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Supplement I

Supplementary material & methods

Acquisition and processing of ^{1}H-NMR spectra

All proton nuclear magnetic resonance (^{1}H-NMR) experiments were acquired on a 600 MHz Bruker Avance II spectrometer (Bruker BioSpin, Karlsruhe, Germany) equipped with a 5 mm TCI cryogenic probe head with Z-gradient system and automatic tuning and matching. All experiments were recorded at 310K. Temperature calibration was done prior to each batch of measurements using the method of Findeisen *et al.*[1] The duration of the π/2 pulses were automatically calibrated for each individual sample using a homonuclear-gated nutation experiment on the locked and shimmed samples after automatic tuning and matching of the probe head.[2]

The stored EDTA plasma and serum samples were thawed at 4°C and were mixed by inverting the tubes 10 times. Next, samples (300 µL) were mixed with 300 µL 75 mM disodium phosphate buffer in H_2O/D_2O (80/20) with a pH of 7.4 containing 6.15 mM NaN_3 and 4.64 mM sodium 3-[trimethylsilyl] d_4-propionate (TSP) using a Gilson 215 liquid handler in combination with a Bruker SampleTrack system. Samples were transferred into 5mm SampleJet NMR tubes in 96 tube racks using a modified Gilson 215 tube filling station and kept at 6°C on a SampleJet sample changer while queued for acquisition.

For water suppression presaturation of the water resonance with an effective field of $\gamma B_1 = 25$ Hz was applied during the relaxation delay.[3] J-resolved spectra (JRES)[4] were recorded with a relaxation delay of 2 s and a total of one scan for each increment in the indirect dimension. A data matrix of 40 x 12,288 data points was collected covering a sweep width of 78 x 10,000 Hz. A sine-shaped window function was applied and the data was zero-filled to 256 x 16,384 complex data points prior to Fourier transformation. The resulting data matrix was tilted along the rows by shifting each row (k) by 0.4992*(128-k) points and symmetrised about the central horizontal lines in order to compensate for the skew of the multiplets in the F1 dimension. For T2-filtered ^{1}H NMR spectra a standard 1D CPMG (Carr–Purcell–Meiboom–Gill) pulse sequence,[5,6] was used with a relaxation delay of 4 seconds. A pulse train of 128 refocusing pulses with individual spin echo delays of 0.6 ms were applied resulting in a total T2 filtering delay of 78 ms. 73,728 data points covering a spectral width of 12,019 Hz were collected using 16 scans. The FID was zero-filled to 131,072 complex data points and an exponential window function was applied with a line broadening factor of 1.0 Hz prior to Fourier transformation. The spectra were automatically phase and baseline corrected.

Quality control, scaling and calibration of the ^{1}H-NMR spectra

Further data processing was performed in Matlab® (R2009a, The Mathworks Inc., Natick, MA, USA). The spectra and associated data were converted into Matlab files using in-house code. First, the spectra were combined into one file while removing superfluous information. For CPMG this included dropping the imaginary part of the spectrum, while for the JRES spectra the sum projection along the indirect dimension was taken. Quality control (QC) on the set of ^{1}H-NMR spectra was carried out by examining a set of spectroscopic parameters such as shim values and intensity of the water signal, and subsequently visually inspecting the spectra. The spectra that failed the QC were not included for further analysis. The spectra were then scaled with respect to the sensitivity of the receiver coil, as determined from the pulse length that was automatically calibrated for each sample.[7] After subtracting a constant value as a simple baseline correction, the spectra were calibrated with respect to the anomeric resonance of α-D-glucose ($\delta = 5.23$ ppm).[8] Since there are small deviations of the peak position in the different ^{1}H-NMR spectra, alignment was performed using the correlation optimized warping algorithm by Tomasi *et al.*[9] This was performed actively for the CPMG spectra, after which the same warping was applied to the JRES projection. Peaks in the JRES projection were picked by finding the signals that were above the surrounding spectral area by more than the estimated noise level. Peaks in different spectra were grouped according to similarity in peak position and intensity. The intensity and the position give good initial guesses for the deconvolution of the peaks by fitting groups of mixed Gaussian-Lorentzian line shapes to isolated spectral areas using a Simplex optimization algorithm. As the fitting algorithm incidentally fails to converge properly, values further from the median than 3 times the interquartile range are discarded. Using PLS regression, the remaining peak intensities were used to build a linear model that predicts all intensities directly from the non-warped spectrum, yielding also values for the cases where the peak picking failed or the deconvolution values were discarded, and eliminating the problem of faulty warping.

