SUPPLEMENTARY DATA

High-density lipoprotein cholesterol and risk of type 2 diabetes: a Mendelian randomization study

Haase CL, Tybjærg-Hansen A, Nordestgaard BG, Frikke-Schmidt R

SUPPLEMENTARY DATA

Supplementary Table S1. Single genotype weights (part A) used for weighted HDL cholesterol decreasing gene score calculation (part B).

A) Single genotype weights for variants in five major HDL cholesterol related genes.

Gene	Variant	Rs. number	Minor allele	Common allele	HDL cholesterol decreasing allele	Single genotype weights
ABCA1	N1800H	rs146292819	C	A	C	-0.4439058
*CETP**	–629C>A+*Taq*1b	rs1800775/rs708272	A, A	C, G	C, G	-0.0569779
LCAT	S208T	rs4986970	T	A	T	-0.0541012
LIPC	–480C>T	rs1800588	T	C	C	-0.0609659
*APOA1***	Carrier	NA	G,A,d,G	T,T,nd,T	G,A,d,G	-0.3025877

The single genotype weights correspond to the per-HDL cholesterol decreasing allele β-coefficients adjusted for age, sex and study cohort. *To account for likely functionality of both -629C>A (rs1800775) and *Taq*1bG>A (rs708272), as well as for high linkage disequilibrium (R^2 0.78) between the two *CETP* variants (1), a combined genotype was constructed summarizing the number of HDL cholesterol decreasing *CETP* alleles. Consequently, each *CETP* allele only contributes once to the score. **The four rare *APOA1* variants (S36A, F71Y, K107del, L144R) were combined into one carrier group. Hardy Weinberg equilibrium was present for all variants (P-values: 0.79, 0.71, 0.22, 0.24. 0.21. 0.95, 0.92, 0.98, and 0.97 for *ABCA1* N1800H, *CETP* -629C>A, *Taq*1b, *LCAT* S208T, *LIPC* -480C>T, *APOA1* S36A, F71Y, K107del, and L144R, respectively). The present internally-derived weights were more conservative than previously reported (2), and are thus unlikely to be inflated. Call-rates for each variant were above 0.99 due to two rounds of rerun analyses. NA=not available. d=deletion. nd=non-deleted.

B) Combined single genotype weights into a weighted HDL cholesterol decreasing gene score.

ABCA1 N1800H	*CETP* (-629+*Taq*1b) HDL decreasing alleles, N	*LCAT* S208T	*LIPC* -480C>T	*APOA1* mutation carriers	Gene score weights	N	Gene score quartile
NH	0	SS	CC	Yes	-0.8684253	1	4
NH	4	SS	CC	No	-0.7937492	12	4
NH	3	SS	CC	No	-0.7367713	3	4
NH	2	ST	CC	No	-0.7338946	1	4
NH	4	SS	CT	No	-0.7327833	18	4
NN	4	ST	CC	Yes	-0.7065323	1	4
NH	2	SS	CC	No	-0.6797934	29	4
NH	3	SS	CT	No	-0.6758054	1	4
NH	2	ST	CT	No	-0.6729287	1	4
NH	4	SS	TT	No	-0.6718174	2	4

NN	4	SS	CC	Yes	-0.6524311	14	4
NN	4	ST	CT	Yes	-0.6455664	1	4
NH	1	SS	CC	No	-0.6228155	6	4
NH	2	SS	CT	No	-0.6188275	15	4
NH	1	ST	CT	No	-0.6159508	1	4
NN	3	SS	CC	Yes	-0.5954532	3	4
NN	2	ST	CC	Yes	-0.5925765	4	4
NN	4	SS	CT	Yes	-0.5914652	4	4
NN	3	ST	CT	Yes	-0.5885885	1	4
NH	0	SS	CC	No	-0.5658376	10	4
NH	1	SS	CT	No	-0.5618496	1	4
NH	2	SS	TT	No	-0.5578616	6	4
NN	2	SS	CC	Yes	-0.5384753	32	4
NN	3	SS	CT	Yes	-0.5344873	3	4
NN	4	SS	TT	Yes	-0.5304993	1	4
NH	0	SS	CT	No	-0.5048717	7	4
NN	1	SS	CC	Yes	-0.4814974	3	4
NN	2	SS	CT	Yes	-0.4775094	7	4
NN	4	TT	CC	No	-0.4580458	8	4
NH	0	SS	TT	No	-0.4439058	2	4
NN	0	SS	CC	Yes	-0.4245195	14	4
NN	1	SS	CT	Yes	-0.4205315	1	4
NN	0	ST	CT	Yes	-0.4176548	2	4
NN	2	SS	TT	Yes	-0.4165435	2	4
NN	4	ST	CC	No	-0.4039446	532	4
NN	3	TT	CC	No	-0.4010679	1	4
NN	4	TT	CT	No	-0.3970799	9	4
NN	0	SS	CT	Yes	-0.3635536	2	4
NN	4	SS	CC	No	-0.3498434	7118	4
NN	3	ST	CC	No	-0.3469667	117	4
NN	2	TT	CC	No	-0.34409	28	4
NN	4	ST	CT	No	-0.3429787	307	4
NN	0	SS	TT	Yes	-0.3025877	1	4
NN	3	SS	CC	No	-0.2928655	1540	4

NN	2	ST	CC	No	-0.2899888	999	4
NN	4	SS	CT	No	-0.2888775	4000	4
NN	1	TT	CC	No	-0.2871121	4	3
NN	3	ST	CT	No	-0.2860008	54	3
NN	2	TT	CT	No	-0.2831241	13	3
NN	4	ST	TT	No	-0.2820128	42	3
NN	2	SS	CC	No	-0.2358876	11933	3
NN	1	ST	CC	No	-0.2330109	97	2
NN	3	SS	CT	No	-0.2318996	816	2
NN	0	TT	CC	No	-0.2301342	9	2
NN	2	ST	CT	No	-0.2290220	491	2
NN	4	SS	TT	No	-0.2279116	536	2
NN	3	ST	TT	No	-0.2250349	9	2
NN	2	TT	TT	No	-0.2221582	1	2
NN	1	SS	CC	No	-0.1789097	1281	2
NN	0	ST	CC	No	-0.176033	391	2
NN	2	SS	CT	No	-0.1749217	6688	2
NN	1	ST	CT	No	-0.172045	57	1
NN	3	SS	TT	No	-0.1709337	112	1
NN	0	TT	CT	No	-0.1691683	2	1
NN	2	ST	TT	No	-0.168057	62	1
NN	0	SS	CC	No	-0.1219318	5111	1
NN	1	SS	CT	No	-0.1179438	719	1
NN	0	ST	CT	No	-0.1150671	190	1
NN	2	SS	TT	No	-0.1139558	913	1
NN	1	ST	TT	No	-0.1110791	6	1
NN	0	SS	CT	No	-0.0609659	2759	1
NN	1	SS	TT	No	-0.0569779	78	1
NN	0	ST	TT	No	-0.0541012	25	1
NN	0	SS	TT	No	0	357	1

For all existing genotype combinations their single genotype weights were summarized into a gene score. These weights were subsequently categorized into four groups of approximate equal size, named “gene score quartiles” for simplicity, and numbered 1 to 4 with decreasing HDL cholesterol. Red font indicates the HDL decreasing allele of the individual genotypes. See distribution of the gene score weights in Supplemental Figure S2.

