Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

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Methods

Measurement of mtDNA-CN

In ARIC, DNA was extracted using the Gentra Puregene Blood Kit (Qiagen) from the buffy coat of whole blood collected in visits 1 (1987–1989), 2 (1990–1992), 3 (1993–1995), and 4 (1996–1998) in 4.2, 79.6, 15.6 and 0.6% of participants, respectively. In CHS, DNA was extracted by salt precipitation following proteinase K digestion of the buffy coat from whole blood collected at visit 1. In MESA, DNA was isolated from peripheral leukocytes from blood collected at visit 1using the Gentra Puregene Blood Kit.

In ARIC and MESA, mtDNA-CN was calculated from probe intensities of mitochondrial single nucleotide polymorphisms (SNPs) on the Affymetrix Genome-Wide Human SNP Array 6.0[1] using the Genvisis software package (www.genvisis.org). This method uses median mitochondrial probe intensity of 25 high quality mitochondrial probes as an initial raw measure of mtDNA-CN. To correct for batch effects, DNA quality, and starting DNA quantity, data decomposition techniques (surrogate variable [SV] analysis[2] in ARIC and principal component [PC] analysis in MESA) were applied to probe intensities of 43,316 autosomal SNPs. Surrogate variable analysis, with a covariate for CVD status, was used in ARIC, as genotyping plates were confounded by CVD status. In ARIC, the raw measure of mtDNA-CN was first stratified by race, and then, using a linear regression, mtDNA-CN was adjusted for the effects of age, sex, collection center, and SVs. In MESA, the raw measure of mtDNA-CN was adjusted for the effects of age, sex, collection center, race and PCs and then a rank-based inverse normal transformation was performed to reduce the impact of outliers. The standardized residuals from these models were used as the mtDNA-CN metric for all analyses (i.e. with a mean of 0 and standard deviation of 1).

Two criteria come into play when deciding on how many PCs to include to correct for confounding variables. The first is what order to add PCs, and the second is how many to include.

Traditionally, one would include PCs in their natural order (i.e. PC1, then PC2, etc…), since the order is dictated by the maximum variance explained. However, for our study, PCs are generated on nuclear SNP probe intensity, but in fact, we are interested in explaining the variance in mtDNA-CN. Thus, we took two approaches with respect to the order in which PCs are added. First, PCs were either taken in their natural order or alternatively, taken from an initial pool of PCs (5% of total sample size) using a stepwise forward regression to select PCs that explain the maximal variance of the raw estimates of mtDNA-CN ("rank order"). With regards to the number of PCs to add, we either used a fixed number (the top 15 PCs) or PCs were added in rank order until they no longer significantly improved the model ($P > 0.05$ after Bonferroni correction for the number of PCs tested; 152 PCs and 95 PCs for ARIC and MESA, respectively). Note that the "rank order" does not apply to SVs, as SVs are not necessarily orthogonal, and the number of SVs is pre-specified for analysis, with all SVs generated included in the model. Thus, we used the number of PCs derived from the forward regression to determine the number of SVs to include. We tested the performance of these 2 approaches in ARIC (SV15 and SV152) and 4 approaches in MESA (PC15 natural, PC15 rank, PC95 natural, PC95 rank) utilizing several criteria: 1) known associations with sex and age; 2) correlation with alternative mtDNA-CN estimates (whole-genome sequence on 400 individuals in ARIC, qPCR for ~5,400 individuals in MESA); 3) the observed association between mtDNA-CN and white blood cell count (WBC). Due to the correlation between mtDNA-CN and WBC, we hypothesized that SNPs associated with WBC count should also be associated with mtDNA-CN (uncorrected for WBC), thus providing us an independent method to evaluate our different estimates of mtDNA-CN. We specifically focused on two extremely strong signals in African Americans (rs2814778 and rs12149261)[3], for which we are adequately powered in our sample sizes. In ARIC, SV15 showed stronger associations across all our metrics. In MESA, the results were not as clear, with PC95 natural showing the best overall performance.

In CHS, mtDNA-CN was measured using multiplexed Taqman-based qPCR as previously described[4]. To correct for batch effects and known influences on mtDNA-CN, the raw measure of

mtDNA-CN was first stratified by race, then we used a linear mixed model to adjust the raw mtDNA-CN measurement for age, sex, and collection site (fixed effects) and qPCR plate (random effect). Standardized residuals from this model were used as the mtDNA-CN metric for all analyses (i.e. with a mean of 0 and standard deviation of 1).

Statistical Analyses

To examine whether mtDNA-CN may improve risk prediction beyond PCE, we compared the discrimination ability of the calculated 10-year risk score vs. the combination of the 10-year risk score and mtDNA-CN using Harrell's C statistics. P-value for the difference in C-statistics was obtained by bootstrapping, using 100 bootstraps (function 'censboot' in the R package 'boot')[5]. For risk prediction models, we pooled participants from ARIC, CHS, and MESA and derived pooled coefficients for mtDNA-CN after multivariable adjustments[5]. This analysis was restricted to individuals with the same criteria as described in the 2013 ACC/AHA Guideline[6] (ages 40-79 years and no prevalent CVD, atrial fibrillation, or heart failure), with follow-up time censored at 12 years. We included identical variables in the model as used in the PCE, with mtDNA-CN being the only addition. As was done for the PCE, coefficients for mtDNA-CN were estimated separately in whites and blacks, stratified by sex. For generating 10-year hard ASCD risk scores incorporating mtDNA-CN, we updated the PCE model by adding the difference between the mean linear predictor calculated in our dataset with and without mtDNA-CN.

We also examined whether adding mtDNA-CN could improve the net reclassification index (NRI) and/or integrated discrimination improvement (IDI) for statin therapy[7], based on the 2013 ACC/AHA recommendations for starting statin therapy, excluding individuals whose risk score would not impact therapy decisions based on the 2013 ACC/AHA guidelines (i.e., prevalent CVD, age >75 years, prevalent diabetes, LDL≥190, or LDL<70). Given that NRI can be biased by poorly calibrated

estimates, we generated recalibrated 10-year hard ASCVD risk scores in the pooled ARIC, CHS, and MESA cohorts, excluding individuals whose risk score would not impact the choice to initiate statin therapy. Consistent with the approach used to generate the PCE, we stratified on race and sex, and included the same variables as used to generate the PCE, including all interaction terms, with the only changes being the removal of diabetes (since this is an exclusion criteria in our analysis) and the addition of hypertension medication status. We also included a term for race in the "whites" analysis, as Hispanics and Chinese were included in this group (per the 2013 ACC/AHA guideline recommendation to use the PCE generated in Whites for Hispanics and Asian populations).

