SUPPLEMENTARY DATA

Epigenetic Link Between Statin Therapy and Type 2 Diabetes

Figures, tables and text in the supplement

SUPPLEMENTARY DATA

SUPPLEMENTARY DATA

Supplementary Table 1. DNA methylation assessment methods of each cohort

	RS-III-1	**RS-BIOS**	**SHIP-Trend**	**ESTHER**	**KORA**	**LOLIPOP**
Methylation assay	Illumina 450K array	Illumina 450K array	Illumina EPIC array	Illumina 450K array	Illumina 450K array	Illumina 450K array
IDAT extraction	Custom script	Beadstudio	Custom script	GenomeStudio	Custom script	minfi
Background correction	separate colors	separate colors	separate colors	separate colors	separate colors	minfi
Detection P value cutoff	0.01	NA	1.00E-16	0.01	0.01	0.01
Sample call rate threshold	99%	99%	95%	95%	95%	NA
Nbeads filter	3	NA	NA	NA	3	3
Normalization	SWAN	DASEN	CPACOR	Illumina	CPACOR	CPACOR

SUPPLEMENTARY DATA

Supplementary Table 2-a. Baseline characteristics of the cohorts included in the discovery panel

	Discovery panel											
	SHIP-Trend			ESTHER			KORA F4			LOLIPOP		
Country	Germany			Germany			Germany			United Kingdom		
Ethnicity	Caucasian			Caucasian			Caucasian			South Asian		
Study Design	Population based			Population-based			Population-based			Prospective type 2 diabetes Case/Control		
	Non-current users	Current users	Total	Non-current users	Current users	Total	Non-current users	Current users	Total	Non-current users	Current users	Total
Sample size	207	40	247	906	92	998	1455	271	1726	3390	459	3849
Age, years, mean (S.D.)	48.8 (13.1)	65.2 (8.3)	51.5 (13.8)	62.0 (6.5)	63.6 (6.6)	62.1 (6.5)	60.02 (8.84)	66.06 (7.21)	60.97 (8.88)	51.3 (10.1)	57.8 (9.4)	52.0 (10.3)
Sex female, n (%)	116 (56.0)	14 (35.0)	130 (52.6)	462 (51.0)	36 (39.1)	498 (49.9)	767 (52.7)	115 (42.4)	882 (51.1)	1325 (39.1)	134 (29.2)	1459 (37.9)
Body mass index, kg/m2,mean (SD)	27 (4.2)	28.7 (2.9)	27.2 (4.0)	27.8 (4.3)	27.2 (3.6)	27.8 (4.3)	27.88 (4.74)	29.35 (4.78)	28.11 (4.78)	27.4 (4.4)	28.0 (4.1)	27.5 (4.4)
Systolic blood pressure, mmHg, mean (SD)	123.6 (16.7)	128.4 (19.3)	124.3 (17.2)	139.5 (19.9)	141.5 (16.8)	139.7 (19.7)	124.79 (18.87)	124.82 (17.98)	124.8 (18.73)	131.7 (19.0)	138.6 (20.0)	132.5 (19.3)
Antihypertensive medication use, n (%) [missing, n (%)]	56 (27.1)	26 (65.0)	82 (33.2)	406 (44.8)	71 (77.2)	477 (47.8)	445 (30.6)	204 (75.3)	649 (37.6)	524 (21.7) [978 (28.8)]	307 (66.9) [0 (0.0)]	831 (28.9) [978 (25.4)]
Coronary heart disease, n (%) [a]	36 (17.4)	29 (72.5)	65 (26.3)	107 (11.8)	37 (40.2)	144 (14.4)	100 (6.9)	83 (30.5)	183 (10.6)	58 (1.7)	166 (36.2)	223 (5.8)
Prevalent type 2 diabetes, n (%) [b] [missing, n (%)]	0	0	0	136 (15.0)	20 (20.7)	156 (15.6)	99 (6.8)	62 (22.9)	161 (9.3)	206 (9.1) [1123 (33.1)]	56 (14.3) [67 (14.6)]	262 (6.8) [1190 (30.9)]
Incident type 2 diabetes, n (%) [b] [missing, n (%)]	NA	NA	NA	161 (17.8)	13 (13.7)	174 (17.4)	83 (5.7)	41 (15.1)	117 (6.8)	802 (35.4) [1123 (33.1)]	270 (68.9) [67 (14.6)]	1072 (40.3) [1190 (30.9)]

