SUPPLEMENTARY MATERIAL

Effect of loss-of-function genetic variants in *PCSK9* on glycemic traits, neurocognitive impairment and hepatobiliary function

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SUPPLEMENTARY MATERIAL

Study population

The UK Biobank (UKB) is a large-scale national cohort study of approximately 500,000 participants aged 40 to 69 years, who visited 22 assessment centers throughout the United Kingdom between 2006 and 2010, with comprehensive baseline clinical assessments and collection of genetic data. The UKB resource was approved by the UK Biobank Research Ethics Committee and all participants provided written informed consent to participate.

Sample quality control and ancestry definition

In the present analysis samples that were outliers for heterozygosity, missingness or excess relativeness were removed. Samples were further excluded if they were not used in the central kinship inference. In addition, individuals with putative sex chromosome aneuploidy or with a mismatch between self-reported and genetically inferred sex were excluded. In order to identify a subset of individuals with European ancestry, we clustered individuals in UKB that had self-reported as "White" (UKB data field 21000; data codes: 1. 1001. 1002 and 1003). We then applied the Bayesian outlier detection algorithm implemented in the R package *aberrant* with principal components 1-2. 3-4 and 5-6, respectively.[1] The intersection of these clustered sets defined "individuals with European ancestry" (**Supplementary Figure 1**).

Sequencing coverage of PCSK9

Sequencing depth of the coding region of *PCSK9* was calculated using Mosdepth v.0.3.1.[2] We selected a random subset of 2.000 exomes and generated coverage data for each. Sequencing depth in each exon is reported as mean values and % of base pairs covered with >30x coverage (**Supplementary Table 8 and Supplementary Figure 2**). A sequencing depth over 20x is generally considered sufficient coverage for calling a variant.

Polygenic risk score

We conducted polygenic risk score (PRS) analyses to determine whether the effect of *PCSK9* pLoF on glycemic traits, hepatobiliary function and neurocognitive traits differed across

polygenic risk score quantiles for LDL-C, coronary artery disease (CAD) or type 2 diabetes (T2D). We used PRSice (URL: http://www.prsice.info/)[3] to generate PRSs, according to standard protocol. Briefly, SNPs reaching genome-wide significance ($P < 5 \times 10^{-8}$) were extracted from three studies; the Global Lipids Genetics Consortium (GLGC) summary statistics on LDL-C[4] (excluding the *PCSK9* locus), the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium summary statistics on T2D[5] and the Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) consortium summary statistics on CAD.[6] To avoid bias, only studies that did not include the UK Biobank cohort were considered. Clumping was applied using an LD $r^2 < 0.10$ and a 250 kb sliding window. We summed sets of alleles, weighted by their betas, into PRSs for each individual in the UKB data set. The resulting PRS was split into quintiles, with the first quintile serving as reference. Only unrelated individuals were retained, i.e. related individuals with *KING* coefficient > 0.0884, indicating 2nd degree or closer relatedness were excluded. To formally test whether the effect of the *PCSK9* pLoF variants on glycemic traits, hepatobiliary function and neurocognitive traits differed across different levels of polygenic risk for LDL-C, CAD and T2D, we tested for effect modification using linear regression. Effect modification was evaluated with likelihood ratio tests comparing a main model to a model with an interaction term. Models were adjusted for age at enrollment, sex, assessment center and the 5 first principal components. A two-sided $P < 0.05$ was considered statistically significant.

SUPPLEMENTARY RESULTS

Test for effect modification between PCSK9 loss-of-function variants and polygenic risk score

In total, 129 SNPs constituted the LDL-C PRS, 40 SNPs comprised the CAD-PRS and 38 SNPs represented the T2D-PRS, respectively. We found significant associations between the LDL-C PRS and LDL-C (0.19 mmol/L per SD increase in PRS; 95% CI 0.19-0.20; $P < 2 \times 10^{-16}$), CAD-PRS and myocardial infarction (OR 1.34 per SD increase in PRS; 95% CI 1.31-1.38; $P < 2 \times 10^{-16}$), T2D-PRS and type 2 diabetes (OR 1.41 per SD increase in PRS; 95% CI 1.38-1.44; $P < 2 \times 10^{-16}$), which confirms the eligibility for the PRSs to serve as proxies for genetically determined risk.

