**SUPPLEMENTAL MATERIAL**

**DNA Methylation of the *LY86* Gene is Associated With Obesity, Insulin Resistance and Inflammation**

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**Study Cohorts**

This study is based on five existing cohorts and one ongoing study (the EpiGO study), which recruited youth and young adults from the same area (Augusta, GA), including African Americans (AA) and European Americans (EA), as well as males and females.

**Lifestyle, Adiposity, and Cardiovascular Health in Youth (LACHY) study:** This is a NIH funded (HL64157) cross-sectional study with the goal ofdetermining the relations of fatness and fitness to cardiovascular disease (CVD) risk factors in the juvenile years (Gutin et al., 2007). LACHY recruited a total of 765 youths from local high schools in the Augusta, Georgia area from 2000 to 2004. It consisted of roughly equal numbers of African-Americans (AAs) and European-Americans (EAs) aged 14–18 years of both genders. The youths were apparently healthy and were not taking any anti-hypertensive, lipid-lowering, anti-diabetic or anti-inflammatory medications.

**Blood Pressure (BP) stress cohort:** BP stress study is a NIH funded (HL041781 and HL069999 project 1) longitudinal cohort which was established in 1989 to study the development of cardiovascular risk factors (X. Wang et al., 2006). It included 396 EA and 349 AA healthy youths (aged to 7 to 16 years at baseline) with evaluations conducted annually from 1989 to 2001 (visit 1–10) and every 1.5 year from 2002 to 2007 (visit 11–14). Two more visits are currently conducted from 2008 to 2012 (visit 15–16).

**Georgia (GA) twin cohort:** The Georgia cardiovascular twin study is another ongoing NIH-funded (HL056622) longitudinal study that was established in 1996 to explore the change in relative influence of genetic and environmental factors on the development of cardiovascular risk factors (Ge et al., 2006). It also includes roughly equal numbers of EA and AA youth and young adults (>500 twin pairs aged 7 to 25 years at baseline) with evaluation conducted every 4 years. All twin pairs were reared together and zygosity has been determined using standard microsatellite markers in DNA collected with buccal swabs.

**Prevention of Hypertension in African American Teens (PHAT) study**: This is a NIH-funded **(**HL077230) cross-sectional study with the goal of identifying genetic predispositions responsible for impaired stress-induced pressure natriuresis (Zhu et al., 2009). A total of 300 AA youths of both genders aged from 14 to 20 years were recruited from local high schools in the Augusta, GA area from 2006 to 2008. All the subjects were apparently healthy and were not taking any prescribed medications.

**Stress Induced Pressure Natriuresis (SIPN) study:** The overall goal of this study is to obtain a more complete understanding of the role that physiologic changes induced by stress play in the development of ethnic differences in essential hypertension. For this purpose, a cross-sectional study with a sample size of 300 was designed and got funded from NIH (HL064225). An expansion on this study including additional samples and additional measurements was also funded by NIH (HL069999 project 2; Hanevold et al., 2008; Harshfield et al., 2007; Harshfield et al., 2003; Wilson et al., 2004). In total, 655 youths aged from 15 to 19, including roughly equal numbers of AAs and EAs of both genders, were recruited from local high schools in the Augusta, GA area from 2002 to 2005. Similar to the LACHY study, all the subjects were apparently healthy and were not taking any prescribed medications.

**EpiGenetic Basis of Obesity Induced Cardiovascular Disease and Type 2 Diabetes (EpiGO study):** This is a NIH-funded (HL105689) ongoing study. It was established in 2011 with the goal of identifying DNA methylation changes involved in the pathogenesis of obesity and its related comorbidities. Currently it is still ongoing and will in total enroll 400 obese and 400 lean youth aged 14–20 years with roughly equal number of AAs and EAs as well as males and females. The inclusion criteria are as follows: (1) age ≥14 but <21; (2) BMI ≥ 30kg/m2 or BMI ≥ 95th percentile for age and sex if age ≤ 20 for obese cases and BMI < 25kg/m2 or BMI<50th percentile for age and sex if age ≤ 20 for lean controls; (3) free of any acute or chronic illness; (4) no daily medication controls for diseases; (5) EAs or AAs with both parents of the subjects reporting being of European or African ancestry, respectively. All the subjects will be recruited from the southeastern United States (Xu et al., 2013).