SUPPLEMENTARY DATA

Fasting glucose, mmol/L, median (I.Q.R.)	NA	NA	NA	5.62 (1.28)	5.73 (0.83)	5.63 (1.22)	5.33 (0.84)	5.61 (1.19)	5.33 (0.89)	5.1 (0.8)	5.5 (1.0)	5.2 (0.9)
Fasting insulin, uU/mL, median (I.Q.R.)	NA	NA	NA	NA	NA	NA	8.9 (6.5)	11 (8.9)	9.3 (7.3)	10.4 (9.0)	11.8 (9.9)	10.6 (9.3)
LDL cholesterol, mmol/L, mean (S.D.)	3.50 (0.90)	2.80 (0.70)	3.40 (0.90)	3.82 (0.97)	3.50 (1.14)	3.78 (1.00)	3.70 (1.17)	3.05 (0.93)	3.57 (1.24)	3.32 (0.88)	2.76 (0.91)	3.24 (0.90)
HDL cholesterol, mmol/L, median (I.Q.R.)	1.36 (0.41)	1.37 (0.48)	1.36 (0.42)	1.32 (0.60)	1.39 (0.57)	1.33 (0.57)	4.42 (0.52)	1.32 (0.43)	1.42 (0.49)	1.31 (0.39)	1.25 (0.30)	1.26 (0.39)
Total cholesterol, mmol/L, mean (S.D.)	5.50 (1.10)	4.80 (0.90)	5.40 (1.10)	4.26 (1.41)	4.33 (1.58)	4.27 (1.42)	5.76 (1.32)	5.12 (1.16)	5.68 (1.34)	5.36 (1.00)	4.83 (1.10)	5.29 (1.03)
Triglycerides, mmol/L, median (I.Q.R.)	1.30 (0.95)	1.52 (0.80)	1.35 (0.93)	1.24 (0.91)	1.41 (0.91)	1.26 (0.91)	1.23 (0.91)	1.49 (1.09)	1.26 (0.93)	1.40 (0.97)	1.62 (0.98)	1.40 (0.99)
Never smoker %	85 (41.0)	14 (42.5)	102 (41.2)	440 (48.6)	39 (42.4)	479 (48.0)	604 (41.5)	118 (43.5)	722 (41.8)	2875 (84.8)	358 (78.0)	3233 (84.0)
Former smoker %	78 (37.7)	15 (37.5)	93 (37.7)	295 (32.5)	40 (43.5)	335 (33.5)	630 (43.3)	124 (45.8)	754 (43.7)	251 (7.4)	61 (13.3)	312 (8.1)
Current smoker %	44 (21.3)	8 (20.0)	52 (21.1)	171 (18.9)	13 (14.1)	184 (18.5)	221 (15.2)	29 (10.7)	250 (14.5)	264 (7.8)	40 (8.7)	304 (7.9)

[a] Coronary heart disease: Revascularization by PCI or CABG; Angiographically severe coronary disease; Documented acute coronary syndrome (ACS; symptoms, ECG change and/or biochemistry).
[b] Type 2 Diabetes: physician diagnosis, fasting glucose ≥ 7.0 mmol/L or HbA1c>6.5%, or self-report

SUPPLEMENTARY DATA

Supplementary Table 2-B. Baseline characteristics of the cohorts participating in the replication panel and in post-ewas analyses

