraction of the heritability in African Americans (Mahajan et al., 2014; Ng et al., 2014). Moreover, most associated variants are located in noncoding genomic regions. Thus, determining how these loci modulate systemic glucose homeostasis at the molecular level remains unclear. Approaches investigating molecular endophenotypes more proximal to gene products may assist in identifying the molecular basis of genetic susceptibility to T2D in African Americans.

Genetic variation can impact transcript abundance. We and others reported that T2D- and related trait-associated variants are enriched for expression-regulatory single nucleotide polymorphisms (eSNPs) in tissues important for glucose homeostasis (Das and Sharma, 2014; GTEx consortium, 2015; Nicolae et al., 2010). Identifying genetic variants associated with transcript expression in metabolically relevant tissues may identify functionally meaningful sets of SNPs involved in T2D, obesity, and related metabolic phenotypes. However, studies on the genetics of gene expression in populations of African ancestry are predominantly limited to lymphocytes/lymphoblastoid cell lines (Storey et al., 2007; Stranger et al., 2012; Zhang et al., 2008). The goal of this study was to identify genetic regulatory variants modulating expression of adipose and muscle transcripts in African Americans at risk for T2D and evaluate their role in susceptibility to T2D, obesity, and related metabolic disorders. A systematic analysis was performed on the genome-wide transcript expression profiles of insulin responsive tissues (subcutaneous adipose and skeletal muscle) and

genome-wide SNP genotypes in a metabolically characterized cohort of 260 non-diabetic African Americans from North Carolina. To our knowledge, this is the largest existing cohort of non-diabetic African Americans characterized for gluco-metabolic phenotypes with available biological samples (DNA and tissue) for conducting an integrative multi-omics approach. These data were used to test two hypotheses: 1) expression levels of a subset of transcripts would associate with genotype and manifest as expression quantitative trait loci (eQTL) in both adipose and muscle, while a subset of transcripts would be modulated by tissue-specific eQTLs; and 2) a subset of the expression regulatory SNPs (eSNPs) would associate with glucose homeostasis related phenotypes, obesity and/or T2D in large GWAS, identifying putative causal SNPs in African Americans.

## MATERIALS AND METHODS

### Human subjects

Participants were healthy, self-reported African Americans residing in North Carolina aged 18–60 years with a body mass index (BMI) between 18 and 42 kg/m<sup>2</sup>. A total of 260 unrelated non-diabetic individuals completed all study visits and are referred to as the “African American Genetics of Metabolism and Expression” (AAGMEx) cohort; subcutaneous adipose tissue (from the abdomen near the umbilicus) and skeletal muscle (from the *vastus lateralis*) biopsies were collected from 256 individuals. Studies were performed at the Wake Forest School of Medicine (WFSM) Clinical Research Unit. This study was approved by the WFSM Institutional Review Board and all participants provided written informed consent.

A standard 75-g oral glucose tolerance test (OGTT) was used to exclude individuals with diabetes and results were analyzed by homeostatic model assessment (HOMA; <http://mmatsuda.diabetes-smc.jp/MIndex.html>) to evaluate insulin sensitivity (Matsuda Index) and insulin resistance (HOMA-IR) (Matsuda and DeFronzo, 1999; Matthews et al., 1985). High quality insulin modified (0.03 U/kg) frequently sampled intravenous glucose tolerance test (FSIGT) data were available in 235 participants. The MINMOD Millennium program was used to analyze FSIGT data to determine insulin sensitivity ( $S_I$ ) and acute insulin response ( $AIR_G$ ) (Bergman et al., 2014). Clinical, anthropometric, and physiological characteristics of the AAGMEx cohort have been described (Sharma et al., 2016).

### Gene expression analysis and genotyping

Genome-wide expression data were generated using HumanHT-12 v4 Expression BeadChip (Illumina, San Diego, CA) whole genome gene expression arrays for quantitative analyses of transcript expression in adipose and muscle samples. Infinium HumanOmni5Exome-4 v1.1 DNA Analysis BeadChips (Illumina) were used to genotype DNA samples based on the manufacturer’s recommendations. Additional technical details of standard gene expression analyses and genotyping methods are described in Supplementary methods.

### Quality control

Detailed data quality control methods are presented in Supplementary methods. In brief, measures of glucose homeostasis and obesity were examined for outliers in a univariate

fashion and as correlated pairs. Genome-wide gene expression data (probe level) for both the adipose and muscle samples were extracted separately using Illumina GenomeStudio V2011.1. Expression level was  $\log_2$  transformed, robust multi-array average normalized (RMA, includes quantile normalization) (Irizarry et al., 2003) and batch-corrected using ComBat (Johnson et al., 2007). The HumanHT-12 v4 Expression BeadChip includes 47,231 probes annotated to transcripts. Significant expression ( $p < 0.05$ ) of 16,010 and 13,118 transcript probes was observed in adipose and muscle RNA, respectively, in 90% of participants. Data from these probes were primarily used for analysis. Probes were further filtered out based on bioinformatic criteria described in the supplementary methods. Genotype data were examined to verify sample and SNP quality. Genotype assays of 4,210,443 SNPs passed technical quality filters. The genotype of 2,296,925 autosomal SNP assays (representing 2,210,735 unique high-quality genotyped SNPs with  $MAF > 0.01$  and  $HWE-p \text{ value} > 1 \times 10^{-6}$ ) was used in eQTL analysis.

### Statistical and Bioinformatic analyses

To identify expression quantitative trait loci (eQTLs), linear regression was computed with the  $\log_2$  transformed expression values as the outcome and an additive genetic model for the SNP as implemented in the R-package MatrixEQTL (Shabalin, 2012); age, gender, and African ancestry proportion were covariates. Analyses scanned for both *cis* and *trans* eQTLs, but partitioned the overall type 1 error rate of  $\alpha = 0.05$  into  $\alpha = 0.04$  for *cis* and  $\alpha = 0.01$  for *trans*. However, we considered as significant any *cis*- and *trans*-eSNPs with a false discovery rate (FDR)-corrected p-value (Q-value)  $< 0.01$  (or 1.0%). Detailed statistical and bioinformatic data analysis methods are presented in Supplementary methods. Sample sizes in each analysis (Supplementary Table 1) varied based on available data after quality control.