Figure Legends

eFigure 1: Association of mtDNA-CN with prevalent CVD. Forest plots are shown for meta-analyses of incident CHD (top panel), incident stroke (middle panel), and incident CVD (bottom panel).

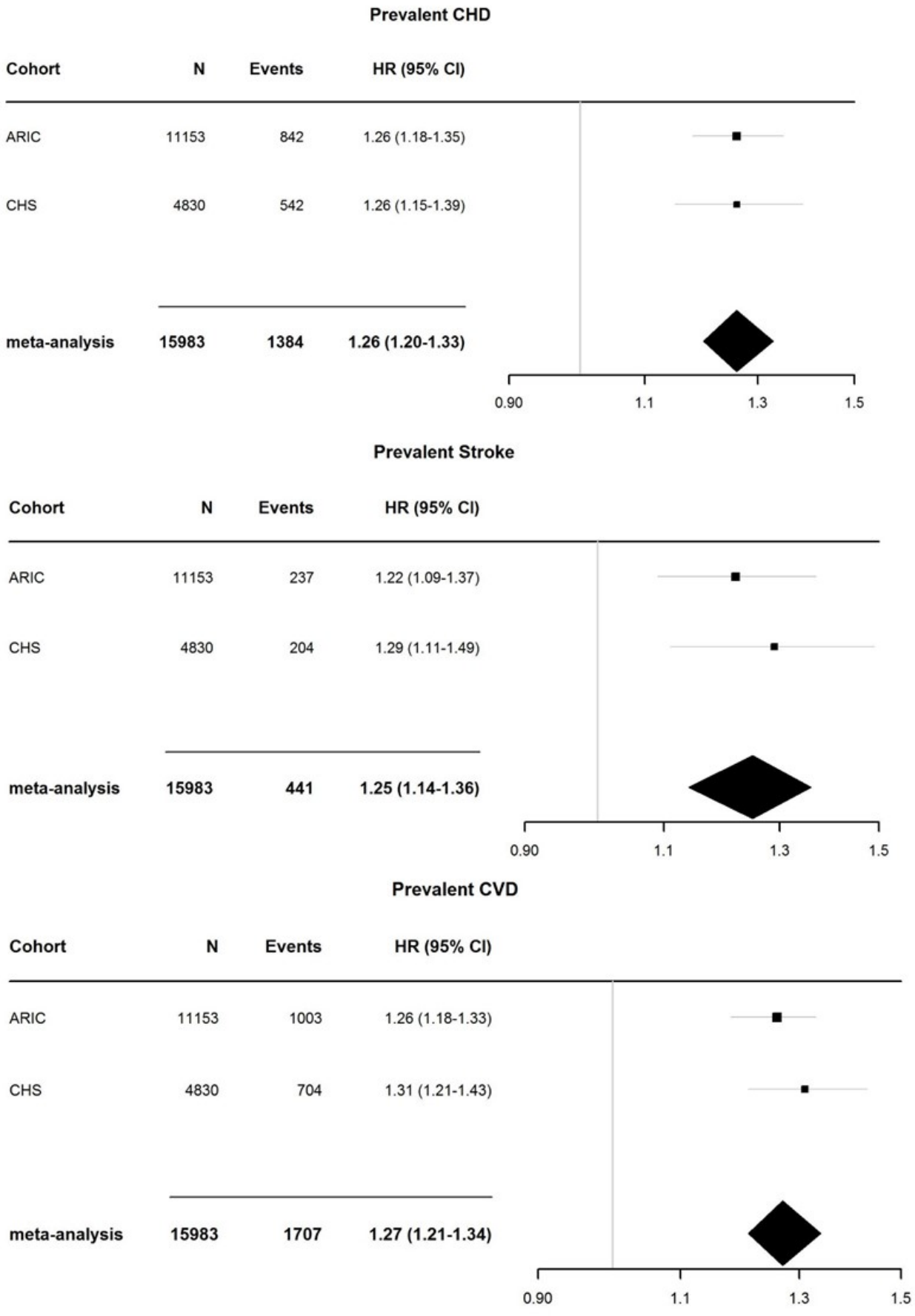
eFigure 2: Association of mtDNA-CN with prevalent CVD, stratified by race. Forest plots are shown for meta-analyses of incident CHD (top panel), incident stroke (middle panel), and incident CVD (bottom panel).

eFigure 3: Association of mtDNA-CN with prevalent CVD, stratified by sex. Forest plots are shown for meta-analyses of incident CHD (top panel), incident stroke (middle panel), and incident CVD (bottom panel).

