Supplemental Data

Genetic and Metabolic Determinants of Plasma Levels of ANGPTL8

Federico Oldoni1*, Kevin Bass1*, Julia Kozlitina2*, Hannah Hudson1, Lisa M. Shihanian3, Viktoria Gusarova3, Jonathan C. Cohen2,4 and Helen H. Hobbs1,2,5

1Department of Molecular Genetics, 2The Eugene McDermott Center of Human Growth and Development, 3Regeneron Pharmaceuticals, Tarrytown, NY 10591, 4The Center for Human Nutrition, and 5Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390

*These authors contributed equally to the work
Figure S1. Analysis of purified A8 used as a standard for the ELISA and of the intra-individual variation of plasma A8 levels. (A) The level of A8 standard was estimated by comparing the band intensity of A8 and dilutions of BSA (bovine serum albumin) using Coomassie blue staining after SDS-PAGE. (B) Representative standard curves from 3 ELISA plates conformed best to a quadratic fit (dotted line), which was used to interpolate sample concentration values. (C) A8 levels in 48 fasted, unrelated individuals from a placebo-treated group in a clinical study (1) were measured by ELISA as described in the Methods. Each box plot represents the variation of A8 levels from a single individual. The bolded horizontal line indicates the median of
4 measurements, and the upper and lower boundaries of each box represent the 25th and 75th percentiles. The dots are color-coded corresponding to the respective visit 1 (blue), 2 (green), 4 (yellow) and 5 (cyan). Visits were 1-2 weeks apart. ICC, Intra-class correlation coefficient.

**Figure S2.** Comparison of results quantitating plasma levels of human A8 obtained by an immunoblotting assay and commercial A8 ELISA kits. Venous blood was collected after a 12 h fast and 4 h after refeeding. Plasma aliquots (1.5 μL) were size-fractionated on a 15% agarose gel and immunoblotted with a mouse mAb (42C1) (top panel). Fibronectin (FN1) was used as
loading control. The relative intensities of bands were determined by LI-COR, quantified relative to FN1 levels and compared to commercial A8 ELISA kits. The assays were performed according to the manufacturers’ instructions.

Figure S3

A

Median = 11.9 ng/mL (IQR) (6.6 - 20.7)

B

Blacks Whites Hispanics

P = 6.6x10⁻⁴

P = 1.3x10⁻⁴

P = 4.4x10⁻⁵

A8 (ng/mL)

|        | n  | Median (IQR) |
|--------|----|--------------|
| Blacks | 1654 | 14.3 (8.6 - 24.5) |
| Whites | 1072 | 8.5 (4.3 - 14.9) |
| Hispanics | 466 | 12.2 (7.1 - 21.4) |
Figure S3. Overall and racial/ethnic distribution of fasting plasma A8 levels in DHS-2. (A) The frequency distribution of plasma A8 levels, as measured by ELISA, with median and inter-quartile range (IQR, 25th - 75th percentile) values. (B) Plasma A8 levels after stratification by race/ethnicity. The median and IQR values for each group are provided.

Figure S4. Genetic variants associated with circulating A8 levels before (upper panel) and after (lower panel) conditioning on rs2278426. Regional plots show the association of sequence variants associated with circulating A8 levels in the Chromosome 19 region including ANGPTL8/DOCK6, KANK2, SPC24, LDLR, TSPAN16 and RAB3D loci. The significance
threshold is denoted by the gray dotted line. The strength of Linkage Disequilibrium (LD) between genetic variants and the reference rs2278426 variant is color-coded with the purple diamond representing the rs2278426 reference variant (Ref Var) located in the ANGPTL8/DOCK6 locus.

**Figure S5.** Plasma A8 levels in racial/ethnic groups after stratification by R59W genotype (rs2278426) in DHS-2 participants. Plasma A8 levels stratified by A8(R59W) genotype in Blacks (n=1,624), Whites (n=1,059) and Hispanics (n=462) in DHS-2 with median and IQR values. Associations were tested using linear regression with adjustment for age, sex, ancestry and BMI (body mass index). The corresponding genotype (RR, RW, WW) is indicated below the number of individuals in each subcategory.
**Figure S6.** Distribution of plasma A3 levels in DHS-1 and correlations between A3 and A8 concentration among A8(59R) and A8(59W) homozygotes. (A) Distribution of plasma A3 levels were measured using a commercial ELISA kit in individuals matched by race/ethnicity and also by A8 levels whom were either homozygous for the A8(59R) or A8(59W) variant. Each bin
corresponds to a range of 25 ng/mL. (B) Comparison of A3 and A8 concentrations in the 59R and 59W genotype groups, and the respective Spearman’s correlation coefficients are shown.