### Replication of eQTL data

Adipose *cis*-eQTL data from the Multiple Tissue Human Expression Resource (MuTHER) project (Grundberg et al., 2012) and muscle *cis*-eQTL data reported by Keildson *et al.* (Keildson et al., 2014) were mined to replicate *cis*-eGenes identified in the AAGMEx cohort (Supplementary Methods). Additionally, replication of adipose and muscle *cis*-eGenes was tested in publically available tissue eQTL data from GTEx project (GTEx\_Analysis\_v6 updated) and lymphoblastoid cell line (LCL) eQTL data from both the Geuvadis RNA sequencing project and the SeeQTL data depository.

### Integration of GWAS data

*Cis*-eSNPs identified in adipose and muscle of African Americans represented a prioritized set of SNPs providing statistical evidence for genotype-dependent variation in transcript abundance. The NHGRI Catalog of Published GWAS (Hindorff et al., 2009) and meta-analysis data from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) (Scott et al., 2012) were mined to identify the role of putatively functional SNPs in T2D susceptibility and gluco-metabolic phenotypes. We also searched for associations of eSNPs with T2D and BMI in the Meta-analysis of Type 2 Diabetes in African Americans (MEDIA) Consortium (Ng et al., 2014) and African Ancestry Anthropometry Genetic Consortium (AAAGC) (Monda et al., 2013) GWAS (Supplementary Methods).

## RESULTS

### eQTLs identified in adipose and muscle

We identified 1971 and 2078 transcripts with at least one significant *cis*-eQTL (top eSNP within  $\pm 500$ kb around the expressed transcript at FDR  $< 0.01$ ) in adipose and muscle, respectively (Figure 1A, 1B, Supplementary Tables 2 and 3). These transcripts were considered *cis*-eGenes.

Overlap between *cis*-eGenes was identified in adipose and muscle. *Cis*-eQTLs for 885 transcripts were identified in both insulin-responsive tissues (Figure 1B). Among these, 317 were most strongly associated with the same *cis*-eSNPs in both tissues and showed the same direction of effect (Figure 1C and Supplementary Table 4). The most significant *cis*-eGenes (FDR  $< 1 \times 10^{-100}$ ) observed in both tissues included churchill domain containing 1 (*CHURC1*), up-regulated during skeletal muscle growth-homolog 5 (*USMG5*), and endoplasmic reticulum aminopeptidase 2 (*ERAP2*) (Table 1).

SNP–transcript expression–level associations for variants located on other chromosomes or outside the defined *cis* boundary ( $\pm 500$ kb around the transcript) were examined to identify *trans*-regulatory variants. Associations were identified (FDR  $< 0.01$ ) for expression of 603 and 943 transcripts with a genotype of at least one *trans*-eSNP in adipose and muscle, respectively (data not shown). Considering the large number of tests performed to identify *trans*-eQTLs, we conservatively considered a transcript associated with  $> 1$  *trans*-eSNP as statistically significant. Using this conservative criterion, 322 and 591 *trans*-eGenes were identified in adipose and muscle, respectively. Overlap of these *trans*-eGenes with *cis*-eGenes is shown in Figure 1B. Summary statistics of all *cis*- and *trans*-eSNPs is publically accessible through a searchable database at <https://mdsetaa.phs.wakehealth.edu>.

### Genomic distribution of *cis*-eSNPs

The distribution of the top *cis*-eSNPs for each transcript was assessed in relation to gene proximity and plotted against the distribution of distances between *cis*-eSNPs with the lowest p-values and transcription start site (TSS). As reported (Stranger et al., 2005; Stranger et al., 2007; Veyrieras et al., 2008), the majority of top *cis*-eSNPs in adipose (62.6%) and muscle (61.7%) were located within  $\pm 50$ kb of the TSS (Figure 2A and 2B). For adipose, 80% and 95% of the top *cis*-eSNPs were within 118.6 kb and 374.4 kb, respectively, of the TSS. For muscle, these distances were 122.8 kb and 371.4 kb, respectively. *Cis*-eSNPs with larger effect sizes were also overrepresented close to TSS (Figure 2C and 2D). Greater than 80% (80.8% in adipose; 84% in muscle) of the highly significant *cis*-eSNPs (P-value  $< 1 \times 10^{-10}$ ) were located within  $\pm 100$  kb of the TSS. To assess potential functional significance, we annotated the genomic locations (based on the Sequence Ontology definitions) of the top *cis*-eSNPs of associated transcripts (FDR  $< 0.01$ ). Interestingly, 79.6% of the top *cis*-eSNPs were located within or near gene regions ( $\pm 5$  kb), and only 20.4% were intergenic. Most of the top *cis*-eSNPs were intronic (43.1%) or in the 3' or 5' untranslated regions (UTR, 16.3%, Figure 3). Utilizing the TRANSFAC Database, SNPnexus (Dayem Ullah et al., 2012) annotation predicted the disruption of transcription factor binding sites by 80 and 77 top *cis*-eSNPs in adipose and muscle, respectively.

### ***In silico* analyses indicate functional significance of *cis*-eGenes**

Gene-annotation enrichment analysis with DAVID (Huang et al., 2009) indicated an enrichment of mitochondrial genes (GO:0005739) among *cis*-eGenes in both adipose ( $p=4.67\times 10^{-11}$ , 141 transcripts) and muscle ( $p=4.84\times 10^{-30}$ , 235 transcripts). Although not strongly enriched, *cis*-eGenes in both adipose (34 genes,  $p=0.03$ , FDR=26.5%) and muscle (47 genes,  $p=0.0079$ , FDR= 7.8%) included genes involved in diabetes based on Reactome pathway annotation (Reactome pathway, REACT\_15380: Diabetes pathways). IPA predicted HNF4A (a transcription factor) as the most common upstream regulatory factor for adipose ( $p$ -value of overlap= $2.23\times 10^{-15}$ , 254 transcripts) and muscle ( $p=3.82\times 10^{-22}$ , 288 genes) *cis*-eGenes identified. Expression of a subset of *cis*-eGenes (362 in adipose; 42 in muscle) was associated with  $S_1$  in this non-diabetic African American cohort (Sharma et al., 2016).