For 76 signals, metabolites were assigned using information from previously reported plasma/serum metabolites,[6,8,10,11] the Human Metabolome Database (HMDB)[12] and the Pearson correlation coefficients between the signal intensities.

References

1. Findeisen M, Brand T, Berger S. A 1H-NMR thermometer suitable for cryoprobes. Magn Reson Chem 2007;45(2):175-8.
2. Wu PS, Otting G. Rapid pulse length determination in high-resolution NMR. J Magn Reson 2005;176(1):115-9.
3. Price WS. Water signal suppression in NMR spectroscopy. Annual Reports on Nmr Spectroscopy, Vol 38 1999;38:289-354.
4. Aue WP. Homonuclear broad band decoupling and two-dimensional J-resolved NMR spectroscopy. The Journal of Chemical Physics 1976;64(10):4226.
5. Meiboom S, Gill D. Modified Spin-Echo Method for Measuring Nuclear Relaxation Times. Review of Scientific Instruments 1958;29(8):688.
6. Nicholson JK, Foxall PJ, Spraul M, et al. 750 MHz 1H and 1H-13C NMR spectroscopy of human blood plasma. Anal Chem 1995;67(5):793-811.
7. Wider G, Dreier L. Measuring protein concentrations by NMR spectroscopy. J Am Chem Soc 2006;128(8):2571-6.
8. MacIntyre DA, Jimenez B, Lewintre EJ, et al. Serum metabolome analysis by 1H-NMR reveals differences between chronic lymphocytic leukaemia molecular subgroups. Leukemia 2010;24(4):788-97.
9. Tomasi G, van den Berg F, Andersson C. Correlation optimized warping and dynamic time warping as preprocessing methods for chromatographic data. Journal of Chemometrics 2004;18(5):231-241.
10. Wevers RA, Engelke U, Heerschap A. High-resolution 1H-NMR spectroscopy of blood plasma for metabolic studies. Clin Chem 1994;40(7 Pt 1):1245-50.
11. Wishart DS. Quantitative metabolomics using NMR. Trac-Trends in Analytical Chemistry 2008;27(3):228-237.
12. Wishart DS, Knox C, Guo AC, et al. HMDB: a knowledgebase for the human metabolome. Nucleic Acids Res 2009;37(Database issue):D603-10.

Table S1 | An overview of all peaks identified in the 2 dimensional J-resolved ^{1}H-NMR spectrum and their association with incident CHD in the primary prospective case-cohort study.