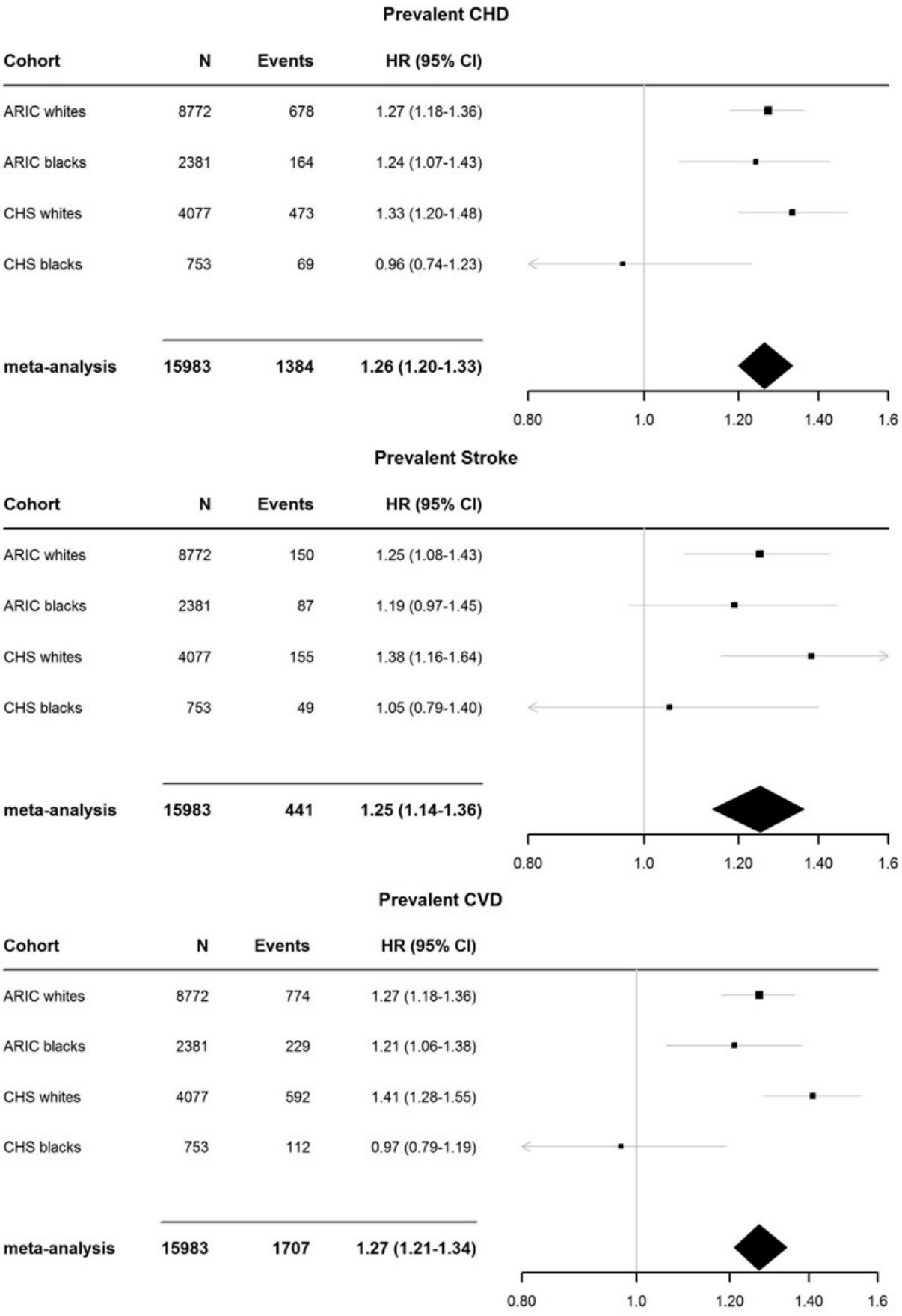
eFigure 4: Association of mtDNA-CN with incident CVD, stratified by race. Forest plots are shown for meta-analyses of incident CHD (top panel), incident stroke (middle panel), and incident CVD (bottom panel).

eFigure 5: Association of mtDNA-CN with incident CVD, stratified by sex. Forest plots are shown for meta-analyses of incident CHD (top panel), incident stroke (middle panel), and incident CVD (bottom panel).

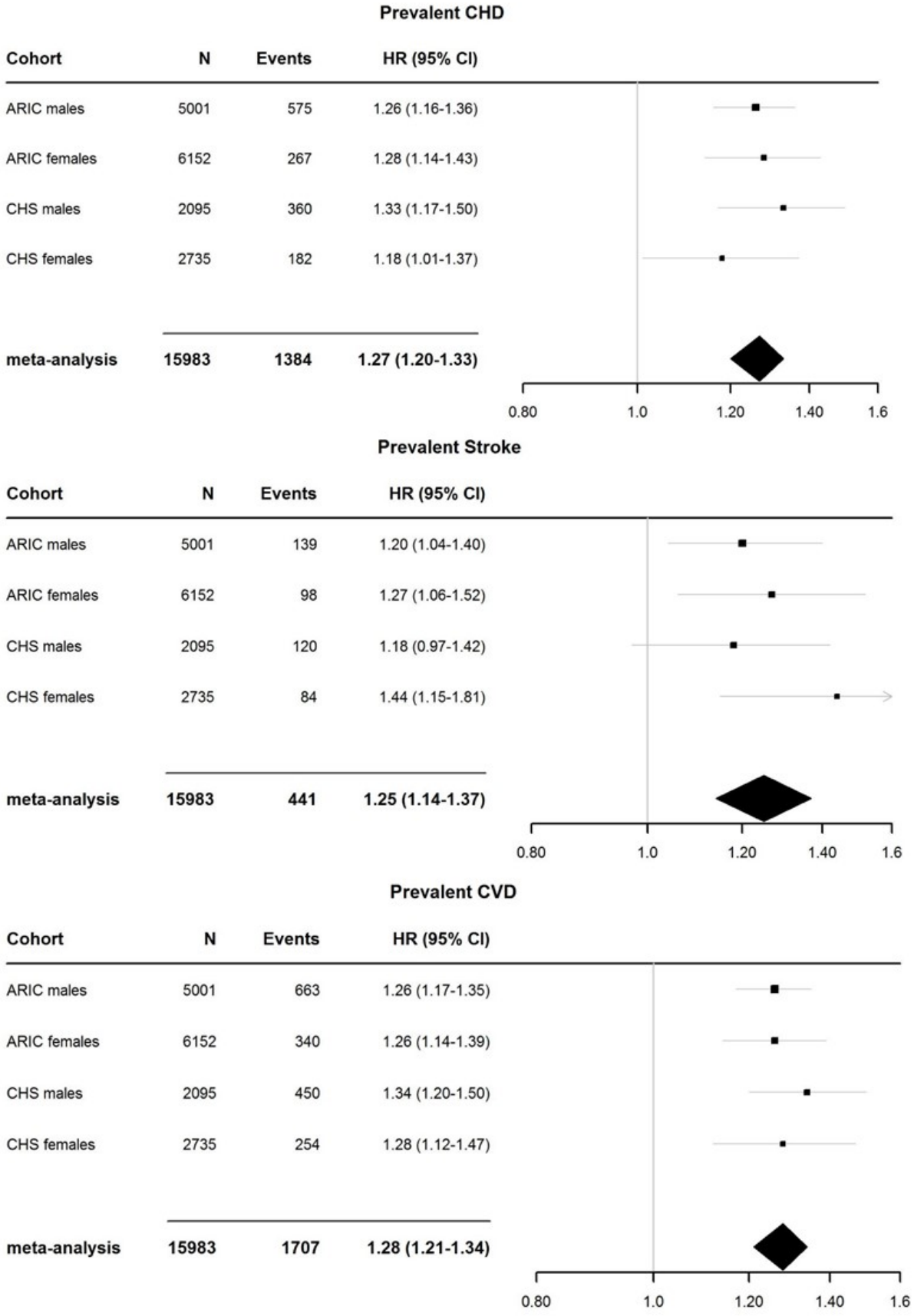
eFigure 1: Association of mtDNA-CN with prevalent CVD