### **Replication of *cis*-eQTLs using publically available data**

Published studies for eQTLs in insulin-responsive tissues in African Americans are not available. Replication of eQTLs was assessed by mining published adipose and muscle eQTLs in Caucasians (populations of European ancestry). Adipose eQTL data from MuTHER (Grundberg et al., 2012) were assessed for replication of 190 top *cis*-eGenes identified in AAGMEx (included probes for 100 top *cis*-eGenes and 100 top  $S_1$ -associated *cis*-eGenes). Information for 155 of the 190 selected probes was available in MuTHER. Comparison of data sets showed an association of 114 *cis*-eGenes (probes) with the same *cis*-eSNPs in both studies (Supplementary Table 5), supporting replication of 73.5% of top adipose *cis*-eQTLs in our AAGMEx cohort.

Muscle *cis*-eQTLs as reported by Keildson *et al.* (Keildson et al., 2014), were searched to assess replication of these *cis*-eGenes ( $n=287$ ) in the AAGMEx cohort, considering all probes representing significant (up to FDR <0.04) *cis*-eGenes. Comparison of the two muscle-eQTL data sets showed an association of 144 *cis*-eGenes (represented by 191 probes, Supplementary Table 6). Despite the small sample and different design of the previous study by Keildson *et al.*, 50.2% of their muscle-eGenes were replicated in our AAGMEx African American cohort. Despite different methods of transcript quantification (RNA-seq) and sample characteristics ( $N=298$  and 361 cadaver donors for subcutaneous adipose and skeletal muscle, respectively), results from GTEx (GTEx\_Analysis\_v6 updated: 2015-06-18, dbGaP Accession phs000424.v6.p1) (GTEx consortium, 2015) supported replication of 965 adipose *cis*-eGenes and 1016 muscle *cis*-eGenes from AAGMEx (data not shown).

To determine the replication of adipose and muscle tissue *cis*-eGenes in surrogate tissues, publically available eQTL data for transformed lymphoblastoid cell lines (LCL) were searched. The LCL-eQTL data from a small African ancestry cohort (Geuvaris RNA sequencing project of 1000 Genomes YRI samples,  $N= 89$ ; <http://www.geuvaris.org/web/geuvaris/RNAseq-project>), replicated 96 and 95 adipose and muscle tissue-identified *cis*-eGenes, respectively. However, replication was much higher when larger LCL-eQTL datasets, e.g. HapMap3 consensus *cis*-eQTL (SeeQTL; [http://www.bios.unc.edu/research/genomic\\_software/seeQTL](http://www.bios.unc.edu/research/genomic_software/seeQTL)) were searched for comparison. Considering *cis*-eGenes at  $q<0.01$  from SeeQTL data, ~24% of AAGMEx adipose and muscle *cis*-eGenes (448 in

adipose; 443 in muscle) were replicated in LCLs. Thus, expression of a subset of transcripts is genetically regulated across tissues, and the eQTL data from LCLs may be used as a proxy for identifying a small subset of adipose and muscle tissue *cis*-eGenes.

### Association of *cis*-eSNPs with T2D, obesity and related metabolic phenotypes

The NHGRI Catalog of Published GWAS (from UCSC table browser) was mined (Hindorff et al., 2009) for association of all significant *cis*-eSNPs (FDR<0.01) for adipose and muscle transcripts to identify potential roles in T2D susceptibility and related phenotypes. This catalog only includes phenotype-associated index SNPs ( $p$ -values  $<1.0 \times 10^{-5}$ ), mostly from people not of recent African descent. Association of *cis*-eSNPs was detected for >50 genes with gluco-metabolic phenotypes including T2D (e.g. *PIK3C2A*, *RBMS1*, *UFSP1*, *ACHE*), fasting plasma glucose (e.g. *NOSTRIN*, *RREB1*), hemoglobin A1c (e.g. *FN3KRP*), and BMI and obesity-related traits (e.g. *POMC*, *MARCH6*, *NINJI*, *RBPI*, *HMBOX1*, *CHURC1*, *CPEB4*; Supplementary Table 7).

GWAS data from Caucasians in the MAGIC cohort (Scott et al., 2012) were assessed for glucose homeostasis phenotypes; Supplementary Table 8 lists *cis*-eSNPs for 54 adipose and 63 muscle genes with evidence for association ( $p < 0.01$ ) with glucose homeostasis phenotypes. An eSNP (both adipose and muscle) for inositol polyphosphate-5-phosphatase (*INPP5E*) was strongly associated with fasting glucose (rs1128905,  $p = 5.81 \times 10^{-9}$ ), and an eSNP for *ERAP2* was strongly associated with 2h-glucose (rs1019503,  $p = 8.97 \times 10^{-9}$ ). Interestingly, rs560887, located in the intron of the glucose-6-phosphatase catalytic subunit 2 gene (*G6PC2*) was strongly associated with fasting glucose ( $p = 1.4 \times 10^{-178}$ ) in Caucasians in the MAGIC cohort, as was a *cis*-eSNP for *NOSTRIN* (nitric oxide synthase trafficker gene) in adipose of people in the AAGMEx cohort. Muscle eSNPs rs2068834 (sorting nexin 17 gene, *SNX17*;  $p = 9.78 \times 10^{-20}$ ) and rs11715915 (macrophage stimulating 1 gene, *MST1*;  $p = 4.90 \times 10^{-8}$ ) associated with fasting glucose; eSNP rs6912327 (UHRF1 binding protein 1 gene, *UHRF1BP1*) associated with BMI-adjusted fasting insulin ( $p = 2.26 \times 10^{-8}$ ).

