Supplemental Material

Methods

Study Population

ARIC is a prospective study of CVD in 15,792 middle-aged adults recruited from four U.S. communities in 1987–1989 (1). The current study was conducted among participants in ARIC visit 4 (1996–1998). Of 11,656 eligible individuals, we excluded those with self-reported race neither white nor black (n=31) and African American participants at the Minnesota and Washington County field centers (n=38) because of small enrollment numbers, individuals missing data for LDL-TG, RLP-C, or other covariates (n=1524), and those with prevalent coronary heart disease (CHD) (n=632) or ischemic stroke (n=97) at visit 4. Therefore, 9334 individuals were included in this analysis (Figure 1).

Prevalent CHD and stroke were defined as self-reported myocardial infarction or stroke before ARIC visit 1; or silent myocardial infarction (diagnosed by electrocardiographic changes), validated myocardial infarction, coronary revascularization, or stroke between ARIC visits 1 and 4. Incident CVD events were a composite of incident CHD and incident ischemic stroke after visit 4 and through December 31, 2013. Methods of assessing incident CHD events and ischemic strokes in ARIC have been described (2,3). Briefly, incident CHD events included fatal CHD, definite or probable myocardial infarction, silent myocardial infarction determined by electrocardiography, and coronary revascularization. Incident stroke events included only ischemic strokes, defined as validated definite or probable hospitalized embolic or thrombotic strokes. The median (25th percentile, 75th percentile) follow-up for CVD, CHD, and ischemic stroke events was 15.6 (10.8, 16.6) years, 15.6 (11.5, 16.6) years, and 15.8 (13.8, 16.7) years,

respectively. The mean follow-up for CVD, CHD, and ischemic stroke events was 13.3±4.83 years, 13.5±4.71 years, and 14.2±4.11 years, respectively.

Medical history, demographic data, anthropometric data, blood pressure measurements, lipid assessments, and blood for RLP-C and LDL-TG measurements were obtained during ARIC visit 4. Research protocols were approved by each field center's institutional review board; all participants provided written informed consent.

Lipoprotein and Lipid Assays

All lipid measurements were performed in the ARIC lipid laboratory at Baylor College of Medicine. Lipids were measured in 12-hour fasting plasma stored at –70°C with ethylenediaminetetraacetic acid. Total cholesterol, HDL-C, and TGs were measured using enzymatic measures (4). RLP-C (5) and LDL-TG (6) were determined by fully automated detergent-based homogeneous methods (Denka Seiken, Tokyo, Japan). Interassay coefficients of variation for the RLP-C and LDL-TG assays were 6.8% and 12.0%, respectively. The automated homogeneous LDL-TG method used for our study was validated against the standard sequential density ultracentrifugation method (7).

Statistical Analysis

Distributions of continuous variables were evaluated for normality. LDL-TG and RLP-C were modeled as both continuous and categorical (quartiles) variables. Associations between both exposure variables and outcomes, including overall CVD and incident CHD or incident ischemic stroke, were determined using Cox proportional-hazards modeling in unadjusted and adjusted models. Linear terms representing quartile number were used to obtain a p-value for trend. The

basic model (model 1) was adjusted for age, gender, and race. Model 2 included all components of model 1 plus traditional cardiovascular risk factors in the Pooled Cohort Risk Equation (8), including total cholesterol, HDL-C, systolic blood pressure, antihypertensive medication use, smoking status (current versus not current), and presence of diabetes (fasting blood glucose ≥126 mg/dL, nonfasting blood glucose ≥200 mg/dL, self-reported physician diagnosis, or diabetes medication use). To assess the extent to which LDL-TG provides incremental value in the prediction of future CVD risk beyond circulating TG and apoB levels we used statistical measures of discrimination including the area under the receiver operating characteristic curve (AUC) (9), net reclassification index (NRI), and integrated discrimination index (IDI) (10) to calculate the incremental value of adding the individual lipid measures separately to the PCE model (including all variables of the PCE risk equation) and then all three lipid measures together to the PCE model. Kaplan–Meier survival curves were calculated for each outcome across RLP-C and LDL-TG quartiles. In primary CVD or stroke survival analyses, we assumed that individuals who died of causes other than CVD or stroke were still at risk of developing CVD or a stroke. To address this biologically untenable assumption, we also performed a sensitivity analysis using the Fine and Gray approach to competing risks (11). The results showed that the conventional Kaplan–Meier survival analysis led to overestimation of the event rates compared with the Fine and Gray approach to competing risks.

Genetic Methods and Analysis

In a targeted gene approach, we investigated candidate genes and well-established variants within those genes (*LPL*, *LIPC*, *LIPG*, *APOC3*, *APOA5*, *ANGPTL3,* and *ANGPTL4*) and *APOE* haplotypes with respect to LDL-TG and RLP-C.