Figure S7. Correlation analysis between plasma A8 levels obtained by in-house A8 ELISA and commercial A8 ELISA kits. The assays were performed according to the manufacturers’ instructions.
| Sample | 5% plasma matrix | 10% plasma matrix |
|--------|-----------------|-------------------|
|        | Expected value  | Observed value    | Recovery (%) | Expected value  | Observed value    | Recovery (%) |
|        | (ng/mL)         | (ng/mL)           | (%)           | (ng/mL)         | (ng/mL)           | (%)           |
| 1      | 37.3            | 36.5              | 97.9          | 37.5            | 33.3              | 89.0          |
|        | 76.0            | 67.5              | 88.8          | 77.1            | 65.5              | 84.9          |
|        | 114.7           | 101.6             | 88.6          | 119.0           | 105.1             | 88.3          |
| 2      | 37.3            | 35.1              | 94.3          | 37.5            | 32.1              | 85.6          |
|        | 76.0            | 69.5              | 91.5          | 77.1            | 62.9              | 81.6          |
|        | 114.7           | 111.5             | 97.2          | 119.0           | 107.7             | 90.5          |
| 3      | 37.3            | 33.4              | 89.8          | 37.5            | 30.4              | 81.1          |
|        | 76.0            | 62.3              | 82.0          | 77.1            | 57.3              | 74.4          |
|        | 114.7           | 94.9              | 82.7          | 119.0           | 94.6              | 79.5          |
| 4      | 37.3            | 33.1              | 88.8          | 37.5            | 32.4              | 86.4          |
|        | 76.0            | 63.5              | 83.5          | 77.1            | 61.7              | 80.0          |
|        | 114.7           | 101.7             | 88.6          | 119.0           | 95.9              | 80.6          |
| 5      | 37.3            | 34.5              | 92.5          | 37.5            | 30.2              | 80.6          |
|        | 76.0            | 66.4              | 87.4          | 77.1            | 60.0              | 77.9          |
|        | 114.7           | 103.3             | 90.0          | 119.0           | 97.5              | 81.9          |
| 6      | 37.3            | 36.9              | 99.0          | 37.5            | 33.2              | 88.6          |
|        | 76.0            | 71.0              | 93.4          | 77.1            | 64.7              | 84.0          |
|        | 114.7           | 112.0             | 97.6          | 119.0           | 100.8             | 84.7          |

Spike and recovery tests using both 5% and 10% plasma were performed as described in the Methods. Experiments were repeated (n=3), and results were equivalent across tests.
| Sample Dilution | Expected value (ng/mL) | Observed value (ng/mL) | Linearity (%) |
|-----------------|------------------------|------------------------|---------------|
| 1:2             | -                      | 91.3                   | -             |
| 1:4             | 45.7                   | 55.7                   | 121.9         |
| 1:8             | 27.8                   | 34.0                   | 122.1         |
| 1:16            | 17.0                   | 22.5                   | 132.5         |
| 1:32            | 11.3                   | 11.7                   | 104.2         |
| 1:64            | 5.9                    | 5.4                    | 91.9          |

Linearity tests were performed as described in the Methods. Experiments were repeated (n=3), and results were equivalent across tests.
| Characteristic               | N (%) | Total     | N (%) | Blacks     | Whites     | Hispanics  | P-value |
|-----------------------------|-------|-----------|-------|------------|------------|------------|---------|
| Age (years)                 | 3283  | 50 ± 11.1 | 3192  | 49.7 ± 11.4| 52.1 ± 10.6| 46.5 ± 10.5| 1.7E-19 |
| Female, N (%)               | 3283  | 13.9 (59.1)| 3192  | 1046 (63.2)| 579 (54)   | 274 (58.8) | 9.7E-06 |
| BMI (kg/m²)                 | 3279  | 31.2 ± 7.4| 3189  | 32.5 ± 8   | 29.5 ± 6.5 | 31 ± 6.5   | 2.3E-23 |
| Systolic BP (mmHg)          | 3275  | 132.6 ± 20.1| 3184  | 137.3 ± 21.1| 127.5 ± 16.9| 127.6 ± 18.4| 4.1E-41 |
| Diastolic BP (mmHg)         | 3275  | 80.8 ± 9.3 | 3184  | 83 ± 9.6   | 78.3 ± 8.2 | 78.8 ± 8.8 | 3.4E-39 |
| Hypertension, N (%)         | 3281  | 1675 (51.1)| 3190  | 1049 (63.5)| 423 (39.5) | 168 (36.1) | 8.6E-45 |
| Glucose (mg/dL)             | 3245  | 94 (87 - 103)| 3154  | 93 (85 - 104)| 94 (88 - 101)| 96 (89 - 106)| 7.4E-05 |
| HbA1c (%)                   | 3201  | 5.5 (5.2 - 5.9)| 3117  | 5.6 (5.3 - 6)| 5.4 (5.2 - 5.6)| 5.5 (5.3 - 5.9)| 2.2E-35 |
| Insulin (mIU/L)             | 3278  | 12.7 (8.4 - 19.6)| 3187  | 13.3 (8.6 - 21)| 11.4 (7.9 - 17.6)| 14 (9.3 - 21)| 1.2E-09 |
| HOMA-IR (mass units)        | 3235  | 3.0 (1.9 - 5) | 3144  | 3.2 (1.9 - 5)| 2.7 (1.8 - 4.4)| 3.5 (2.1 - 5.6)| 1.6E-09 |
| Diabetic, N (%)             | 3283  | 529 (16.1) | 3192  | 329 (19.9) | 103 (9.6) | 80 (17.2)  | 1.2E-12 |
| GFR MDRD (mL/min/1.73m²)    | 3233  | 95.9 ± 29.9| 3142  | 100.2 ± 36.1| 87.5 ± 18.6| 101.4 ± 23 | 8.0E-56 |
| Total Cholesterol (mg/dL)   | 3276  | 192.1 ± 39.5| 3185  | 189.2 ± 38.8| 196.1 ± 39.7| 193 ± 40.1 | 7.1E-05 |
| LDL-C (mg/dL)               | 3276  | 115.2 ± 35.8| 3185  | 114.4 ± 36.1| 116.9 ± 35.9| 114.6 ± 34.4| 0.25    |
| HDL-C (mg/dL)               | 3276  | 52.5 ± 15 | 3185  | 53.8 ± 14.9 | 52.6 ± 15.9 | 48.1 ± 12.4 | 9.5E-14 |
| Triglycerides (mg/dL)       | 3276  | 102 (74 - 146)| 3185  | 90 (68 - 125)| 112 (80 - 160.8)| 129.5 (93 - 177.2)| 3.2E-47 |
| A8 (ng/mL)                  |       |           |       |            |            |            |         |
| Men                         | 1344  | 11.5 (6.6 - 19.8)| 1293  | 13.5 (8.3 - 23.2)| 9 (5.4 - 14.9)| 12.5 (7.8 - 20.5)| 6.4E-17 |
| Women                       | 1939  | 12.1 (6.5 - 21.3)| 1899  | 14.7 (8.9 - 24.9)| 7.8 (3.7 - 14.9)| 11.9 (6.3 - 22.5)| 1.2E-40 |