A search for association of eSNPs (FDR <0.01) with T2D and BMI was performed in GWAS from MEDIA (Ng et al., 2014) and AAAGC (Monda et al., 2013). *Cis*-eSNPs for 72 genes in adipose and 80 genes in muscle showed nominal evidence ( $p < 0.01$ ) of association with T2D in African Americans in the MEDIA cohort (Supplementary Table 9). Three *cis*-eSNPs show stronger association ( $p < 1.0 \times 10^{-4}$ ) with T2D (Table-2A). A *cis*-eSNP for the transcript of ATP synthase subunit s-like protein (*ATP5SL*) gene was most significantly associated with T2D (rs7259208,  $p = 1.20 \times 10^{-5}$ ). *Cis*-eSNPs for 65 genes in adipose and 91 genes in muscle showed nominal evidence of association ( $p < 0.01$ ) with BMI in African Americans in the AAAGC cohort (Supplementary Table 10). Four *cis*-eSNPs show stronger association ( $p < 1.0 \times 10^{-4}$ ) with BMI (Table-2B). Among the selected subset of *cis*-eSNPs, rs4074110 (methylcrotonoyl-CoA carboxylase 1, *MCCCI*) showed the most significant association with BMI ( $p = 6.11 \times 10^{-6}$ ) in meta-analyses from AAAGC data. Thus, *cis*-eSNPs may modulate the risk for T2D and obesity in African Americans.

Integration of AAGMEx eQTL results and GWAS of gluco-metabolic traits suggested putative target genes for GWAS-identified SNPs. A total of 216 and 249 target *cis*-eGenes in adipose and muscle, respectively, were identified. Among these target *cis*-eGenes, mRNA



expression of 55 genes in adipose, and 20 genes in muscle were significantly associated ( $p < 0.001$ ) with the glucose homeostasis traits ( $S_I$  and  $AIR_G$  derived from FSIGT; HOMA-IR and Matsuda index derived from OGTT), or obesity (BMI) phenotypes of AAGMEx participants (Supplementary Table 11).

## DISCUSSION

Despite successes in GWAS, the majority of loci accounting for T2D heritability remain unknown and the diversity of its pathophysiology, molecular mechanisms, and variants explaining enhanced susceptibility in African Americans are poorly understood. The present study combined gene expression in tissues important to insulin action, and genome-wide genotype data in African Americans to fill these gaps. Results provide a comprehensive map of genetically regulated transcripts in African Americans, which is critical for prioritizing GWAS-identified SNPs in replication studies and detecting functional roles of variants involved in T2D and related traits.

Integration of genome-wide expression and genotype data enabled mapping of loci involved in the regulation of gene expression. Association of SNPs with transcript levels of nearby (*cis*) or distal (*trans*) genes were identified. Compared to adipose, slightly more *cis*-eQTL-transcripts (*cis*-eGenes) were found in muscle. Overlap of 885 *cis*-eQTL transcripts (~45% of *cis*-eGenes) was seen in both tissues indicating tissue-independent expression regulatory elements. Significant *cis*-eGenes observed in both tissues included *USMG5* and *ERAP2*. The *USMG5* gene, also known as the diabetes-associated protein in insulin-sensitive tissue gene (*DAPIT*), is differentially modulated in insulin-responsive tissues of streptozotocin-treated diabetic rats (Kontro et al., 2012; Paivarinne and Kainulainen, 2001). *ERAP2* is involved in maturation of many proteins in the endoplasmic reticulum (ER), and has been implicated in regulation of angiogenesis and blood pressure (Cifaldi et al., 2012). An eSNP for *ERAP2* was strongly associated with 2h-glucose in the MAGIC cohort. Consistent with published eQTL studies (Stranger et al., 2005; Stranger et al., 2007; Veyrieras et al., 2008), top *cis*-eSNPs for 60% of transcripts in both tissues were within  $\pm 50$  kb of the TSS. The genomic distribution of *cis*-eSNPs fits with existing knowledge on the genetic regulatory architecture of transcript expression. Further bioinformatic annotation of these loci indicated the disruption of transcription factor binding by eSNPs and provided evidence for regulatory motifs. Thus, the identified eQTLs support the concept that functional regulatory genomic regions exist in glucose homeostasis-regulating tissues. Many *cis*-eQTLs identified in this African American cohort were replicated in non-African cohorts. Thus, a subset of genetic regulatory mechanisms of transcript expression is common between African Americans and non-Africans. Further studies will be required to confirm, whether other subsets of genetic regulatory mechanisms of transcript expression predominately influence particular ancestral groups.

Enrichment (DAVID analysis) of mitochondrial genes was identified among adipose and muscle *cis*-eGenes, indicating a role for genetic factors in modulation of this pathway. IPA revealed enrichment of pathways involved in mitigating oxidative stress (including glutathione-mediated detoxification,  $p = 8.32 \times 10^{-3}$ – $3.72 \times 10^{-5}$ ; NRF2-mediated oxidative

stress response,  $p=0.02-4.47\times 10^{-4}$ ) among *cis*-eGenes. These biological pathways may play key roles in modulating insulin sensitivity in African Americans.

Compared to *cis*-eQTLs, the effect sizes of *trans*-eQTLs are generally small, requiring larger sample sizes for robust detection of *trans*-eQTLs (Grundberg et al., 2012). A recent eQTL analysis in adipose tissue from Caucasian female twins (MuTHER Project, N=856) identified 3,529 *cis*-eQTLs (at FDR 1%) and 639 *trans*-eQTLs (at FDR 10%) (Grundberg et al., 2012). A stringent threshold (FDR<1%, with corresponding uncorrected p-values  $<2.6\times 10^{-9}$  in adipose tissue) was used to account for the large number of tests performed for *trans*-eQTL analysis in the AAGMEx cohort, and it identified 322 and 591 *trans*-eGenes in adipose and muscle, respectively. Thus, the number of *trans*-eGenes identified in AAGMEx is consistent with expectations and comparable to published studies on adipose and other tissues.

Mining of the NHGRI catalogue of GWAS (Hindorff et al., 2009) and MAGIC GWAS meta-analysis (Scott et al., 2012) results revealed association of *cis*-eSNPs in this study with T2D and related phenotypes. Although these SNP-disease association results are primarily from cohorts of individuals not of African descent, integration of eQTL data from our African American participants suggests molecular mechanisms that are putatively regulated by these SNPs and sequentially modulating disease susceptibility.