**In the present study,** the subjects used for the initial EWAS panel, two replication panels and the general youth population panel were selected from the 5 existing cohorts and the subjects used for the second EWAS panel were selected from the EpiGO study.

**Initial EWAS panel:** The genome-wide methylation analysis was conducted in 7 obese and 7 age-matched lean controls. (X. Wang et al., 2010) These 14 subjects were identified from the participants in the LACHY study using the following inclusion criteria: (1) AA ancestry; (2) male; (3) having leukocyte DNA available; (4) obese cases having a body mass index (BMI) ≥ 99th percentile for age and gender and lean controls having BMI ≤ 10th percentile for age and gender.

**Second EWAS panel:** This genome wide methylation analysis was conducted in 48 obese (24 males and 24 females) and 48 age- and gender-matched lean AA participants from the EpiGO study (Xu et al., 2013).

**Replication panel 1:** This panel included 46 obese (BMI ≥ 30 kg/m2 or BMI ≥ 95th percentile for age and gender if age ≤ 18) and 46 lean (BMI ≤ 22 kg/m2 or BMI ≤ 40th percentile for age and gender if age ≤ 18) AA males selected from three cohorts, the BP stress study, the GA twin study and the PHAT study. (X. Wang et al., 2010) The obese and lean subjects were identified based on the following criteria: (1) having leukocyte DNA available; (2) AA males; (3) only one twin subject from a twin pair was selected if both twins met the criteria; (4) if multiple visits (with multiple leukocyte DNA) were available for a subject, this subject had to be obese or lean on all the visits to be included in the replication sample and the leukocyte DNA collected at the visit when the subject were the most obese or most lean was used.

**Replication panel 2:** This panel included 231 obese (BMI ≥ 30 kg/m2 or BMI ≥ 95th percentile for age and gender if age ≤ 18) and 412 lean (BMI ≤ 25 kg/m2 or BMI ≤ 50th percentile for age and gender if age ≤ 18) subjects selected from three cohorts, the LACHY study, the BP stress study and the SIPN study, including both AAs (*N* = 298) and EAs (*N* = 345), as well as males (*N* = 289) and females (*N* = 354). The other inclusion criteria were same to the 1st and 4th criteria used for the replication panel 1.

**General population panel of youth:** This panel involved all participants who had DNA available in the LACHY study, which represented a general population of youth recruited from the same area. It involved 703 subjects (aged 13.8-19, 372 EAs, 331 AAs, 351 males and 352 females) with BMI ranging from 14.6 to 45.9 kg/m2, of which 12 subjects (6 obese vs. 6 lean) have been included in the initial stage (2 subjects excluded because of no DNA available for this panel) and 221 subjects (53 obese vs. 168 lean) were included in the second replication panel, respectively.

All subjects from these panels were overtly healthy, free of any acute or chronic illness on the basis of self-report and parental report (if subjects were younger than 18) and were not on anti-hypertensive, lipid lowering, anti-diabetic and anti-inflammatory medications. The Institutional Review Board at the Medical College of Georgia approved the studies. Informed consent was obtained from all subjects and by parents if subjects were less than 18 years of age.

**Measurement of Obesity-Related Metabolic Traits**

**Anthropometric measures:** For all the cohorts, anthropometric measurements were conducted in an examination room in the following order: height (via a wall-mounted stadiometer, to nearest 0.1 cm), weight (via a digital scale, to nearest 0.1 kg), waist circumference (via meter, to nearest 0.1 cm at the narrowest point of the torso below the rib cage and above the umbilicus) and hip circumference (via meter, to nearest 0.1 cm at the maximum circumference of the buttocks). All measurements were obtained twice and if there was a big difference (>1 cm for height, >0.25 kg for weight and >0.5 cm for waist and hip circumference) between the first two measures, a third measurement will be conducted. The average of the two close readings will be used. BMI was calculated as weight/height2 (kg/m2).