Data are shown as N (%), mean ± standard deviation, or median (25th – 75th percentile). Abbreviations: A8, ANGPTL8; BMI, Body mass index; BP, Blood pressure; GFR MDRD, Glomerular filtration rate using modification of diet in renal disease; HbA1c, Hemoglobin A1C; HDL-C, High-density lipoprotein-cholesterol; HOMA-IR, Homeostatic model assessment of insulin resistance; LDL-C, Low-density lipoprotein-cholesterol.
### Supplementary Table 4. Correlations of A8 levels with metabolic phenotypes in DHS-1

| Phenotype class    | Phenotype                                | N    | Spearman's correlation coefficient | P-value | Partial correlation, rho (adjusted for age, sex, race/ethnicity and BMI) | P-value |
|--------------------|------------------------------------------|------|------------------------------------|---------|------------------------------------------------------------------------|---------|
| Demographic        | Age (years)                              | 3531 | 0.046                              | 0.0066  | -                                                                      | -       |
| Anthropometric     | Height (cm)                              | 3058 | -0.063                             | 4.9E-04 | -0.014                                                               | 0.44    |
|                    | Weight (kg)                              | 3058 | 0.201                              | 3.4E-29 | 0.031                                                                | 0.084   |
|                    | Waist circumference (cm)                 | 3056 | 0.243                              | 1.8E-42 | 0.136                                                                | 4.5E-14 |
|                    | Hip circumference (cm)                   | 3054 | 0.208                              | 4.1E-31 | 0.013                                                                | 0.47    |
|                    | BMI (kg/m²)                              | 3057 | 0.254                              | 4.2E-46 | -                                                                    | -       |
| Blood Pressure     | Body surface area (m²)                   | 3052 | 0.163                              | 1.4E-19 | 0.012                                                                | 0.51    |
|                    | Systolic BP (mmHg)                       | 3527 | 0.186                              | 1.1E-28 | 0.078                                                                | 4.0E-06 |
|                    | Diastolic BP (mmHg)                      | 3527 | 0.188                              | 2.2E-29 | 0.090                                                                | 8.0E-08 |
|                    | Heart rate (bpm)                         | 3527 | 0.131                              | 5.3E-15 | 0.106                                                                | 2.5E-10 |
| Lipids             | Total cholesterol (mg/dL)                | 3530 | 0.091                              | 5.2E-08 | 0.104                                                                | 5.5E-10 |
|                    | LDL-cholesterol (mg/dL)                  | 3529 | 0.020                              | 0.23    | 0.016                                                                | 0.35    |
|                    | HDL-cholesterol (mg/dL)                  | 3530 | -0.121                             | 5.0E-13 | -0.135                                                               | 7.9E-16 |
|                    | Triglycerides (mg/dL)                    | 3530 | 0.300                              | 2.4E-74 | 0.349                                                                | 6.5E-102|
|                    | Lipoprotein(a) (nmol/L)                  | 3529 | 0.031                              | 0.066   | -0.061                                                               | 3.0E-04 |
| Glucose            | Glucose (mg/dL)                          | 3530 | 0.160                              | 9.3E-22 | 0.101                                                                | 1.9E-09 |
|                    | Insulin (µU/L)                           | 3058 | 0.369                              | 1.5E-99 | 0.289                                                                | 4.3E-60 |
|                    | HOMA-IR (mass units)                     | 2975 | 0.369                              | 8.5E-97 | 0.290                                                                | 7.5E-59 |
| Liver fat          | Liver fat, H-MRS (%)                     | 2278 | 0.264                              | 1.6E-37 | 0.235                                                                | 6.9E-30 |
| Liver function tests| ALT (IU/L)                               | 3530 | 0.069                              | 3.8E-05 | 0.100                                                                | 2.3E-09 |
|                    | AST (IU/L)                               | 3530 | 0.064                              | 1.3E-04 | 0.093                                                                | 3.4E-08 |
