Electronic supplementary material (ESM)

Dose-response relationship between genetically-proxied average blood glucose levels and incident coronary heart disease in individuals without diabetes mellitus

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ESM Methods

UK Biobank

The UK Biobank is made up of approximately 500,000 participants (of which 94% are of self-reported European-ancestry), aged 40 to 69 at recruitment between 2006 and 2010 across 22 assessment centres in the UK. Ethical approval for the UK Biobank study was obtained from the North West Multicenter Research Ethics Committee. All participants provided written informed consent. In this study, UK Biobank data was accessed through application 29202 and follow up was performed to 31 March 2020 or the date of death. Participant information was available for genotype, clinical measurements, biological assays, and self-reported health behaviours, with further linkage to electronic health records (1). To derive our initial analytic sample, we excluded participants having non-European ancestry (self-report or judged by genetics), low call rate or excess heterozygosity (>3 standard deviations from the mean) as described previously (2). We included only one of each set of related participants (third-degree relatives or closer). We also excluded participants without a valid HbA_{1c} measurement.

HbA_{1c} was measured in packed red blood cells using the Bio-Rad Variant II Turbo analyser (Bio-Rad Laboratories, Inc), which employs a High Performance Liquid Chromatography method. Results are expressed in mmol/mol units, with an analytical range of 15-184mmol/mol.

Our analyses only included participants who were judged as unlikely to have any type of diabetes mellitus. Possible diabetes was identified based on self-reported information, hospital episode statistics, and information on prescription medication as previously described (3). Only those judged as diabetes 'unlikely' were included in the analysis. Additionally, we excluded from analysis all those with residual HbA_{1c} (defined below) above

47.5 mmol/mol (6.5%), the threshold defined by the American Diabetes Association as a diagnostic criterion for diabetes (4).

International Classification of Diseases 9th Revision (ICD-9) codes 410-414, and ICD-10 codes I20-I25 were used to identify incident coronary heart disease cases.

Linear Mendelian randomization

The ratio of coefficients method was used to perform Mendelian randomization analyses that assumed a linear relationship between genetically-proxied average blood glucose levels and risk of incident coronary heart disease (CHD) (5). This represents the association of the average blood glucose level allele score with CHD divided by the association of the allele score with HbA_{1c} (6). We used linear regression to estimate the association of the allele score with HbA_{1c}, incorporating age, sex, principal components 1-10 of genetic ancestry, genotyping chip and assessment centre as covariates.

We calculated the proportion of variance in HbA_{1c} explained by the allele score and its F-statistic to estimate instrument strength (7). We used Cox proportional hazard regression to estimate the association of the allele score with CHD risk, incorporating sex, principal components 1-10 of genetic ancestry, genotyping chip and assessment centre as covariates. Age was used as the time variable in the time-to-event analyses. In sensitivity analyses, each variant in the allele score was considered as a separate instrumental variable using Mendelian randomization methods that differ in their requisite assumptions on the inclusion of pleiotropic variants: fixed-effects inverse-variance weighted, random-effects inverse-variance weighted, Egger, weighted median, contamination-mixture and PRESSO Mendelian randomization (8). An intercept term in the Egger method that differs from zero can be used

to provide evidence of directional pleiotropy (9). Statistics measuring heterogeneity in the Mendelian randomization estimates generated by different variants were further calculated to measure potential pleiotropy (10).

Non-linear Mendelian randomization

The fractional polynomial method was used to investigate for a non-linear relationship between genetically-proxied average blood glucose levels and risk of incident CHD (11-13). In this approach, we stratified the population into trigintiles (30 equal groups) based on residual HbA_{1c}, which is defined as a participant's HbA_{1c} minus the genetic contribution to HbA_{1c} from the average blood glucose level allele score. Thus, we aimed to compare individuals in the population who would have a similar average blood glucose levels (in the same trigintile stratum) if they also had the same genetic predisposition. Stratifying on HbA_{1c} itself would introduce collider bias and potentially distort estimates, as average blood glucose levels may be on the causal pathway from the genetic variants to CHD (13; 14). For each trigintile of the population, a linear Mendelian randomization estimate for the association of genetically-proxied HbA_{1c} with CHD was calculated using the ratio of coefficients method, as detailed above (6). A meta-regression of the linear Mendelian randomization estimates obtained for each trigintile against the mean HbA_{1c} in that centile was then performed using a flexible semiparametric framework (11; 13). We used a fractional polynomial test to investigate whether a non-linear model fit this meta-regression better than a linear model (11-13). A significant *p* value for this test is evidence against the null hypothesis that the linear model fits the data as well as the best-fitting fractional polynomial model. Hence a significant *p* value suggests that a non-linear model fits the data better than a linear model. Pre-specified

subgroup analyses considering males and females separately were also performed to investigate potential sex-specific effects.