**Blood pressure:** In the LACHY study, supine BP was obtained with Dinamap monitors during a 15-minute supine relaxation period. (Gutin et al., 2007) By using an appropriately sized BP cuff placed on the subject’s right arm, three BP measurements were taken at 11, 13, and 15 minutes. The average of the last 2 readings will be used to represent systolic blood pressure (SBP) and diastolic blood pressure (DBP) values.

**Fasting insulin, glucose and lipid profile:** In the LACHY study, fasting glucose levels were measured using Ektachem DT II system (Johnson and Johnson Clinical Diagnostics, Rochester, NY, USA) and fasting insulin was assayed in duplicate by specific radioimmunoassay (Linco Research, Inc., St Charles, MO, USA). (Gutin et al., 2007) Cross-reactivity with proinsulin is <0.2%. Assay sensitivity was 3.41 mU/mL. The intra-assay coefficient of variation was 3.7%. Plasma concentrations of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured at the Emory University Lipid Research Center using homogeneous enzymatic assays (Equal Diagnostics, Exton, PA). Triglycerides (TGs) were measured using enzymatic methods on the Beckman CX7 chemistry autoanalyzer (Beckman Coulter Diagnostics, Fullerton, CA).

**Fibrinogen and C-reactive protein (CRP):** These two inflammatory markers have been measured in the LACHY study. (Gutin et al., 2007) For fibrinogen, citrated plasma was assayed in duplicate using a BBL Fibrometer and reagents purchased from Biomerieux (St. Louis, MO). Standard curves were made using 1:5, 1:10, 1:20, and 1:40 dilutions of Fibrinogen Calibration Reference (Biomerieux 235530) in Owrens Veronal buffer (Biomerieux 235532). Plasma was diluted 1:10 in Owrens Veronal buffer. Two hundred microliters of diluted plasma or Calibration Reference was added to a sample cup at 37 °C and warmed for 3 minutes, at which time the cup was moved to a well under the fibrometer probe. One hundred microliters of thrombin reagent (Biomerieux 233531) was added to the cup, and the timer was activated. The time it took for samples to clot was recorded and averaged. Fibrinogen concentrations were determined from the standard curve. Plasma CRP was assayed in duplicate using ELISAs.

**Measurement of Accurate Indices of Adiposity**

**Dual-energy X-ray absorptiometry:** In the LACHY study, total percentage body fat (%BF) was measured using DXA (Hologic QDR-4500W, software version 6.0, Waltham, MA, USA). (Gutin et al., 2007) DXA provides reliable values for %BF. Repeat measurements were performed using the QDR-4500W machine with 219 adolescents and the intraclass correlation coefficient (ICC) for %BF was found to be 0.99. For some subjects, DXA values were only available from the Hologic QDR-1000W, but not from the Hologic QDR-4500W model. For these individuals, %BF values were derived from prediction equations based on 284 youths who were assessed on both instruments, using linear regression; ethnicity, gender and the QDR-1000W measurement were the predictor variables. The multiple R2 value for %BF was 0.99.

**Magnetic resonance imaging:** In the LACHY study, visceral adipose tissue (VAT) and subcutaneous abdominal adipose tissue (SAAT) were determined using MRI (1.5 T General Electric Medical Systems, Milwaukee, WI) as described previously. (Gutin et al., 2007) Briefly, with subjects in the supine position, a series of five, 1-cm-thick, transverse images was acquired beginning at the inferior border of the fifth lumbar vertebra and proceeding toward the head. A gap is left between the slices to avoid cross-talk. Values for VAT and SAAT from a single image were calculated in terms of surface area (cm2) and the volume (cm3) estimated by multiplying the surface area by the image width (1 cm) and then summing the five images. VAT and SAAT were measured in the Department of Radiology on equipment dedicated to patient care. VAT and SAAT measures were obtained in those subjects who underwent testing on days when the MRI equipment was available for the research study.