SUPPLEMENTARY DATA

Supplementary Table S2. Characteristics of individuals in the Copenhagen City Heart Study and the Copenhagen General Population Study by type 2 diabetes status.

	No type 2 diabetes	Type 2 diabetes
No. of individuals	45,040	2,587
Age (years)	56.7±0.07	63.7±0.21‡
Females (%)	56	44‡
Glucose	5.3±0.005	7.8±0.07‡
HbA1c (%)	5.8±0.008	7.2±0.08‡
HbA1c (mmol/mol)	40±0.1	55±0.9
HDL cholesterol (mmol/L)	1.6±0.002	1.4±0.009‡
Total cholesterol (mmol/L)	5.7±0.005	5.6±0.03‡
LDL cholesterol (mmol/L)	3.3±0.005	3.1±0.02‡
Triglycerides (mmol/L)*	1.5±0.01	2.1±0.04‡
Body mass index (kg/m^2)	25.8±0.02	29.6±0.10‡
Hypertension (%)	57	82‡
Smoking (%)	27	32‡
Alcohol consumption (%)	18	18
Physical inactivity (%)	54	67‡
Postmenopausal (%)†	65	88‡
Hormonal replacement therapy (%)†	14	12§
Lipid-lowering therapy (%)	6	28‡

Education < 8 years (%)	16	34‡

Values are mean (± standard error of the mean) or percent. Measurements of HbA1c were available on 5,625 individuals. Hypertension was use of anti-hypertensive medication and/or a systolic blood pressure of 140 mm Hg or greater, and/or a diastolic blood pressure of 90 mm Hg or greater. Smoking was current smoking. Alcohol consumption was >14/21 units per week for women/men (1 unit=12 g alcohol, equivalent to one glass of wine or one beer (33cL)). Physical inactivity was ≤4 hours per week of light physical activity in leisure time. Women reported menopausal status and use of hormonal replacement therapy. Lipid-lowering therapy was primarily statins (yes/no). Education was less than eight years of education. Missing values (≤0.3%) were imputed (continuous covariates) or assigned a dummy variable (categorical covariates). *Geometric mean; †In women only. ‡P<0.001 and §P<0.05 by Student's t-test or Pearson's χ^2-test. HbA1c=glycated hemoglobin; HDL= high-density lipoprotein; LDL=low-density lipoprotein. To convert cholesterol values to mg/dL, divide by 0.0259. To convert triglycerides to mg/dL, divide by 0.0113. Results were similar when individuals with type 2 diabetes before blood sampling were excluded.

SUPPLEMENTARY DATA

Supplementary Table S3. Characteristics of individuals in the Copenhagen City Heart Study and the Copenhagen General Population Study separately by type 2 diabetes status.

	Copenhagen City Heart Study		Copenhagen General Population Study	
	No type 2 diabetes	Type 2 diabetes	No type 2 diabetes	Type 2 diabetes
No. of individuals	9,236	930	35,804	1,657
Age (years)	55.8±0.17	62.5±0.36‡	56.9±0.07	64.3±0.26‡
Females (%)	57	44‡	55	44‡
Glucose	5.5±0.01	8.6±0.14‡	5.2±0.005	7.3±0.08‡
HbA1c (%)	5.8±0.008	7.2±0.08‡	NA	NA
HbA1c (mmol/l)	40±0.1	55±0.9‡	NA	NA
HDL cholesterol (mmol/L)	1.6±0.005	1.3±0.02‡	1.7±0.003	1.4±0.01‡
Total cholesterol (mmol/L)	6.0±0.01	6.3±0.04‡	5.7±0.006	5.2±0.03‡
LDL cholesterol (mmol/L)	3.7±0.01	3.8±0.04‡	3.3±0.005	2.8±0.03‡
Triglycerides (mmol/L)*	1.5±0.02	2.3±0.06‡	1.5±0.009	2.0±0.04‡
Body mass index (kg/m^2)	25.2±0.04	29.1±0.16‡	26.0±0.02	29.9±0.13‡
Hypertension (%)	49	76‡	58	84‡
Smoking (%)	47	46	22	24
Alcohol consumption (%)	15	17	18	19
Physical inactivity (%)	62	72‡	52	64‡
Postmenopausal (%)†	67	90‡	65	88‡

Hormonal replacement therapy (%)†	16	12§	14	12
Lipid-lowering therapy (%)	1	2‡	8	42‡
Education < 8 years (%)	30	47‡	13	27‡

Values are mean (± standard error of the mean) or percent. Measurements of HbA1c were available on 5,625 individuals in the Copenhagen City Heart Study. Hypertension was use of anti-hypertensive medication and/or a systolic blood pressure of 140 mm Hg or greater, and/or a diastolic blood pressure of 90 mm Hg or greater. Smoking was current smoking. Alcohol consumption was >14/21 units per week for women/men (1 unit=12 g alcohol, equivalent to one glass of wine or one beer (33cL)). Physical inactive was ≤4 hours per week of light physical activity in leisure time. Women reported menopausal status and use of hormonal replacement therapy. Lipid-lowering therapy was primarily statins (yes/no), and education was less than eight years of education. Missing values (≤0.3%) were imputed (continuous covariates) or assigned a dummy variable (categorical covariates). *Geometric mean; †In women only. ‡P<0.001 and §P<0.05 by Student's t-test or Pearson's χ^2-test. HbA1c=glycated hemoglobin; HDL= high-density lipoprotein; LDL=low-density lipoprotein. NA=not available. To convert cholesterol values to mg/dL, divide by 0.0259. To convert triglycerides to mg/dL, divide by 0.0113.

SUPPLEMENTARY DATA

Supplementary Table S4. Characteristics of individuals in the Copenhagen City Heart Study and the Copenhagen General Population Study by gene score quartile.

