Supplemental data:

Type 2 Diabetes (T2D) Associated Polymorphisms Regulate Expression of Adjacent Transcripts in Transformed Lymphocytes, Adipose and Muscle from Caucasian and African American Subjects

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[#] Dr Steven C Elbein passed away unexpectedly during the preparation of this manuscript.

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Supplemental Methods:

Genotyping: Genomic DNA isolated from TLs or total blood samples were used for genotyping. Sequenom MassARRAY system (Sequenom Inc. SanDiego,CA) was used for genotyping 15 SNPs . Genotyping procedures were performed according to the manufacturer's iPLEX application guide at the genotyping laboratory of WFUHS Center for Genomics. Additional seven SNPs were genotyped by pre-designed Taqman SNP genotyping assays (Applied Biosystems Inc., Foster City, CA) using ABI-7500 Fast real time PCR system. One SNP was genotyped by Pyrosequencing (PSQ96, Qiagene Inc, Valencia, CA, USA; formerly Biotage, Sweden) using a modification of manufacturer's protocol that used a biotinylated universal primer (15). Details of all selected SNPs and genotyping assays are described in supplemental table-2. Genotyping was confirmed by 100% concordance in 10% blinded duplicates.

RNA isolation: Total RNA from TLs grown under standard conditions was extracted using RNeasy mini kit (Qiagen Inc., Valancia, CA). We extracted total RNA from subcutaneous adipose by using the RNeasy Lipid Tissue Mini kit (Qiagen Inc.) and from muscle using the Ultraspec RNA kit (Biotecx Laboratories, Inc, Houston, TX). The quantity and quality of the isolated total RNA samples were determined by ultraviolet spectrophotometry (Nanodrop, Thermo Scientific, USA) and electrophoresis (Experion nucleic acid analyzer, BioRad Laboratories, Inc., Hercules, CA) respectively.

Supplemental Tables:

Supplemental Table-1: Demographics of study population

	All	European American	African American
N (T2D/Non-diabetic)	168 (13/155)	122(6/166)	46 (7/39)
Gender (M/F)	73/95	44/78	29/17
Age (Yrs)	40.9±10.4	40.0±10.7	43.5±9.3
WHR	0.9±0.2	0.9±0.2	0.9±0.1
BMI (kg/m2)	29.5±5.9	29.4±5.8	29.8±6.1
% FAT	33.0±9.9	34.5±9.4	29.3±10.2
Glucose AUC (mmol-min/L)¶	626±411	636±419	599±391.2
Insulin AUC (mmol-min/L)¶	18098±22588	18968±22512	15723±22936
S_I ($\times 10^{-4}.min^{-1}$ $[\mu U/ml]^{-1}$)*	3.7±2.2	3.8±2.3	3.4±1.9
AIR_G (pmol/L)*	3287±2578	2824±2156	4560±3188
DI*	1636±1142	1470±1056	2091±1258
S_G (min-1)*	0.017±0.007	0.017±0.007	0.016±0.008

Continuous variables are shown as mean ± SD. ¶, Metabolic measurements from OGTT; *, Metabolic traits from 135 non-diabetic individuals completed FSIGT evaluation. S_I, insulin sensitivity index, Units are taken from MINMOD program. To convert values to SI units ($X10^{-4}$ $min^{-1}[pmol/l]^{-1}$) multiply by 0.167. AIR_G, Acute insulin response to glucose; DI, Disposition index and Sg, is the measure of the ability of glucose to promote its own uptake.

Supplemental Table-2: Novel T2D associated SNPs from GWAS studies and Genotyping assays

Refer supplementary MS-Excel file (tab S.table-2)

Supplemental Table-3: Primer sequences for quantitative real time PCR (qRT-PCR) assays

Refer supplementary MS-Excel file (tab S.table-3)

Supplemental Table-4: Primer sequences of pyrosequencing assay for allele specific expression imbalance analyses

Refer supplementary MS-Excel file (tab S.table-4)

Supplemental Table- 5: Correlation of metabolic traits with expression of transcripts adjacent to T2D GWAS associated SNPs in Human Subcutaneous Adipose and Skeletal Muscle