	Replication panel					
	The Rotterdam Study (RS)					
	RS-III-1			**RS-BIOS (RS-II-3 and RS-III-2)**		
Country	the Netherlands			the Netherlands		
Ethnicity	Caucasian			Caucasian		
Study Design	Population based			Population based		
	Non-current users	Current users	Total	Non-current users	Current users	Total
Sample size	641	90	731	546	173	719
Age, years, mean (S.D.)	59.1 (7.9)	65.1 (8.5)	59.9 (8.2)	67.14 (6.2)	69.1 (0.4)	67.6 (6.0)
Sex female, n (%)	355 (55.4)	40 (44.4)	395 (54.0)	326 (59.7)	92 (53.2)	414 (57.6)
Body mass index, kg/m2,mean (SD)	27.3 (4.8)	29.0 (4.5)	27.52 (4.8)	27.57 (4.0)	28.1 (4.2)	27.7 (4.1)
Systolic blood pressure, mmHg, mean (SD)	134.0 (19.9)	137.4 (18.4)	134.4 (4.5)	144.7 (22.1)	144.5 (21.3)	144.7 (21.9)
Antihypertensive medication, n (%)	147 (22.9)	70 (77.8)	217 (29.7)	187 (34.2)	123 (71.1)	310 (43.1)
Coronary heart disease, n (%) [a]	19 (3.0)	26 (29.0)	45 (6.2)	15 (2.7)	44 (25.4)	59 (8.2)
Prevalent type 2 diabetes, n (%) [b]	45 (7.0)	29 (32.2)	74 (10.1)	55 (10.1)	52 (30.1)	107 (14.9)
Incident type 2 diabetes, n (%) [b]	16 (2.5)	10 (11.1)	26 (3.6)	NA	NA	NA
Fasting glucose, mmol/L, median (I.Q.R.)	5.3 (0.8)	5.6 (1.7)	5.4 (0.7)	5.30 (0.80)	5.65 (1.22)	5.40 (0.90)
Fasting insulin, uU/mL, median (I.Q.R.)	10.8 (7.8)	15.6 (14.3)	11.2 (7.8)	9.50 (7.24)	12.31 (8.86)	10.08 (7.78)
LDL cholesterol, mmol/L, mean (S.D.)	3.61 (0.93)	2.51 (0.69)	3.48 (0.98)	3.58 (0.85)	2.57 (0.70)	3.33 (0.92)
HDL cholesterol, mmol/L, median (I.Q.R.)	1.37 (0.52)	1.17 (0.48)	1.37 (0.52)	1.49 (0.62)	1.37 (0.47)	1.46 (0.59)
Total cholesterol, mmol/L, mean (S.D.)	5.70 (1.04)	4.56 (0.76)	5.56 (1.04)	5.77 (0.87)	4.68 (0.92)	5.51 (1.02)
Triglycerides, mmol/L, median (I.Q.R.)	1.28 (0.83)	1.59 (0.97)	1.28 (0.81)	1.26 (0.72)	1.41 (0.83)	1.30 (0.76)

SUPPLEMENTARY DATA

Never smoker, n (%)	192 (30.0)	21 (23.3)	213 (29.1)	192 (35.2)	56 (32.4)	248 (34.5)
Former smoker, n (%)	275 (42.9)	46 (51.1)	321 (43.9)	297 (54.4)	97 (56.1)	394 (54.8)
Current smoker, n (%)	174 (27.1)	23 (25.6)	197 (26.9)	57 (10.4)	20 (11.6)	77 (10.7)

[a] Coronary heart disease: Revascularization by PCI or CABG; Angiographically severe coronary disease; Documented acute coronary syndrome (ACS; symptoms, ECG change and/or biochemistry).
[b] Type 2 Diabetes: physician diagnosis, fasting glucose ≥ 7.0 mmol/L or HbA1c>6.5%, or self-report

SUPPLEMENTARY DATA

Supplementary Table 3. Pearson correlation among the replicated cpgs in two the sub-cohorts of the replication panel (RS)

RS-III-1	cg06500161	cg27243685	cg17901584	cg10177197	cg05119988
cg06500161	1				
cg27243685	0.244	1			
cg17901584	0.176	0.289	1		
cg10177197	0.065	0.353	0.174	1	
cg05119988	-0.173	-0.092	-0.187	-0.107	1
RS-BIOS	cg06500161	cg27243685	cg17901584	cg10177197	cg05119988
cg06500161	1				
cg27243685	0.477	1			
cg17901584	-0.011	0.270	1		
cg10177197	0.090	0.303	0.143	1	
cg05119988	-0.201	-0.420	-0.222	-0.371	1

SUPPLEMENTARY DATA

Supplementary Table 4. Replication of DNA methylation sites associated with current statin use in a subset of the replication sample, excluding former statin users

CpG	Chr	Position	location	Gene	Effect	SE	P-value
cg17901584	1	55353706	TSS1500	***DHCR24***	**-0.0179**	**0.0037**	**1.34E-06**
cg06500161	21	43656587	gene body	***ABCG1***	**0.0123**	**0.0027**	**3.63E-06**
cg05119988	4	166251189	5'UTR	***SC4MOL***	**-0.0121**	**0.0039**	**1.94 E-03**
cg27243685	21	43642366	gene body	***ABCG1***	**0.0072**	**0.0018**	**5.06E-05**
cg10177197	1	55316481	gene body	***DHCR24***	**-0.0045**	**0.0010**	**1.35E-05**

Model adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases, prevalent type 2 diabetes.
Bonferroni correction for significance for replication P < 7.14E-03.
Sample size n=1205 (RS)
Abbreviations: Chr, chromosome; SE, standard error. Bold text indicates statistically significant associations.