*Cis*-eSNPs for many adipose and muscle transcripts showed association with T2D and BMI in MEDIA (Ng et al., 2014) and AAAGC (Monda et al., 2013) African Americans, supporting roles for these transcripts in T2D. Genes modulated by disease-associated *cis*-eSNPs (e.g., *CD36*, *CAMK2A*, *IRS2*, *POMC*, *TLR4*, *XBPI*) are involved in the pathophysiology of T2D, obesity and related traits; whereas the roles of other *cis*-eGenes (e.g. *ADAL*, *ATP5SL*, *MCCCI*) are unknown. Interestingly, among the target *cis*-eGenes for these GWAS-identified SNPs, mRNA expression of 55 genes in adipose, and 20 genes in muscle was significantly associated with glucose homeostasis or obesity phenotypes in AAGMEx African Americans. This observation suggests a putative role for these GWAS-identified SNPs and respective *cis*-eGenes in the pathophysiology of T2D and related metabolic diseases. Association summary statistics were available from the MEDIA and AAAGC cohorts for directly genotyped SNPs and HapMap reference panel imputed SNPs. Association results for a subset of *cis*-eSNPs or their proxies (those that were not among the HapMap SNPs) were not available from these GWAS. Thus, the role of *cis*-eSNPs of several transcripts identified in this study cannot be evaluated in the MEDIA and AAAGC cohorts.

Target *cis*-eGenes of T2D-associated SNPs from MEDIA African Americans overlapped with target *cis*-eGenes of glucose homeostasis trait-associated SNPs from MAGIC Caucasians. *Cis*-eSNPs for 18 genes (*ACAD10*, *CUL3*, *G3BP2*, *GINI1*, *HLA-DPA1*, *HLA-DPB1*, *HSPA1B*, *KCTD10*, *NOTCH4*, *PFDN1*, *PPM1M*, *RNF41*, *SNX17*, *ST7L*, *STARD10*, *TIPARP*, *TMEM116*, and *WDR6*) were associated with T2D in MEDIA African Americans and were also associated with glucose homeostasis phenotypes (e.g., fasting glucose, 2h-OGTT glucose and fasting insulin) in Caucasians from MAGIC. Similarly, target *cis*-eGenes of BMI-associated SNPs from AAAGC African Americans overlapped with target *cis*-eGenes of glucose homeostasis trait-associated SNPs from MAGIC

Caucasians. *Cis*-eSNPs for 12 genes (*APIP*, *ASAP3*, *BCS1L*, *GIN1*, *HSPA1B*, *PPP1CB*, *RABEP1*, *RPP40*, *SNX17*, *TMEM60*, *TOM1*, and *UHRF1BPI*) were associated with BMI in AAAGC African Americans and were also associated with glucose homeostasis phenotypes in MAGIC Caucasians. Thus, regulatory SNP-mediated modulation of the transcript expression of some target genes may modulate susceptibility to T2D and related gluco-metabolic phenotypes in individuals with either African or European ancestry.