Multivariable Mendelian randomization

Associations of the allele score were assessed using two-sample Mendelian randomization implemented by the inverse-variance weighted method with a random-effects model. Genetic associations with two-hour (post-load) glucose, fasting glucose, and fasting insulin were obtained from the MAGIC consortium (15; 16). Genetic associations with low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were obtained from the Global Lipids Genetics Consortium (GLGC) (17). Multivariable Mendelian randomization was performed by first creating an allele score for genetically-proxied LDL-cholesterol using genetic associations with LDL-cholesterol from the GLGC as weights. We then adjusted for genetically-proxied LDL-cholesterol in the calculation of the stratum-specific estimates, before combining in the non-linear model as described above.

Exclusion of variants associated with LDL-cholesterol

As a further sensitivity analysis, we performed Mendelian randomization analysis that excluded variants associated with LDL-cholesterol at $p<0.01$.

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ESM Table 1. The genetic variants used as instruments for average blood glucose levels, and their associations with type 2 diabetes liability and HbA_{1c}. Genetic variants were selected based on their association with type 2 diabetes liability ($p<5x10^{-8}$) in a genome-wide association study of 228,499 cases and 1,178,783 controls (79% European ancestry) that included UK Biobank participants and their association with HbA_{1c} ($p<0.001$ and concordant direction of association) in an independent study of 100,880 European ancestry participants (no overlap with UK Biobank) that were free of diabetes mellitus (as defined by physician diagnosis, medications, or fasting glucose ≥7 mmol/L).

						Type 2 diabetes			HbA_{1c}		
Single-nucleotide polymorphism	Chromosome	Position	Effect allele	Other allele	Effect allele frequency	Beta (log odds ratio)	Standard error	*p* value	Beta (%)	Standard error	*p* value
rs10923360	1	118166877	T	C	0.3299	0.0286	0.004	5.99E-13	0.007	0.0019	1.73E-04
rs3020781	1	155269776	G	A	0.3719	0.0294	0.0043	7.29E-12	0.0069	0.002	4.53E-04
rs340874	1	214159256	C	T	0.4991	0.0543	0.0039	6.47E-45	0.0079	0.0016	3.65E-07
rs1260326	2	27730940	C	T	0.5805	0.0625	0.0039	2.16E-57	0.0059	0.0017	3.69E-04
rs17334919	2	43707385	T	C	0.0916	-0.1207	0.0075	2.27E-58	-0.012	0.003	6.44E-05
rs10184004	2	165508389	T	C	0.4049	-0.0636	0.0041	4.39E-54	-0.0063	0.0018	3.45E-04
rs11708067	3	123065778	G	A	0.2229	-0.0804	0.005	1.63E-57	-0.013	0.0019	1.42E-12
rs9873519	3	124921457	T	C	0.4712	0.0373	0.0038	2.53E-22	0.0071	0.0019	1.39E-04
rs16851397	3	141134818	G	A	0.0656	-0.0684	0.0093	2.17E-13	-0.014	0.0037	1.25E-04
rs8192675	3	170724883	C	T	0.3167	-0.0452	0.0042	3.85E-27	-0.011	0.0017	1.38E-11
rs9859406	3	185534482	A	G	0.3524	0.1117	0.004	2.01E-169	0.0064	0.0019	5.95E-04
rs1996617	4	52798624	C	T	0.3914	0.0289	0.004	6.54E-13	0.0078	0.002	7.35E-05
rs735949	4	185716232	C	T	0.1332	-0.0555	0.0064	2.78E-18	-0.0098	0.0022	6.58E-06
rs6878122	5	76427311	A	G	0.7058	-0.0517	0.0045	2.00E-30	-0.0091	0.0019	1.05E-06
rs10440833	6	20688121	T	A	0.7028	-0.1286	0.0041	4.51E-215	-0.01	0.002	1.65E-07
rs3117189	6	32033944	G	A	0.8469	0.0573	0.0057	3.87E-24	0.013	0.0031	1.61E-05