SUPPLEMENTARY DATA

Supplementary Table 5. Causal mediation analysis on the significant associations between the statin-related CpG cg06500161 and glycemic traits and incident type 2 diabetes, with a model adjusted for sex, age, cohort and body mass index

Outcomes	ACME estimate of mediator cg06500161 (95% CI)	ADE estimate (95% CI)	Total Effect (95% CI)	Proportion mediated by cg06500161 (95% CI)
log Glucose [a]	0.004 (0.001, 0.010)	0.006 (-0.009, 0.020)	0.010 (-0.005, 0.030)	0.404 (-3.201, 3.920)
log Insulin [a]	**0.025 (0.011, 0.04)**	**0.205 (0.131, 0.280)**	**0.229 (0 .1562, 0.30)**	**0.106 (0.047, 0.190)**
log HOMA-IR [a]	**0.029 (0.014, 0.050)**	**0.212 (0.132, 0.290)**	**0.240 (0.160, 0.32)**	**0.119 (0.057, 0.210)**
Incident type 2 diabetes [b]	0.007 (-0.002, 0.020)	0.116 (0.024, 0.22)	0.123 (0.030, 0.230)	0.060 (-0.024, 0.230)

Models adjusted for sex, age, cohort and body mass index
Bold text indicates statistically significant results.
[a] Sample n=1,180 (RS) complete cases, of which 178 were current statin users. Non-fasting samples (n=25), prevalent type 2 diabetes cases (n=181) and former statin users (n=74) were excluded from the analysis.
[b] Sample n=640 (RS) complete cases, of which 23 were cases of incident type 2 diabetes.
Abbreviations: CI, confidence interval; ACME, average causal mediator effect; ADE, average direct effect; HOMA-IR, homeostatic model assessment insulin resistance.

SUPPLEMENTARY DATA

Supplementary Table 6. Causal mediation analysis on the significant associations between the statin-related CpG cg06500161 and glycemic traits and incident type 2 diabetes, with a model adjusted for sex, age, cohort, body mass index and high density lipoprotein cholesterol

Outcomes	ACME estimate of mediator cg06500161 (95% CI)	ADE estimate (95% CI)	Total Effect (95% CI)	Proportion mediated by cg06500161 (95% CI)
log Glucose [a]	0.003 (0.001, 0.010)	0.005 (-0.010, 0.020)	0.008 (-0.007, 0.020)	0.396 (-3.231, 3.63)
log Insulin [a]	**0.014 (0.004, 0.030)**	**0.184 (0.111, 0.260)**	**0.198 (0.125, 0.27)**	**0.071 (0.017, 0.15)**
log HOMA-IR [a]	**0.018 (0.006, 0.030)**	**0.189 (0.114, 0.260)**	**0.207 (0.131, 0.280)**	**0.085 (0.028, 0.17)**
Incident type 2 diabetes [b]	0.006 (-0.003, 0.020)	0.110 (0.019, 0.210)	0.116 (0.024, 0.220)	0.051 (-0.039, 0.230)

Models adjusted for sex, age, cohort, body mass index and high density lipoprotein cholesterol.
Bold text indicates statistically significant results.
[a] Sample n=1,180 (RS) complete cases, of which 178 were current statin users. Non-fasting samples (n=25), prevalent type 2 diabetes cases (n=181) and former statin users (n=74) were excluded from the analysis.
[b] Sample n=640 (RS) complete cases, of which 23 were cases of incident type 2 diabetes.
Abbreviations: CI, confidence interval; ACME, average causal mediator effect; ADE, average direct effect; HOMA-IR, homeostatic model assessment insulin resistance.

SUPPLEMENTARY DATA

Supplementary Table 7. Causal mediation analysis on the significant associations between the statin-related CpG (cg06500161) and glycemic traits and incident type 2 diabetes, with a comprehensive assessment of confounder factors

Outcomes	ACME estimate of mediator cg06500161 (95% CI)	ADE estimate (95% CI)	Total Effect (95% CI)	Proportion mediated by cg06500161 (95% CI)
log Glucose [a]	0.004 (0.001, 0.010)	-0.003 (-0.019, 0.010)	0.002 (-0.015, 0.020)	2.382 (-6.352, 6.180)
log Insulin [a]	**0.024 (0.011, 0.040)**	**0.151 (0.069, 0.230)**	**0.174 (0.093, 0.260)**	**0.136 (0.060, 0.290)**
log HOMA-IR [a]	**0.028 (0.013, 0.050)**	**0.149 (0.061, 0.240)**	**0.176 (0.088, 0.270)**	**0.156 (0.069, 0.340)**
Incident type 2 diabetes [b]	0.004 (-0.004, 0.010)	0.090 (-0.004, 0.190)	0.094 (-0.001, 0.200)	0.047 (-0.123, 0.320)

Models adjusted for sex, age, cohort, body mass index, smoking status, systolic blood pressure, anti-hypertensive medication and presence of coronary heart disease.
Bold text indicates statistically significant results.
a Sample n=1,160 (RS) complete cases, of which 178 were current statin users. Non-fasting samples (n=25), prevalent type 2 diabetes cases (n=181) and former statin users (n=74) were excluded from the analysis.
[b] Sample n=622 (RS) complete cases, of which 23 were cases of incident type 2 diabetes.
Abbreviations: CI, confidence interval; ACME, average causal mediator effect; ADE, average direct effect; HOMA-IR, homeostatic model assessment insulin resistance.