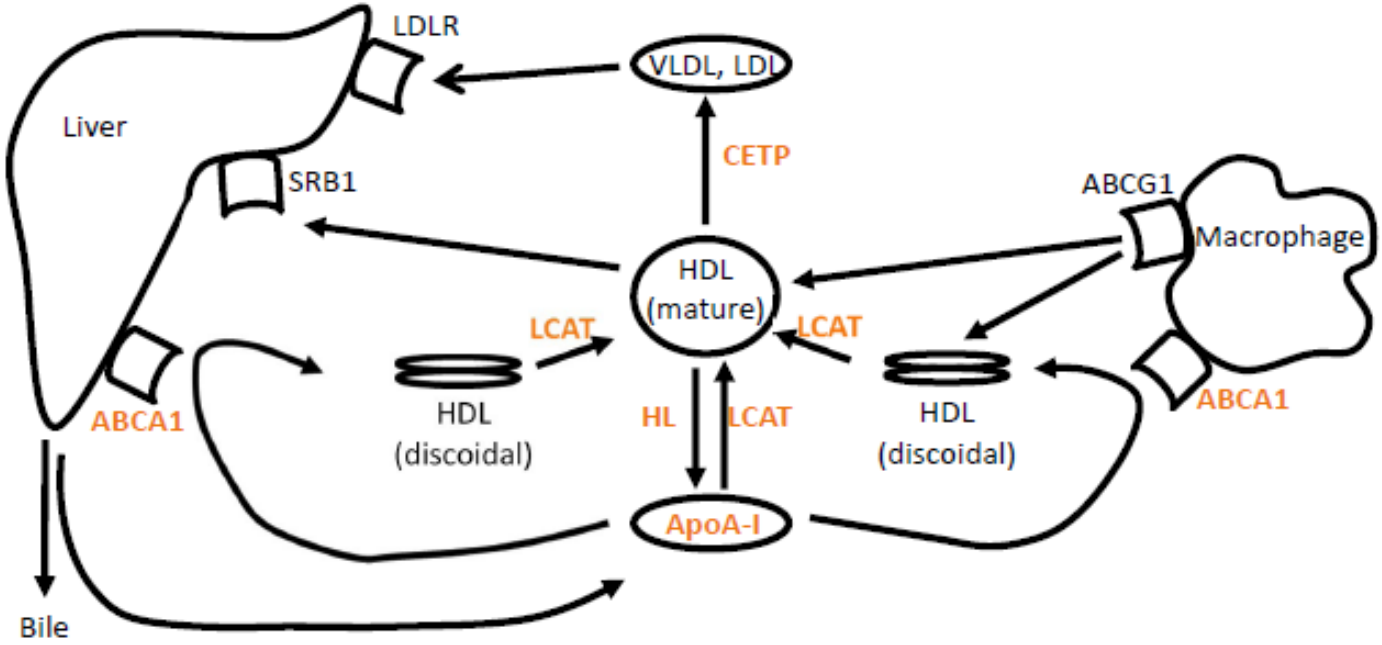
	Gene score quartiles			
	1st	2nd	3rd	4th
No. of individuals	10,391	10,319	12,046	14,871
Age (years)	56.9±0.14	56.8±0.14	56.9±0.13	57.3±0.12
Females (%)	55	54	55	55
Glucose	5.4±0.01	5.4±0.01	5.4±0.01	5.4±0.01
HbA1c (%)	5.9±0.02	5.9±0.02	5.9±0.02	5.9±0.02
HbA1c (mmol/mol)	41±0.2	41±0.2	41±0.2	41±0.2
HDL cholesterol (mmol/L)	1.8±0.005	1.7±0.005	1.6±0.005	1.5±0.004
Total cholesterol (mmol/L)	5.8±0.01	5.8±0.01	5.7±0.01	5.7±0.009
LDL cholesterol (mmol/L)	3.3±0.01	3.3±0.01	3.3±0.009	3.4±0.008
Triglycerides (mmol/L)*	1.5±0.02	1.5±0.02	1.5±0.02	1.5±0.01
Body mass index (kg/m^2)	26.0±0.04	26.0±0.04	26.1±0.04	26.0±0.04
Hypertension (%)	58	58	58	58
Smoking (%)	27	29	28	27
Alcohol consumption (%)	18	18	18	17
Physical inactivity (%)	54	54	55	54
Postmenopausal (%)†	65	66	66	67
Hormonal replacement therapy (%)†	14	14	15	14

Lipid-lowering therapy (%)	7	8	7	8
Education < 8 years (%)	17	17	17	18

Values are mean (± standard error of the mean) or percent. Measurements of HbA1c were available on 5,625 individuals in the Copenhagen City Heart Study. Hypertension was use of anti-hypertensive medication and/or a systolic blood pressure of 140 mm Hg or greater, and/or a diastolic blood pressure of 90 mm Hg or greater. Smoking was current smoking. Alcohol consumption was >14/21 units per week for women/men (1 unit=12 g alcohol, equivalent to one glass of wine or one beer (33cL)). Physical inactive was ≤4 hours per week of light physical activity in leisure time. Women reported menopausal status and use of hormonal replacement therapy. Lipid-lowering therapy was primarily statins (yes/no), and education was less than eight years of education. Missing values (≤0.3%) were imputed (continuous covariates) or assigned a dummy variable (categorical covariates). *Geometric mean; †In women only. HbA1c=glycated hemoglobin; HDL= high-density lipoprotein; LDL=low-density lipoprotein. NA=not available. To convert cholesterol values to mg/dL, divide by 0.0259. To convert triglycerides to mg/dL, divide by 0.0113.