|                    | ALP (IU/L)                               | 3530 | 0.118                              | 2.4E-12 | 0.060                                                                | 3.6E-04 |
|                    | GGT (IU/L)                               | 3530 | 0.177                              | 3.8E-26 | 0.125                                                                | 9.8E-14 |
|                    | Bilirubin (mg/dL)                        | 3530 | -0.230                             | 1.6E-43 | -0.185                                                               | 1.8E-28 |
| Renal function     | Microalbumin (mg/dL)                     | 3407 | 0.052                              | 0.0025  | -0.003                                                               | 0.88    |
|                    | Urine albumin/creatinine ratio           | 3402 | 0.060                              | 4.2E-04 | 0.015                                                                | 0.37    |
|                    | eGFR MDRD (mL/min/1.73m²)                | 3530 | 0.080                              | 2.1E-06 | 0.054                                                                | 0.0012  |
| NMR Lipoproteins                                      | Value        | Unit        | Value        | Unit        | Value        |
|------------------------------------------------------|--------------|-------------|--------------|-------------|--------------|
| eGFR MDRD4 (mL/min/1.73m²)                           | 3530         | 0.087       | 2.5E-07      | 0.050       | 0.0030       |
| Total VLDL particles (nmol/L)                         | 3402         | 0.077       | 6.9E-06      | 0.138       | 7.4E-16      |
| Large VLDL/Chylomicrons (nmol/L)                     | 3402         | 0.328       | 3.4E-06      | 0.373       | 9.4E-113     |
| Medium VLDL (nmol/L)                                 | 3402         | 0.083       | 1.4E-06      | 0.161       | 4.4E-21      |
| Small VLDL (nmol/L)                                  | 3402         | -0.026      | 0.12         | -0.010      | 0.54         |
| Total LDL particles (nmol/L)                          | 3402         | 0.193       | 8.6E-03      | 0.195       | 1.9E-30      |
| IDL particles (nmol/L)                               | 3402         | 0.188       | 2.0E-03      | 0.149       | 2.1E-18      |
| Large LDL (nmol/L)                                   | 3402         | -0.159      | 9.5E-03      | -0.183      | 4.9E-27      |
| Small LDL (nmol/L)                                   | 3402         | 0.229       | 1.3E-02      | 0.250       | 1.3E-49      |
| Medium small LDL (nmol/L)                            | 3402         | 0.237       | 1.5E-02      | 0.260       | 1.7E-53      |
| Very small LDL (nmol/L)                              | 3402         | 0.225       | 2.1E-02      | 0.245       | 7.6E-48      |
| Total HDL particles (nmol/L)                          | 3402         | 0.060       | 5.2E-02      | 0.090       | 1.5E-07      |
| Large HDL (nmol/L)                                   | 3402         | -0.180      | 2.7E-02      | -0.214      | 1.7E-36      |
| Medium HDL (nmol/L)                                  | 3402         | 0.114       | 2.1E-02      | 0.151       | 9.6E-19      |
| Small HDL (nmol/L)                                   | 3402         | 0.087       | 4.2E-02      | 0.085       | 6.4E-07      |
| Mean VLDL size (nm)                                  | 3402         | 0.351       | 2.1E-01      | 0.333       | 7.3E-89      |
| Mean LDL size (nm)                                   | 3402         | -0.223      | 1.3E-01      | -0.251      | 7.7E-50      |
| Mean HDL size (nm)                                   | 3402         | -0.207      | 2.8E-01      | -0.241      | 4.2E-46      |
| Total VLDL in triglyceride MCU (mg/dL)               | 3402         | 0.238       | 5.8E-03      | 0.315       | 3.3E-79      |
| Large VLDL in triglyceride MCU (mg/dL)               | 3402         | 0.351       | 6.1E-03      | 0.399       | 3.6E-130     |
| Medium VLDL in triglyceride MCU (mg/dL)              | 3402         | 0.101       | 3.0E-03      | 0.178       | 1.4E-25      |
| Small VLDL in triglyceride MCU (mg/dL)               | 3402         | -0.035      | 0.042        | -0.018      | 0.29         |
| Total LDL in cholesterol MCU (mg/dL)                 | 3402         | 0.103       | 1.5E-03      | 0.092       | 8.0E-08      |
| IDL in cholesterol MCU (mg/dL)                       | 3402         | 0.189       | 1.0E-03      | 0.151       | 7.2E-19      |
| Large LDL in cholesterol MCU (mg/dL)                 | 3402         | -0.160      | 7.2E-03      | -0.183      | 4.1E-27      |
| Total small LDL in cholesterol MCU (mg/dL)           | 3402         | 0.232       | 1.2E-03      | 0.254       | 4.4E-51      |
| Medium small LDL in cholesterol MCU (mg/dL)          | 3402         | 0.237       | 1.4E-03      | 0.260       | 1.7E-53      |
| Very small LDL in cholesterol MCU (mg/dL)            | 3402         | 0.226       | 1.4E-03      | 0.246       | 3.8E-48      |
| Total HDL in cholesterol MCU (mg/dL)                 | 3402         | -0.086      | 5.8E-03      | -0.077      | 7.0E-06      |
| Large HDL in cholesterol MCU (mg/dL)                 | 3402         | -0.184      | 3.6E-03      | -0.216      | 2.8E-37      |