SUPPLEMENTARY DATA

Supplementary Table 8. Significant results of the gene expression-DNA methylation association analyses between the statin-related CpGs and expression probes

CpG	Chr	Position	Location	Gene at CpG	Illumina Probe ID	Gene at expression probe	Effect	P-value
cg06500161	21	43656587	gene body	***ABCG1***	**ILMN_1794782**	*ABCG1*	-13.000	**8.85E-13**
					ILMN_2329927	*ABCG1*	-8.504	**4.85E-12**
cg27243685	1	43642366	gene body	***ABCG1***	**ILMN_1794782**	*ABCG1*	-15.974	**1.31E-07**
cg17901584	1	55353706	TSS1500	***DHCR24***	**ILMN_1794782**	*ABCG1*	9.319	**2.21E-10**
					ILMN_2329927	*ABCG1*	4.975	6.15E-07

Sample size n=731, from cohort RS (RSIII-1)
Bonferroni correction p < 4.7E-07
Bold text indicates significant associations.

SUPPLEMENTARY DATA

Supplementary Table 9. association between the CpGs-associated expression probes for *ABCG1* and current statin use as exposure

ILMN_1794782 (*ABCG1*)			ILMN_2329927 (*ABCG1*)		
Effect	SE	P-value	Effect	SE	P-value
-0.7999	**0.1648**	**1.54E-06**	**-0.3750**	**0.1186**	**0.0016**

Model: gene-expression ~ statin use, adjusted for age, sex, body mass index, coronary heart disease, systolic blood pressure, high-density lipoprotein cholesterol; n=631 (RS).
Cases of prevalent type 2 diabetes and former users of statins were excluded.
Bold text indicates significant associations.

SUPPLEMENTARY DATA

Supplementary Table 10. Association between significant outcomes found in the causal mediation analyses (fasting insulin and HOMA-IR) and the CpGs-associated expression probes for gene *ABCG1*

		log Insulin [b]			log HOMA-IR [b]		
Illumina Probe	Gene at probe	Effect	SE	P-value	Effect	SE	P-value
ILMN_1794782	***ABCG1***	**-0.0290**	**0.0137**	**0.0355**	**-3.130E-02**	**1.5E-2**	**0.0354**
ILMN_2329927	*ABCG1*	-0.0252	0.019774	0.2038	-2.921E-02	2.1E-2	0.1718

Model: glycemic trait (insulin or HOMA-IR) ~ statin use, adjusted for age, sex, body mass index, coronary heart disease, systolic blood pressure, high-density lipoprotein cholesterol; n=616 (RS).
Cases of prevalent type 2 diabetes and former users of statins were excluded.
Bold text indicates significant associations.

SUPPLEMENTARY DATA

Supplementary Table 11. association between DNA methylation at the identified statin-related CpGs and statin dose among current users of statins

CpG	Chr	Position	location	Gene	Effect	SE	P-value
cg17901584	1	55353706	TSS1500	*DHCR24*	-0.0058	0.0019	**2.104E-03**
cg06500161	21	43656587	gene body	*ABCG1*	0.0037	0.0014	**9.579E-03**
cg05119988	4	166251189	5'UTR	*SC4MOL*	-0.0068	0.0021	**1.317E-03**
cg27243685	21	43642366	gene body	*ABCG1*	0.0025	0.0009	**5.012E-03**
cg10177197	1	55316481	gene body	*DHCR24*	0.0025	0.0013	**9.510E-03**

Model adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases and prevalent type 2 diabetes.
Bonferroni correction for significance of $P < 0.05/5$ replicated CpGs = 0.01.
Sample size n=303 current statin users with dose data, from cohorts SHIP-Trend and RS.
Abbreviations: Chr, chromosome; SE, standard error.