		Cases		Sub-cohort		Association with CHD	
Chemical shift	**Assignment**	**Mean (sd)**	**Min : Max**	**Mean (sd)**	**Min : Max**	**HR (95% CI)**	***P-value***
0.873 ppm[*]	lipids (CH3)[†]	0.45 (1.48)	-0.93 : 6.67	-0.05 (0.94)	-0.97 : 6.67	1.40 (1.13-1.74)	0.002
0.929 ppm[*]	isoleucine	0.27 (0.99)	-1.60 : 2.89	-0.04 (1.00)	-2.02 : 5.08	1.14 (0.90-1.45)	0.282
0.947 ppm[*]	leucine	0.30 (1.05)	-1.67 : 3.83	-0.04 (1.00)	-1.72 : 4.98	1.23 (0.98-1.55)	0.074
0.952 ppm[*]	unknown	0.01 (1.24)	-3.14 : 3.00	0.00 (0.97)	-4.29 : 3.21	0.99 (0.77-1.26)	0.921
0.957 ppm[*]	leucine	0.24 (1.00)	-1.78 : 2.79	-0.04 (0.99)	-2.10 : 4.07	1.17 (0.92-1.50)	0.207
0.965 ppm	unknown	0.07 (1.13)	-3.56 : 2.45	-0.02 (1.00)	-3.56 : 4.76	0.97 (0.75-1.24)	0.780
0.973 ppm[*]	unknown	0.03 (1.00)	-2.35 : 2.93	-0.01 (1.00)	-3.13 : 3.77	0.93 (0.71-1.20)	0.568
0.981 ppm[*]	valine	0.33 (0.98)	-1.65 : 3.26	-0.05 (0.99)	-2.37 : 4.43	1.23 (0.97-1.57)	0.090
0.988 ppm[*]	unknown	0.24 (0.87)	-1.88 : 2.69	-0.03 (1.01)	-7.36 : 4.19	1.22 (0.96-1.55)	0.102
1.000 ppm[*]	isoleucine	0.27 (0.96)	-1.79 : 2.48	-0.03 (1.00)	-2.07 : 4.31	1.17 (0.92-1.49)	0.214
1.032 ppm[*]	valine	0.29 (1.02)	-1.88 : 3.26	-0.04 (0.99)	-2.35 : 4.36	1.18 (0.92-1.51)	0.193
1.054 ppm	unknown	0.26 (1.38)	-1.94 : 5.02	-0.04 (0.93)	-1.66 : 3.99	1.21 (0.95-1.55)	0.131
1.063 ppm[*]	ketoisovalerate	0.12 (1.27)	-1.89 : 7.24	-0.01 (0.96)	-1.90 : 4.89	1.09 (0.84-1.41)	0.509
1.163 ppm[*]	unknown	0.14 (1.77)	-0.18 : 15.48	-0.02 (0.83)	-0.21 : 14.04	1.11 (0.94-1.31)	0.235
1.174 ppm[*]	ethanol	0.14 (1.63)	-0.13 : 14.20	-0.02 (0.87)	-0.13 : 14.52	1.10 (0.93-1.29)	0.280
1.191 ppm[*]	3-hydroxybutyrate	0.08 (0.96)	-0.57 : 6.77	-0.02 (1.00)	-0.58 : 9.75	1.10 (0.90-1.34)	0.362
1.212 ppm[*]	unknown	0.15 (1.44)	-1.33 : 10.34	-0.02 (0.92)	-2.03 : 5.48	1.17 (0.91-1.52)	0.228
1.264 ppm[*]	lipids (CH2)[†]	0.42 (1.39)	-0.91 : 5.79	-0.05 (0.95)	-0.94 : 6.23	1.36 (1.10-1.69)	0.005
1.319 ppm[*]	lactate	0.20 (1.06)	-1.51 : 4.43	-0.02 (0.99)	-1.90 : 4.41	1.22 (0.95-1.56)	0.120
1.391 ppm[*]	unknown	-0.14 (1.02)	-1.99 : 2.69	0.02 (1.00)	-2.60 : 4.35	0.78 (0.60-1.00)	0.053
1.407 ppm[*]	unknown	0.01 (1.00)	-1.78 : 2.19	0.00 (1.00)	-2.53 : 3.24	1.02 (0.79-1.32)	0.904
1.425 ppm[*]	unknown	0.17 (1.06)	-1.93 : 2.78	-0.02 (1.00)	-1.98 : 3.51	1.18 (0.92-1.52)	0.198
1.471 ppm[*]	alanine	0.22 (0.93)	-1.72 : 2.54	-0.03 (1.00)	-2.35 : 3.45	1.13 (0.88-1.45)	0.344
1.706 ppm[*]	unknown	0.24 (1.20)	-1.77 : 4.62	-0.03 (0.98)	-2.67 : 4.62	1.21 (0.92-1.58)	0.172
1.908 ppm[*]	acetate	0.13 (0.88)	-0.77 : 3.84	-0.02 (1.01)	-0.81 : 16.58	1.04 (0.87-1.24)	0.656
2.001 ppm[*]	lipids (CH*2CH=CH)[†]	0.49 (1.72)	-1.01 : 8.67	-0.05 (0.91)	-1.19 : 8.67	1.37 (1.13-1.67)	0.001
2.035 ppm[*]	glycoproteins	0.52 (1.18)	-1.51 : 3.85	-0.06 (0.96)	-2.05 : 4.88	1.32 (1.03-1.69)	0.028
2.066 ppm[*]	glycoproteins	0.32 (1.12)	-1.59 : 4.33	-0.04 (0.99)	-2.51 : 4.33	1.20 (0.93-1.55)	0.159
2.099 ppm[*]	glutamine/ glutamate	-0.01 (0.88)	-1.85 : 2.77	-0.01 (1.01)	-2.43 : 3.58	0.97 (0.76-1.22)	0.773
2.125 ppm[*]	glutamine/ glutamate	0.00 (1.05)	-2.41 : 3.66	0.00 (1.01)	-2.48 : 3.66	1.03 (0.81-1.32)	0.791
2.221 ppm[*]	lipids (CH2CO)[†]	0.40 (1.55)	-0.76 : 7.24	-0.05 (0.93)	-0.84 : 7.24	1.35 (1.10-1.66)	0.004
2.260 ppm[*]	valine	0.11 (1.15)	-2.10 : 2.71	-0.02 (0.98)	-2.11 : 4.41	1.01 (0.78-1.30)	0.954
2.301 ppm[*]	3-hydroxybutyrate	0.01 (0.63)	-0.55 : 2.71	0.00 (1.03)	-0.64 : 11.41	1.04 (0.82-1.30)	0.766
2.349 ppm[*]	glutamate	0.50 (1.21)	-0.91 : 4.24	-0.06 (0.96)	-1.68 : 4.24	1.38 (1.09-1.75)	0.007
2.364 ppm[*]	pyruvate	-0.19 (0.99)	-1.47 : 3.13	0.02 (1.00)	-1.66 : 3.91	0.96 (0.73-1.26)	0.768