SUPPLEMENTARY TABLES

Supplementary Table 1. UK Biobank data fields used to infer study outcomes

Trait (unit)	Data field
Lipid and lipoprotein traits	
LDL cholesterol (mmol/L)	30780
HDL cholesterol (mmol/L)	30760
Triglyceride (mmol/L)	30870
Apolipoprotein A (g/L)	30630
Apolipoprotein B (g/L)	30640
Lipoprotein A (nmol/L)	30790
Glycemic traits and type 2 diabetes	
Blood glucose (mmol/L)	30740
Hemoglobin A1c (mmol/mol)	30750
Type 2 diabetes	130708
Hepatobiliary traits	
Alanin aminotransferase (U/L)	30620
Gamma glutamyltransferase (U/L)	30730
Bilirubin (µmol/L)	30840
Neurocognitive function	
Trail making test	20156
Fluid intelligence score	20240
Numeric memory	20240
Gray matter volume (cm^3)	25005
White matter volume (cm3)	25007

Supplementary Table 2. Loss-of-function variants in *PCSK9*

Chromosome	Position	Reference allele	Alternate allele	Mutation	Variant type	Carriers
1	55039838	A	G	p.Met1?	Start lost	2
1	55039902	T	TG	p.Leu23fs	Frameshift variant	2
1	55039925	G	GCGCA	p.Gln31fs	Frameshift variant	22
1	55039951	C	A	p.Tyr38Ter	Stop gained	1
1	55040006	G	T	p.Glu57Ter	Stop gained	3
1	55040024	AC	A	p.Phe64fs	Frameshift variant	3
1	55040045	G	A	c.207+1G>A	Splice donor variant	1
1	55043851	G	A	p.Trp72Ter	Stop gained	1
1	55043903	C	T	p.Gln90Ter	Stop gained	1
1	55044012	T	A	p.Met1?	Start lost	9
1	55046570	CT	C	p.Phe150fs	Frameshift variant	1
1	55046570	CTT	C	p.Phe150fs	Frameshift variant	1
1	55046646	G	A	p.Asp175Asn	Missense variant and Splice region variant	3
1	55052273	GA	G	c.524-2delA	Splice acceptor variant and Intron variant	1
1	55052374	ATG	A	p.Val208fs	Frameshift variant	1
1	55052412	G	T	c.657+1G>T	Splice donor variant and Intron variant	41
1	55052648	A	C	c.658-2A>C	Splice acceptor variant and Intron variant	1
1	55052661	T	A	p.Cys223Ter	Stop gained	1
1	55052704	G	GA	p.Asp238fs	Frameshift variant	2
1	55055992	G	A	c.800-1G>A	Splice acceptor variant and Intron variant	1
1	55056006	TC	T	p.Arg272fs	Frameshift variant	2
1	55056009	GA	G	p.Ser274fs	Frameshift variant	6
1	55057364	C	T	p.Gln344Ter	Stop gained	4
1	55057478	C	T	p.Gln382Ter	Stop gained	8
1	55057514	G	A	p.Gly394Ser	Missense variant and Splice region variant	37

1	55058036	G	T	p.Gly394Val	Missense variant and Splice region variant	10
1	55058113	GC	G	p.Asp422fs	Frameshift variant	12
1	55058139	G	A	p.Trp428Ter	Stop gained	1
1	55058597	A	AG	p.Ser485fs	Frameshift variant	1
1	55058649	T	C	c.1503+2T>C	Splice donor variant and Intron variant	1
1	55059491	A	AG	p.Lys506fs	Frameshift variant	1
1	55059573	C	T	p.Gln531Ter	Stop gained	1
1	55061368	G	GC	c.1682-3dup	Splice acceptor variant and Intron variant	19
1	55061374	G	C	c.1682-1G>C	Splice acceptor variant and Intron variant	3
1	55061437	C	T	p.Arg582Ter	Splice acceptor variant and Intron variant	2
1	55061473	GC	G	p.Ser470fs	Frameshift variant	2
1	55061517	CA	C	p.Lys609fs	Frameshift variant	1
1	55061557	G	A	c.1863+1G>A	Splice donor variant and Intron variant	97
1	55063542	C	A	p.Cys679Ter	Stop gained	64
1	55063570	C	T	p.Gln689Ter	Stop gained	3
1	55063582	T	G	p.Ter693Glyext	Stop lost	1
Total						**374**

Supplementary Table 3. Association between individual loss-of-function variants in *PCSK9* and outcomes