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**Supplemental Table 1: 117 Genes Identified From the Literature**

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|  | Gene | Chromosome | Associated phenotypes | References |
| Monogenic obesity |  |  |
|  | BDNF | 11 | Obesity, childhood-onset | (Han et al., 2008) |
|  | LEP | 7 | Obesity, severe, due to leptin deficiency  | (Montague et al., 1997) |
|  | LEPR | 11 | Obesity, morbid, with hypogonadism | (Farooqi et al., 2007) |
|  | MC4R | 18 | Obesity, autosomal dominant  | (Farooqi et al., 2003) |
|  | NTRK2 | 9 | Obesity, hyperphagia, with developmental delay  | (Yeo et al., 2004) |
|  | PCSK1 | 5 | Obesity, impaired prohormone processing  | (Jackson et al., 1997) |
|  | POMC | 2 | Obesity, severe early-onset, due to POMC deficiency | (Krude et al., 1998) |
|  | SH2B1 | 7 | Obesity, early-onset, with developmental delay | (Bachmann-Gagescu et al., 2010) |
|  | SIM1 | 6 | Obesity, severe | (Holder et al., 2000) |
| Syndromic obesity |  |  |
|  | ALMS1 | 2 | Alstrom syndrome  | (Collin et al., 2002) |
|  | BBS1 | 11 | Bardet-Biedl syndrome | (Mykytyn et al., 2002) |
|  | GNAS1 | 20 | Pseudohypoparathyroidism type Ic | (Thiele et al., 2011) |
|  | SNRPN | 15 | Prader-Willi syndrome  | (Gillessen-Kaesbach et al., 1999) |
| Genome Wide Association Study |  |  |
|  | AC138894.2 | 16 | BMI | (Speliotes et al., 2010) |
|  | ADAMTS9 | 3 | WHR | (Heid et al., 2010) |
|  | ADCY3 | 2 | BMI | (Speliotes et al., 2010) |
|  | AIF1 | 6 | Weight | (Thorleifsson et al., 2009) |
|  | APBB2 | 4 | Obesity | (K. Wang et al., 2011) |
|  | APOB48R | 16 | BMI | (Speliotes et al., 2010) |
|  | ARG1 | 6 | WHR | (K. Wang et al., 2011) |
|  | ASAH1 | 8 | BMI, weight | (K. Wang et al., 2011) |
|  | ATP2A1 | 16 | BMI | (Thorleifsson et al., 2009) |
|  | ATXN2L | 16 | BMI | (Speliotes et al., 2010) |
|  | BAT2 | 6 | Weight | (Thorleifsson et al., 2009) |
|  | BCDIN3D | 12 | BMI, weight | (Thorleifsson et al., 2009) |
|  | BDNF | 11 | BMI, weight, obesity | (Jiao et al., 2011; Speliotes et al., 2010; Thorleifsson et al., 2009) |
|  | BTNL2 | 6 | WHR | (Heid et al., 2010) |
|  | CADM2 | 3 | BMI | (Speliotes et al., 2010) |
|  | CHST8 | 19 | BMI, weight | (Thorleifsson et al., 2009) |
|  | CPEB4 | 5 | WHR | (Heid et al., 2010) |
|  | CUGBP1 | 11 | BMI | (Speliotes et al., 2010) |
|  | DGKG | 3 | BMI, weight | (Thorleifsson et al., 2009) |
|  | DNM3 | 1 | WHR | (Heid et al., 2010) |
|  | ETV5 | 3 | BMI, weight | (Speliotes et al., 2010; Thorleifsson et al., 2009) |