rs679582	6	139831180	A	G	0.5485	-0.0262	0.0039	2.28E-11	-0.009	0.0019	1.37E-06
rs2191349	7	15064309	T	G	0.5668	0.067	0.0038	2.57E-71	0.0086	0.0017	2.09E-07
rs2267716	7	30716643	C	T	0.2741	-0.0364	0.0044	2.21E-16	-0.0074	0.0022	6.66E-04
rs1799884	7	44229068	T	C	0.1716	0.0553	0.005	9.45E-29	0.029	0.0026	1.06E-29
rs13266634	8	118184783	T	C	0.3186	-0.1024	0.0041	4.23E-136	-0.015	0.0017	4.53E-20
rs4237150	9	4290085	C	G	0.4292	0.0433	0.0037	6.98E-31	0.0064	0.0018	2.77E-04
rs10811661	9	22134094	C	T	0.2442	-0.1471	0.0048	9.59E-206	-0.014	0.0024	4.14E-09
rs505922	9	136149229	C	T	0.3613	0.0413	0.0039	1.61E-26	0.0072	0.0017	1.39E-05
rs11257655	10	12307894	T	C	0.2956	0.0946	0.0056	1.44E-63	0.0082	0.0021	7.51E-05
rs1111875	10	94462882	T	C	0.451	-0.0928	0.0038	1.44E-128	-0.0068	0.0016	1.20E-05
rs17747324	10	114752503	C	T	0.2198	0.2493	0.005	<1.00E-299	0.015	0.0023	6.12E-11
rs2403221	11	9852475	A	G	0.5516	0.0263	0.0041	1.16E-10	0.0065	0.0019	4.88E-04
rs757110	11	17418477	A	C	0.6296	-0.0599	0.0039	4.59E-52	-0.0056	0.0017	7.25E-04
rs174541	11	61565908	C	T	0.3493	-0.0277	0.0041	2.39E-11	-0.0076	0.002	1.12E-04
rs1552224	11	72433098	C	A	0.1452	-0.0917	0.0058	2.53E-56	-0.012	0.0021	1.61E-08
rs10830963	11	92708710	G	C	0.3057	0.0731	0.0042	1.29E-66	0.02	0.002	2.23E-23
rs2732480	12	48736303	A	C	0.4201	-0.0314	0.0042	6.40E-14	-0.012	0.002	2.00E-09
rs12910361	15	77782335	G	A	0.614	0.0686	0.004	5.19E-65	0.008	0.002	4.78E-05
rs2290202	15	91512267	T	G	0.2533	0.0576	0.005	6.31E-31	0.011	0.0029	1.29E-04
rs6600191	16	295795	C	T	0.2491	-0.0428	0.0046	2.97E-20	-0.01	0.0026	5.88E-05
rs1421085	16	53800954	C	T	0.367	0.1182	0.004	1.29E-189	0.0085	0.0017	2.89E-07
rs2297508	17	17715317	G	C	0.5583	-0.0305	0.004	2.39E-14	-0.0068	0.0019	2.64E-04
rs12603589	17	65825248	C	T	0.3128	0.0421	0.0046	1.06E-19	0.0075	0.0023	9.94E-04
rs10408179	19	46157004	C	T	0.4257	-0.0517	0.0039	1.86E-40	-0.006	0.0017	2.93E-04

ESM Table 2. Baseline characteristics of UK Biobank participants. Individuals with possible diabetes mellitus were excluded. CHD: coronary heart disease; LDL-C: low-density lipoprotein cholesterol; SD: standard deviation.