		Cases		Sub-cohort		Association with CHD	
Chemical shift	**Assignment**	**Mean (sd)**	**Min : Max**	**Mean (sd)**	**Min : Max**	**HR (95% CI)**	***P-value***
2.393 ppm*	3-hydroxybutyrate	0.16 (0.86)	-0.94 : 3.82	-0.02 (1.01)	-0.96 : 10.08	1.13 (0.92-1.39)	0.242
2.412 ppm	unknown	0.32 (0.99)	-2.37 : 2.67	-0.04 (0.99)	-2.64 : 3.11	1.22 (0.96-1.55)	0.110
2.430 ppm*	glutamine	-0.22 (1.04)	-1.69 : 2.10	0.02 (0.99)	-1.79 : 3.30	0.81 (0.63-1.05)	0.107
2.530 ppm*	citrate	-0.10 (0.81)	-1.52 : 2.13	0.01 (1.02)	-2.56 : 4.43	0.77 (0.59-0.99)	0.045
2.645 ppm*	citrate	-0.19 (0.76)	-1.81 : 1.84	0.02 (1.02)	-2.53 : 4.56	0.73 (0.56-0.95)	0.017
2.690 ppm	Mg-EDTA	0.11 (1.03)	-1.47 : 2.79	-0.01 (0.99)	-1.80 : 3.88	1.05 (0.82-1.33)	0.710
2.737 ppm	sarcosine	0.44 (1.57)	-1.45 : 9.23	-0.05 (0.96)	-1.45 : 9.23	1.32 (1.08-1.61)	0.007
2.801 ppm	aspartate	-0.04 (1.00)	-1.70 : 4.01	0.01 (1.00)	-2.36 : 4.75	0.84 (0.65-1.09)	0.191
2.858 ppm	asparagine	0.13 (0.98)	-1.50 : 5.23	-0.02 (1.00)	-2.95 : 10.18	1.18 (0.94-1.47)	0.148
2.914 ppm*	dimethylglycine	0.17 (1.04)	-2.07 : 2.53	-0.02 (0.99)	-2.42 : 3.57	1.02 (0.79-1.32)	0.875
3.024 ppm*	lysine	-0.08 (0.83)	-2.04 : 1.85	0.01 (1.02)	-2.24 : 3.89	0.81 (0.63-1.06)	0.119
3.034 ppm*	creatine + creatinine	0.17 (1.18)	-1.72 : 6.82	-0.03 (0.97)	-2.05 : 4.07	0.96 (0.75-1.23)	0.754
3.050 ppm*	ornithine	0.24 (1.11)	-2.03 : 2.55	-0.03 (0.99)	-2.49 : 3.42	1.07 (0.82-1.40)	0.614
3.090 ppm	Ca-EDTA	0.07 (1.13)	-2.09 : 4.19	0.00 (1.01)	-2.25 : 4.19	0.95 (0.75-1.22)	0.700
3.117 ppm	Ca-EDTA	0.08 (1.12)	-3.84 : 4.04	-0.02 (0.99)	-3.84 : 4.55	1.01 (0.79-1.28)	0.957
3.145 ppm*	unknown	0.02 (1.15)	-2.57 : 5.57	-0.01 (0.98)	-2.57 : 6.04	0.88 (0.66-1.16)	0.348
3.200 ppm	EDTA	0.11 (0.84)	-2.88 : 2.04	-0.01 (1.03)	-3.39 : 9.57	1.16 (0.91-1.48)	0.228
3.240 ppm*	glucose	0.32 (1.49)	-1.38 : 9.56	-0.05 (0.90)	-2.24 : 7.29	1.20 (0.98-1.47)	0.078