	Variant														
	p.Gln31fs			**c.657+1G>T**			**p.Gly394Ser**			**c.1863+1G>A**			**p.Cys679Ter**		
	n=22 heterozygotes			**n=41 heterozygotes**			**n=37 heterozygotes**			**n=97 heterozygotes**			**n=64 heterozygotes**		
	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*	β	SE	*P*
Lipids and lipoprotein traits															
LDL cholesterol, mmol/L	-0.85	0.16	1.03E-07	-0.67	0.12	8.33E-09	-0.45	0.14	9.43E-04	-0.83	0.08	1.14E-23	-0.71	0.11	1.10E-10
HDL cholesterol, mmol/L	-0.01	0.07	0.92	0.07	0.05	0.17	-0.02	0.06	0.77	0.01	0.03	0.75	0.00	0.05	0.96
Triglyceride, mmol/L	0.05	0.09	0.60	-0.07	0.07	0.32	0.01	0.08	0.95	-0.07	0.05	0.13	0.01	0.06	0.91
Apolipoprotein A, g/L	0.04	0.05	0.38	0.08	0.04	0.04	0.01	0.04	0.87	0.01	0.02	0.77	0.04	0.03	0.20
Apolipoprotein B, g/L	-0.22	0.05	1.10E-06	-0.17	0.03	3.40E-07	-0.13	0.04	6.60E-04	-0.22	0.02	3.38E-23	-0.19	0.03	3.39E-10
Lipoprotein A, nmol/L	0.02	0.24	0.93	-0.10	0.18	0.59	0.31	0.20	0.11	-0.05	0.12	0.68	0.25	0.16	0.13
Glycemic traits															
Blood glucose, mmol/L	0.05	0.03	0.12	-0.01	0.02	0.74	-0.01	0.03	0.79	-0.01	0.02	0.50	-0.03	0.02	0.22
Hemoglobin A1c, mmol/mol	2.48	1.06	0.02	-0.01	0.02	0.71	0.01	0.02	0.55	-0.01	0.01	0.50	-0.58	0.75	0.44
Hepatobiliary traits															
Alanin aminotransferase, U/L	0.22	0.08	3.94E-03	-0.06	0.06	0.27	-0.14	0.07	0.04	0.05	0.04	0.21	0.00	0.05	0.96
Gamma glutamyltransferase, U/L	0.15	0.11	0.15	-0.06	0.08	0.44	-0.08	0.09	0.37	0.02	0.06	0.77	-0.04	0.07	0.57
Bilirubin, µmol/L	0.03	0.07	0.62	0.02	0.05	0.72	-0.08	0.06	0.18	-0.03	0.04	0.37	-0.03	0.05	0.58
Neurocognitive function															
Trail making test	-0.05	0.11	0.67	-0.01	0.06	0.84	0.06	0.10	0.57	-0.03	0.06	0.65	0.04	0.07	0.56
Fluid intelligence score	-0.62	0.64	0.33	0.00	0.43	1.00	0.86	0.61	0.16	0.18	0.36	0.62	0.12	0.44	0.78
Numeric memory	-0.68	0.48	0.16	-0.04	0.30	0.90	0.09	0.46	0.84	0.33	0.27	0.21	0.62	0.33	0.06
Gray matter volume, cm^3	-9.33	16.15	0.56	-7.01	13.65	0.61	-2.13	16.14	0.89	12.88	9.01	0.15	26.76	14.75	0.07
White matter volume, cm^3	-0.66	17.24	0.97	-12.53	14.58	0.39	7.55	17.23	0.66	9.03	9.57	0.35	9.91	15.76	0.53

P-values were obtained using the Generalized Linear Mixed Model Association Test (GMMAT), after adjustment for age, sex, assessment center and the first 5 principal components of ancestry. A genetic-relatedness matrix was included as a random-effects covariate. Values for triglyceride, lipoprotein A, alanin aminotransferase, gamma glutamyltransferase, bilirubin, HbA1c, glucose and trail making test were log-transformed prior to association testing to obtain normality of residuals. Abbreviations: β, regression coefficient; SE, standard error; P, P-value.

Supplementary Table 4. Evaluation of the effect of *PCSK9* loss-of-function variants on study outcomes across different LDL-C polygenic risk score quintiles.