In an unbiased approach, genotypes were obtained from the Illumina HumanExome BeadChip, capturing suggestively functional exonic variants for 8003 European Americans and 2153 African Americans. Associations between RLP-C and LDL-TG levels and nonsynonymous common coding genomic variants (minor allele frequency [MAF] >1%) were evaluated using single-variant analysis and gene-based burden tests, aggregating variants with MAF ≤1%. Only genes with cumulative minor allele count ≥3 in both European Americans and African Americans (13,690 genes) were included in the analysis. Race-specific analyses were performed, followed by a meta-analysis using R seqMeta (12). The inverse variance–weighted fixed effects method was used for the single-variant meta-analyses (13). Gene-based analyses was performed using the T1 count method (12,14). All analyses were adjusted for age, gender, and population stratification (using the first three principal components). In single-variant analysis, associations reaching the predefined threshold of 2.5×10^{-8} (accounting for 1,000,000 independent variants and 2 traits) were considered statistically significant. In gene-based analysis, gene–trait pairs reaching the threshold of 1.83×10^{-6} (accounting for 13,690 genes and 2 traits) were considered statistically significant.

Whole exome sequencing for 5847 European Americans and 1915 African Americans was completed at Baylor College of Medicine Human Genome Sequencing Center (HGSC). Exomes were captured using the HGSC VCRome 2.1 reagent (15) (42Mb, NimbleGen), and all samples were paired-end sequenced using Illumina GAII or HiSeq instruments. Variant calling was done using Atlas2 (16) suite.

Whole exome variants were annotated using ANNOVAR (17) and dbNSFP v2.0 (18) according to the reference genome GRCh37 and National Center for Biotechnology Information RefSeq. Coding variants were annotated to a unique gene as well as to splicing or

nonsynonymous categories for single-variant tests. Detailed methods for sequencing, variant calling, and variant quality control are published (19)**.**

Both exome chip and whole exome sequencing were available in 5767 European Americans and 1857 African Americans. rs2070895 was imputed in ARIC participants using the 1000 Genomes Project reference panel (20,21). The imputation quality was 0.929 and 0.971 for African Americans and European Americans, respectively.

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Online Table 1. Common variants (MAF >1%) significantly associated in meta-analysis (p ≤ 2.5E-8)

						Meta-Analysis				African-Americans				European-Americans						
Trait	Closest Gene	Chr:Position	SNP name	Ref	Alt	MAF	p-value	Beta	SE	MAF	p-value	Beta	SE	MAF	p-value	Beta	SE	Function	Amino-acid ref/alt	CADD_phred
log(LDL-TG)	GCKR	2:27730940	rs1260326	T	C	0.353	8.63E-09	0.037	0.006	0.142	0.113701	0.031	0.020	0.409	2.99E-08	0.037	0.007	splice	[L|X]/[P|R]	0.108
log(LDL-TG)	APOE	19:45412079	rs7412	C	T	0.087	5.68E-39	-0.139	0.011	0.109	2.35E-10	-0.140	0.022	0.081	3.39E-30	-0.138	0.012	nonsynonymous	R/C	30
log(RLPC)	APOB	2:21225281	rs1042034	C	T	0.205	6.04E-10	-0.098	0.016	0.156	0.002332	-0.110	0.036	0.219	6.63E-08	-0.096	0.018	nonsynonymous	S/N	0.005
log(RLPC)	APOB	2:21231524	rs676210	G	A	0.204	4.83E-10	-0.099	0.016	0.156	0.001708	-0.113	0.036	0.217	6.88E-08	-0.096	0.018	nonsynonymous	P/L	27.1
log(RLPC)	GCKR	2:27730940	rs1260326	T	C	0.351	4.72E-16	0.113	0.014	0.142	0.020241	0.088	0.038	0.408	5.58E-15	0.117	0.015	splice	[L|X]/[P|R]	0.108
log(RLPC)	MLXIPL	7:73012042	rs35332062	G	A	0.114	1.52E-10	-0.130	0.020	0.072	0.230246	-0.061	0.051	0.126	1.02E-10	-0.144	0.022	splice	A/V	18.4
log(RLPC)	MLXIPL	7:73020337	rs3812316	C	G	0.107	5.48E-12	-0.146	0.021	0.042	0.390677	-0.057	0.067	0.125	2.92E-12	-0.156	0.022	nonsynonymous	Q/H	19.07
log(RLPC)	LPL	8:19819724	rs328	C	G	0.097	3.44E-15	-0.173	0.022	0.073	0.003146	-0.149	0.050	0.103	2.51E-13	-0.179	0.024	stop	S/X	43
log(RLPC)	ZNF259	11:116655600	rs35120633	G	A	0.058	1.11E-25	0.289	0.028	0.026	0.000857	0.276	0.083	0.067	2.98E-23	0.290	0.029	nonsynonymous	A|P/V|S	21.9
log(RLPC)	APOA5	11:116662407	rs3135506	G	C	0.065	1.93E-25	0.271	0.026	0.058	0.001998	0.175	0.057	0.067	3.97E-24	0.296	0.029	nonsynonymous	S/W	25.2
log(RLPC)	APOE	19:45412079	rs7412	C	T	0.087	2.64E-32	0.267	0.023	0.108	6.10E-11	0.275	0.042	0.081	6.01E-23	0.264	0.027	nonsynonymous	R/C	30