| Medium HDL in cholesterol MCU (mg/dL)                | 3402         | 0.114       | 2.1E-03      | 0.151       | 9.6E-19      |
| Small HDL in cholesterol MCU (mg/dL)                 | 3402         | 0.086       | 5.2E-03      | 0.085       | 8.0E-07      |
| Blood Chemistries | NMR-calculated triglycerides (mg/dL) | 3402 | 0.252 | 1.6E-50 | 0.323 | 3.0E-83 |
|-------------------|--------------------------------------|------|--------|---------|--------|--------|
|                    | NMR-calculated total cholesterol (mg/dL) | 3402 | 0.111 | 8.7E-11 | 0.132 | 1.3E-14 |
|                    | Homocystine (μmol/L)                   | 3397 | 0.005 | 0.76    | 0.010 | 0.55   |
|                    | Sodium (mEq/L)                         | 3530 | -0.004 | 0.80    | 0.035 | 0.039  |
|                    | Potassium (mEq/L)                      | 3420 | -0.018 | 0.28    | 0.038 | 0.028  |
|                    | Creatinine (mg/dL)                     | 3530 | -0.028 | 0.096   | -0.051| 0.0025 |
|                    | Chloride (mEq/L)                       | 3527 | 0.012  | 0.46    | 0.019 | 0.25   |
|                    | Blood urea nitrogen (mg/dL)            | 3530 | -0.063 | 1.7E-04 | -0.036| 0.034  |
|                    | Albumin (g/dL)                         | 3530 | -0.131 | 4.4E-15 | -0.020| 0.24   |
|                    | Lactate dehydrogenase (IU/L)           | 3530 | 0.098  | 5.8E-09 | 0.022 | 0.18   |
|                    | Carbon dioxide (mEq/L)                 | 3530 | -0.074 | 1.0E-05 | -0.065| 1.0E-04|
|                    | Total protein (g/dL)                   | 3530 | 0.054  | 0.0013  | -0.007| 0.69   |
|                    | Calcium (mg/dL)                        | 3529 | -0.032 | 0.054   | 0.011 | 0.53   |
|                    | Creatine kinase (IU/L)                 | 3530 | 0.130  | 8.3E-15 | 0.038 | 0.025  |
|                    | Uric acid (mg/dL)                      | 3530 | 0.127  | 3.8E-14 | 0.090 | 9.1E-08|
|                    | Phosphorus (mg/dL)                     | 3529 | 0.068  | 5.8E-05 | 0.064 | 1.6E-04|
|                    | Iron (μg/dL)                           | 3521 | -0.169 | 4.8E-24 | -0.095| 1.6E-08|
|                    | Magnesium (mg/dL)                      | 3530 | -0.031 | 0.062   | 0.030 | 0.073  |
|                    | Sodium, urine (nmol/L)                 | 3513 | 0.061  | 2.7E-04 | 0.033 | 0.050  |
|                    | Potassium, urine (nmol/L)              | 3522 | 0.010  | 0.56    | -0.006| 0.70   |
|                    | Creatinine, urine (mg/dL)              | 3522 | 0.002  | 0.92    | -0.023| 0.18   |
|                    | Leptin (μg/L)                          | 3057 | 0.263  | 1.1E-49 | 0.202 | 1.2E-29|
|                    | PCSK9 (ng/mL)                          | 3433 | 0.274  | 3.9E-60 | 0.269 | 5.3E-58|
|                    | PCSK9 (ng/mL)                          | 3433 | 0.274  | 3.9E-60 | 0.269 | 5.3E-58|
|                    | Biomarkers                             |      |        |         |       |        |
|                    | ApoB IgG (RLU)                         | 3489 | 0.030  | 0.076   | -0.006| 0.73   |
|                    | ApoB IgM (RLU)                         | 3489 | -0.069 | 4.1E-05 | -0.056| 8.9E-04|
|                    | Adiponectin (μg/mL)                    | 3512 | -0.242 | 3.9E-48 | -0.161| 9.4E-22|
|                    | ADMA (mmol/L)                          | 3507 | -0.033 | 0.052   | -0.049| 0.0037 |
|                    | Aldosterone (ng/dL)                    | 3033 | 0.002  | 0.92    | 0.024 | 0.19   |
|                    | ANA (ELISA units)                      | 3524 | 0.022  | 0.19    | -0.015| 0.36   |
|                    | Angiogenin (ng/mL)                     | 3279 | 0.088  | 4.6E-07 | 0.058 | 8.4E-04|
|                    | Arginine (mmol/L)                      | 3456 | 0.020  | 0.23    | 0.002 | 0.90   |
|                    | Beta-Crosslaps (pg/mL)                 | 3506 | -0.127 | 5.4E-14 | -0.098| 5.4E-09|
|                      | Value 1     | Value 2     | Value 3     | Value 4     |
|----------------------|-------------|-------------|-------------|-------------|
| proBNP (pg/mL)       | 3506        | -0.108      | 1.5E-10     | -0.107      | 2.5E-10     |
| Caspase 3 (ng/mL)    | 3202        | 0.083       | 2.9E-06     | 0.046       | 0.0099      |
| CCL11 (pg/mL)        | 3268        | -0.027      | 0.12        | 0.029       | 0.094       |
| CD40 ligand (ng/mL)  | 2795        | 0.009       | 0.62        | 0.003       | 0.86        |
| CKMB (ng/mL)         | 3275        | 0.051       | 0.0037      | -0.004      | 0.80        |
| CRP (mg/L)           | 3507        | 0.165       | 7.2E-23     | 0.057       | 7.9E-04     |
| CT-1 (pg/mL)         | 3281        | 0.009       | 0.63        | -0.005      | 0.77        |
| Cu-LDL IgG (RLU)     | 3485        | 0.021       | 0.21        | -0.028      | 0.099       |
| Cu-LDL IgM (RLU)     | 3485        | -0.014      | 0.41        | -0.040      | 0.017       |
| CXCL1 (ng/mL)        | 3188        | 0.034       | 0.054       | -0.019      | 0.28        |
| CXCL10 (ng/mL)       | 3275        | -0.002      | 0.89        | -0.043      | 0.014       |