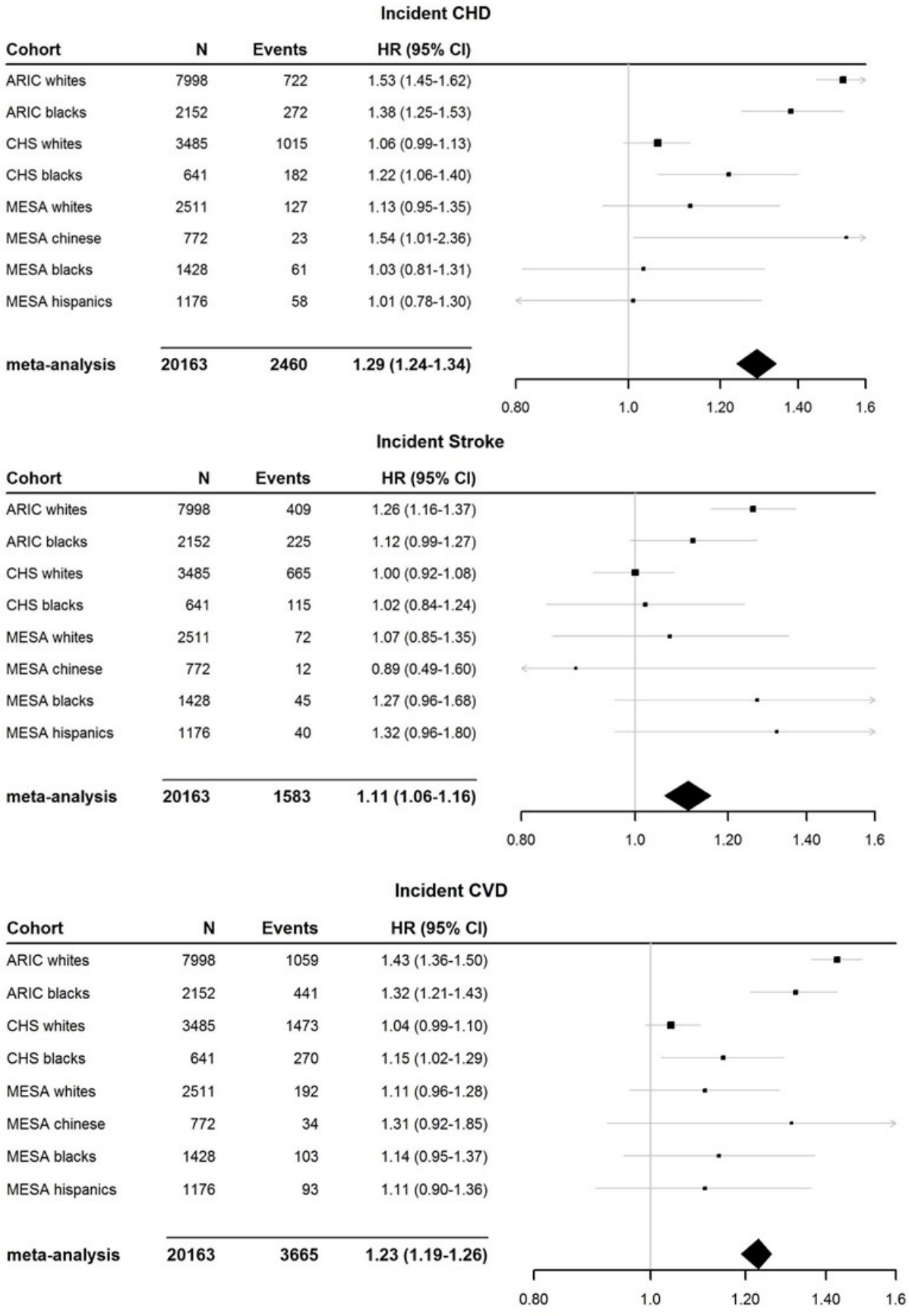
eFigure 2: Association of mtDNA-CN with prevalent CVD, stratified by race



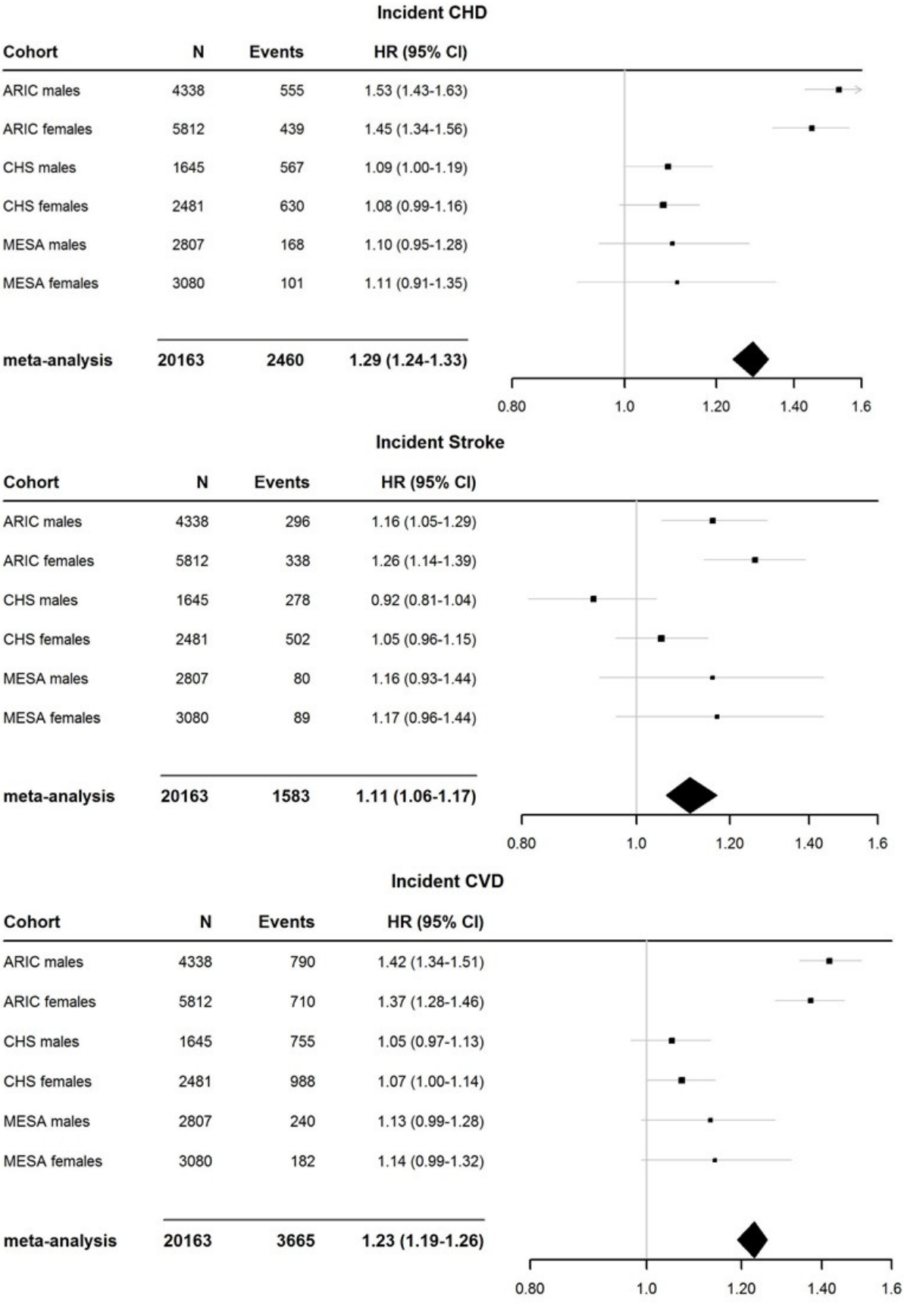
eFigure 3: Association of mtDNA-CN with prevalent CVD, stratified by sex