	Overall	Males	Females	No CHD event	CHD event	Possible diabetes mellitus (excluded)
Number of participants (n)	324,830	145,472	179,358	318,824	6006	26,562
Mean (SD) age at survey / years	56.9 (8.0)	57.1 (8.2)	56.8 (7.9)	56.9 (8.1)	60.5 (6.9)	60.1 (7.1)
Number (%) of females	179,358 (55.2)	-	-	177,758 (55.8)	1600 (26.6)	10,493 (39.5)
Mean (SD) body mass index / kg/m^2	27.0 (4.5)	27.5 (3.9)	26.7 (4.9)	27.0 (4.5)	28.0 (4.3)	31.6 (5.8)
Mean (SD) HbA$_{1c}$ / mmol/mol	34.8 (3.6)	34.8 (3.9)	34.9 (3.6)	34.8 (3.6)	35.9 (3.7)	49.9 (14.0)
Mean (SD) HbA$_{1c}$ / %	5.3 (0.3)	5.3 (0.3)	5.3 (0.3)	5.3 (0.3)	5.4 (0.3)	6.7 (1.3)
Mean (SD) LDL-cholesterol / mmol/L	3.6 (0.8)	3.6 (0.8)	3.7 (0.9)	3.6 (0.8)	3.7 (1.0)	3.0 (0.9)
Mean (SD) systolic blood pressure / mmHg	137.3 (18.6)	140.6 (17.4)	134.7 (19.2)	137.2 (18.6)	144.8 (19.6)	142.1 (18.0)
Mean (SD) diastolic blood pressure / mmHg	81.9 (10.1)	84.0 (10.0)	80.3 (9.9)	81.9 (10.1)	84.5 (10.7)	82.3 (10.3)
Number of current smokers (%)	33,052 (10.2)	17,566 (12.1)	15,486 (8.6)	31,834 (10.0)	1218 (20.3)	3213 (12.1)
Number of current alcohol drinkers (%)	304,235 (93.7)	138,544 (95.2)	165,691 (92.4)	298,726 (93.7)	5509 (91.7)	23,208 (87.4)

ESM Table 3. Results of non-linear Mendelian randomization analyses investigating the association of genetically-proxied average blood glucose levels with incident coronary heart disease in the total analytic sample (all) and in quintiles of the sample based on residual HbA_{1c}. Hazard ratios are given per 1 mmol/mol increase in genetically-proxied HbA_{1c}. HR: hazard ratio.

Strata	Overall		Males		Females	
	Mean HbA_{1c} (mmol/mol / %)	**HR (95% CI)**	**Mean HbA_{1c} (mmol/mol / %)**	**HR (95% CI)**	**Mean HbA_{1c} (mmol/mol / %)**	**HR (95% CI)**
All	34.8 / 5.3%	1.11 (1.05-1.18) $p=2\times10^{-4}$	34.8 / 5.3%	1.12 (1.05-1.19) $p=4\times10^{-4}$	34.9 / 5.3%	1.08 (0.96-1.20) $p=0.20$
Quintile 1	29.8 / 4.9%	1.18 (1.01-1.37) $p=0.037$	29.8 / 4.9%	1.22 (1.03-1.45) $p=0.023$	29.9 / 4.9%	1.07 (0.78-1.48) $p=0.67$
Quintile 2	33.0 / 5.2%	1.10 (0.95-1.26) $p=0.21$	33.0 / 5.2%	1.13 (0.96-1.32) $p=0.13$	33.1 / 5.2%	1.05 (0.77-1.42) $p=0.77$
Quintile 3	34.8 / 5.3%	1.27 (1.11-1.45) $p=4\times10^{-4}$	34.8 / 5.3%	1.22 (1.05-1.41) $p=0.010$	34.9 / 5.3%	1.28 (0.98-1.66) $p=0.066$
Quintile 4	36.6 / 5.5%	1.10 (0.98-1.24) $p=0.12$	36.6 / 5.5%	1.08 (0.95-1.24) $p=0.24$	36.7 / 5.5%	1.15 (0.91-1.45) $p=0.26$
Quintile 5	39.8 / 5.8%	1.01 (0.91-1.12) $p=0.90$	39.8 / 5.8%	1.04 (0.92-1.17) $p=0.53$	39.8 / 5.8%	0.93 (0.76-1.13) $p=0.46$

ESM Table 4. Results of linear Mendelian randomization (MR) sensitivity analyses investigating the association of genetically-proxied average blood glucose levels with incident coronary heart disease. Hazard ratios are given per 1mmol/mol increase in genetically-proxied HbA_{1c}. HR: hazard ratio.