SUPPLEMENTARY DATA

Supplementary Table 12. Association between gene expression and statin use

Statin use (exposure)	Illumina Probe	Gene at probe	Effect	SE	P-value
Current use	**ILMN_1794782**	***ABCG1***	**-0.0490**	**0.0177**	**5.92 E-03**
	ILMN_2329927	*ABCG1*	-0.0496	0.0256	0.0529

Models adjusted for age, sex, body mass index, coronary heart disease, systolic blood pressure, high-density lipoprotein cholesterol.
Cases of prevalent type 2 diabetes and former users of statins were excluded.
Sample size n=631 (RS)
Bold text indicates significant associations.

SUPPLEMENTARY DATA

Supplementary Table 13. DNA methylation sites associated with current statin use (compared to non-current use) in the discovery panel and replication panel (EWAS) in models further adjusted for blood lipids

Model additionally adjusted for serum total cholesterol [a]					Discovery panel [b]			Replication panel [c]			Both panels combined		
CpG	Chr	Position	location	Gene	Effect	SE	P-value	Effect	SE	P-value	Effect	SE	P-value
cg17901584	**1**	55353706	TSS1500	***DHCR24***	-0.0204	0.0021	3.69E-22	-0.0108	0.0034	1.66E-03	**-0.0178**	**0.0018**	**4.18E-23**
cg06500161	**21**	43656587	gene body	***ABCG1***	0.01	0.001	1.05E-21	0.0102	0.0024	1.73E-05	**0.01**	**0.001**	**9.44E-26**
cg05119988	**4**	166251189	5'UTR	***SC4MOL***	-0.0111	0.0018	2.63E-10	-0.0107	0.0034	1.61E-03	**-0.011**	**0.0016**	**1.65E-12**
cg05575921	5	373378	gene body	*AHRR*	-0.0136	0.0022	1.06E-09	-0.0009	0.0059	8.79E-01	**-0.012**	**0.0021**	**8.49E-09**
cg27243685	**21**	43642366	gene body	***ABCG1***	0.0045	0.0008	7.65E-09	0.0065	0.0016	6.32E-05	**0.0048**	**0.0007**	**4.01E-12**
cg10177197	**1**	55316481	gene body	***DHCR24***	0.0036	0.0007	3.90E-07	0.0042	0.0017	1.41E-02	**0.0037**	**0.0007**	**1.82E-08**
Model additionally adjusted for HDL-C, LDL-C and TG [d]					Discovery panel [b]			Replication panel [c]			Both panels combined		
CpG	Chr	Position	location	Gene	Effect	SE	P-value	Effect	SE	P-value	Effect	SE	P-value
cg17901584	**1**	55353706	TSS1500	***DHCR24***	**-0.0188**	**0.0022**	**2.27E-18**	**-0.0108**	**0.0035**	**1.79E-03**	**-0.0166**	**0.0018**	**1.15E-19**
cg06500161	**21**	43656587	gene body	***ABCG1***	**0.0074**	**0.001**	**1.21E-12**	**0.0093**	**0.0023**	**7.32E-05**	**0.0077**	**0.001**	**5.37E-16**
cg15348274	12	123451191	TSS200	*ABCB9*	**-0.0148**	**0.0026**	**1.08E-08**	0.0028	0.0055	6.05E-01	**-0.0116**	**0.0023**	**7.62E-07**
cg05575921	5	373378	gene body	*AHRR*	**-0.013**	**0.0023**	**2.05E-08**	-0.0002	0.0059	9.79E-01	**-0.0113**	**0.0022**	**1.68E-07**
cg05119988	**4**	166251189	5'UTR	***SC4MOL***	**-0.0098**	**0.0018**	**7.73E-08**	**-0.0092**	**0.0034**	**7.03E-03**	**-0.0097**	**0.0016**	**1.86E-09**
cg27243685	**21**	43642366	gene body	***ABCG1***	0.0035	0.0008	1.08E-05	**0.0063**	**0.0016**	**7.70E-05**	**0.004**	**0.0007**	**1.24E-08**
cg10177197	**1**	55316481	gene body	***DHCR24***	0.0037	0.0007	3.66E-07	0.0041	0.0017	1.71E-02	**0.0038**	**0.0007**	**1.98E-08**

[a] Model adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases, prevalent type 2 diabetes, serum total cholesterol.
Bonferroni correction for significance for discovery P < 1.0E-07 and P < 7.14E-03 for replication.
[b] Discovery panel (n=6,820): Cohorts KORA-F4, SHIP-Trend, ESTHER, LOLIPOP.
[c] Replication panel (n=1,450): Sub-cohorts from The Rotterdam Study (RSIII-1 and RS-Bios)
[d] Model adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases, prevalent type 2 diabetes, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides.
Abbreviations: Chr, chromosome; SE, standard error. Bold text indicates statistically significant associations.