eFigure 4: Association of mtDNA-CN with incident CVD, stratified by race



eFigure 5: Association of mtDNA-CN with incident CVD, stratified by sex



eTable 1a: Baseline Characteristics of Pooled ARIC, CHS, and MESA Study Participants by Quintiles of mtDNA-CN.

	Q1	Q2	Q3	Q4	Q5	Total	P
no.	4374	4374	4374	4374	4374	21870	
Mean age (SD) - yr	62.6 ± 9.4	62.7 ± 9.4	62.2 ± 9.2	61.8 ± 9.1	62.5 ± 9.2	62.4 ± 9.3	0.70
Male sex - no. (%)	1960 (44.8)	1993 (45.6)	2016 (46.1)	1948 (44.5)	1986 (45.4)	9903 (45.3)	0.96
Race							0.97
Race,Whites - no. (%)	3048 (69.7)	3105 (71.0)	3133 (71.6)	3052 (69.8)	3022 (69.1)	15360 (70.2)	
Race,Blacks - no. (%)	922 (21.1)	874 (20.0)	856 (19.6)	953 (21.8)	957 (21.9)	4562 (20.9)	
Race,Chinese - no. (%)	159 (3.6)	151 (3.5)	151 (3.5)	164 (3.7)	147 (3.4)	772 (3.5)	
Race,Hispanics - no. (%)	245 (5.6)	244 (5.6)	234 (5.3)	205 (4.7)	248 (5.7)	1176 (5.4)	
Prevalent diabetes - no. (%)	773 (17.7)	634 (14.5)	562 (12.8)	590 (13.5)	536 (12.3)	3095 (14.2)	<0.001
Mean SBP (SD) - mm Hg	127.5 ± 21.3	127.4 ± 21.6	126.1 ± 21.1	125.8 ± 20.5	125.8 ± 21.4	126.5 ± 21.2	<0.001
Hypertension medication - no. (%)	1793 (41.0)	1700 (38.9)	1604 (36.7)	1539 (35.2)	1537 (35.1)	8173 (37.4)	<0.001
Current smoker - no. (%)	954 (21.8)	837 (19.1)	783 (17.9)	694 (15.9)	624 (14.3)	3892 (17.8)	<0.001
Mean HDL (SD) - mg/dl	50.1 ± 16.0	51.2 ± 16.3	50.9 ± 16.2	51.8 ± 16.6	52.4 ± 16.4	51.3 ± 16.3	<0.001
Mean total cholesterol (SD) - mg/dl	205.1 ± 40.5	205.4 ± 39.3	206.5 ± 38.7	206.0 ± 38.1	207.3 ± 39.2	206.1 ± 39.2	0.19

SBP=systolic blood pressure; HDL=high-density lipoprotein. P value is from regression analyses using the continuous mtDNA-CN value, not quintiles.

eTable 1b: Baseline Characteristics of ARIC Study Participants by Quintiles of mtDNA-CN.

	Q1	Q2	Q3	Q4	Q5	Total	P
no.	2231	2230	2231	2230	2231	11153	
Mean age (SD) - yr	57.9 ± 6.0	58.0 ± 6.0	58.0 ± 6.0	58.0 ± 5.9	57.9 ± 5.8	57.9 ± 6.0	0.96
Male sex - no. (%)	982 (44.0)	1002 (44.9)	1003 (45.0)	1009 (45.2)	1005 (45.0)	5001 (44.8)	0.83
Race							0.86
Race,Whites - no. (%)	1764 (79.1)	1774 (79.6)	1754 (78.6)	1747 (78.3)	1733 (77.7)	8772 (78.7)	
Race,Blacks - no. (%)	467 (20.9)	456 (20.4)	477 (21.4)	483 (21.7)	498 (22.3)	2381 (21.3)	
Prevalent diabetes - no. (%)	446 (20.0)	363 (16.3)	304 (13.6)	292 (13.1)	281 (12.6)	1686 (15.1)	<0.001
Mean SBP (SD) - mm Hg	123.4 ± 20.3	122.9 ± 19.9	122.0 ± 18.8	122.1 ± 18.6	121.3 ± 18.5	122.3 ± 19.2	<0.001
Hypertension medication - no. (%)	840 (37.7)	794 (35.6)	692 (31.0)	692 (31.0)	723 (32.4)	3741 (33.5)	<0.001
Current smoker - no. (%)	689 (30.9)	567 (25.4)	511 (22.9)	413 (18.5)	386 (17.3)	2566 (23.0)	<0.001
Mean HDL (SD) - mg/dl	48.6 ± 17.0	49.6 ± 16.7	49.9 ± 16.9	51.2 ± 17.6	51.3 ± 17.2	50.1 ± 17.1	<0.001
Mean total cholesterol (SD) - mg/dl	209.7 ± 41.9	208.6 ± 39.6	210.0 ± 38.3	209.4 ± 38.7	211.5 ± 39.6	209.8 ± 39.7	0.94

SBP=systolic blood pressure; HDL=high-density lipoprotein. P value is from regression analyses using the continuous mtDNA-CN value, not quintiles.

eTable 1c: Baseline Characteristics of CHS Study Participants by Quintiles of mtDNA-CN.

