SUPPLEMENTAL MATERIAL

Supplemental Table 1. Sample quality control metrics and exclusions.

Statistic	Q1*	Mean	Q3*	Lower Bound†	Upper Bound†	Broad	UW	Baylor
Samples jointly called						2317	481	475
Total # of variants	19289	19480	19696	18068	20917	16	2	1
# Singletons	56	94.54	135	0	372	1	0	0
# Doubletons	54	65.583	77	0	146	6	2	2
TiTv	3.22	3.252	3.282	3.034	3.468	0	0	0
Het/Hom	5.912	6.215	6.538	4.031	8.419	12	3	1
Missingness	0.014	0.022	0.028	0	0.069	6	2	1
Poor concordance						10	0	1
Total Excluded (%)						36 (1.6%)	7 (1.5%)	7 (1.5%)
Total Remaining						2281	474	468

* Q1 and Q3 refer to the 1st and 3rd quartiles, respectively

† Lower and Upper Bounds are the bounds used for excluding outlier individuals (> ± 3 x interquartile range)

Supplemental Table 2. Variant descriptive statistics.

Sites were limited to those labeled as VQSR PASS and quality depth (QD) ≥ 2.

# of variant sites	MAF* < 5%	MAF ≥ 5%
Total	729,666	55,015
Nonsense	7,791	128
Frameshift	6,574	285
Splice-site	2,898	59
Damaging missense†	49,929	343
Non-damaging missense	354,017	21,701
Non-protein altering	308,457	32,499

* MAF, minor allele frequency.

† Damaging missense variants were classified according to the following 7 in silico prediction algorithms: LRT, Mutation Taster, PolyPhen2 (HumDiv), PolyPhen2 (HumVar), SIFT, MutationAssessor and FATHMM

Supplemental Table 3. All gene-based association results reaching a significance level of 1.1×10^{-7}.

Outcome	Gene	Set of variants*	# variants†	# Carriers‡	Beta±SE	P-value
LDL-C	*PCSK9*	Null	3	72	-44.1±4.7	1.61×10^{-20}
(mg/dl)	*PCSK9*	Null+≥7/7 damaging	3	72	-44.1±4.7	1.61×10^{-20}
	PCSK9	Null+≥6/7 damaging	7	116	-35.8±3.8	2.86×10^{-21}
	PCSK9	Null+≥5/7 damaging	10	156	-27.0±3.3	2.49×10^{-16}
Total cholesterol	*PCSK9*	Null	2	60	-38.6±5.6	6.55×10^{-12}
(mg/dl)	*PCSK9*	Null+≥7/7 damaging	2	60	-38.6±5.6	6.55×10^{-12}
	PCSK9	Null+≥6/7 damaging	6	94	-30.3±4.5	2.52×10^{-11}
	PCSK9	Null+≥5/7 damaging	9	126	-22.8±3.9	7.35×10^{-9}
MCH (pg)	*HBQ1*	Null+≥6/7 damaging	1	85	-2.0±0.3	8.44×10^{-13}
	HBQ1	Null+≥5/7 damaging	1	85	-2.0±0.3	8.44×10^{-13}
MCV (fL)	*HBQ1*	Null+≥6/7 damaging	1	85	-4.9±0.7	4.40×10^{-12}
	HBQ1	Null+≥5/7 damaging	1	85	-4.9±0.7	4.40×10^{-12}
Red blood cell distribution width [RDW]	*VPS13A*	Null+≥5/7 damaging	9	34	1.3±0.2	7.13×10^{-8}

* Null variants are defined as nonsense, splice-site, and frameshift variants. Damaging missense variants were classified according to the following 7 in silico prediction algorithms: LRT, Mutation Taster, PolyPhen2 (HumDiv), PolyPhen2 (HumVar), SIFT, MutationAssessor and FATHMM.