	Polygenic risk score, quintile (Q1-Q5)										
	***PCSK9* pLoF carriers vs. non-carriers**										
	Q1 (59 carriers; 32,014 non-carriers)		Q2 (62 carriers; 32,319 non-carriers)		Q3 (64 carriers; 31,722 non-carriers)		Q4 (79 carriers; 32,348 non-carriers)		Q5 (74 carriers; 32,113 non-carriers)		
	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	*P* for interaction
Lipids and lipoprotein traits											
LDL cholesterol, mmol/L	-0.70 [-0.92; -0.48]	<0.001	-0.65 [-0.86; -0.44]	<0.001	-0.59 [-0.81; -0.38]	<0.001	-0.78 [-0.97; -0.60]	<0.001	-0.75 [-0.95; -0.55]	<0.001	0.691
HDL cholesterol, mmol/L	0.02 [-0.07; 0.12]	0.630	0.00 [-0.09; 0.09]	0.953	0.04 [-0.04; 0.13]	0.333	0.04 [-0.04; 0.12]	0.388	0.04 [-0.05; 0.13]	0.391	0.957
Triglyceride, mmol/L	-0.07 [-0.20; 0.06]	0.266	-0.13 [-0.26; -0.00]	0.045	-0.02 [-0.14; 0.11]	0.762	0.03 [-0.08; 0.14]	0.548	-0.09 [-0.21; 0.02]	0.120	0.331
Apolipoprotein A, g/L	0.01 [-0.06; 0.08]	0.744	-0.02 [-0.09; 0.04]	0.470	0.08 [0.01; 0.14]	0.017	0.07 [0.01; 0.13]	0.014	0.04 [-0.02; 0.11]	0.182	0.144
Apolipoprotein B, g/L	-0.19 [-0.25; -0.13]	<0.001	-0.17 [-0.23; -0.11]	<0.001	-0.16 [-0.22; -0.10]	<0.001	-0.20 [-0.25; -0.15]	<0.001	-0.23 [-0.29; -0.18]	<0.001	0.372
Lipoprotein A, nmol/L	-0.14 [-0.46; 0.19]	0.414	0.02 [-0.30; 0.34]	0.902	0.08 [-0.27; 0.42]	0.662	0.05 [-0.24; 0.34]	0.740	-0.05 [-0.36; 0.25]	0.730	0.896
Glycemic traits											
Blood glucose, mmol/L	0.03 [-0.02; 0.07]	0.216	0.02 [-0.03; 0.06]	0.465	-0.02 [-0.06; 0.02]	0.252	-0.01 [-0.05; 0.03]	0.582	-0.02 [-0.06; 0.02]	0.315	0.339
Hemoglobin A1c, mmol/mol	0.03 [-0.01; 0.06]	0.121	0.00 [-0.03; 0.04]	0.806	-0.01 [-0.04; 0.03]	0.617	0.01 [-0.02; 0.04]	0.580	-0.00 [-0.03; 0.03]	0.952	0.656
Hepatobiliary traits											
Alanin aminotransferase, U/L	0.01 [-0.10; 0.12]	0.871	-0.06 [-0.17; 0.05]	0.280	0.02 [-0.09; 0.12]	0.770	0.10 [0.01; 0.19]	0.036	-0.08 [-0.18; 0.02]	0.103	0.079
Gamma glutamyltransferase, U/L	0.05 [-0.10; 0.20]	0.539	-0.11 [-0.26; 0.03]	0.130	0.01 [-0.14; 0.15]	0.903	0.08 [-0.05; 0.21]	0.229	-0.09 [-0.23; 0.04]	0.184	0.218
Bilirubin, µmol/L	0.03 [-0.07; 0.12]	0.570	-0.02 [-0.11; 0.07]	0.685	0.00 [-0.09; 0.09]	0.978	0.01 [-0.07; 0.09]	0.855	-0.03 [-0.12; 0.05]	0.437	0.896
Neurocognitive function											
Trail making test	-0.10 [-0.27; 0.07]	0.258	-0.02 [-0.14; 0.11]	0.758	0.04 [-0.11; 0.19]	0.585	0.10 [-0.04; 0.24]	0.150	-0.07 [-0.22; 0.07]	0.313	0.314

Fluid intelligence score	0.61 [-0.49; 1.71]	0.275	-0.01 [-0.84; 0.81]	0.973	-0.02 [-0.93; 0.89]	0.961	-0.47 [-1.28; 0.34]	0.254	-0.20 [-1.06; 0.67]	0.653	0.639
Numeric memory	-0.36 [-1.12; 0.40]	0.352	-0.12 [-0.73; 0.49]	0.696	0.29 [-0.40; 0.98]	0.408	-0.03 [-0.64; 0.58]	0.922	0.13 [-0.54; 0.80]	0.696	0.761
Gray matter volume, cm^3	24.17 [-4.71; 53.05]	0.101	9.10 [-15.93; 34.12]	0.476	7.10 [-17.92; 32.11]	0.578	-23.36 [-46.94; 0.23]	0.052	8.95 [-14.63; 32.54]	0.457	0.115
White matter volume, cm^3	0.71 [-30.21; 31.62]	0.964	6.85 [-19.94; 33.64]	0.616	11.60 [-15.18; 38.38]	0.396	-5.41 [-30.66; 19.84]	0.674	1.83 [-23.42; 27.08]	0.887	0.920

Effect modification was evaluated with likelihood ratio tests comparing a main model to a model with an interaction term. Models were adjusted age, sex, 5 first principal components and assessment center. A two-sided $P < 0.05$ was considered statistically significant. Abbreviations: β, regression coefficient; CI, confidence interval; P, P-value.

Supplementary Table 5. Evaluation of the effect of *PCSK9* loss-of-function variants on study outcomes across different coronary artery disease polygenic risk score quintiles.