In conclusion, this study identified genetic loci influencing the expression of several genes in adipose and muscle of African Americans. Additionally, this study provides data on molecular mechanisms putatively regulated by eSNPs and sequentially modulating susceptibility for T2D and related metabolic phenotypes in African Americans.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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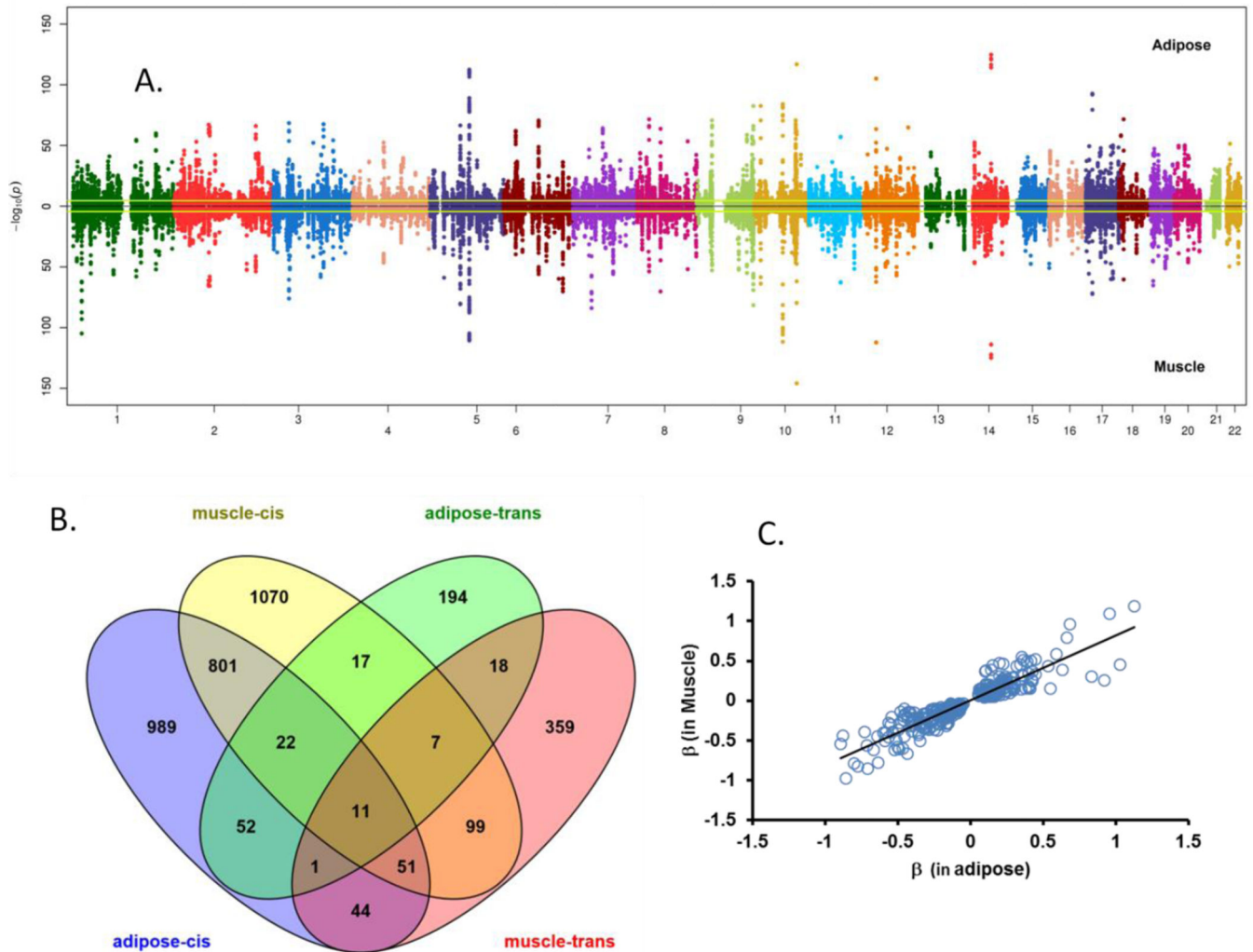
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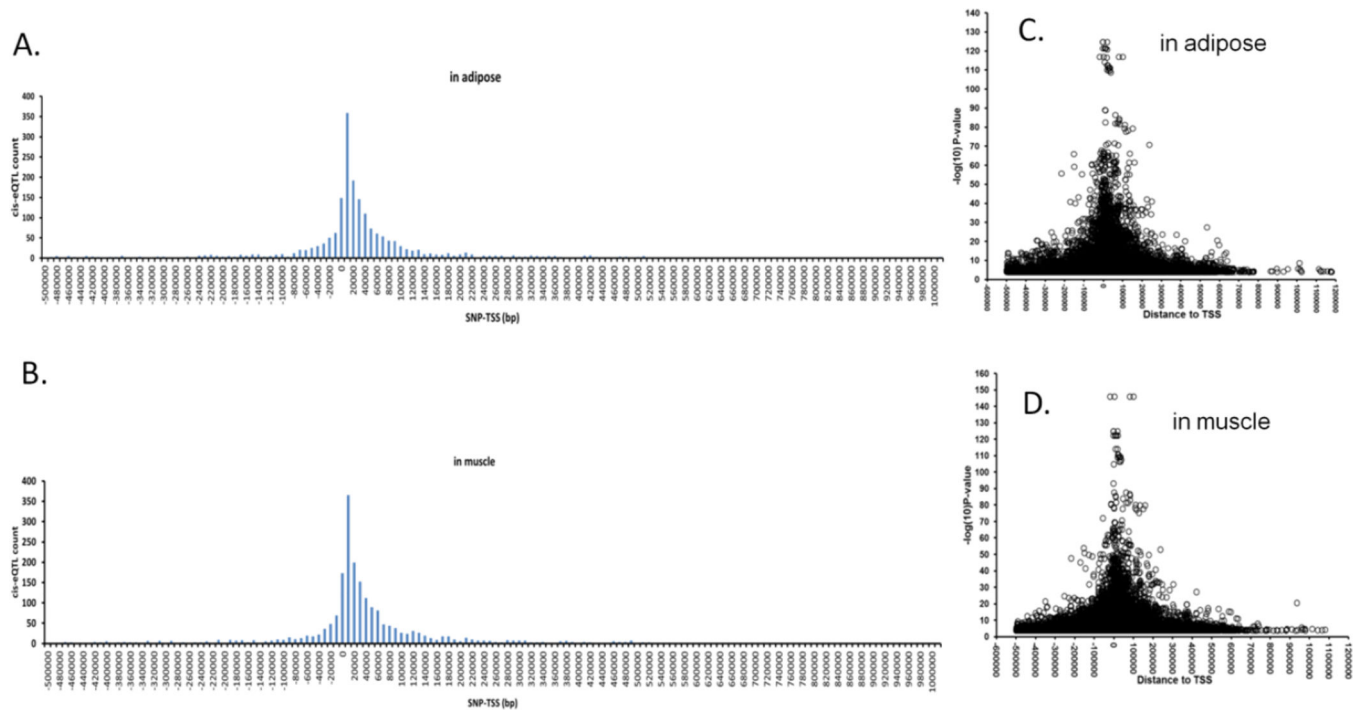
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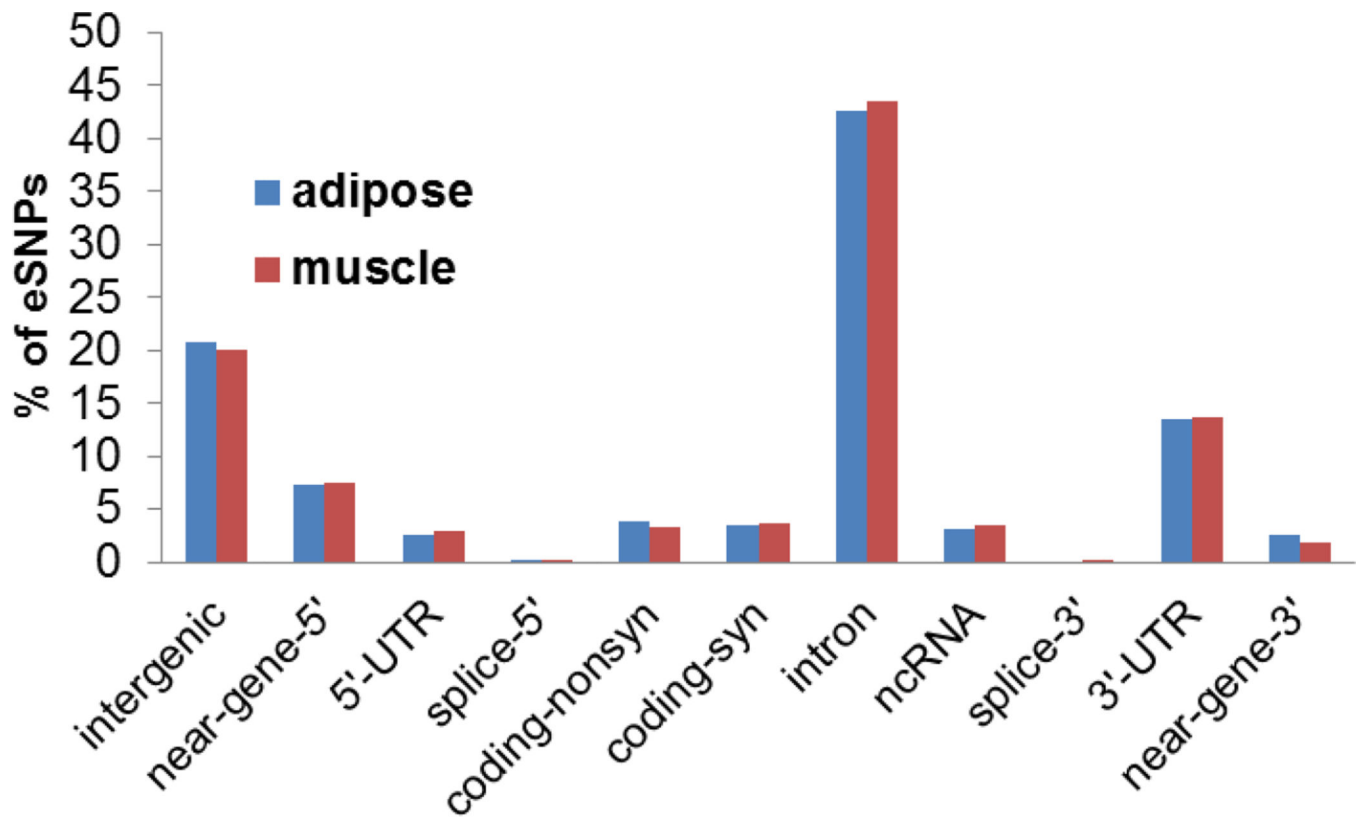