SUPPLEMENTARY DATA

Supplementary Table 14. replication of the identified statin-related CpGs in an independent sample excluding type 2 diabetes and pre-type 2 diabetes cases in the replication panel

CpG	Chr	Position	location	Gene	Effect	SE	P-value
cg17901584	1	55353706	TSS1500	*DHCR24*	-0.0165	0.0041	**5.21E-05**
cg06500161	21	43656587	gene body	*ABCG1*	0.0086	0.0028	**2.08E-03**
cg05119988	4	166251189	5'UTR	*SC4MOL*	-0.0136	0.0039	**5.36E-04**
cg27243685	21	43642366	gene body	*ABCG1*	0.0068	0.0019	**3.53E-04**
cg10177197	1	55316481	gene body	*DHCR24*	0.0081	0.0020	**4.24E-05**

Model adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases
Sample from RS (n=1065)
Bonferroni correction P < 7.14E-0
Abbreviations: Chr, chromosome; SE, standard error. Bold text indicates statistically significant associations.

SUPPLEMENTARY DATA

Supplementary Table 15. Replication of the association between current statin use and DNA methylation at the five identified CpGs in a set of participants from the replication panel with high LDL-C levels (≥ 70 mg/dl and ≥ 100 mg/dl)

			All participants from the replication panel [a]			Participants from the replication panel with LDL-C levels ≥ 70 mg/dL only [b]			Participants from the replication panel with LDL-C levels ≥ 100 mg/dL only [c]		
CpG site	Chr	Gene	Effect	SE	P-value	Effect	SE	P-value	Effect	SE	P-value
cg17901584	1	*DHCR24*	-0.0145	0.0035	3.06E-05	-0.0156	0.0036	1.38E-05	-0.0202	0.0046	9.12E-06
cg06500161	21	*ABCG1*	0.0139	0.0024	7.84E-09	0.0129	0.0025	2.72E-07	0.0143	0.0031	4.44E-06
cg05119988	4	*SC4MOL*	-0.0118	0.0035	6.81E-04	-0.0085	0.0036	0.0188	-0.0133	0.0045	3.33E-03
cg27243685	21	*ABCG1*	0.0074	0.0016	6.08E-06	0.0077	0.0017	7.74E-06	0.0067	0.0021	1.83E-03
cg10177197	1	*DHCR24*	0.0060	0.0017	5.10E-04	0.0051	0.0018	4.85E-03	0.0061	0.0024	9.79E-03

All analyses adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases, prevalent type 2 diabetes.
Bonferroni correction for replication P < 7.14E-03.
[a] Sample size n=1320 (RS) (RS), from which n=247 participants were current statin users and n=1320 were never users.
[b] Sample size participants LDL ≥ 70 mg/dL only, n=1267 (RS), from which n=216 participants were current statin users and n=1051 were never users.
[c] Sample size participants LDL ≥ 100 mg/dL only, n=1065 (RS), from which n=110 participants were current statin users and n=955 were never users.
Abbreviations: Chr, chromosome; SE, standard error; mg/dL, milligrams per deciliter.

SUPPLEMENTARY DATA

Supplementary Table 16. Trans-ethnic replication of the association of current statin use (compared to non-current users)

					Caucasian group as discovery panel [a]			British-Asian group as replication panel [b]		
CpG	Chr	Position	location	Gene	Effect	SE	P-value	Effect	SE	P-value
cg06500161	21	43656587	TSS1500	*ABCG1*	0.0129	0.0013	4.60E-22	**0.008**	**0.001**	**1.01E-09**
cg17901584	1	553153706	gene body	*DHCR24*	-0.0199	0.0022	2.42E-19	**-0.019**	**0.003**	**4.09E-11**
cg27243685	21	43642366	5'UTR	*ABCG1*	0.0063	0.0009	3.69E-12	**0.003**	**0.001**	**2.76E-03**
cg10177197	1	55316481	gene body	*DHCR24*	0.005	0.0008	1.68E-09	**0.003**	**0.001**	**6.99E-03**
cg05119988	4	166251189	gene body	*SC4MOL*	-0.0118	0.0021	1.59E-08	**-0.012**	**0.002**	**3.26E-08**
cg17475467	1	55316769	location	*DHCR24*	0.0048	0.0009	3.96E-08	0.002	0.001	0.027

Model adjusted for leukocyte proportions, batch effects, sex, age, smoking status, body mass index, systolic blood pressure, anti-hypertensive medication, presence of coronary heart diseases, prevalent type 2 diabetes. Bonferroni correction for significance for discovery $P < 1.0E-07$ and $P < 8.33E-03$ for replication.