	Polygenic risk score, quintile (Q1-Q5)										
	***PCSK9* pLoF carriers vs. non-carriers**										
	Q1 (62 carriers; 31,649 non-carriers)		Q2 (57 carriers; 32,405 non-carriers)		Q3 (74 carriers; 33,049 non-carriers)		Q4 (76 carriers; 32,004 non-carriers)		Q5 (69 carriers; 31,404 non-carriers)		
	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	*P* for interaction
Lipids and lipoprotein traits											
LDL cholesterol, mmol/L	-0.83 [-1.05;-0.62]	<0.001	-0.55 [-0.78;-0.32]	<0.001	-0.64 [-0.84;-0.44]	<0.001	-0.72 [-0.92;-0.52]	<0.001	-0.70 [-0.91;-0.50]	<0.001	0.479
HDL cholesterol, mmol/L	0.05 [-0.04;0.15]	0.273	0.09 [-0.01;0.18]	0.071	0.04 [-0.04;0.13]	0.310	-0.00 [-0.08;0.08]	0.924	-0.02 [-0.11;0.07]	0.644	0.442
Triglyceride, mmol/L	-0.06 [-0.18;0.07]	0.357	-0.06 [-0.18;0.07]	0.357	-0.12 [-0.26;0.01]	0.071	-0.07 [-0.19;0.05]	0.233	0.02 [-0.10;0.13]	0.793	0.622
Apolipoprotein A, g/L	0.04 [-0.03;0.11]	0.233	0.06 [-0.01;0.13]	0.099	0.07 [0.01;0.13]	0.030	0.02 [-0.04;0.08]	0.549	0.01 [-0.05;0.08]	0.62895	0.692
Apolipoprotein B, g/L	-0.24 [-0.30;-0.18]	<0.001	-0.17 [-0.23;-0.11]	<0.001	-0.15 [-0.21;-0.10]	<0.001	-0.18 [-0.23;-0.13]	<0.001	-0.20 [-0.25;-0.14]	<0.001	0.277
Lipoprotein A, nmol/L	-0.20 [-0.53;0.12]	0.216	-0.02 [-0.35;0.30]	0.888	0.04 [-0.25;0.33]	0.769	-0.06 [-0.34;0.23]	0.699	0.15 [-0.17;0.47]	0.368	0.638
Glycemic traits											
Blood glucose, mmol/L	-0.02 [-0.06;0.02]	0.358	-0.00 [-0.05;0.04]	0.973	0.00 [-0.04;0.04]	0.937	0.00 [-0.04;0.04]	0.946	-0.00 [-0.04;0.04]	0.865	0.949
Hemoglobin A1c, mmol/mol	0.02 [-0.02;0.05]	0.313	0.01 [-0.03;0.04]	0.717	-0.03 [-0.06;0.01]	0.100	0.02 [-0.01;0.05]	0.239	0.02 [-0.02;0.05]	0.356	0.240
Hepatobiliary traits											
Alanin aminotransferase, U/L	-0.02 [-0.12;0.09]	0.759	0.00 [-0.11;0.11]	0.990	-0.03 [-0.13;0.07]	0.596	0.02 [-0.08;0.11]	0.737	0.03 [-0.08;0.13]	0.624	0.948
Gamma glutamyltransferase, U/L	0.06 [-0.08;0.21]	0.398	-0.06 [-0.21;0.10]	0.467	-0.02 [-0.16;0.11]	0.731	-0.01 [-0.14;0.13]	0.928	-0.03 [-0.17;0.11]	0.637	0.831
Bilirubin, µmol/L	-0.02 [-0.12;0.07]	0.633	-0.01 [-0.11;0.09]	0.889	0.04 [-0.05;0.13]	0.352	-0.03 [-0.12;0.05]	0.431	-0.00 [-0.09;0.09]	0.989	0.793
Neurocognitive function											
Trail making test	-0.03 [-0.19;0.12]	0.692	0.08 [-0.10;0.25]	0.382	-0.07 [-0.19;0.06]	0.307	0.04 [-0.08;0.17]	0.488	-0.02 [-0.18;0.13]	0.790	0.644
Fluid intelligence score	-0.00 [-1.02;1.02]	0.996	-0.01 [-1.00;0.98]	0.980	-0.29 [-1.07;0.48]	0.457	0.09 [-0.68;0.87]	0.813	-0.16 [-1.12;0.80]	0.740	0.968

Numeric memory	-0.49 [-1.20;0.22]	0.176	0.19 [-0.63;1.01]	0.655	-0.14 [-0.70;0.42]	0.625	0.44 [-0.17;1.04]	0.159	-0.08 [-0.77;0.60]	0.809	0.366
Gray matter volume, cm^3	11.10 [-17.78;39.99]	0.451	-25.39 [-60.76;9.97]	0.159	26.20 [4.86;47.54]	0.016	-16.51 [-43.25;10.24]	0.226	0.52 [-19.92;20.97]	0.960	0.052
White matter volume, cm^3	-2.90 [-33.81;28.01]	0.854	-8.80 [-46.65;29.06]	0.649	8.31 [-14.53;31.16]	0.476	-11.70 [-40.32;16.93]	0.423	13.69 [-8.19;35.57]	0.220	0.618

Effect modification was evaluated with likelihood ratio tests comparing a main model to a model with an interaction term. Models were adjusted age, sex, 5 first principal components and assessment center. A two-sided $P < 0.05$ was considered statistically significant. Abbreviations: β, regression coefficient; CI, confidence interval; P, P-value.

Supplementary Table 6. Evaluation of the effect of *PCSK9* loss-of-function variants on study outcomes across different type 2 diabetes polygenic risk score quintiles.