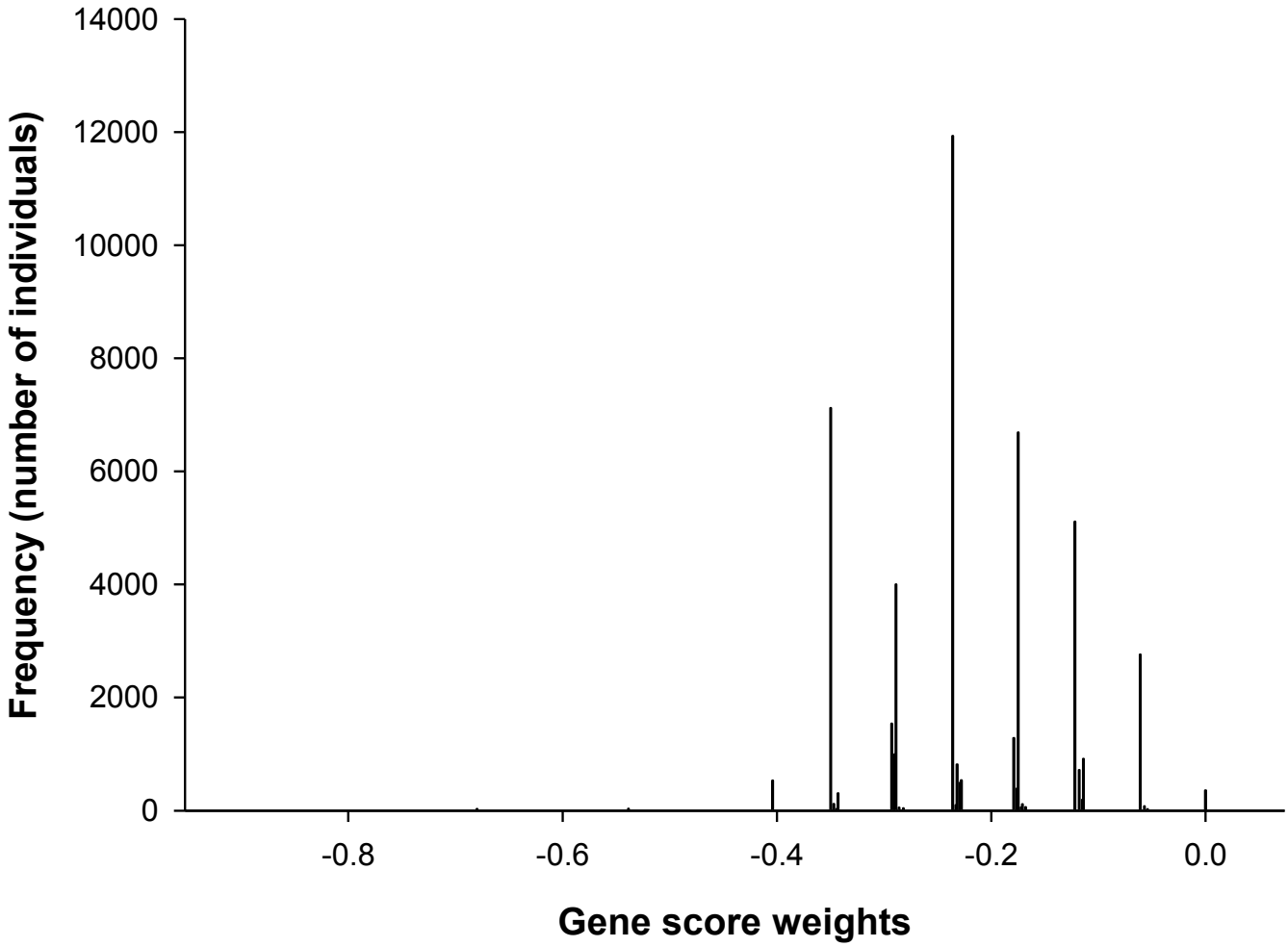
SUPPLEMENTARY DATA

Supplementary Figure S1. Schematic illustration of HDL cholesterol metabolism. Lipid-poor apoA-I is secreted by the liver and acquires cholesterol via the hepatocyte ABCA1 transporter. Nascent discoidal HDL particles are formed acquiring additional free cholesterol primarily from macrophage ABCA1 and ABCG1. Free cholesterol is esterified to cholesteryl esters by LCAT to form mature HDL, which transfers its cholesterol to apolipoprotein B-containing lipoproteins, such as VLDL and LDL in exchange for triglycerides, via CETP mediated transfer. This cholesterol is subsequently taken up by the liver via the LDL receptor. Alternatively, selective uptake of cholesterol from mature HDL is mediated by SRB1. The HDL particle can be remodeled by hepatic lipase (HL) (gene name *LIPC*). In orange, proteins encoded by genes in which nine genetic variants were genotyped in the present study (1,3-5). ABCA1=ATP-binding-cassette transporter A1; ABCG1=ATP-binding cassette transporter G1; apoA-I=apolipoprotein A-I; CETP=cholesteryl-ester transfer protein; HDL=high-density lipoprotein; HL=hepatic lipase; LCAT=lecithin-cholesterol acyltransferase; LDL=low-density lipoprotein; LDLR=LDL receptor; SRB1=scavenger receptor B1; VLDL=very-low density lipoprotein.