3.252 ppm	TMAO	0.32 (0.93)	-2.60 : 3.46	-0.04 (1.00)	-3.26 : 6.43	1.17 (0.93-1.47)	0.174
3.267 ppm*	1,5-anhydrosorbitol	-0.15 (1.07)	-5.08 : 1.98	0.01 (0.99)	-4.19 : 3.42	1.01 (0.80-1.28)	0.927
3.312 ppm	unknown	0.20 (0.88)	-1.80 : 3.71	-0.02 (1.01)	-2.00 : 11.23	1.09 (0.82-1.44)	0.555
3.333 ppm*	proline	0.17 (0.91)	-1.44 : 2.67	-0.03 (1.01)	-1.80 : 4.80	1.02 (0.80-1.29)	0.902
3.342 ppm*	1,5-anhydrosorbitol	-0.16 (0.92)	-1.96 : 2.10	0.01 (1.01)	-1.85 : 3.53	0.86 (0.66-1.13)	0.287
3.348 ppm	unknown	-0.02 (0.90)	-1.75 : 3.21	0.01 (1.01)	-2.67 : 3.57	1.00 (0.79-1.27)	0.978
3.354 ppm*	unknown	0.11 (1.53)	-1.15 : 10.50	-0.02 (0.90)	-1.15 : 9.50	1.21 (0.95-1.53)	0.124
3.362 ppm	unknown	0.17 (1.31)	-2.79 : 4.62	-0.02 (0.95)	-2.79 : 3.72	1.21 (0.93-1.56)	0.154
3.401 ppm*	glucose	0.38 (1.48)	-1.69 : 9.93	-0.05 (0.90)	-1.95 : 7.82	1.23 (1.01-1.49)	0.036
3.458 ppm	glucose	0.30 (1.47)	-1.53 : 9.22	-0.04 (0.91)	-1.71 : 6.45	1.26 (1.01-1.57)	0.040
3.487 ppm*	glucose	0.37 (1.43)	-1.64 : 9.52	-0.05 (0.91)	-2.03 : 7.82	1.22 (1.01-1.47)	0.042
3.531 ppm*	glucose	0.39 (1.48)	-1.64 : 9.76	-0.05 (0.90)	-1.83 : 7.82	1.23 (1.02-1.49)	0.033
3.551 ppm*	unknown	-0.18 (0.90)	-1.53 : 3.77	0.02 (1.01)	-4.31 : 4.53	0.81 (0.62-1.05)	0.111
3.599 ppm	EDTA	0.12 (0.84)	-2.86 : 2.52	-0.01 (1.02)	-3.41 : 9.90	1.18 (0.93-1.50)	0.175
3.633 ppm	unknown	0.00 (0.51)	-2.47 : 0.65	0.00 (1.05)	-22.4 : 2.58	0.97 (0.81-1.16)	0.755
3.649 ppm*	ethanol	0.13 (1.59)	-0.41 : 13.79	-0.02 (0.88)	-0.41 : 14.69	1.09 (0.92-1.29)	0.303
3.712 ppm*	glucose	0.33 (1.46)	-1.71 : 9.30	-0.05 (0.91)	-1.76 : 7.51	1.19 (0.98-1.45)	0.078
3.721 ppm*	glucose	0.36 (1.45)	-1.68 : 9.67	-0.05 (0.91)	-2.31 : 7.73	1.23 (1.01-1.49)	0.040
3.735 ppm	3-phosphoglycerate	0.22 (1.12)	-1.53 : 3.37	-0.04 (0.98)	-1.53 : 2.95	1.19 (0.94-1.52)	0.156
3.747 ppm*	unknown	0.12 (1.02)	-2.19 : 2.28	-0.01 (1.00)	-3.09 : 2.77	1.09 (0.85-1.39)	0.509