	Q1	Q2	Q3	Q4	Q5	Total	P
no.	966	966	966	966	966	4830	
Mean age (SD) - yr	72.3 ± 5.5	72.8 ± 5.5	72.5 ± 5.4	72.7 ± 5.4	72.4 ± 5.5	72.5 ± 5.5	0.98
Male sex - no. (%)	422 (43.7)	417 (43.2)	449 (46.5)	388 (40.2)	419 (43.4)	2095 (43.4)	0.90
Race							0.84
Race,Whites - no. (%)	817 (84.6)	829 (85.8)	824 (85.3)	805 (83.3)	802 (83.0)	4077 (84.4)	
Race,Blacks - no. (%)	149 (15.4)	137 (14.2)	142 (14.7)	161 (16.7)	164 (17.0)	753 (15.6)	
Prevalent diabetes - no. (%)	185 (19.2)	152 (15.7)	124 (12.8)	151 (15.6)	148 (15.3)	760 (15.7)	0.035
Mean SBP (SD) - mm Hg	135.5 ± 22.0	136.6 ± 21.9	137.0 ± 21.5	136.3 ± 21.6	135.9 ± 21.6	136.3 ± 21.7	0.85
Hypertension medication - no. (%)	481 (49.8)	448 (46.4)	459 (47.5)	461 (47.7)	409 (42.3)	2258 (46.7)	0.001
Current smoker - no. (%)	132 (13.7)	114 (11.8)	116 (12.0)	113 (11.7)	99 (10.2)	574 (11.9)	0.09
Mean HDL (SD) - mg/dl	52.8 ± 15.5	54.6 ± 16.2	54.3 ± 15.9	54.4 ± 15.1	55.2 ± 15.8	54.2 ± 15.7	0.009
Mean total cholesterol (SD) - mg/dl	209.3 ± 39.8	210.2 ± 38.1	212.7 ± 39.5	212.4 ± 39.6	213.4 ± 38.6	211.6 ± 39.1	0.048

SBP=systolic blood pressure; HDL=high-density lipoprotein. P value is from regression analyses using the continuous mtDNA-CN value, not quintiles.

eTable 1d: Baseline Characteristics of MESA Study Participants by Quintiles of mtDNA-CN.

	Q1	Q2	Q3	Q4	Q5	Total	P
no.	1178	1177	1177	1177	1178	5887	
Mean age (SD) - yr	62.6 ± 10.4	62.1 ± 10.4	62.4 ± 10.2	62.5 ± 10.4	62.5 ± 10.1	62.4 ± 10.3	0.72
Male sex - no. (%)	551 (46.8)	566 (48.1)	575 (48.9)	557 (47.3)	558 (47.4)	2807 (47.7)	0.94
Race							0.96
Race,Whites - no. (%)	503 (42.7)	520 (44.2)	493 (41.9)	502 (42.7)	493 (41.9)	2511 (42.7)	
Race,Blacks - no. (%)	311 (26.4)	271 (23.0)	258 (21.9)	277 (23.5)	311 (26.4)	1428 (24.3)	
Race,Chinese - no. (%)	142 (12.1)	148 (12.6)	167 (14.2)	173 (14.7)	142 (12.1)	772 (13.1)	
Race,Hispanics - no. (%)	222 (18.8)	238 (20.2)	259 (22.0)	225 (19.1)	232 (19.7)	1176 (20.0)	
Prevalent diabetes - no. (%)	151 (12.8)	122 (10.4)	130 (11.0)	130 (11.0)	116 (9.8)	649 (11.0)	0.037
Mean SBP (SD) - mm Hg	127.9 ± 20.8	127.1 ± 21.8	126.0 ± 21.3	126.4 ± 22.2	125.3 ± 22.4	126.5 ± 21.7	0.024
Hypertension medication - no. (%)	469 (39.8)	445 (37.8)	445 (37.8)	415 (35.3)	400 (34.0)	2174 (36.9)	0.001
Current smoker - no. (%)	162 (13.8)	159 (13.5)	147 (12.5)	149 (12.7)	135 (11.5)	752 (12.8)	0.084
Mean HDL (SD) - mg/dl	51.0 ± 14.6	50.4 ± 14.9	50.1 ± 14.1	51.4 ± 15.3	52.3 ± 15.3	51.0 ± 14.9	0.008
Mean total cholesterol (SD) - mg/dl	194.3 ± 36.4	193.7 ± 36.8	195.0 ± 35.5	193.9 ± 33.3	194.9 ± 36.3	194.4 ± 35.7	0.50

SBP=systolic blood pressure; HDL=high-density lipoprotein. P value is from regression analyses using the continuous mtDNA-CN value, not quintiles.

eTable 2. Adjusted Incidence Rates, Incidence Rate Ratios, and Incidence Rate Differences for Cardiovascular disease (CVD) Endpoints by Quintiles of mtDNA Copy Number in the ARIC, CHS, and MESA studies.