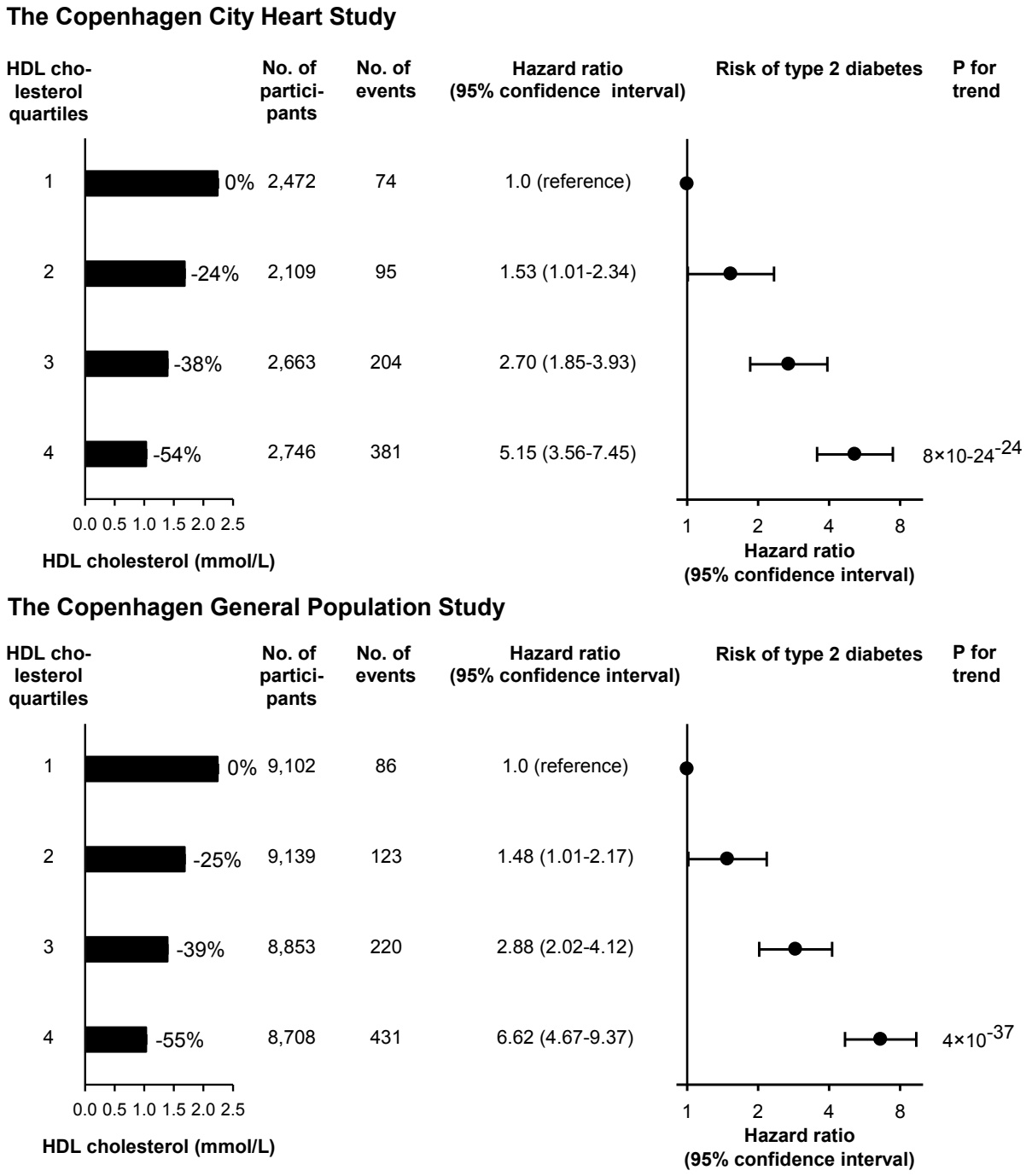
SUPPLEMENTARY DATA

Supplementary Figure S2. Distribution of HDL decreasing gene score weights. The distribution is skewed to the left which would be expected when rare loss-of function variants with large HDL cholesterol decreasing effects are included in the gene score.



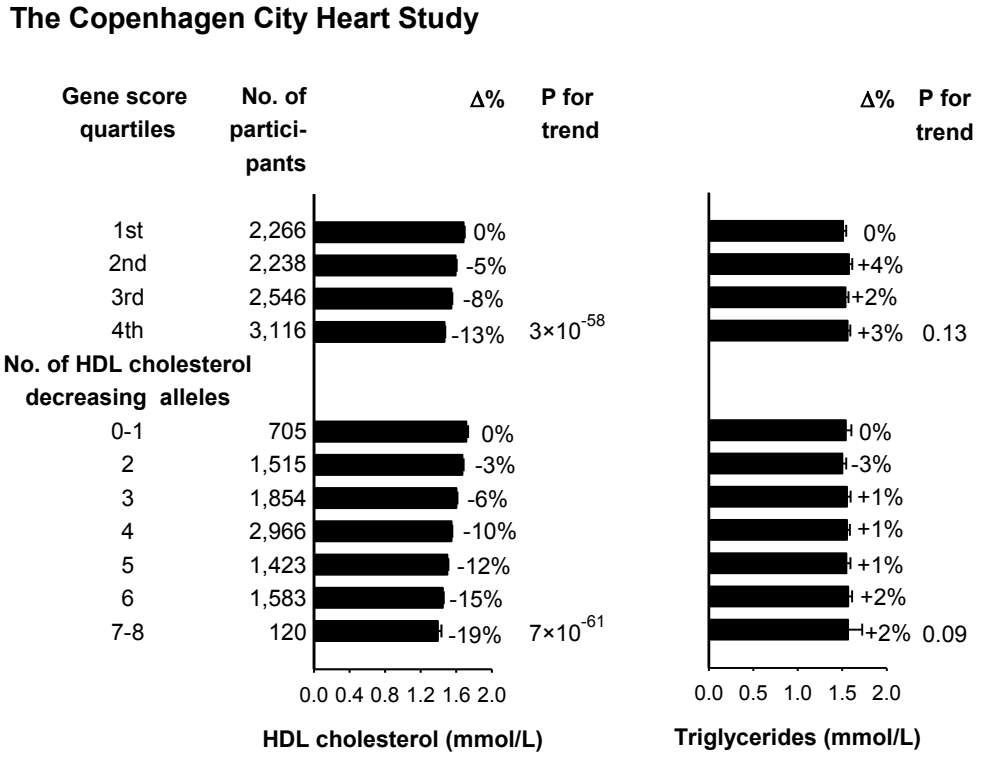
SUPPLEMENTARY DATA

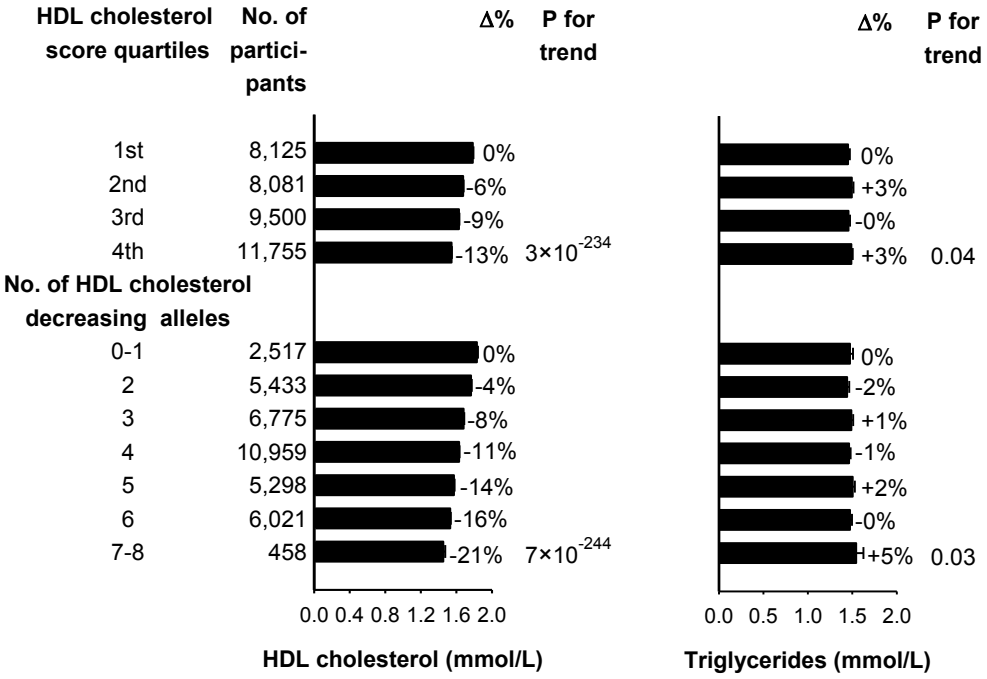
Supplementary Figure S3. Risk of type 2 diabetes as a function of plasma HDL cholesterol levels in quartiles in the Copenhagen City Heart Study and the Copenhagen General Population Study. Individuals with type 2 diabetes before blood sampling were excluded, leaving, respectively, 9,990 and 36,662 individuals for analyses. Multifactorial adjustment was for age (as time scale), sex, body mass index, hypertension, smoking, alcohol intake, physical inactivity, postmenopausal status and hormonal replacement therapy in women, lipid-lowering therapy and educational level. Hazard ratios including confidence intervals were corrected for regression dilution bias. *P*-values from Cox regression trend test. HDL=high-density lipoprotein. To convert HDL cholesterol to mg/dL, divide by 0.0259.