|  | FAIM2 | 12 | BMI, weight | (Paternoster et al., 2011; Speliotes et al., 2010; Thorleifsson et al., 2009) |
|  | FANCL | 2 | BMI | (Speliotes et al., 2010) |
|  | FAT1 | 4 | Obesity | (K. Wang et al., 2011) |
|  | FDX1 | 11 | Obesity | (K. Wang et al., 2011) |
|  | FHIT | 3 | Obesity | (K. Wang et al., 2011) |
|  | FLJ35779 | 5 | BMI | (Speliotes et al., 2010) |
|  | FTO | 16 | BMI, weight, obesity | (Meyre et al., 2009; Paternoster et al., 2011; Scherag et al., 2010; Speliotes et al., 2010; Thorleifsson et al., 2009; K. Wang et al., 2011; Willer et al., 2009) |
|  | GIPR | 19 | BMI | (Speliotes et al., 2010) |
|  | GNPDA2 | 4 | BMI | (Paternoster et al., 2011; Speliotes et al., 2010; Willer et al., 2009) |
|  | GPRC5B | 16 | BMI | (Speliotes et al., 2010) |
|  | GRB14 | 2 | WHR | (Heid et al., 2010) |
|  | GTF3A | 13 | BMI | (Speliotes et al., 2010) |
|  | HMGA1 | 6 | BMI | (Speliotes et al., 2010) |
|  | HMGCR | 5 | BMI | (Speliotes et al., 2010) |
|  | HOXC13 | 12 | WHR | (Heid et al., 2010) |
|  | INHBB | 2 | WHR | (K. Wang et al., 2011) |
|  | INSIG2 | 2 | Obesity | (Herbert et al., 2006) |
|  | IQCK | 16 | BMI | (Speliotes et al., 2010) |
|  | ITPR2 | 12 | WHR | (Heid et al., 2010) |
|  | KCNMA1 | 10 | Obesity | (Jiao et al., 2011) |
|  | KCTD15 | 19 | BMI, weight | (Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2009) |
|  | KREMEN1 | 22 | WHR | (Heid et al., 2010) |
|  | LBXCOR1 | 15 | BMI | (Speliotes et al., 2010) |
|  | LGR4 | 11 | BMI, weight | (Thorleifsson et al., 2009) |
|  | LHFPL3 | 7 | Hip | (K. Wang et al., 2011) |
|  | LIN7C | 11 | BMI, weight | (Thorleifsson et al., 2009) |
|  | LRP1B | 2 | BMI | (Speliotes et al., 2010) |
|  | LRRN6C | 9 | BMI | (Speliotes et al., 2010) |
|  | LY86 | 6 | WHR | (Heid et al., 2010) |
|  | LYPLAL1 | 1 | WHR | (Heid et al., 2010) |
|  | MAF | 16 | Obesity | (Meyre et al., 2009) |
|  | MAP2K5 | 15 | BMI | (Speliotes et al., 2010) |
|  | MC4R | 18 | BMI, weight, obesity | (Meyre et al., 2009; Paternoster et al., 2011; Scherag et al., 2010; Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2009) |
|  | MSRA | 8 | Obesity | (Scherag et al., 2010) |
|  | MTCH2 | 11 | BMI | (Speliotes et al., 2010; Willer et al., 2009) |
|  | MTIF3 | 13 | BMI | (Speliotes et al., 2010) |
|  | MTNR1A | 4 | Obesity | (K. Wang et al., 2011) |
|  | MYLIP | 6 | Obesity | (K. Wang et al., 2011) |
|  | NCAM2 | 21 | Waist | (K. Wang et al., 2011) |
|  | NCR3 | 6 | Weight | (Thorleifsson et al., 2009) |
|  | NDUFS3 | 11 | BMI | (Speliotes et al., 2010) |