† # variant sites, number of sites going into the gene-based test

‡ # Carriers, number of individuals carrying a null or damaging missense variant tested

Supplemental Table 4. Single variant results contributing into the gene-based tests reaching a significance level of 1.1 x 10^{-7}.

Single variant regression results not reported for variants with less than 3 carriers.

Trait	Gene	chr:pos_REF_ALT*	Protein Change	N	MAC	Call rate	MAF	Beta±SE	p-value
LDL-C	*PCSK9*	1:55512222_C/G	p.Y142*	2950	12	1	0.20%	-51.4±11.4	$5x10^{-6}$
(mg/dl)		1:55512262_T/C	p.W156R	2950	1	1	0.02%		
		1:55518064_G/A	p.G213R	2950	1	1	0.02%		
		1:55518419_G/A	p.V252M	2945	2	1	0.03%		
		1:55518422_C/T	p.L253F	2945	40	1	0.68%	-21.6±6.4	$8x10^{-04}$
		1:55527221_C/T	p.Q619*	2680	1	0.91	0.02%		
		1:55529215_C/A	p.C679*	2928	62	0.99	1.06%	-43.2±5.1	$7x10^{-17}$
MCH (pg)	*HBQ1*	16:230724_G/C	p.G52A	2103	88	0.75	2.09%	-1.8±0.3	$7x10^{-12}$
RDW	*VPS13A*	9:79792694_C/T	p.S25F	2780	1	1	0.02%		
(%)		9:79816302_A/G	p.K56E	2779	1	1	0.02%		
		9:79852982_C/T	p.L554F	2779	2	1	0.04%		
		9:79867171_C/T	p.R731*	2780	1	1	0.02%		
		9:79917908_G/A	p.R1297Q	2780	1	1	0.02%		
		9:79959197_G/GTAA	p.E2385L 2386ins*	2778	1	1	0.02%		
		9:79973335_G/C	p.K2672N	2780	22	1	0.40%	1.0±0.3	$9x10^{-04}$
		9:79973337_C/T	p.S2673L	2780	4	1	0.07%	5.0±0.7	$5x10^{-13}$
		9:80022452_C/T	p.R3135*	2777	1	1	0.02%		

* Chr:pos_REF_ALT is in hg19.

Supplemental Table 5. Comparison of phenotypes for compound heterozygous null *PCSK9* carriers, heterozygous null *PCSK9* carriers, and non-carriers.

Values reported as means for each category of *PCSK9* carrier status. Regression results (beta, SE, p-value) compare carriers of *PCSK9* null mutations (n=80) to non-carriers (n=3,143) adjusting for age and sex. Wilcoxon rank sum test compares the distribution of *PCSK9* compound nulls (n=3) to individuals carrying one null mutation in PCSK9 (n=77).

Trait	Non-carrier (n=3143)	Frame shift (n=1)	p.C679X (n=64)	p.Q619X (n=2)	p.Y142X (n=10)	p.Y142X & p.Q679X (n=3)	Beta±SE	p-value	Wilcoxon p-value
LDL-C (mg/dl)	132.9	--	89.80	71	89.60	55.93	-44.7±4.6	$2.5x10^{-22}$	0.04
Total Cholesterol (mg/dl)	207.0	--	170.64	--	159.89	161.50	-38.4±5.5	$4.2x10^{-12}$	0.73
HDL-C (mg/dl)	51.5	--	55.41	53	49.33	67.33	3.8±1.7	0.02	0.45
MCH (pg)	28.9	31.8	29.50	30.25	28.54	29.30	0.6±0.3	0.05	0.92
MCV (fL)	87.0	94.3	88.44	91.8	86.21	87.90	1.4±0.8	0.06	0.77
QRS interval (msec)	92.0	94	93.51	90	95.71	100.00	1.9±1.1	0.09	0.44
DBP (mmHg)	81.5	80	83.72	91.5	79.00	84.33	1.9±1.2	0.11	1