SUPPLEMENTARY DATA

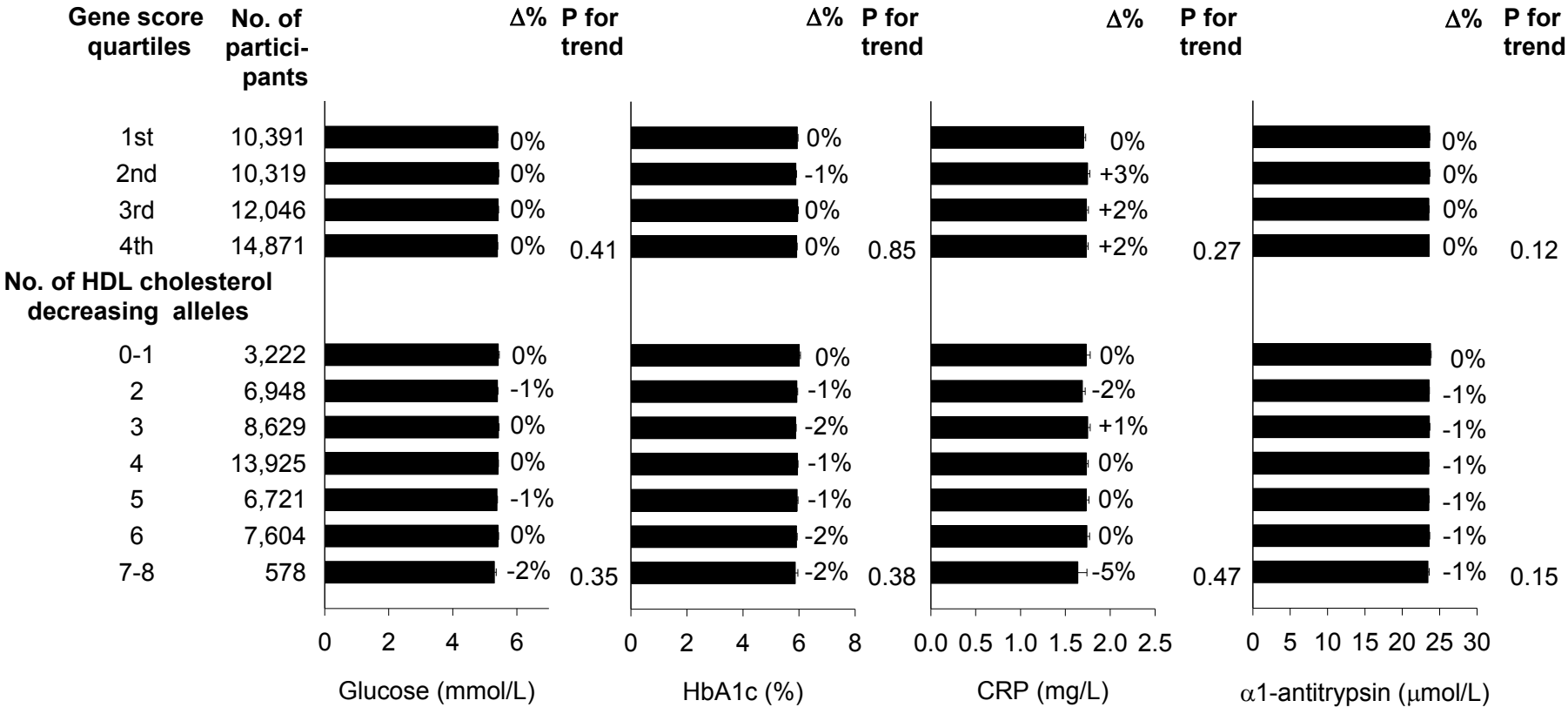
Supplementary Figure S4. Plasma levels of HDL cholesterol and triglycerides as a function of HDL cholesterol gene score quartiles/number of HDL decreasing cholesterol alleles in the Copenhagen City Heart Study and the Copenhagen General Population Study. Values are mean or geometric mean (± standard error of the mean). Percentages are changes in mean level from 1st quartile and 0-1 decreasing alleles, respectively. P-values are test for trend by linear regression. HDL=high-density lipoprotein. To convert HDL cholesterol to mg/dL, divide by 0.0259.