	Number	Events / Person-years	Crude incidence rate, per 10^4 p-y (95% CI)	Adjusted* incidence rate, per 10^4 p-y (95% CI)	Adjusted* incidence rate ratio (95% CI)	Adjusted* incidence rate difference, per 10^4 p-y (95% CI)
ARIC						
Q1	2,030	435 / 30,449.4	142.9 (130.0-156.9)	140.2 (126.9-153.4)	1.97 (1.68-2.31)	69.1 (53.0-85.2)
Q2	2,030	336 / 31,891.1	105.4 (94.7-117.2)	103.7 (92.5-114.8)	1.46 (1.23-1.72)	32.6 (18.2-47.0)
Q3	2,030	278 / 32,524.1	85.5 (76.0-96.1)	87.4 (77.1-97.7)	1.23 (1.03-1.46)	16.4 (2.7-30.1)
Q4	2,030	215 / 33,344.6	64.5 (56.4-73.7)	69.0 (59.8-78.3)	0.97 (0.81-1.17)	-2.0 (-15.0-10.9)
Q5	2,030	236 / 33,877.7	69.7 (61.3-79.1)	71.0 (62.0-80.1)	1.00 (reference)	0.0 (reference)
CHS						
Q1	825	350 / 9,751.6	358.9 (323.2-398.6)	381.4 (340.6-422.1)	1.21 (1.04-1.41)	66.3 (12.6-120.0)
Q2	825	344 / 10,019.6	343.3 (308.9-381.6)	347.5 (310.6-384.4)	1.10 (0.95-1.29)	32.4 (-18.1-83.0)
Q3	825	354 / 10,299.8	343.7 (309.7-381.4)	341.0 (305.3-376.8)	1.08 (0.93-1.26)	26.0 (-23.5-75.4)
Q4	825	365 / 10,563.2	345.5 (311.9-382.9)	352.5 (316.0-388.9)	1.12 (0.96-1.30)	37.4 (-12.4-87.2)
Q5	826	330 / 10,662.8	309.5 (277.8-344.7)	315.1 (280.8-349.4)	1.00 (reference)	0.0 (reference)
MESA						
Q1	1,177	98 / 11,585.1	84.6 (69.4-103.1)	83.9 (67.2-100.6)	1.42 (1.04-1.93)	24.6 (2.7-46.5)
Q2	1,177	83 / 11,797.3	70.4 (56.7-87.2)	71.3 (55.9-86.8)	1.20 (0.87-1.66)	12.1 (-9.0-33.1)
Q3	1,178	84 / 11,977.9	70.1 (56.6-86.9)	70.0 (55.0-85.1)	1.18 (0.86-1.63)	10.7 (-10.0-31.5)
Q4	1,177	90 / 11,814.6	76.2 (62.0-93.7)	75.7 (60.0-91.4)	1.28 (0.93-1.76)	16.4 (-4.8-37.6)
Q5	1,178	67 / 11,965.6	56.0 (44.1-71.1)	59.3 (45.0-73.5)	1.00 (reference)	0.0 (reference)

* Adjusted incidence rates were marginally adjusted using Poisson regression models with time split in 1-year intervals and adjusting for age (continuous), sex, race, center, total cholesterol (continuous), HDL-cholesterol

(continuous), systolic blood pressure (continuous), use of antihypertensive medication, current smokers, and diabetes. Calculation of quintiles and marginal adjustments were performed in each study separately.

eTable 3. Hazards Ratio for Incident Cardiovascular Disease (CVD) by Quintiles of mtDNA Copy Number in MESA.										
	All				Age <65 yrs				Age ≥ 65 yrs	
	Events / Total N	Model 1	Model2		Events / Total N	Model 1	Model2		Events / Total N	Model 1	Model2
CHD										
Q1	59 / 1178	1.38 (0.93-2.04)	1.30 (0.88-1.93)		19 / 648	1.58 (0.78-3.20)	1.29 (0.64-2.63)		40 / 531	1.34 (0.83-2.15)	1.29 (0.80-2.07)
Q2	47 / 1177	1.13 (0.75-1.71)	1.06 (0.70-1.61)		18 / 647	1.44 (0.70-2.94)	1.22 (0.60-2.51)		28 / 530	0.94 (0.56-1.58)	0.89 (0.53-1.50)
Q3	64 / 1177	1.47 (1.00-2.16)	1.40 (0.95-2.07)		21 / 647	1.70 (0.85-3.40)	1.46 (0.73-2.94)		44 / 530	1.41 (0.88-2.25)	1.37 (0.86-2.19)
Q4	56 / 1177	1.28 (0.86-1.90)	1.21 (0.81-1.80)		15 / 647	1.24 (0.59-2.61)	1.02 (0.48-2.17)		41 / 530	1.28 (0.80-2.06)	1.24 (0.77-1.99)
Q5	43 / 1178	reference	reference		13 / 647	reference	reference		30 / 530	reference	reference
Continuous	269 / 5887	1.11 (0.98-1.25)	1.09 (0.97-1.23)		86 / 3236	1.28 (1.04-1.59)	1.22 (0.98-1.52)		183 / 2651	1.04 (0.90-1.20)	1.03 (0.89-1.19)
P-trend		0.10	0.16			0.022	0.07			0.63	0.74
Stroke										
Q1	43 / 1178	1.67 (1.02-2.71)	1.58 (0.97-2.57)		14 / 648	2.00 (0.84-4.77)	1.71 (0.71-4.10)		27 / 531	1.48 (0.82-2.70)	1.42 (0.78-2.60)
Q2	37 / 1177	1.50 (0.91-2.47)	1.38 (0.83-2.28)		13 / 647	1.82 (0.75-4.40)	1.64 (0.67-3.99)		26 / 530	1.45 (0.79-2.64)	1.33 (0.72-2.43)
Q3	25 / 1177	0.97 (0.56-1.67)	0.91 (0.53-1.58)		12 / 647	1.63 (0.67-4.01)	1.46 (0.59-3.61)		13 / 530	0.71 (0.35-1.46)	0.68 (0.33-1.40)
Q4	38 / 1177	1.45 (0.88-2.39)	1.39 (0.84-2.30)		10 / 647	1.42 (0.56-3.62)	1.31 (0.52-3.34)		28 / 530	1.47 (0.81-2.65)	1.41 (0.78-2.56)
Q5	26 / 1178	reference	reference		8 / 647	reference	reference		18 / 530	reference	reference
Continuous	169 / 5887	1.17 (1.01-1.36)	1.15 (0.99-1.34)		57 / 3236	1.34 (1.03-1.73)	1.28 (0.98-1.66)		112 / 2651	1.09 (0.91-1.31)	1.09 (0.90-1.31)
P-trend		0.042	0.07			0.027	0.07			0.34	0.38
CVD										
Q1	98 / 1178	1.49 (1.09-2.03)	1.41 (1.03-1.93)		33 / 648	1.73 (1.00-3.00)	1.43 (0.83-2.49)		63 / 531	1.39 (0.95-2.04)	1.34 (0.91-1.96)
Q2	83 / 1177	1.30 (0.94-1.79)	1.21 (0.87-1.67)		30 / 647	1.53 (0.88-2.68)	1.33 (0.76-2.34)		54 / 530	1.19 (0.80-1.77)	1.11 (0.75-1.65)
Q3	84 / 1177	1.25 (0.90-1.72)	1.18 (0.86-1.63)		33 / 647	1.68 (0.97-2.91)	1.45 (0.84-2.52)		52 / 530	1.10 (0.74-1.64)	1.06 (0.71-1.58)