**Figure 1. Expression quantitative trait locus (eQTL) analysis identified regulatory polymorphisms for adipose and muscle tissue transcripts in African Americans**  
Opposing Manhattan plot showing chromosomal distribution of  $-\log_{10}(p)$ -values for association of all *cis*-SNPs ( $\pm 500$  kb of the transcript start and end) tested for transcripts (all probes representing refSeq genes) expressed in adipose and muscle. Significance threshold (Q-value  $< 0.01$ ) is marked by fluorescent yellow color lines. (A). A Venn diagram (B) shows common and tissue specific *cis*- and *trans*-eGenes (FDR $< 0.01$  and selected clean probes representing known genes) in adipose and muscle. Top *cis*-eSNPs for 317 transcripts showed the same direction of effect in both tissues (C)



**Figure 2. Physical location of cis-eSNPs with respect to transcription start sites of each gene** Distance distribution of each transcript's most strongly associated *cis*-eSNPs (FDR<0.01) to its TSS in adipose (**A**) and muscle (**B**). Each bar represents the count of top *cis*-eSNPs in 10kb bins. Plots **C** and **D** show distribution of significance level ( $-\log_{10}$  p-values) of all genotyped *cis*-eSNPs (FDR<0.01) relative to TSS in adipose and muscle.





**Figure 3. Genomic distribution and functional annotation of *cis*-eSNPs**

Bar graph shows functional annotation of each adipose and muscle tissue transcript's most strongly associated *cis*-eSNPs (FDR<0.01) in the genome.

Table 1

Top 20 *cis*-eQTLs common in adipose and muscle tissue of African Americans

<i>cis</i> -eSNP	Chr	A1	MAF	Adipose			Muscle			Probe ID	Entrez Gene ID	Symbol
				$\beta$	P-value	Q-value	$\beta$	P-value	Q-value			
rs7143432	14	C	0.391	0.96	1.97E-125	7.97E-119	1.10	1.58E-125	2.23E-119	ILMN_1798177	91612	<i>CHURCI</i>
rs11191642	10	G	0.091	1.13	1.59E-117	1.61E-111	1.18	1.36E-146	3.36E-140	ILMN_1773313	84833	<i>USMG5</i>
rs74345571	5	G	0.440	1.03	4.60E-113	3.49E-107	0.45	2.52E-111	1.18E-105	ILMN_1743145	64167	<i>ERAP2</i>
rs1055340	10	A	0.251	-0.41	2.91E-83	8.03E-78	-0.32	1.58E-64	1.44E-59	ILMN_1795336	9317	<i>PTER</i>
rs8413	9	G	0.365	-0.40	3.42E-83	9.02E-78	-0.28	2.75E-82	4.74E-77	ILMN_1811301	56623	<i>INPP5E</i>
rs200198999	18	G	0.191	-0.60	3.01E-72	5.63E-67	-0.40	4.18E-61	3.24E-56	ILMN_1776515	65258	<i>MPPEI</i>
rs2137471	8	G	0.396	-0.40	3.45E-72	6.34E-67	-0.23	1.79E-54	9.46E-50	ILMN_1720059	79618	<i>HMBBOX1</i>
rs59347416	9	T	0.153	-0.54	2.44E-71	4.28E-66	-0.21	1.55E-53	7.87E-49	ILMN_1744980	84186	<i>ZCCHC7</i>
rs2232745	2	T	0.457	-0.34	8.32E-68	1.23E-62	-0.29	2.54E-66	2.63E-61	ILMN_1655340	51255	<i>RNF181</i>
rs6873912	5	A	0.271	0.39	3.47E-67	5.02E-62	0.49	5.95E-81	9.44E-76	ILMN_2103295	10412	<i>TINP1</i>
rs7021977	9	A	0.327	0.43	3.66E-67	5.23E-62	0.37	2.42E-49	9.58E-45	ILMN_1808661	401505	<i>TOMM5</i>
rs28405687	2	T	0.375	0.40	1.11E-66	1.50E-61	0.34	1.73E-54	9.22E-50	ILMN_1770020	53938	<i>PPIL3</i>
rs6663	12	T	0.165	0.55	1.75E-65	2.13E-60	0.15	1.18E-39	2.66E-35	ILMN_1719064	83892	<i>KCTD10</i>
rs6873912	5	A	0.271	0.42	2.41E-57	1.80E-52	0.50	2.02E-81	3.26E-76	ILMN_1694259	10412	<i>NSA2</i>
rs3796683	4	T	0.366	-0.58	3.31E-53	2.09E-48	-0.40	2.36E-47	8.22E-43	ILMN_1656560	25849	<i>PARMI</i>
rs73992309	17	G	0.064	-0.47	9.31E-50	5.00E-45	-0.61	8.54E-61	6.46E-56	ILMN_1685112	51204	<i>TACO1</i>
rs73366229	17	C	0.270	0.38	4.57E-49	2.36E-44	0.15	7.24E-21	3.47E-17	ILMN_1720708	1453	<i>CSNK1D</i>
rs17856037	14	T	0.178	-0.73	1.37E-44	5.73E-40	-0.39	1.11E-30	1.33E-26	ILMN_3243744	55837	<i>EAPP</i>
rs3211938	7	G	0.100	-0.86	9.59E-44	3.78E-39	-0.98	2.37E-35	4.01E-31	ILMN_1796094	948	<i>CD36</i>
rs2908700	12	C	0.415	0.40	2.72E-43	1.04E-38	0.18	1.73E-23	1.11E-19	ILMN_1784608	9976	<i>CLEC2B</i>