	Polygenic risk score, quintile (Q1-Q5)										
	***PCSK9* pLoF carriers vs. non-carriers**										
	Q1 (77 carriers; 32,104 non-carriers)		Q2 (67 carriers; 31,682 non-carriers)		Q3 (66 carriers; 32818 non-carriers)		Q4 (69 carriers; 31,774 non-carriers)		Q5 (59 carriers; 32,139 non-carriers)		
	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	β [95% CI]	*P*	*P* for interaction
Lipids and lipoprotein traits											
LDL cholesterol, mmol/L	-0.73 [-0.93;-0.53]	<0.0001	-0.68 [-0.89;-0.47]	<0.0001	-0.69 [-0.91;-0.48]	<0.0001	-0.62 [-0.83;-0.41]	<0.0001	-0.72 [-0.94;-0.49]	<0.0001	0.960
HDL cholesterol, mmol/L	0.04 [-0.04;0.12]	0.309	0.09 [0.00;0.17]	0.046	0.02 [-0.06;0.11]	0.584	-0.05 [-0.13;0.04]	0.288	0.03 [-0.07;0.12]	0.603	0.303
Triglyceride, mmol/L	-0.04 [-0.15;0.08]	0.516	-0.01 [-0.13;0.11]	0.847	-0.11 [-0.23;0.01]	0.081	-0.03 [-0.15;0.09]	0.635	-0.06 [-0.19;0.06]	0.325	0.829
Apolipoprotein A, g/L	0.06 [0.00;0.12]	0.044	0.10 [0.04;0.16]	0.002	0.02 [-0.04;0.09]	0.496	-0.01 [-0.07;0.06]	0.840	0.00 [-0.07;0.07]	0.907	0.123
Apolipoprotein B, g/L	-0.19 [-0.25;-0.14]	<0.0001	-0.18 [-0.24;-0.12]	<0.0001	-0.18 [-0.24;-0.12]	<0.0001	-0.18 [-0.24;-0.13]	<0.0001	-0.20 [-0.26;-0.14]	<0.0001	0.978
Lipoprotein A, nmol/L	0.00 [-0.29;0.30]	0.981	0.12 [-0.20;0.45]	0.453	-0.10 [-0.43;0.23]	0.561	-0.10 [-0.41;0.21]	0.516	0.07 [-0.26;0.40]	0.686	0.829
Glycemic traits											
Blood glucose, mmol/L	-0.00 [-0.04;0.04]	0.915	-0.02 [-0.06;0.02]	0.434	0.02 [-0.02;0.06]	0.282	-0.00 [-0.04;0.04]	0.867	-0.02 [-0.06;0.03]	0.514	0.706
Hemoglobin A1c, mmol/mol	0.01 [-0.02;0.04]	0.547	0.01 [-0.03;0.04]	0.631	0.04 [0.01;0.08]	0.017	-0.02 [-0.05;0.02]	0.335	-0.01 [-0.05;0.03]	0.622	0.157
Hepatobiliary traits											
Alanin aminotransferase, U/L	0.00 [-0.10;0.10]	0.990	0.02 [-0.08;0.12]	0.715	0.01 [-0.10;0.11]	0.868	-0.02 [-0.13;0.08]	0.645	0.00 [-0.11;0.11]	0.995	0.985
Gamma glutamyltransferase, U/L	0.01 [-0.12;0.15]	0.844	0.06 [-0.08;0.20]	0.426	0.10 [-0.04;0.24]	0.178	-0.13 [-0.27;0.01]	0.062	-0.10 [-0.25;0.05]	0.196	0.110
Bilirubin, µmol/L	0.04 [-0.05;0.12]	0.412	-0.02 [-0.11;0.07]	0.625	-0.07 [-0.16;0.02]	0.135	0.06 [-0.03;0.15]	0.205	-0.03 [-0.13;0.06]	0.518	0.274
Neurocognitive function											
Trail making test	0.03 [-0.10;0.16]	0.643	-0.03 [-0.17;0.10]	0.642	-0.17 [-0.33;-0.01]	0.042	0.03 [-0.15;0.20]	0.762	0.08 [-0.05;0.22]	0.225	0.191
Fluid intelligence score	0.68 [-0.17;1.52]	0.116	-0.81 [-1.64;0.01]	0.053	-0.29 [-1.25;0.67]	0.555	-0.55 [-1.51;0.40]	0.257	0.48 [-0.39;1.34]	0.279	0.065

Numeric memory	0.05 [-0.55;0.66]	0.863	-0.62 [-1.22;-0.01]	0.046	0.59 [-0.16;1.35]	0.125	0.15 [-0.58;0.89]	0.680	0.15 [-0.58;0.89]	0.680	0.161
Gray matter volume, cm^3	-5.25 [-27.63;17.13]	0.646	26.01 [2.42;49.60]	0.031	30.03 [-1.59;61.65]	0.063	-19.13 [-44.15;5.88]	0.134	-3.86 [-28.87;21.15]	0.762	0.054
White matter volume, cm^3	4.23 [-19.72;28.19]	0.729	15.89 [-9.36;41.14]	0.217	23.22 [-10.63;57.06]	0.179	-24.16 [-50.94;2.61]	0.077	0.91 [-25.86;27.69]	0.947	0.174