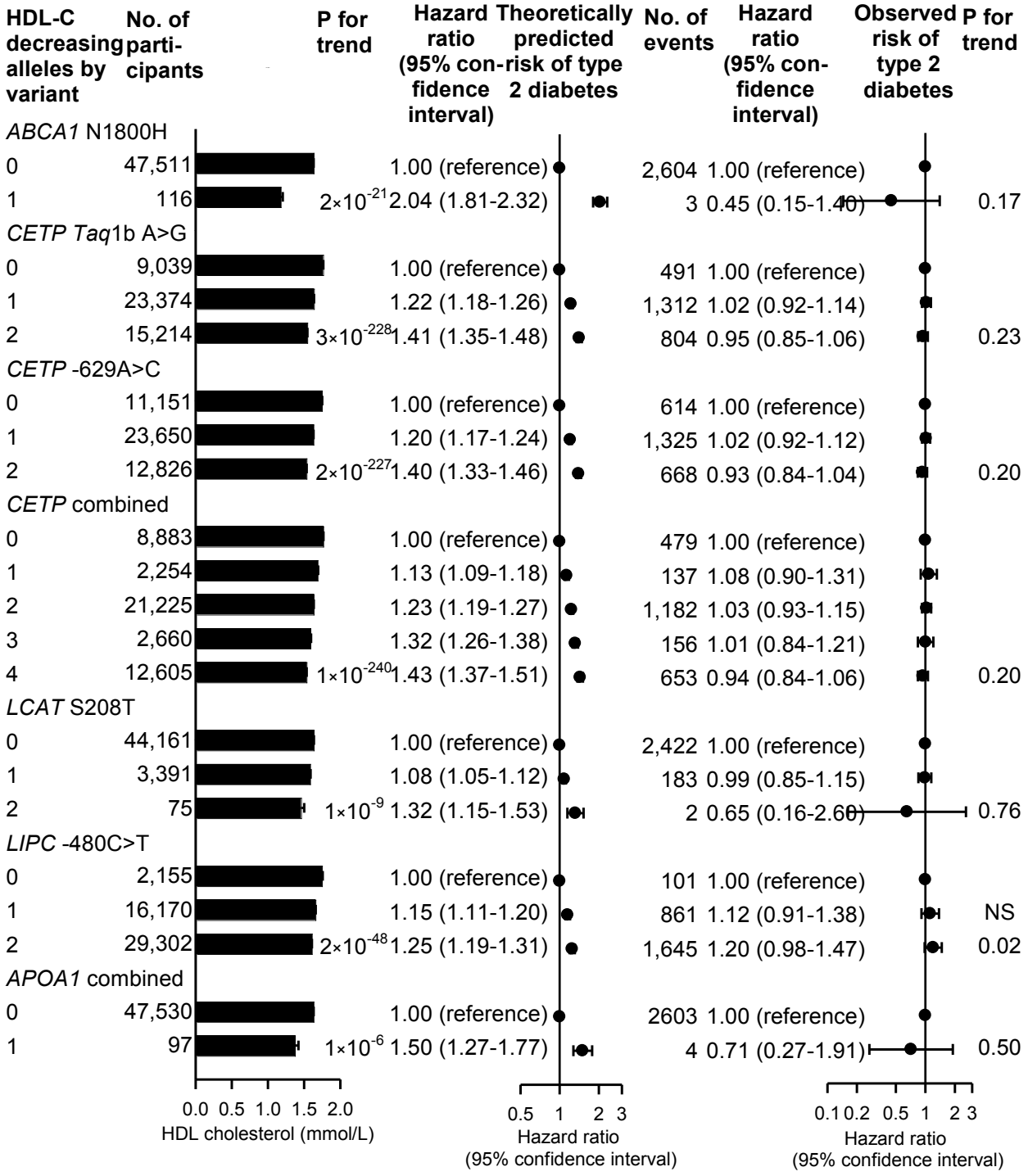
SUPPLEMENTARY DATA

Supplementary Figure S5. Plasma levels of nonfasting-glucose, HbA1c, CRP and α1-antitrypsin as a function of HDL cholesterol gene score quartiles/number of HDL cholesterol decreasing alleles in the Copenhagen City Heart Study and the Copenhagen General Population Study combined (n=47,627). Values are mean (± standard error of the mean). Percentages are changes in mean level from 1st quartile and 0-1 decreasing alleles, respectively. Measurements of HbA1c were available for 5,625 individuals. P-values are tests for trend by linear regression. HbA1c=glycated hemoglobin; CRP=C-reactive protein; HDL= high-density lipoprotein. HbA1c units: 4%=20 mmol/mol, 6%=42 mmol/mol, 8%=64 mmol/mol.