Shown are the *cis*-eSNPs (genotyped SNP within  $\pm 500$ kb of the 5' and 3' end of the transcript) that are most strongly associated (Q-value < 0.01) and in the same direction for the same transcripts. Chr, Chromosome; A1, Minor Allele; MAF, Minor Allele Frequency;  $\beta$ , effect size of minor allele (A1); P-value, significance in additive model (MatrixEQTL analysis); Q-value, false discovery rate. Results for all 317 *cis*-eGenes associated with same *cis*-eSNPs and showing the same allele direction of effect in both tissues are provided in Supplementary Table 4.

*cis*-eSNPs for adipose and muscle tissue transcripts associated with T2D and BMI in GWAS meta-analysis in African American Subjects

**Table 2**

A) eSNPs (FDR 1%) from the AAGMEx cohort are associated with T2D (p 0.0001) in the "Meta-analysis of Type 2 Diabetes in African Americans" Consortium cohorts.

SNP	SNP Chr	SNP Pos	AI	MAF	Illumina probe_ID	Tissue	$\beta$	P Value <sup>a</sup>	Symbol	Entrez Gene_ID	Allele1	Freq1	OR (95%CI)	P Value <sup>b</sup>	N
rs1594	2	202025621	C	0.443	ILMN_1770020	Ad	-0.166	5.90E-09	<i>PP1L3</i>	53938	A	0.527	0.912 (0.873–0.953)	4.17E-05	23737
					ILMN_1789830	Ad	0.119	5.07E-07	<i>CFLAR</i>	8837					
					ILMN_1770020	Mu	-0.114	1.01E-05	<i>PP1L3</i>	53938					
					ILMN_1789830	Mu	0.086	2.45E-05	<i>CFLAR</i>	8837					
rs2930532	15	44133890	C	0.191	ILMN_1725043	Ad	-0.068	1.08E-05	<i>ADAL</i>	161823	T	0.813	1.137 (1.069–1.210)	4.89E-05	21261
					ILMN_1725043	Mu	-0.065	1.32E-07							
rs7259208	19	41894678	A	0.094	ILMN_1809027	Ad	0.163	5.64E-09	<i>ATP5SL</i>	55101	A	0.096	1.184 (1.098–1.277)	1.20E-05	22755
					ILMN_1809027	Mu	0.156	1.18E-06							

B) eSNPs (FDR 1%) from the AAGMEx cohort are associated with BMI (p 0.0001) in the "African Ancestry Anthropometry Genetic Consortium" cohorts

SNP	SNP Chr	SNP Pos	AI	MAF	Illumina probe_ID	Tissue	$\beta$	P Value <sup>a</sup>	Symbol	Entrez Gene_ID	Allele1	Freq1	Effect (SE)	P Value <sup>b</sup>	N
rs4074110	3	182728669	C	0.140	ILMN_1760174	Mu	0.109	6.65E-08	<i>MCC1</i>	56922	A	0.830	-0.051 (0.011)	6.11E-06	37033
rs6005881	22	29183133	A	0.336	ILMN_1809433	Mu	0.082	2.60E-06	<i>XBP1</i>	7494	A	0.351	-0.034 (0.008)	3.62E-05	38497
rs7018469	9	114195274	C	0.140	ILMN_1704531	Ad	0.429	2.81E-18	<i>PTGR1</i>	22949	T	0.870	-0.058 (0.013)	1.86E-05	29370
					ILMN_2225537	Ad	0.463	4.50E-22	<i>PTGR1</i>						
					ILMN_1704531	Mu	0.173	9.68E-17	<i>PTGR1</i>						
					ILMN_2225537	Mu	0.205	7.85E-20	<i>PTGR1</i>						

B) eSNPs (FDR 1%) from the AAGME<sub>x</sub> cohort are associated with BMI (p 0.0001) in the "African Ancestry Anthropometry Genetic Consortium" cohorts

SNP	SNP Chr	SNP Pos	A1	MAF	Illumina probe_ID	Tissue	$\beta$	P Value <sup>a</sup>	Symbol	Entrez Gene_ID	Allele1	Freq1	Effect (SE)	P Value <sup>b</sup>	N
rs7949567	11	85251005	G	0.346	ILMN_1784847	Ad	-0.071	2.17E-07	CREBZF	58487	A	0.633	0.036 (0.008)	7.10E-06	39130
					ILMN_2336609	Mu	-0.225	4.21E-10	SYTL2	54843					
					ILMN_2217809		0.211	6.42E-24	TMEM126A	84233					

In *eQTL analysis* A1, Minor Allele; MAF, Minor Allele Frequency;  $\beta$ , effect size of minor allele (A1);

P-value<sup>a</sup>, significance in additive model; Q-value, false discovery rate. In *T2D and BMI GWAS meta-analysis* Allele1, effect allele; Freq1, frequency of effect allele;

P-value<sup>b</sup>, GWAS Meta-analysis p-values. Ad, subcutaneous adipose tissue; Mu, skeletal muscle tissue. Other T2D or BMI associated (p<0.01) eSNPs are shown in supplementary table 9 and 10.