Online Table 2. T1 results (MAF ≤1%, MAC ≥3 in both AA and EA) significantly associated in meta-analysis (p ≤1.83E-6)

		Meta-analysis						African-Americans						European-Americans					
Trait	Gene	cMAF	p-value	Beta	SE	cMAC	#SNPs	cMAF	p-value	Beta	SE	cMAC	#SNPs	cMAF	p-value	Beta	SE	cMAC	#SNPs
log(RLPC)	APOC3	0.0019	9.35E-07	-0.715	0.146	37	3	0.0047	0.011546	-0.486	0.192	20	3	0.0011	4.56E-06	-1.024	0.223	17	3
log(LDL-TG)	TARM1	0.0003	4.04E-07	-0.939	0.185	20	1	0.0036	0.376152	0.096	0.108	15	2	0.0003	4.04E-07	-0.939	0.185	5	1

Online Table 3. Rare nonsynonymous and splicing exonic variants in TARM1 and APOC3.

						Meta-Analysis					African-Americans					European-Americans							
Trait	Gene	Chr:Position	SNP name	Ref	Alt	MAF	p-value	Beta	SE	MAC	MAF	p-value	Beta	SE	MAC	MAF	p-value	Beta	SE	MAC	Function	Amino-acid ref/alt	CADD_phred
log(LDL-TG)	TARM1	19:54578196	rs2361558	C	T	0.0003	4.04E-07	-0.939	0.185	5	NA	NA	NA	NA	NA	0.0003	4.04E-07	-0.939	0.185	5	nonsynonymous	E/K	8.402
log(LDL-TG)	TARM1	19:54578328	rs17305269	A	G	0.0115	0.370159	-0.025	0.027	228	0.0014	0.196382	0.235	0.182	6	0.0142	0.269437	-0.031	0.028	222	nonsynonymous	S/P	16.61
log(LDL-TG)	TARM1	19:54573300	rs139802953	C	T	0.0104	0.725614	0.010	0.027	206	0.0022	0.884562	0.019	0.134	9	0.0126	0.742774	0.009	0.028	197	nonsynonymous	R/Q	0.039
log(RLPC)	APOC3	11:116701353	rs76353203	C	T	0.0005	0.014926	-0.323	0.133	10	0.0005	0.066444	-0.577	0.314	2	0.0005	0.067271	-0.268	0.147	8	stop	R/X	32
log(RLPC)	APOC3	11:116701560	rs147210663	G	A	0.0011	0.179266	-0.126	0.094	21	0.0029	0.359734	-0.118	0.128	12	0.0006	0.323477	-0.136	0.138	9	nonsynonymous	A/T	23.6
log(RLPC)	APOC3	11:116701613	rs140621530	G	T	0.0003	0.003655	-0.520	0.179	6	0.0012	0.004211	-0.568	0.199	5	0.0001	0.451172	-0.312	0.414	1	splice	-	25

Online Table 4. Comparisons of PCE model and PCE plus apoB, triglycerides, or LDL-TG with differences in AUC, NRI, and IDI for risk prediction of CVD						
	C-statistics Primary Model (95% CI)	C-statistics Extended Model (95% CI)	ΔAUC (95% CI)	NRI (95% CI)	Continuous NRI (95% CI)	IDI (95% CI)
PCE vs PCE+apoB	0.7196 (0.7098, 0.7320)	0.7202 (0.7109, 0.7321)	0.0007 (0.00001, 0.0020)	0.0031 (-0.0127, 0.0205)	0.0976 (0.0156, 0.1764)	0.0005 (-0.0001, 0.0019)
PCE vs PCE+log(TG)	0.7196 (0.7098, 0.7320)	0.7199 (0.7100, 0.7323)	0.0003 (-0.0001, 0.0015)	0.0030 (-0.0142, 0.0208)	0.0519 (-0.0013, 0.1173)	0.0007 (0.00002, 0.0022)
PCE vs PCE+log(LDL-TG)	0.7196 (0.7098, 0.7320)	0.7216 (0.7117, 0.7335)	0.0021 (0.0006, 0.0041)	0.0079 (-0.0106, 0.0308)	0.0801 (0.0116, 0.1442)	0.0019 (0.0006, 0.0041)
PCE+apoB+log(TG) vs PCE+apoB+log(TG)+ log(LDL-TG)	0.7209 (0.7119, 0.7328)	0.7219 (0.7127, 0.7347)	0.0010 (0.0001, 0.0028)	0.0106 (-0.0108, 0.0196)	0.0509 (-0.0081, 0.1070)	0.0009 (0.0001, 0.0023)