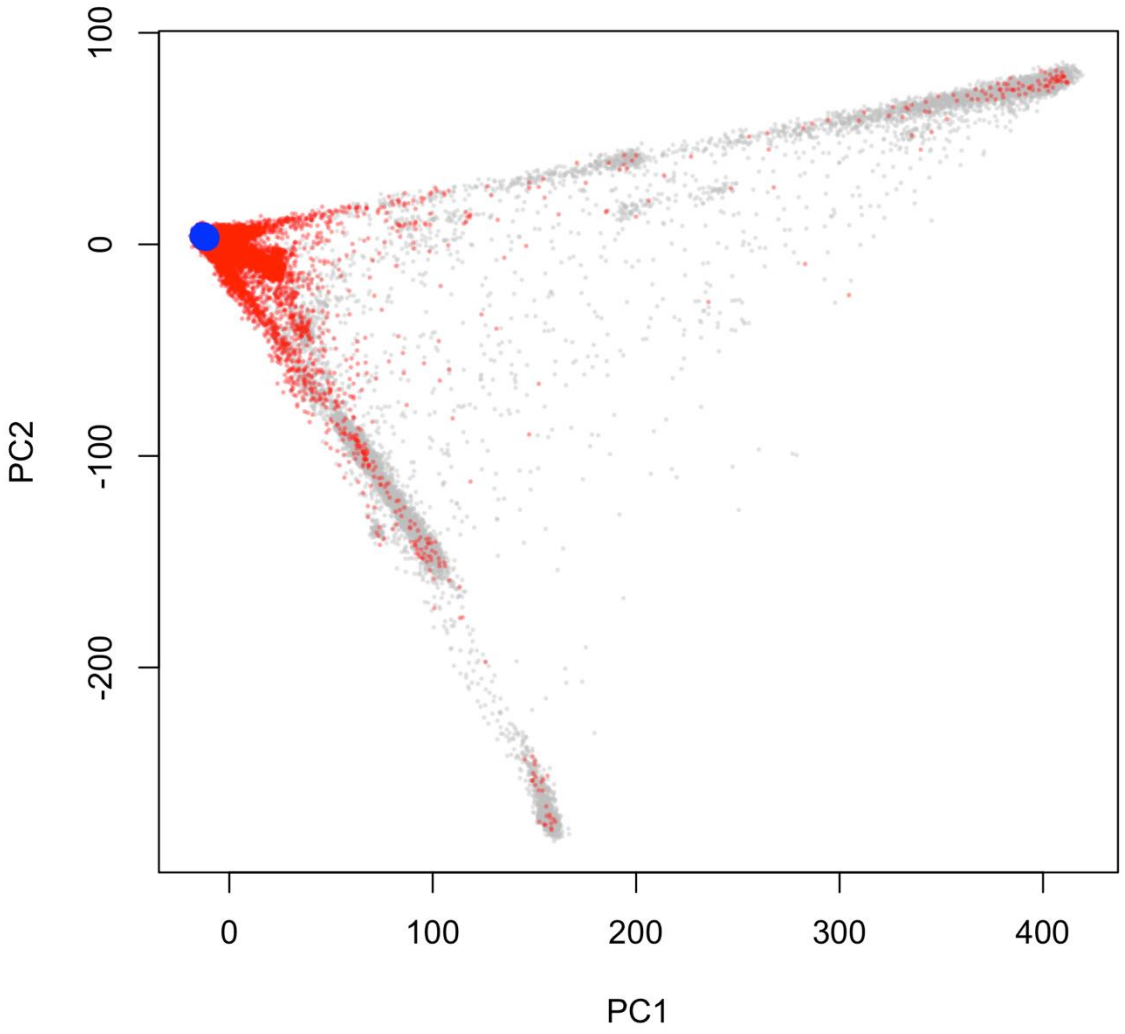
Effect modification was evaluated with likelihood ratio tests comparing a main model to a model with an interaction term. Models were adjusted age, sex, 5 first principal components and assessment center. A two-sided $P < 0.05$ was considered statistically significant. Abbreviations: β, regression coefficient; CI, confidence interval; P, P-value.

Supplementary Table 7. Summarized sequencing depth for each exon in *PCSK9* (n = 2.000). First column lists exon number. Second column lists percentage of bases in exon covered with more than 30x coverage. Third column lists mean coverage for each exon.