|  | NEGR1 | 1 | BMI, weight | (Heid et al., 2010; Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2009) |
|  | NFE2L3 | 7 | WHR | (Heid et al., 2010) |
|  | NISCH | 3 | WHR | (Heid et al., 2010) |
|  | NPC1 | 18 | Obesity | (Meyre et al., 2009) |
|  | NRXN3 | 14 | BMI, WHR | (Speliotes et al., 2010; K. Wang et al., 2011) |
|  | NUDT3 | 6 | BMI | (Hong & Oh, 2012) |
|  | OTOL1 | 3 | Obesity | (K. Wang et al., 2011) |
|  | PCDH9 | 13 | Weight | (K. Wang et al., 2011) |
|  | PIGC | 1 | WHR | (Heid et al., 2010) |
|  | PITPNB | 22 | Hip | (K. Wang et al., 2011) |
|  | POMC | 2 | BMI | (Speliotes et al., 2010) |
|  | PRKD1 | 14 | BMI | (Speliotes et al., 2010) |
|  | PRL | 6 | Obesity | (Meyre et al., 2009) |
|  | PTBP2 | 1 | BMI | (Speliotes et al., 2010) |
|  | PTER | 10 | Obesity | (Meyre et al., 2009) |
|  | QPCTL | 19 | BMI | (Speliotes et al., 2010) |
|  | RASAL2 | 1 | BMI, weight | (Thorleifsson et al., 2009) |
|  | RBJ | 2 | BMI | (Speliotes et al., 2010) |
|  | RPGRIP1L | 16 | BMI, weight | (Thorleifsson et al., 2009) |
|  | RPL27A | 11 | BMI | (Speliotes et al., 2010) |
|  | RSPO3 | 6 | WHR | (Heid et al., 2010) |
|  | SDCCAG8 | 1 | Obesity | (Scherag et al., 2010) |
|  | SEC16B | 1 | BMI, weight | (Speliotes et al., 2010; Thorleifsson et al., 2009) |
|  | SFRS10 | 2 | BMI, weight | (Thorleifsson et al., 2009) |
|  | SH2B1 | 16 | BMI, weight | (Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2009) |
|  | SLC39A8 | 4 | BMI | (Speliotes et al., 2010) |
|  | SSPN | 12 | WHR | (Heid et al., 2010) |
|  | STAB1 | 3 | WHR | (Heid et al., 2010) |
|  | SULT1A2 | 16 | BMI | (Speliotes et al., 2010) |
|  | TBX15 | 1 | WHR | (Heid et al., 2010) |
|  | TFAP2B | 6 | BMI | (Speliotes et al., 2010) |
|  | TMEM160 | 19 | BMI | (Speliotes et al., 2010) |
|  | TMEM161B | 5 | Obesity | (K. Wang et al., 2011) |
|  | TMEM18 | 2 | BMI, weight, obesity | (Scherag et al., 2010; Speliotes et al., 2010; Thorleifsson et al., 2009; Willer et al., 2009) |
|  | TNKS | 8 | Obesity | (Scherag et al., 2010) |
|  | TNNI3K | 1 | BMI | (Speliotes et al., 2010) |
|  | TUB | 11 | BMI | (Speliotes et al., 2010) |
|  | TUFM | 16 | BMI | (Speliotes et al., 2010) |
|  | UBE2E3 | 2 | Obesity | (K. Wang et al., 2011) |
|  | VEGFA | 6 | WHR | (Heid et al., 2010) |
|  | WARS2 | 1 | WHR | (Heid et al., 2010) |
|  | WWOX | 16 | Hip | (K. Wang et al., 2011) |
|  | ZC3H4 | 19 | BMI | (Speliotes et al., 2010) |
|  | ZEB1 | 10 | WHR | (Heid et al., 2010) |
|  | ZNF608 | 5 | BMI | (Speliotes et al., 2010) |
|  | ZNRF3 | 22 | WHR | (Heid et al., 2010) |