| CXCL2 (ng/mL)        | 3189        | -0.018      | 0.30        | -0.027      | 0.13        |
| Cystatin C (mg/L)    | 3342        | -0.028      | 0.11        | -0.074      | 1.7E-05     |
| D-Dimer (µg/mL)      | 3275        | 0.055       | 0.0018      | -0.034      | 0.055       |
| ECM1 (ng/mL)         | 3279        | 0.111       | 1.9E-10     | 0.087       | 5.7E-07     |
| Soluble Endoglin (ng/mL) | 3275 | -0.020      | 0.26        | -0.009      | 0.61        |
| ESAM (ng/mL)         | 3203        | 0.009       | 0.61        | -0.004      | 0.83        |
| FGF21 (ng/mL)        | 3275        | 0.022       | 0.21        | 0.019       | 0.29        |
| Folate receptor alpha C (ng/mL) | 3275 | -0.055      | 0.0016      | -0.020      | 0.26        |
| Fructosamine (µmol/L) | 3507        | -0.003      | 0.85        | -0.007      | 0.66        |
| FSH (mIU/mL)         | 1956        | 0.040       | 0.080       | -0.007      | 0.75        |
| Diluted FSH (mIU/mL) | 1033        | 0.008       | 0.79        | -0.069      | 0.028       |
| GDF-15 (ng/mL)       | 3275        | 0.008       | 0.66        | -0.031      | 0.081       |
| Homoarginine (µmol/L)| 3494        | 0.159       | 3.5E-21     | 0.087       | 2.3E-07     |
| ICAM-1 (ng/mL)       | 3281        | 0.038       | 0.030       | 0.024       | 0.18        |
| IL-18 (mg/L)         | 2215        | 0.042       | 0.048       | 0.051       | 0.017       |
| IL-6 (pg/mL)         | 3281        | -0.001      | 0.96        | 0.000       | 0.99        |
| IL1AP (ng/mL)        | 3279        | 0.007       | 0.68        | 0.039       | 0.025       |
| LH (mIU/mL)          | 924         | -0.041      | 0.21        | -0.021      | 0.52        |
| Lp-PLA2 activity (nmol/min/mL) | 3529 | -0.130      | 7.8E-15     | -0.059      | 4.9E-04     |
| Lp-PLA2 mass (ng/mL) | 3376        | -0.165      | 3.7E-22     | -0.105      | 8.9E-10     |
| LPLUNC1 (ng/mL)      | 3279        | -0.033      | 0.056       | -0.026      | 0.14        |
| Protein            | Value    | Variation | Limit Low | Limit High | P Value |
|--------------------|----------|-----------|-----------|------------|---------|
| LTBR (ng/mL)       | 3196     | 0.018     | 0.32      | -0.015     | 0.41    |
| Lumican (ng/mL)    | 3279     | 0.025     | 0.15      | -0.008     | 0.64    |
| MCP-1 (pg/mL)      | 3480     | -0.004    | 0.84      | -0.003     | 0.84    |
| MDA-LDL IgG (RLU)  | 3489     | 0.031     | 0.064     | -0.028     | 0.098   |
| MDA-LDL IgM (RLU)  | 3489     | -0.046    | 0.0061    | -0.045     | 0.0078  |
| Mesothelin (ng/mL) | 3275     | -0.059    | 7.4E-04   | -0.019     | 0.27    |
| MIP3 (ng/mL)       | 3188     | -0.139    | 4.0E-15   | -0.130     | 2.0E-13 |
| MMP9 (ng/mL)       | 3281     | 0.073     | 2.6E-05   | 0.044      | 0.012   |
| MPO (ng/mL)        | 3281     | -0.026    | 0.14      | -0.065     | 1.9E-04 |
| Myoglobin (ng/mL)  | 3275     | 0.028     | 0.11      | 0.009      | 0.60    |
| NGAL (ng/mL)       | 3206     | -0.119    | 1.6E-11   | -0.084     | 2.1E-06 |
| NTproCNP (ng/mL)   | 3275     | 0.004     | 0.82      | 0.024      | 0.16    |
| Osteoprotegerin (pg/mL) | 3509 | 0.122     | 3.6E-13   | 0.096      | 1.1E-08 |
| Osteopontin (ng/mL)| 3275     | 0.018     | 0.30      | -0.013     | 0.47    |
| Oxidized LDL (RLU)| 3489     | 0.047     | 0.0058    | -0.036     | 0.036   |
| Free PAPPA (ng/mL) | 3275     | 0.026     | 0.13      | 0.016      | 0.36    |
| PAPPA (ng/mL)      | 3281     | -0.040    | 0.024     | 0.003      | 0.85    |
| Pentraxin 3 (ng/mL)| 3275     | -0.072    | 4.0E-05   | -0.050     | 0.0039  |
| Periostin (ng/mL)  | 3275     | -0.007    | 0.70      | -0.018     | 0.32    |
| PGRP-S (ng/mL)     | 3203     | -0.041    | 0.021     | -0.060     | 6.9E-04 |
| PIGR (ng/mL)       | 3275     | -0.095    | 4.7E-08   | -0.076     | 1.5E-05 |
| PLA2 (ng/mL)       | 3279     | 0.091     | 1.7E-07   | 0.064      | 2.5E-04 |
| PLGF (pg/mL)       | 3281     | -0.013    | 0.44      | 0.029      | 0.093   |
| PSAP-B (ng/mL)     | 3275     | 0.032     | 0.068     | 0.005      | 0.77    |
| i-PTH (pg/mL)      | 3431     | 0.121     | 1.3E-12   | 0.039      | 0.023   |
| RAGE (ng/mL)       | 3281     | -0.112    | 1.4E-10   | 0.011      | 0.51    |
| SDMA (μmol/L)      | 3509     | -0.147    | 2.5E-18   | -0.137     | 2.9E-16 |
| SHBG (nmol/L)      | 925      | -0.163    | 6.5E-07   | -0.066     | 0.044   |
| ST2 (ng/mL)        | 3275     | 0.103     | 3.2E-09   | 0.036      | 0.041   |
| Syndecan-1 (ng/mL) | 3275     | -0.055    | 0.0018    | -0.025     | 0.15    |
| TNFR1A (ng/mL)     | 3275     | 0.000     | 1.00      | -0.019     | 0.27    |
| Troponin (ng/mL)   | 3506     | -0.007    | 0.67      | -0.006     | 0.73    |
High-sensitivity cardiac Troponin T (ng/L)  3527  0.032  0.059 -0.015  0.37
TROY (ng/mL)  3275 -0.006  0.74 -0.036  0.039
Calculated free testosterone (pmol/L)  923  0.189  7.3E-09  0.107  0.0011
Free Testosterone (pg/mL)  925  0.138  2.5E-05  0.105  0.0015
Total Testosterone (ng/mL)  925  0.111  7.4E-04  0.088  0.0077
VCAM-1 (ng/mL)  3281  0.073  2.9E-05  0.0077  0.31
VEGF-R1 (ng/mL)  3275  0.047  0.0077  0.018  0.50
WAP4C (ng/mL)  3275 -0.012  0.50 -0.012  0.48