Adiponectin* (ng/ml)	5360.4	2670.9	5082.54	3308.65	3511.22	3539.13	-0.1±0.07	0.13	0.63
Endothelin* (pg/ml)	1.3	1	1.39	1.3	1.35	1.97	0.07±0.06	0.15	0.17
RDW (%)	13.7	12.8	13.41	14	13.91	13.63	-0.2±0.2	0.15	0.70
Renin* (ng/ml/hr)	1.7	1.7	0.42	--	1.89	1.00	-0.3±0.2	0.17	0.28
MCHC (%)	33.2	33.7	33.32	32.9	33.03	33.30	0.1±0.1	0.23	0.90
Height (cm)	169.1	168	170.09	172	166.00	164.67	0.7±0.7	0.36	0.32
hsCRP* (mg/dl)	0.5	2.7	0.55	0.4745	0.55	2.21	0.1±0.1	0.36	0.86
Red cell count (m/cmm)	4.5	3.5	4.46	3.98	4.63	4.85	-0.05±0.05	0.37	0.34
Glucose (mg/dl)	90.6	--	88.92	87	94.67	91.00	-0.9±1.1	0.42	0.28
SBP (mmHg)	132.1	134	133.42	146.5	131.70	134.00	1.6±2.0	0.44	0.79
QT interval (msec)	413.4	378	412.34	389	425.50	369.33	-2.6±3.4	0.44	0.006
Aldosterone* (ng/dl)	5.8	1.9	6.23	2.4	6.42	4.03	0.06±0.08	0.47	0.50
Hemoglobin	13.0	11.2	13.09	12.05	13.20	14.10	0.09±0.1	0.51	0.13

(g/dl))									
sCort (ug/dl)	9.9	5.3	9.49	11.2	10.20	9.07	-0.3±0.5	0.55	0.99
Hematocrit (%)	39.2	33.3	39.33	36.6	40.00	42.53	0.2±0.4	0.55	0.25
Weight (kg)	91.3	74.7	94.53	79.7	87.97	80.13	1.3±2.4	0.58	0.32
Leptin* (ng/ml)	28.4	19.6	30.03	19	23.22	27.33	-0.03±0.08	0.69	0.90
Insulin (IU/ml)	15.7	--	16.60	7	14.00	12.50	0.3±1.1	0.77	0.68
HOMA-B (mmol/l)	215.6	--	232.40	107.146	170.66	170.25	3.9±13.6	0.78	0.66
Waist (cm)	101.3	94	100.48	107.5	102.90	98.67	-0.5±1.8	0.79	0.80
Neck (cm)	38.7	36	38.80	36.5	39.40	36.67	0.09±0.4	0.81	0.23
BMI (kg/m^2)	31.9	26.5	32.17	27.84	32.11	29.56	-0.1±0.8	0.89	0.55
HOMA-IR (mmol/l)	3.6	--	3.70	1.50	3.37	2.80	0.04±0.3	0.90	0.79
Triglycerides* (mg/dl)	107.4	--	103.56	39	104.78	91.00	-0.002±0.06	0.97	0.85
HbA1c (%)	5.51	--	5.47	5.9	5.74	5.40	-0.001±0.06	0.98	0.77

* Regression model output (beta, standard error, and p-value) are based on log(trait) as outcome due to skewed distributions of raw trait.

Supplemental Table 6. Hematological trait association results for *VPS13A*.

Outcome	# Carriers	Beta	SE	P-value
Red blood cell distribution width (%)	34	1.30	0.24	7.13×10^{-08}
Hemoglobin (g/dl)	37	-0.72	0.21	6.98×10^{-04}
Mean corpuscular hemoglobin (pg)	34	-1.44	0.44	1.22×10^{-03}
Hematocrit level (%)	37	-1.85	0.59	1.86×10^{-03}
Mean corpuscular volume (fL)	34	-3.41	1.13	2.63×10^{-03}
Mean corpuscular hemoglobin concentration (%)	34	-0.45	0.16	5.19×10^{-03}
Red cell count (m/cmm)	34	-0.09	0.08	0.25

Supplemental Table 7. Evidence of association in JHS data for genes reported in Li et al.