Q4	90 / 1177	1.34 (0.98-1.84)	1.28 (0.93-1.75)		25 / 647	1.32 (0.74-2.36)	1.18 (0.66-2.11)		65 / 530	1.34 (0.92-1.96)	1.29 (0.88-1.89)
Q5	67 / 1178	reference	reference		21 / 647	reference	reference		46 / 530	reference	reference
Continuous	422 / 5887	1.14 (1.03-1.25)	1.12 (1.02-1.24)		142 / 3236	1.30 (1.10-1.53)	1.23 (1.04-1.45)		280 / 2651	1.06 (0.95-1.20)	1.06 (0.94-1.19)
P-trend		0.009	0.02			0.002	0.016			0.29	0.36
Model 1 is adjusted for age, sex, collection center, and race. Model 2 is Model 1 additionally adjusted for total cholesterol, HDL, systolic blood pressure, current smoking status, hypertension medication status, and type 2 diabetes status.											

eTable 4: Change in C statistic with mtDNA-CN over base AHA Risk Score model

	Combined Cohorts	ARIC	CHS	MESA
CHD				
AHA Risk Score	0.747 (0.733-0.759)	0.745 (0.722-0.764)	0.688 (0.669-0.711)	0.714 (0.686-0.750)
AHA Risk Score + mtDNA-CN	0.760 (0.745-0.774)	0.771 (0.751-0.791)	0.684 (0.662-0.706)	0.714 (0.682-0.748)
Delta Cstatistic	1.2% (0.8-1.6%)	2.6% (2.1-3.2%)	-0.4% (-1.0-0.5%)	0.0% (-1.2-0.9%)
Stroke				
AHA Risk Score	0.763 (0.747-0.780)	0.762 (0.740-0.785)	0.679 (0.651-0.707)	0.739 (0.703-0.772)
AHA Risk Score + mtDNA-CN	0.762 (0.745-0.777)	0.765 (0.737-0.793)	0.670 (0.639-0.701)	0.740 (0.705-0.787)
Delta Cstatistic	-0.2% (-0.5-0.2%)	0.3% (-0.4-1.1%)	-0.9% (-1.7-0.0%)	0.2% (-0.6-1.2%)
CVD				
AHA Risk Score	0.752 (0.742-0.761)	0.752 (0.737-0.768)	0.682 (0.662-0.714)	0.723 (0.699-0.756)
AHA Risk Score + mtDNA-CN	0.759 (0.751-0.769)	0.769 (0.754-0.789)	0.676 (0.657-0.697)	0.724 (0.694-0.749)
Delta Cstatistic	0.7% (0.4-1.0%)	1.8% (1.3-2.3%)	-0.6% (-1.2- -0.1%)	0.2% (-0.7-0.6%)

eTable 5: Net Reclassification Index (NRI) and Integrated Discrimination Improvement (IDI) Comparing AHA Risk Score with and without mtDNA-CN in the Pooled Cohorts Stratified by CVD Type.

	CHD				Stroke				CVD		
	Estimate	**95% CI**	**P**		**Estimate**	**95% CI**	**P**		**Estimate**	**95% CI**	**P**
Categorical NRI*	0.043	0.023-0.064	<0.001		0.008	-0.017-0.032	0.53		0.032	0.015-0.049	<0.001
Continuous NRI	0.232	0.154-0.311	<0.001		0.095	-0.002-0.191	0.05		0.194	0.130-0.258	<0.001
IDI	0.012	0.007-0.016	<0.001		0.002	-0.001-0.006	0.19		0.009	0.005-0.012	<0.001
Categorical NRI* with <5.0% and 5%-<7.5% risk cutoffs											

eTable 6: Comparison of mtDNA-CN derived from Affymetrix 6.0 arrays vs. qPCR for variables known to be associated with mtDNA-CN in MESA (n = 5467 for age and sex comparisons and n = 1238 for SNP comparisons).

	Affymetrix 6.0 Array				qPCR		
	Estimate	**SE**	**P**		**Estimate**	**SE**	**P**
Age	-0.010	0.0013	8.87E-16		-0.004	0.0013	0.001
Sex	-0.314	0.0266	2.00E-16		-0.219	0.0269	5.38E-16
rs2814778	0.289	0.0460	3.18E-10		0.146	0.0499	0.003
rs12149261	-0.155	0.0459	7.55E-04		-0.080	0.0493	0.10

References:

1. Tin A, Grams ME, Ashar FN, et al. Association between Mitochondrial DNA Copy Number in Peripheral Blood and Incident CKD in the Atherosclerosis Risk in Communities Study. J Am Soc Nephrol 2016;ASN.2015060661.

2. Leek JT, Johnson WE, Parker HS, Jaffe AE, Storey JD. The sva package for removing batch effects and other unwanted variation in high-throughput experiments. Bioinforma Oxf Engl 2012;28(6):882–3.

3. Keller MF, Reiner AP, Okada Y, et al. Trans-ethnic meta-analysis of white blood cell phenotypes. Hum Mol Genet 2014;23(25):6944–60.

4. Ashar FN, Moes A, Moore AZ, et al. Association of mitochondrial DNA levels with frailty and all-cause mortality. J Mol Med 2014;93(2):177–86.

5. Canty A, support) BR (author of parallel. boot: Bootstrap Functions (Originally by Angelo Canty for S) [Internet]. 2015 [cited 2016 Jan 15]. Available from: https://cran.r-project.org/web/packages/boot/index.html

6. Goff DC, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA Guideline on the Assessment of Cardiovascular Risk A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation 2013;01.cir.0000437741.48606.98.

7. Uno H, Cai T. survIDINRI: IDI and NRI for comparing competing risk prediction models with censored survival data [Internet]. 2013 [cited 2016 Dec 22]. Available from: https://cran.r-project.org/web/packages/survIDINRI/index.html