[a] Sensitivity analysis-Discovery panel (n=4,349): Cohorts KORA, SHIP-Trend, ESTHER and RS.

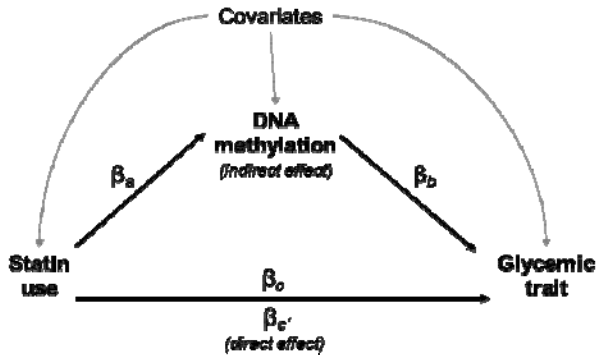
[b] Sensitivity analysis-Replication panel (n=3,849): Cohort LOLIPOP.

Abbreviations: Chr, chromosome; SE, standard error.

Bold text indicates the CpGs that replicated the independent panel.

SUPPLEMENTARY DATA

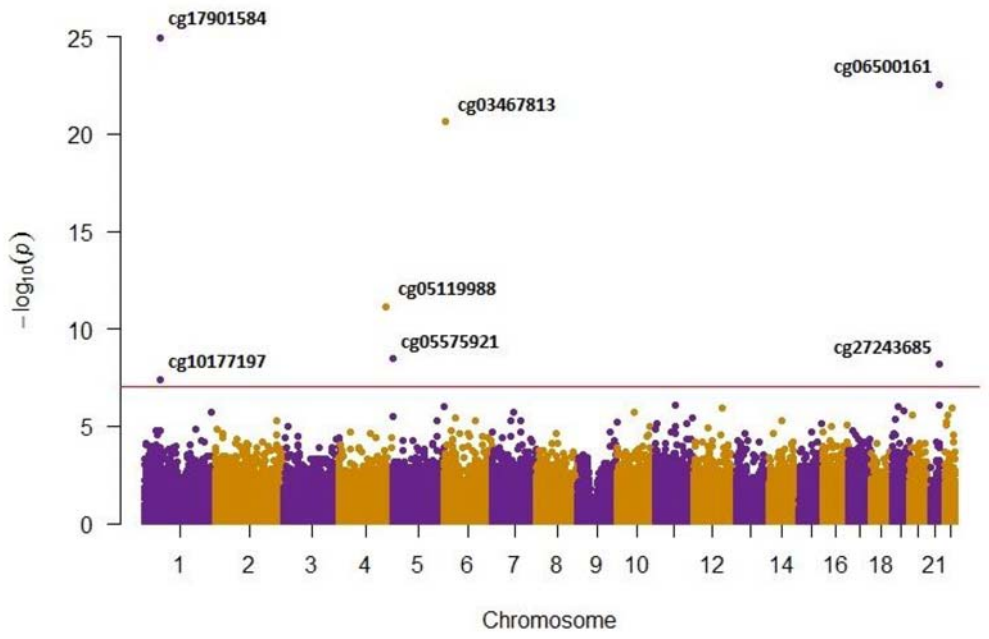
Supplementary Figure 1. Causal Mediation Analysis



Schematic representation of the causal mediation analysis. βc' represents the causal direct effect of statin use (exposure) on the glycemic traits and type 2 diabetes (outcome). βc represents a non-causal pathway. βa represents the effect of statin use on DNA methylation. βb represents the effect of DNA methylation (mediator) on the outcome, which would be mediating an indirect effect of statins on the outcome. Covariates that may confound these associations are included in the model.

SUPPLEMENTARY DATA

Supplementary Figure 2. Manhattan Plot of the epigenome-wide associations between current statin use and DNA methylation (compared to non-current use)



Using a model adjusted for batch effects, leukocyte proportions and cardiometabolic risk factors, we found genome-wide associations of seven methylation sites annotated to *DHCR24* (cg17901584 and cg10177197, chromosome 1), *SC4MOL* (cg05119988, chromosome 4), *ABCG1* (cg06500161 and cg27243685, chromosome 21), *AHRR* (cg05575921, chromosome 5) and *FAM50B* (cg03467813, chromosome 6). Of these, five CpGs, annotated to *DHCR24, ABCG* and *SC4MOL*. From them, five could be replicated in an independent cohort.