**Supplemental Table 2: Correlations Among Six CpG Sites of *LY86* Gene in the Third Panel
(*N* = 703) Representing the General Population of Youth**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | LY86\_1 | LY86\_2 | LY86\_3 | LY86\_4 | LY86\_5 | LY86\_6 |
| LY86\_1 | 1.000 |  |  |  |  |  |
| LY86\_2 | 0.888 | 1.000 |  |  |  |  |
| LY86\_3 | 0.903 | 0.899 | 1.000 |  |  |  |
| LY86\_4 | 0.891 | 0.863 | 0.884 | 1.000 |  |  |
| LY86\_5 | 0.884 | 0.869 | 0.880 | 0.977 | 1.000 |  |
| LY86\_6 | 0.829 | 0.840 | 0.835 | 0.968 | 0.961 | 1.000 |

**Supplemental Table 3: Associations Between the Methylation Levels of the
*LY86* Gene and Obesity-Related Metabolic Traits and Inflammation in the
Third Panel (*N* = 703) Representing the General Population of Youth**

|  |  |  |
| --- | --- | --- |
| Metabolic traitsa | Model 1b | Model 2c |
| Partial correlation | *p* | Partial correlation | *p* |
|  | LY86\_1d |
| Fasting glucose | .099 | .01 | .081 | .04 |
| Fasting insulin | .143 | <.001 | .093 | .02 |
| QUICKI | -.148 | <.001 | -.103 | .009 |
| SBP | .094 | .01 | .058 | .13 |
| DBP | .76 | .04 | .070 | .06 |
| TC | .013 | .74 | .003 | .94 |
| HDL-C | -.082 | .04 | -.049 | .21 |
| LDL-C | .058 | .14 | .035 | .37 |
| TG | .044 | .27 | .010 | .80 |
| Fibrinogen | .161 | <.001 | .124 | .002 |
| CRP | .152 | <.001 | .102 | .01 |
|  | LY86\_2 d |
| Fasting glucose | .084 | .03 | .071 | .07 |
| Fasting insulin | .113 | .004 | .077 | .05 |
| QUICKI | -.116 | .003 | -.083 | .03 |
| SBP | .083 | .03 | .056 | .14 |
| DBP | .052 | .17 | .047 | .21 |
| TC | .037 | .35 | .029 | .46 |
| HDL-C | -.080 | .04 | -.054 | .17 |
| LDL-C | .078 | .05 | .061 | .12 |
| TG | .049 | .21 | .023 | .55 |
| Fibrinogen | .134 | <.001 | .105 | .008 |
| CRP | .134 | <.001 | .101 | .01 |
|  | LY86\_3 d |
| Fasting glucose | .093 | .02 | .081 | .04 |
| Fasting insulin | .110 | .005 | .077 | .05 |
| QUICKI | -.114 | .003 | -.084 | .03 |
| SBP | .076 | .04 | .053 | .17 |
| DBP | .057 | .13 | .052 | .17 |
| TC | .047 | .23 | .041 | .30 |
| HDL-C | -.056 | .15 | -.033 | .40 |
| LDL-C | .078 | .05 | .063 | .11 |
| TG | .077 | .05 | .056 | .15 |
| Fibrinogen | .147 | <.001 | .123 | .002 |
| CRP | .128 | .001 | .101 | .01 |
|  | LY86\_4 d |
| Fasting glucose | .098 | .01 | .085 | .03 |
| Fasting insulin | .114 | .004 | .007 | .05 |
| QUICKI | -.116 | .003 | -.083 | .03 |
| SBP | .096 | .01 | .071 | .06 |
| DBP | .069 | .07 | .065 | .09 |
| TC | .031 | .43 | .024 | .54 |
| HDL-C | -.036 | .36 | -.011 | .77 |
| LDL-C | .052 | .19 | .036 | .36 |
| TG | .042 | .29 | .018 | .64 |
| Fibrinogen | .172 | <.001 | .149 | <.001 |
| CRP | .131 | .001 | .101 | .01 |
|  | LY86\_5 d |
| Fasting glucose | .107 | .006 | .095 | .01 |
| Fasting insulin | .113 | .004 | .080 | .04 |
| QUICKI | -.116 | .003 | -.085 | .03 |
| SBP | .109 | .004 | .087 | .02 |
| DBP | .084 | .03 | .079 | .04 |
| TC | .030 | .45 | .024 | .55 |
| HDL-C | -.039 | .32 | -.016 | .68 |
| LDL-C | .058 | .14 | .043 | .27 |
| TG | .037 | .35 | .015 | .70 |
| Fibrinogen | .189 | <.001 | .170 | <.001 |
| CRP | .152 | <.001 | .128 | .001 |
|  | LY86\_6 d |
| Fasting glucose | .080 | .04 | .071 | .07 |
| Fasting insulin | .107 | .007 | .082 | .04 |
| QUICKI | -.106 | .007 | -.083 | .04 |
| SBP | .095 | .01 | .079 | .04 |
| DBP | .086 | .02 | .083 | .03 |
| TC | .014 | .73 | .009 | .83 |
| HDL-C | -.028 | .48 | -.010 | .81 |
| LDL-C | .040 | .31 | .029 | .47 |
| TG | .028 | .48 | .011 | .78 |
| Fibrinogen | .167 | <.001 | .151 | <.001 |
| CRP | .122 | .002 | .105 | .009 |