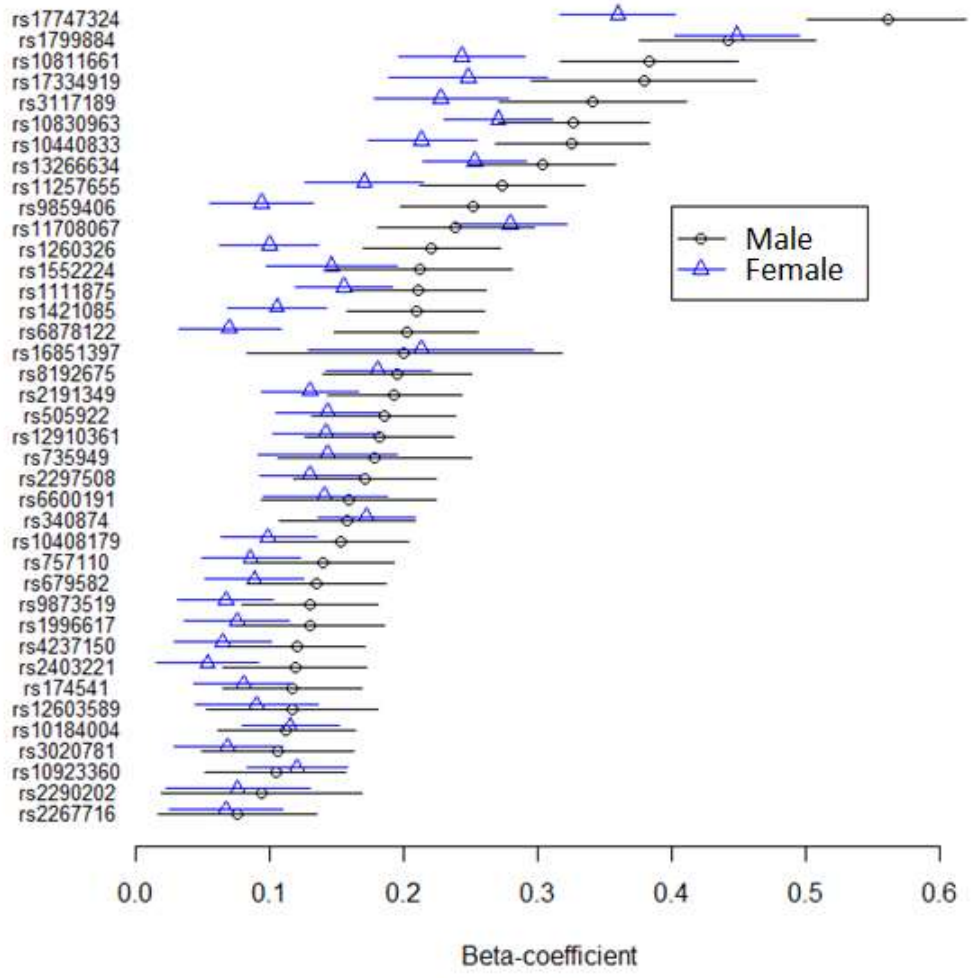
Method	**Hazard ratio (95% CI)**	***p* value**
Inverse-variance weighted method (fixed-effects)	1.11 (1.05, 1.18)	2×10^{-4}
Inverse-variance weighted method (random-effects)	1.11 (1.03, 1.21)	0.007
Weighted median method	1.12 (1.03, 1.23)	0.009
MR-Egger method	1.11 (0.91, 1.35)	0.30
(intercept)	0.000 (-0.022, 0.022)	0.97
MR-PRESSO method	1.10 (1.02, 1.18)	0.014
Contamination mixture method	1.15 (1.06, 1.24)	4×10^{-4}
Heterogeneity test	**Statistic**	***p* value**
Q statistic	73.1	8×10^{-4}
I^2 statistic	46.7%	

One variant was excluded from analysis in the MR-PRESSO method (rs505922).