We did not analyze lactate levels, reported in Li et al to be associated with *WDR62*. We did not find any carriers of rare loss of function variation in *PLEKHG1*, reported in Li et al to be associated with creatinine.

MAC (minor allele count), number of minor alleles across the variant sites; % Carriers, percent of individuals carrying a null or damaging missense variant tested; Beta, SE (standard error), p-value are from a regression model adjusting for age, sex, and PCs.

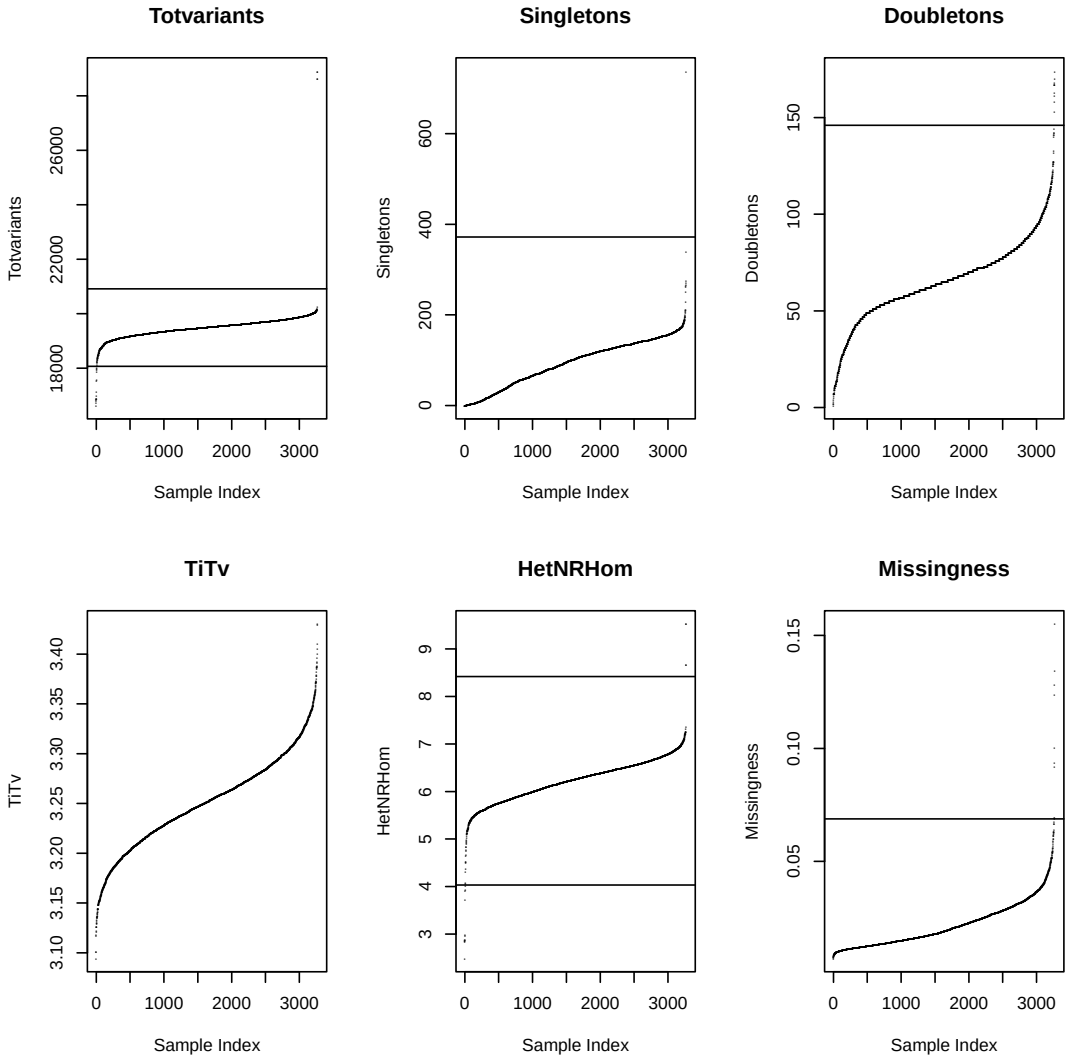
Outcome	Gene	Mutations	MAC	% Carriers	Beta±SE	P-value
Total Cholesterol (mg/dl)	*PCSK9*	p.Y142* p.Q619*	60	2.51%	-38.6±5.6	$6.6x10^{-12}$
Log(triglycerides) (log[mg/dl])	*APOC3*	p.R19* c.55+1G>G c.179+1G>T p.W62* p.T94Lfs*	7	0.24%	-0.9±0.2	$1.0x10^{-05}$
Fasting glucose* (mg/dl)	*TXNDC5*	p.T297Rfs*19	3	0.13%	9.4±5.2	0.07
Fasting glucose* (mg/dl)	*GLIPR1L2*	p.E204Dfs*56	1	0.04%	-3.3±8.6	0.70
Log(triglycerides) (log[mg/dl])	*TIGIT*	p.Q139*	1	0.03%	-0.1±0.5	0.85