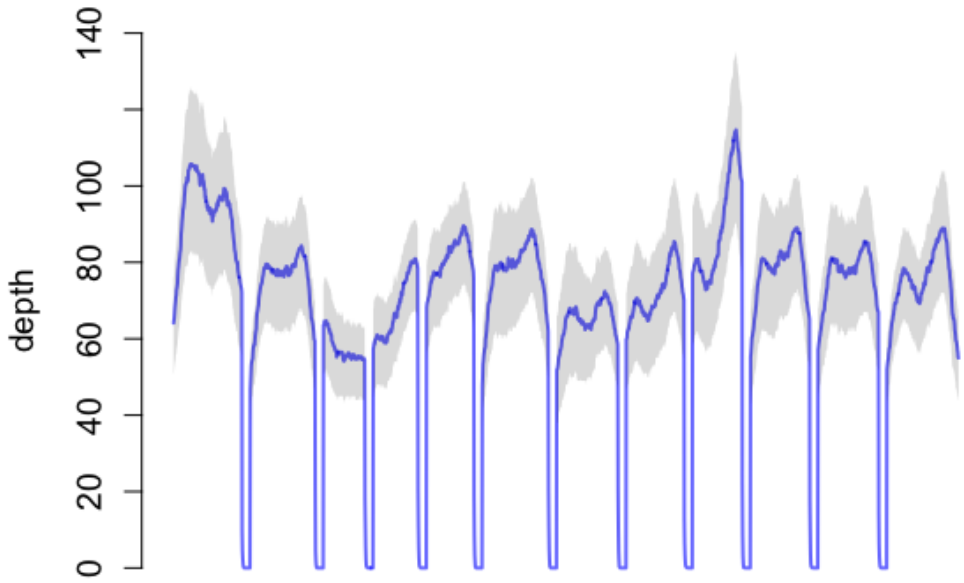
Exon	Percent bases covered with >30x coverage	Mean exon coverage
exon 1	99.8	93.0
exon 2	99.8	75.3
exon 3	98.1	57.2
exon 4	99.4	68.0
exon 5	100	81.2
exon 6	99.4	77.9
exon 7	94.2	65.2
exon 8	98.4	72.0
exon 9	99.6	89.0
exon 10	99.5	78.7
exon 11	99.8	77.1
exon 12	99.7	74.6

SUPPLEMENTARY FIGURES

Supplementary Figure 1. Principal component plots. Population substructure shown by principal components (PC). We plotted PC 1 and 2 from all participants with available exomes (n = 200,643). Dots in grey represents individuals who did not self-report as "White". Dots in red are individuals who self-reported as "White" but was not clustered by aberrant. Dots in blue represent the set of individuals with European ancestry (n = 175,336).



Supplementary Figure 2. Sequencing depth in PCSK9. Sequencing depth in each of the 12 exons in *PCSK9*. Mean depth for each base pair in blue. IQR 1-3 is shown by shaded grey outline.



REFERENCES

1. Bellenguez C, Strange A, Freeman C, Donnelly P, Spencer CCA. A robust clustering algorithm for identifying problematic samples in genome-wide association studies. *Bioinformatics* 2012;**28**:134–135.
2. Pedersen BS, Quinlan AR. Mosdepth: quick coverage calculation for genomes and exomes. *Bioinformatics* 2018;**34**:867–868.
3. Euesden J, Lewis CM, O'Reilly PF. PRSice: Polygenic Risk Score software. *Bioinforma Oxf Engl* 2015;**31**:1466–1468.
4. Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang H-Y, Demirkan A, Den Hertog HM, Do R, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkilä K, Hyppönen E, Isaacs A, Jackson AU, et al. Discovery and refinement of loci associated with lipid levels. *Nat Genet* 2013;**45**:1274–1283.
5. Scott RA, Scott LJ, Mägi R, Marullo L, Gaulton KJ, Kaakinen M, Pervjakova N, Pers TH, Johnson AD, Eicher JD, Jackson AU, Ferreira T, Lee Y, Ma C, Steinthorsdottir V, Thorleifsson G, Qi L, Zuydam NRV, Mahajan A, Chen H, Almgren P, Voight BF, Grallert H, Müller-Nurasyid M, Ried JS, Rayner NW, Robertson N, Karssen LC, Leeuwen EM van, Willems SM, et al. An Expanded Genome-Wide Association Study of Type 2 Diabetes in Europeans. *Diabetes* American Diabetes Association; 2017;**66**:2888–2902.
6. Nikpay M, Goel A, Won H-H, Hall LM, Willenborg C, Kanoni S, Saleheen D, Kyriakou T, Nelson CP, Hopewell JC, Webb TR, Zeng L, Dehghan A, Alver M, Armasu SM, Auro K, Bjonnes A, Chasman DI, Chen S, Ford I, Franceschini N, Gieger C, Grace C, Gustafsson S, Huang J, Hwang S-J, Kim YK, Kleber ME, Lau KW, Lu X, et al. A comprehensive 1000 Genomes–based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015;**47**:1121–1130.