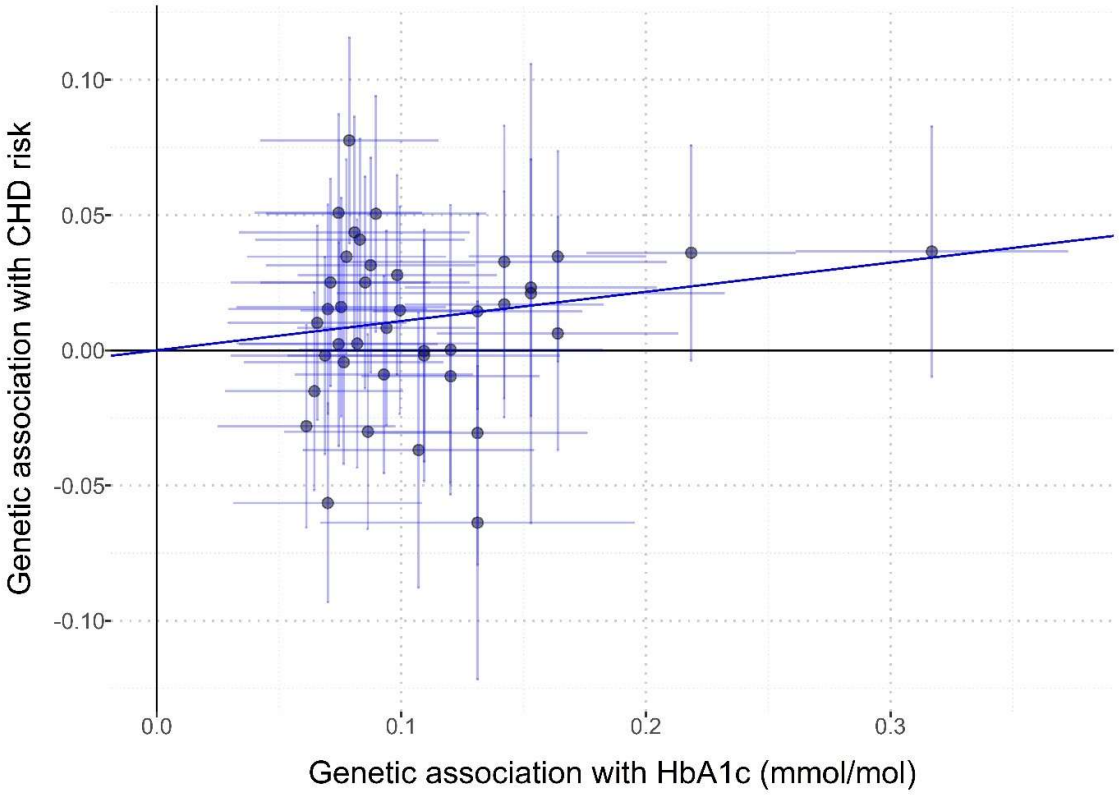
ESM Table 5. Results of Mendelian randomization (MR) analyses investigating the association of genetically-proxied average blood glucose levels with glycaemic and lipid traits. Estimates are given per 1mmol/mol increase in genetically-proxied HbA_{1c}.

Trait	Estimate (95% CI)	*p* value
Fasting glucose (mmol/L)	1.72 (1.36, 2.07)	<0.001
Two-hour glucose (mmol/L)	3.13 (1.95, 4.32)	<0.001
Fasting insulin (nmol/L, log-transformed)	-0.133 (-0.347, 0.080)	0.22
Low-density lipoprotein-cholesterol (SD units)	0.433 (0.034, 0.831)	0.033
High-density lipoprotein-cholesterol (SD units)	-0.031 (-0.378, 0.316)	0.86
Triglycerides (SD units)	0.000 (-0.794, 0.794)	0.99