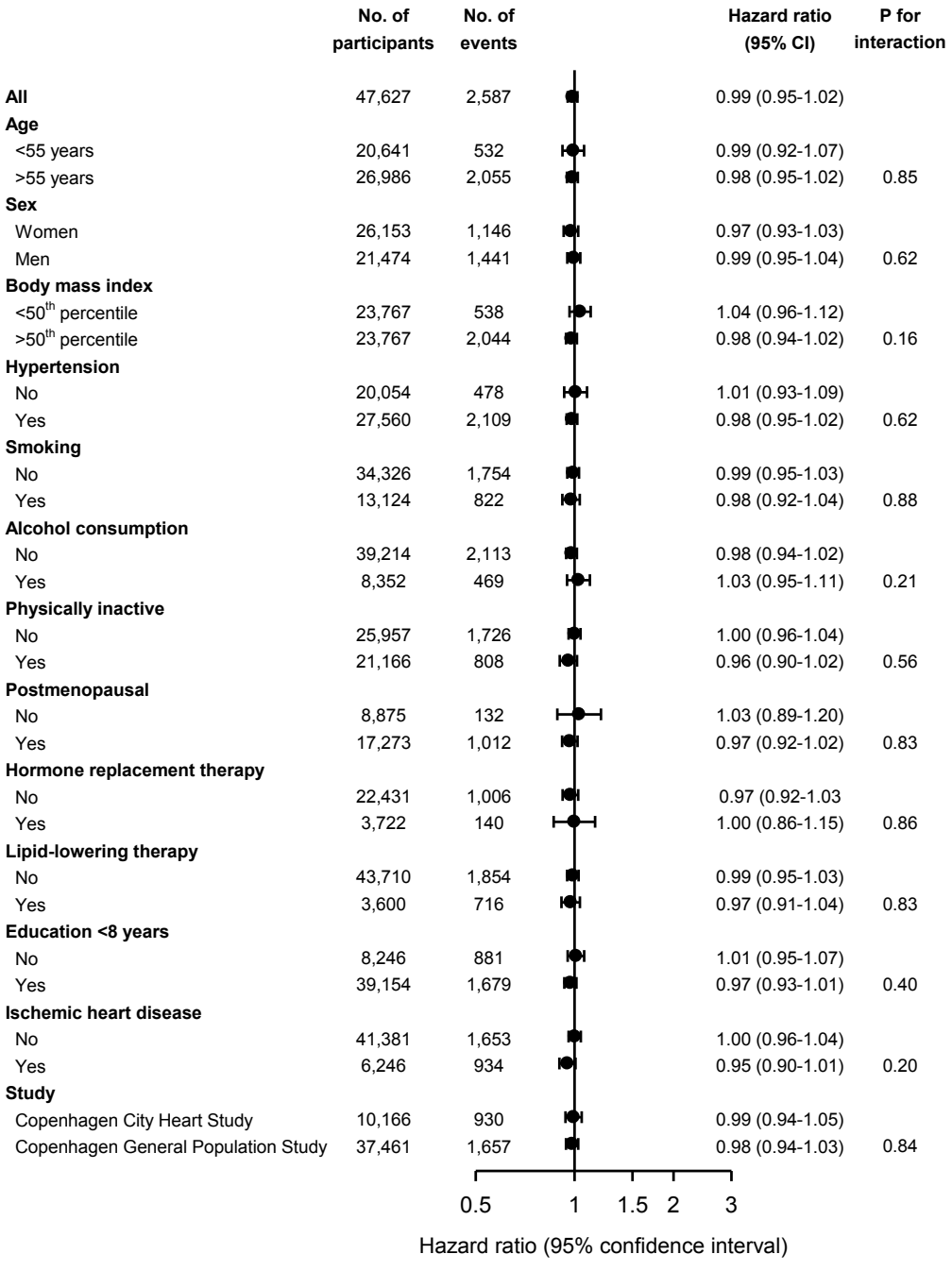
SUPPLEMENTARY DATA

Supplementary Figure S6. Plasma levels of HDL cholesterol, theoretically predicted hazard ratios and observed hazard ratios for type 2 diabetes as a function of individual HDL cholesterol decreasing genetic variants in the Copenhagen City Heart Study and the Copenhagen General Population Study combined (n=47,627). Hazard ratios were multifactorially adjusted for age (as time scale), sex, study, body mass index, hypertension, smoking, alcohol intake, physical inactivity, postmenopausal status and hormonal replacement therapy in women, lipid-lowering therapy and educational level. Theoretically predicted hazard ratios including 95% confidence intervals were corrected for regression dilution bias. HDL cholesterol values are mean (± standard error of the mean). P-values are test for trend. HDL=high-density lipoprotein. To convert HDL cholesterol to mg/dL, divide by 0.0259. NS=not significant after correction for seven multiple comparisons (Bonferroni corrected p-value=0.05/7=0.007).



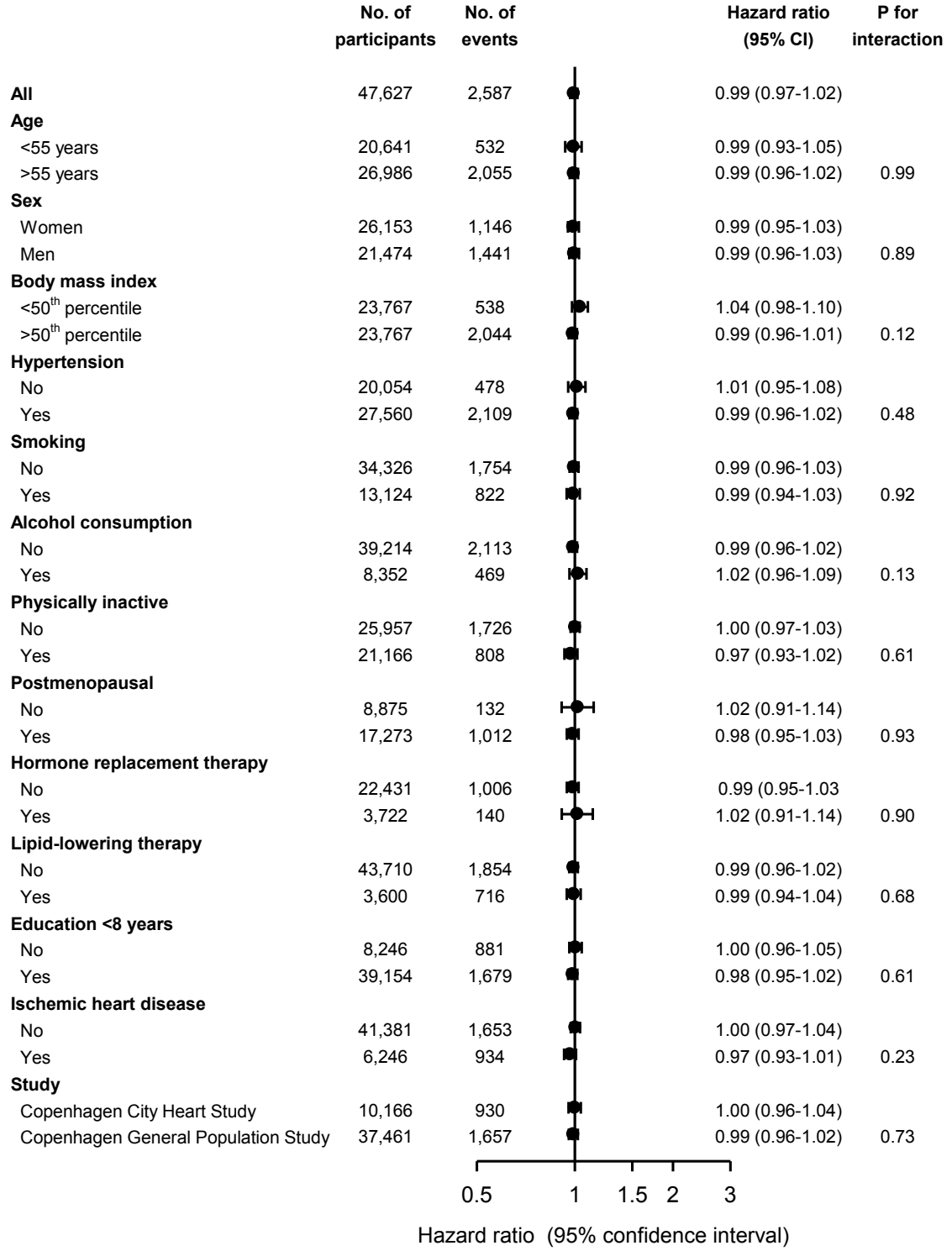
SUPPLEMENTARY DATA

Supplementary Figure S7. Risk of type 2 diabetes as a function of HDL cholesterol gene score quartiles (on a continuous scale) stratified by risk factors for type 2 diabetes in the Copenhagen City Heart Study and Copenhagen General Population Study combined (n=47,627), and stratified by ischemic heart disease and study cohort (bottom). Multifactorial adjustment was for age (as time scale), sex, study, body mass index, hypertension, smoking, alcohol intake, physical inactivity, postmenopausal status and hormonal replacement therapy in women, lipid-lowering therapy and educational level. P-values are for interaction between gene score quartiles and the covariate on risk of type 2 diabetes. HDL=high-density lipoprotein.



SUPPLEMENTARY DATA

Supplementary Figure S8. Risk of type 2 diabetes as a function of number of HDL cholesterol decreasing alleles (on a continuous scale) stratified by risk factors for type 2 diabetes in the Copenhagen City Heart Study and Copenhagen General Population Study combined (n=47,627), and stratified by ischemic heart disease and study cohort (bottom). Multifactorial adjustment was for age (as time scale), sex, study, body mass index, hypertension, smoking, alcohol intake, physical inactivity, postmenopausal status and hormonal replacement therapy in women, lipid-lowering therapy and educational level. P-values are for interaction between number of HDL cholesterol decreasing alleles and the covariate on risk of type 2 diabetes. HDL=high-density lipoprotein.



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