**Imaging**

**DXA: Whole-body**

| Metric                          | Value   | SEM    | 95% CI   | P       |
|---------------------------------|---------|--------|----------|---------|
| **Fat mass (g)**                | 2974    | 0.224  | 5.3E-35  | 0.074   | 5.3E-05 |
| **Lean mass (g)**               | 2974    | 0.085  | 3.3E-06  | -0.018  | 0.34    |
| **Total mass (g)**              | 2974    | 0.193  | 2.2E-26  | 0.015   | 0.41    |
| **Fat percent (%)**             | 2974    | 0.164  | 1.9E-19  | 0.068   | 2.2E-04 |
| **Total area (cm²)**            | 2974    | -0.010 | 0.59     | -0.051  | 0.0056  |
| **Bone mineral content (g)**    | 2974    | 0.015  | 0.41     | -0.062  | 7.9E-04 |
| **Bone mineral density (g/cm²)**| 2974    | 0.044  | 0.018    | -0.051  | 0.0055  |

**MRI: Abdominal**

| Metric                          | Value   | SEM    | 95% CI   | P       |
|---------------------------------|---------|--------|----------|---------|
| **Subcutaneous fat (kg)**       | 2747    | 0.237  | 3.0E-36  | 0.090   | 2.3E-06 |
| **Total Intraperitoneal fat (kg)**| 2743    | 0.203  | 7.2E-27  | 0.179   | 2.8E-21 |
| **Retroperitoneal fat (kg)**    | 2747    | 0.174  | 4.7E-20  | 0.165   | 2.7E-18 |
| **Total Abdominal fat (kg)**    | 2748    | 0.253  | 1.7E-41  | 0.144   | 2.8E-14 |
| **Visceral fat (kg)**           | 2743    | 0.199  | 8.3E-26  | 0.191   | 6.4E-24 |

**MRI: Aortic**

| Metric                          | Value   | SEM    | 95% CI   | P       |
|---------------------------------|---------|--------|----------|---------|
| **Aortic compliance (µL/mmHg)**  | 2664    | -0.125 | 8.4E-11  | -0.095  | 8.6E-07 |
| **Aortic pulse wave velocity (m/s)** | 2619    | 0.059  | 0.0025   | 0.018   | 0.36    |
| **Radial aortic wall thickness (mm)** | 2507    | 0.079  | 7.9E-05  | 0.064   | 0.0014  |
| **Plaque area (mm²)**           | 2492    | 0.045  | 0.024    | 0.050   | 0.013   |
| **Aortic wall thickness by area formula (mm)** | 2507    | 0.077  | 1.1E-04  | 0.063   | 0.0017  |

**MRI: Cardiac**

| Metric                          | Value   | SEM    | 95% CI   | P       |
|---------------------------------|---------|--------|----------|---------|
| **Left ventricular mass (g)**   | 2792    | 0.083  | 1.1E-05  | -0.019  | 0.32    |
| **Left ventricular end diastolic volume (mL)** | 2792    | -0.030 | 0.12    | -0.076  | 5.6E-05 |
| **Left ventricular end systolic volume (mL)** | 2792    | -0.050 | 0.0078  | -0.060  | 0.0015  |
| **Stroke volume (mL)**          | 2791    | -0.007 | 0.72     | -0.062  | 1.0E-03 |
| **Ejection fraction (%)**       | 2792    | 0.043  | 0.022    | 0.009   | 0.63    |
| **Cardiac output (L/min)**      | 2791    | 0.079  | 2.9E-05  | 0.018   | 0.33    |
| ECG   | PR interval (msec) | 3032 | 0.075 | 3.5E-05 | 0.023 | 0.20 |
|-------|-------------------|------|-------|---------|-------|------|
|       | QRS duration (msec) | 3052 | -0.052 | 0.0038 | -0.009 | 0.63 |
|       | QT interval (msec) | 3050 | -0.113 | 3.8E-10 | -0.092 | 4.0E-07 |