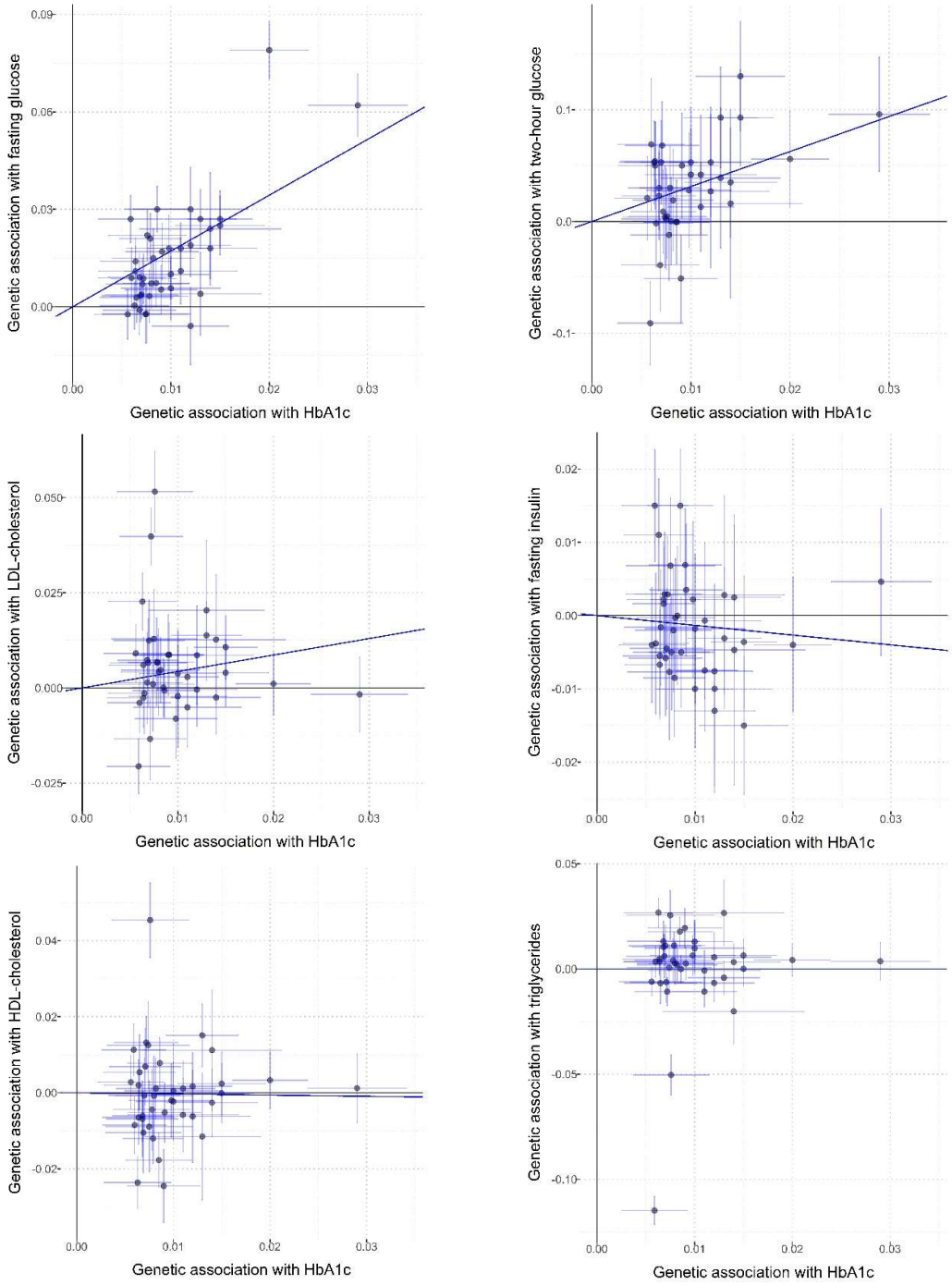
ESM Figure 1. Genetic associations of the instrument variants with HbA_{1c} (mmol/mol units) in male and female participants of the UK Biobank respectively, obtained from analyses performed by the Neale Lab (available at http://www.nealelab.is/uk-biobank). Association estimates for the rs2732480 variant were missing. Error bars represent 95% confidence intervals.