		Adipose			Muscle		
Gene Name		**BMI**	**S_I**	**AIR_G**	**BMI**	**S_I**	**AIR_G**
ADAMTS9	r	-0.060	0.074	-0.079	0.054	0.149	0.018
	p	0.468	0.412	0.378	0.507	0.102	0.843
CAMK1D	r	0.114	0.028	-0.037	-0.003	0.059	-0.004
	p	0.165	0.755	0.677	0.970	0.517	0.968
CDC123	r	0.039	0.003	-0.010	-0.046	0.126	0.082
	p	0.638	0.978	0.912	0.574	0.168	0.370
CDKAL1	r	0.028	0.030	-0.079	-0.077	0.153	0.072
	p	0.734	0.736	0.379	0.340	0.092	0.429
DNAJC11	r	-0.061	0.093	-0.071	0.035	0.144	0.077
	p	0.460	0.303	0.428	0.670	0.113	0.399
FTO	r	0.054	-0.013	0.0004	-0.050	0.154	0.058
	p	0.512	0.881	0.997	0.535	0.090	0.524
HHEX	r	0.138	-0.112	-0.015	ND		
	p	0.094	0.210	0.870			
IDE	r	0.147	-0.058	0.061	ND		
	p	0.073	0.517	0.495			
JAZF1	r	0.102	0.005	-0.039	-0.074	0.165	0.050
	p	0.214	0.957	0.663	0.365	0.069	0.586
NOTCH2	r	0.059	-0.045	-0.022	ND		
	p	0.476	0.621	0.809			
PKN2	r	-0.092	0.085	-0.125	-0.076	0.140	0.087
	p	0.264	0.342	0.164	0.352	0.123	0.339
PPARG	r	-0.147	0.151	-0.061	ND		
	p	0.073	0.090	0.500			
SMARCAD1	r	0.040	0.046	-0.079	-0.097	**0.179**	0.074
	p	0.625	0.606	0.378	0.229	**0.048**	0.415
SYN2	r	-0.062	0.064	0.001	ND		
	p	0.455	0.473	0.993			
TCF7L2 (13a+)	r	-0.012	0.025	-0.126	ND		
	p	0.883	0.782	0.161			
THADA	r	0.090	0.021	-0.038	-0.060	0.097	0.067
	p	0.275	0.819	0.674	0.458	0.287	0.463
TSPAN8	r	0.007	-0.018	-0.060	-0.055	0.049	0.026
	p	0.933	0.839	0.503	0.503	0.591	0.779
VEGFA	r	**-0.305**	**0.230**	-0.134	-0.120	**0.228**	0.031
	p	**0.0002**	**0.010**	0.135	0.139	**0.012**	0.731
WFS1	r	0.037	-0.029	0.007	-0.057	0.131	-0.017
	p	0.657	0.745	0.934	0.483	0.150	0.849

r, partial correlation coefficient and p, statistical significance of partial correlation measures between metabolic traits with gene expressions after controlling for age, gender, and ethnicity.
ND, not detected

Supplemental Table-6: Association of SNPs showing AEI with total transcript expression of T2D GWAS implicated genes

Gene name	AI -SNP	Major/Minor Allele (D/d)	Genotype count (DD/Dd/dd)	p*	p(adj)**
CAMK1D	rs1644394	G/A	49/27/6	0.253	0.210
CDC123	rs1051055	G/A	40/34/8	0.630	0.576
DNAJC11	rs1043681	A/G	36/38/9	**0.045**	**0.013**
SMARCAD1	rs8336	G/A	28/39/17	0.951	0.781
SMARCAD1	rs8026	A/G	28/35/20	0.275	0.267
THADA	rs11899823	T/C	38/36/9	0.940	0.930
VEGFA	rs2010963	G/C	38/35/9	0.354	0.454

*, p, statistical significance of genotypic association from analysis of variance analysis (ANOVA); **, p(adj), statistical significance of genotypic association from mixed effects, general linear regression models and included genotype, gender, and T2D diagnosis as fixed factors.

Supplemental figures:

Supplemental Figure-1: Genotypic association of SNP rs9472138 with the expression of vascular endothelia growth factor A (VEGFA) transcript in transformed lymphocytes. The box plot shows transcript level expression of VEGFA for different genotypes for SNP rs9472138. VEGFA expression is shown after 18S normalization and ln transformation. N=no. of samples. The box represents the interquartile range, which contains 50% of the values. The whiskers are lines that extend the box to the highest and lowest values, excluding outliers. A line across the box indicates the median.