* The analysis of fasting glucose excluding individuals with type 2 diabetes.

Supplemental Table 8. Results for Mendelian lipid genes. Best Test indicates the group of variants that provided the most significant results for the gene. # variant sites, number of sites going into the gene-based test; MAC (minor allele count), number of minor alleles across the variant sites; % Carriers, percent of individuals carrying a null or damaging missense variant tested; Beta, SE (standard error), p-value for association.

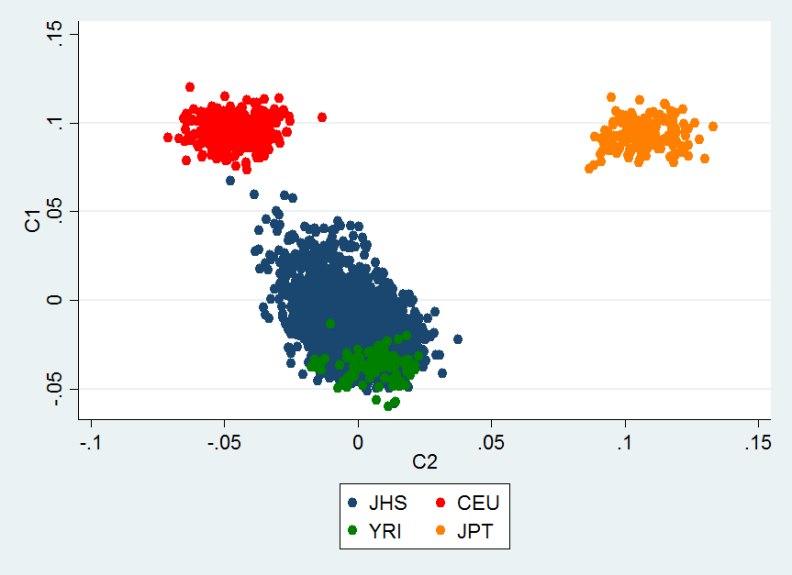
			Best Test					Null variants only				
Outcome	**Gene**	**Test**	**# variant sites**	**MAC**	**% Carriers**	**Beta±SE**	**P-value**	**# variant sites**	**MAC**	**% Carriers**	**Beta±SE**	**P-value**
LDL-C (mg/dl)	PCSK9	Null+≥6/7 damaging	7	116	3.9%	-35.8±3.8	$2.8x10^{-21}$	3	72	2.4%	-44.1±4.7	$1.6x10^{-20}$
	LDLR	Null+≥7/7 damaging	4	6	0.2%	63.9±15.7	$4.7x10^{-05}$			NA		
	LDLRAP1	Null+≥5/7 damaging	3	3	0.1%	58.8±21.9	0.007	1	1	0.0%	65.2±38.5	0.090
	ABCG5	Null+≥7/7 damaging	2	2	0.1%	50.5±26.4	0.056	2	2	0.1%	50.5±26.4	0.056
	MTTP	Null+≥5/7 damaging	3	4	0.1%	-31.6±19.0	0.096	1	2	0.1%	-33.8±27.2	0.215
	ABCG8	Null+≥5/7 damaging	12	141	4.8%	-2.1±3.4	0.538	2	5	0.2%	4.7±17.2	0.787
	ANGPTL3	Null+≥5/7 damaging	6	15	0.5%	-3.9±10.3	0.704	4	13	0.4%	-1.5±11.2	0.895
	APOB	Null+≥7/7 damaging	1	1		NA		1	1		NA	
HDL-C (mg/dl)	CETP	Null+≥5/7 damaging	3	7	0.2%	20.9±5.4	0.0001	3	7	0.2%	20.9±5.4	0.0001
	ABCA1	Null+≥6/7 damaging	13	15	0.5%	-7.0±3.6	0.052	4	4	0.1%	-9.6±6.9	0.166
Log(TG) (log(mg/dl))	LIPC	Null+≥5/7 damaging	17	43	1.4%	0.1±0.1	0.144	3	9	0.3%	0.03±0.2	0.880
	LPL	Null+≥5/7 damaging	2	2	0.1%	0.02±0.4	0.946			NA		