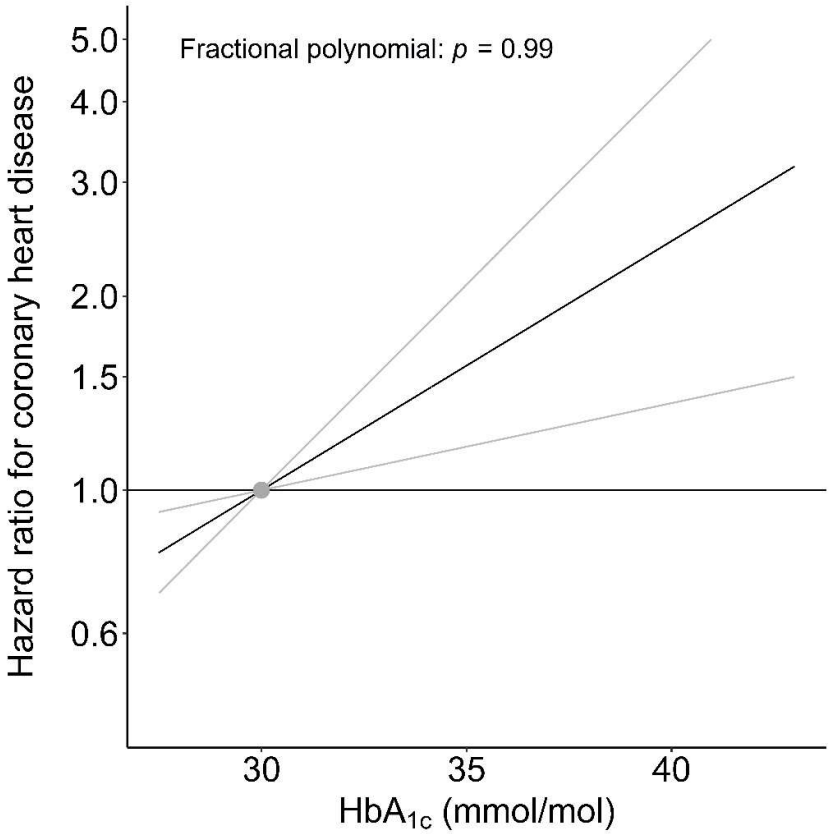
ESM Figure 2. Scatter plot of genetic association estimates for HbA_{1c} and incident coronary heart disease (CHD) risk. The gradient of the blue line depicts the random effects inverse-variance weighted Mendelian randomization estimate. For each variant (N=40), the genetic association and its 95% confidence interval with the exposure (HbA_{1c}; x-axis) and with the outcome (CHD risk; y-axis, log odds ratio) are plotted.



ESM Figure 3. Scatter plots of genetic association estimates for HbA_{1c} and glycaemic and lipid traits, for each variant (N=40).



ESM Figure 4. Multivariable Mendelian randomization (adjusting for genetically-proxied low-density lipoprotein cholesterol) investigating the relationship between genetically-proxied average blood glucose levels, as measured by HbA_{1c}, and risk of incident coronary heart disease in individuals without diabetes mellitus in males and females combined. The x-axis depicts HbA_{1c} levels in mmol/mol. The y-axis depicts the hazard ratio for coronary heart disease with respect to the reference. Reference is set to an HbA_{1c} of 30mmol/mol (4.9%). The grey lines represent the 95% confidence intervals. The fractional polynomial test is a goodness-of-fit test that assesses whether any improvement of fit when using a non-linear function to model the association, as compared to a linear function, is greater than would be expected due to chance (a significant *p* value indicates that a non-linear model is preferred to a linear model).



ESM Figure 5. Non-linear Mendelian randomization investigating the relationship between genetically-proxied average blood glucose levels, as measured by HbA_{1c}, and risk of incident coronary heart disease in individuals without diabetes mellitus in males and females combined. The five variants that associated with low-density lipoprotein cholesterol at $p<0.01$ (rs1260326, rs10184004, rs11708067, rs505922 and rs174541) were excluded. Three of these variants (s10184004, rs505922 and rs174541) associated with low-density lipoprotein cholesterol at $p<5x10^{-8}$). The x-axis depicts HbA_{1c} levels in mmol/mol. The y-axis depicts the hazard ratio for coronary heart disease with respect to the reference. Reference is set to an HbA_{1c} of 30mmol/mol (4.9%). The grey lines represent the 95% confidence intervals. The fractional polynomial test is a goodness-of-fit test that assesses whether any improvement of fit when using a non-linear function to model the association, as compared to a linear function, is greater than would be expected due to chance (a significant p value indicates that a non-linear model is preferred to a linear model).