Note: aAll traits are log-transformed except QUICKI and DBP
bAdjusted for age, gender and race
cAdjusted for age, gender, race and obesity
dAdjusted for batch.

|  |
| --- |
| **Supplemental Table 4: Cell Population Estimates in Cases Versus Controls in the Initial Panel (7 Obese vs. 7 Lean Using 27K)**  |
|   | Est | SE0 | SE1 | SE2 | *p* value |
| <Intercept> | 0.96 | 1.08 | 0.74 | 0.73 | .189 |
| T Cell (cd8+) | -2.29 | 5.13 | 4.46 | 4.36 | .600 |
| T Cell (cd4+) | -6.97 | 5.11 | 5.39 | 5.17 | .177 |
| NK | -4.42 | 1.46 | 1.52 | 1.55 | .004 |
| B Cell | 0.70 | 0.78 | 1.01 | 1.00 | .487 |
| Monocyte | -0.70 | 1.31 | 1.41 | 1.39 | .617 |
| Granulocyte | 12.25 | 1.30 | 3.69 | 3.65 | .0008 |

Note: Est = Regression coefficient estimate (× 100%)
SE0 = Naïve standard error (× 100%)
SE1 = Single-bootstrap standard error (× 100%)
SE2 = Double-bootstrap standard error (× 100%)
*p* values were computed using SE2.

|  |
| --- |
| **Supplemental Table 5: Cell Population Estimates in Cases Versus Controls in the Second EWAS panel (48 obese vs. 48 lean using 450K)**  |
|   | Est | SE0 | SE1 | SE2 | *p* value |
| <Intercept> | 0.66 | 0.25 | 0.32 | 0.32 | .039 |
| T Cell (cd8+) | -0.29 | 1.14 | 0.81 | 0.86 | .737 |
| T Cell (cd4+) | -0.20 | 1.12 | 1.59 | 1.48 | .892 |
| NK | -1.28 | 0.33 | 0.33 | 0.34 | .0001 |
| B Cell | 0.97 | 0.18 | 0.44 | 0.44 | .026 |
| Monocyte | -0.19 | 0.31 | 0.54 | 0.53 | .714 |
| Granulocyte | 2.18 | 0.31 | 1.90 | 1.88 | .246 |
| Note: Est = Regression coefficient estimate (× 100%) |
| SE0 = Naïve standard error (× 100%) |
| SE1 = Single-bootstrap standard error (× 100%) |
| SE2 = Double-bootstrap standard error (× 100%) |
| *p* values were computed using SE2 |

**Supplemental Table 6: Association Between Selected CpG Sites From the Initial Panel
and Obesity in the Second EWAS Panel After the Adjustment for Cell Composition.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **CpG site** | **Methylation level (%)****difference** | ***p*** |
|  *SNRPN* | cg26033681 | -0.8 | .267 |
|   *KREMEN1* | cg01791232 | 1.2 | .013 |
|  *LY86* | cg02212836 | 3.4 | 3.49×10-7 |

Note: a Adjusted for age, gender and cell composition.



Supplemental Figure 1: Methylation level of LY86 CpG site 5 (cg02212836) in different cell subtypes of peripheral leukocytes.