Bolded text are phenotypes used in the manuscript. Abbreviations: ADMA, Asymmetric dimethylarginine; ALP, Alkaline phosphatase; ALT, Alanine transaminase; ANA, Anti-nuclear antibody; ApoB, Apolipoprotein B; AST, Aspartate aminotransferase; BMI, Body mass index; BP, Blood pressure; BPIFB1, Bactericidal/permeability-increasing fold containing family B member 1; CCL11, C-C Motif Chemokine Ligand 11; CD40, Cluster of differentiation 40; CKMB, Creatine kinase myocardial band; CRP, C-reactive protein; CT-1, Cardiotrophin-1; Cu-LDL, Copper-oxidized low-density lipoprotein; CXCL, C-X-C motif chemokine ligand; DXA, Dual energy x-ray absorptiometry; ECG, Electrocardiogram; ECM1, Extracellular matrix protein 1; eGFR MDRD, estimated Glomerular filtration rate using modification of diet in renal disease equation with albumin and blood urea nitrogen; eGFR MDRD4, estimated Glomerular filtration rate using the 4 variable modification of diet in renal disease equation without albumin and blood urea nitrogen; ELISA, Enzyme-linked immunosorbent assay; ESAM, Endothelial cell-selective adhesion molecule; FGF21, Fibroblast growth factor 21; FSH, Follicle-stimulating hormone; GDF-15, Growth differentiation factor 15; GGT, Gamma-glutamyl transferase; HDL, High-density lipoprotein; H-MRS, Proton magnetic resonance spectroscopy; HOMA-IR, Homeostatic model assessment of insulin resistance; ICAM-1, Intracellular adhesion molecule 1; IDL, Intermediate-density lipoprotein; IL, Interleukin; IL1AP, Interleukin 1 receptor accessory protein; i-PTH, intact Parathyroid hormone; LDL, Low-density lipoprotein; LH, Luteinizing hormone; Lp-PLA2, Lipoprotein-associated phospholipase A2; LTBR, Lymphotxin beta receptor; MCP-1, Monocyte chemoattractant protein 1; MCU, Mass concentration units; MDA-LDL, Malondialdehyde-modified low density lipoprotein; MIP3, Macrophage inflammatory protein 3; MMP9, Matrix metalloproteinase 9; MPO, Myeloperoxidase; MRI, Magnetic resonance imaging; NGAL, Neutrophil gelatinase-associated lipocalin; NMR, Nuclear magnetic resonance; NTproCNP, N-terminal propeptide of C-type natriuretic peptide; PAPP-A, Pregnancy-associated plasma protein A; PCSK9, Proprotein convertase subtilisin/kexin type 9; PGRP-S, Peptidoglycan recognition protein S; PLGR, Polymeric immunoglobulin receptor; PLA2, Phospholipase A2; PLGF, Placental growth factor; proBNP, Prohormone of brain natriuretic peptide; PSAP-B, Prosaposin B; RAGE, Receptor for advanced glycation end products; RLU, Relative light unit; SDMA, Symmetrical dimethylarginine; SHBG, Sex hormone binding globulin; ST2, Soluble interleukin 1 receptor-like 1; TNFR1A, Tumor necrosis factor receptor 1A; TROY, Tumor necrosis factor receptor superfamily member 19; VCAM-1, Vascular cell adhesion molecule 1; VEGF-R1, Vascular endothelial growth factor receptor 1; VLDL, Very low density lipoprotein; WAP4C, Whey acidic protein 4 disulfide core.
| Variable               | Beta (SE)     | P-value   | Partial R-squared (%) |
|------------------------|---------------|-----------|-----------------------|
| Female sex             | 0.069 (0.626) | 0.040     | 0.13                  |
| Race/Ethnicity         |               |           |                       |
| Whites                 | Ref           | -         | -                     |
| Blacks                 | 0.626 (0.255) | 1.0E-65   | -                     |
| Hispanics              | 0.255 (0.371) | 3.3E-07   | -                     |
| Other                  | 0.371 (-0.005)| 1.2E-04   | -                     |
| Age (years)            | -0.005 (0.009)| 1.8E-04   | 0.43                  |
| BMI (kg/m²)            | 0.009 (0.011) | 1.6E-04   | 0.44                  |
| Insulin (IU)           | 0.011 (0.003) | 3.8E-20   | 2.56                  |
| TG (mg/dL)             | 0.003 (-0.005)| 3.7E-47   | 6.19                  |
| HDL-C (mg/dL)          | -0.005 (0.001)| 2.7E-05   | 0.54                  |

Multiple R-squared (adjusted): 22.0%. Abbreviations: BMI, Body mass index; HDL-C, High-density lipoprotein-cholesterol; SE, Standard error; TG, Triglycerides.
| SNP       | Position, Bp (GRCh37) | Gene          | SNP effect       | Amino acid change | Alleles | Single-SNP analysis | Conditional analysis | Alt allele frequency | LD R² with rs2278426 |
|-----------|------------------------|---------------|------------------|-------------------|---------|---------------------|----------------------|---------------------|----------------------|
| rs200269584 | 19 11348329            | DOCK6         | SNP             | P653P             | G A     | Beta (SE) P-value   | Beta (SE) P-value   | Global/B/W           | B/W/H                |
| rs35464191  | 19 11361620            | DOCK6         | SNP             | R217Q             | C T     | 0.793 (0.174) 5.7E-06 | 1.002 (0.159) 3.3E-10 | 0.004/0.007/0.003     | 0.001                |
| rs145750006 | 19 11352323            | C19orf80/ANGPTL8 | Intron        | T G               | 0.804 (0.181) 9.0E-06 | 0.997 (0.165) 1.5E-09 | 0.004/0.007/0.003     | 0.001                |
| rs115059172 | 19 11354398            | DOCK6         | SNP             | C A               | 0.093 (0.053) 0.08267 | 0.231 (0.049) 2.4E-06 | 0.048/0.088/0.001   | 0.016/0.002/0.001   |
| rs1541922  | 19 11350433            | C19orf80/ANGPTL8 | H40H          | T C               | 0.150 (0.060) 0.01296 | 0.214 (0.055) 9.8E-05 | 0.038/0.069/0.001   | 0.02/0.002/0.004    |
| rs59168178  | 19 11350326            | C19orf80/ANGPTL8 | SNP             | A5T              | 0.006 (0.052) 0.80965 | 0.156 (0.048) 0.00115 | 0.052/0.096/0.001   | 0.017/0.004/0.005   |
| rs755238   | 19 11304498            | KANK2         | SNP             | S86S             | C T     | -0.037 (0.043) 0.39065 | 0.120 (0.040) 0.00251 | 0.076/0.064/0.007   | 0.02/0.01/0.047    |
| rs755237   | 19 11304404            | KANK2         | SNP             | G118S            | C T     | -0.040 (0.042) 0.34902 | 0.118 (0.039) 0.00266 | 0.078/0.064/0.086   | 0.022/0.0/0.047    |

**Abbreviations:** Alt, Alternate; ANGPTL8, Angiopoietin-like 8; B, Black; Bp, Base pair; DOCK6, Dedicator of cytokinesis 6; H, Hispanic; KANK2, KN motif and ankyrin repeat domain-containing protein 2; LD, Linkage disequilibrium; Ref, Reference; SE, Standard error; SNP, Single nucleotide polymorphism; W, Whites.
1. Lakoski SG, Xu F, Vega GL, Grundy SM, Chandalia M, Lam C, Lowe RS, et al. Indices of cholesterol metabolism and relative responsiveness to ezetimibe and simvastatin. J Clin Endocrinol Metab 2010;95:800-809.