Supplemental Figure 1. Distribution of sample QC metrics.

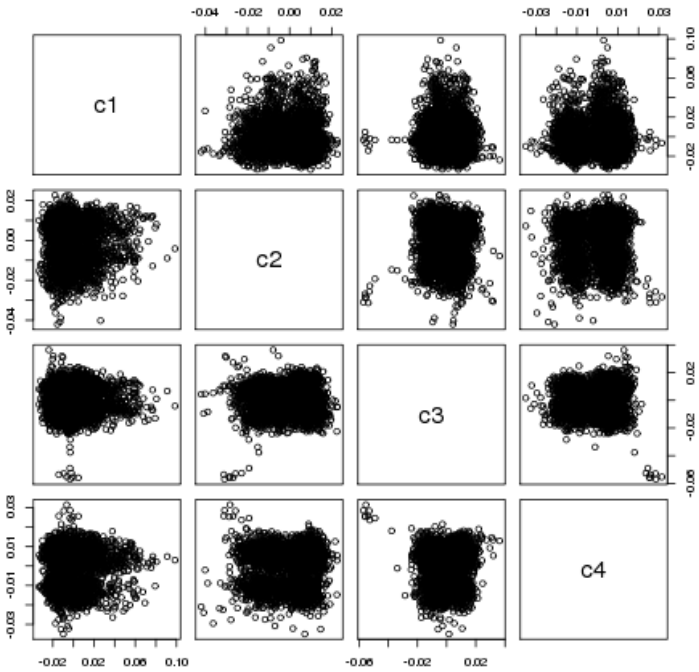


Supplemental Figure 2. Principal components (PCs) of ancestry.

A. PCs of Jackson Heart Study samples with HapMap samples



B. PCs of Jackson Heart Study samples only.



Supplemental Figure 3. Distribution of LDL-C in *PCSK9* null carriers and non-carriers. Distribution of LDL-C in individuals carrying a null mutation in *PCSK9* (n=77; purple) is lowered compared to individuals that do not carry a null mutation in *PCSK9* (n=3,143; pink). Individuals carrying two null mutations in *PCSK9* (n=3; green lines) have lower LDL-C compared with individuals carrying a single null mutation in *PCSK9* (p=0.044 assessed with a Wilcoxon rank sum test).