		Cases		Sub-cohort		Association with CHD	
Chemical shift	**Assignment**	**Mean (sd)**	**Min : Max**	**Mean (sd)**	**Min : Max**	**HR (95% CI)**	***P-value***
3.759 ppm*	glucose	0.32 (1.46)	-1.47 : 9.83	-0.04 (0.91)	-2.29 : 7.89	1.21 (0.99-1.47)	0.061
3.765 ppm	3-phosphoglycerate	0.25 (1.01)	-2.35 : 2.15	-0.04 (0.99)	-3.13 : 4.02	1.24 (0.97-1.58)	0.087
3.772 ppm	3-phosphoglycerate	0.23 (1.07)	-1.33 : 2.90	-0.03 (0.98)	-1.35 : 3.08	1.21 (0.95-1.53)	0.119
3.779 ppm*	alanine	-0.01 (0.97)	-2.80 : 2.26	0.01 (1.01)	-3.58 : 3.92	1.00 (0.78-1.27)	0.977
3.818 ppm*	unknown	0.04 (1.05)	-1.82 : 4.81	0.00 (0.99)	-2.09 : 6.76	1.00 (0.79-1.28)	0.983
3.824 ppm*	glucose	0.39 (1.40)	-2.49 : 7.92	-0.05 (0.92)	-1.89 : 6.32	1.29 (1.04-1.59)	0.019
3.838 ppm*	glucose	0.37 (1.5)	-1.69 : 9.94	-0.05 (0.89)	-2.07 : 7.37	1.25 (1.02-1.52)	0.031
3.876 ppm*	1,5-anhydrosorbitol	0.00 (1.23)	-5.59 : 2.69	0.00 (0.96)	-5.37 : 5.01	1.02 (0.80-1.29)	0.888
3.888 ppm	unknown	0.03 (1.10)	-2.28 : 4.80	-0.01 (0.99)	-2.50 : 3.68	0.96 (0.75-1.23)	0.745
3.893 ppm*	glucose	0.36 (1.47)	-1.57 : 9.91	-0.05 (0.90)	-1.96 : 7.83	1.22 (1.01-1.49)	0.042
3.924 ppm*	unknown	0.00 (0.93)	-2.30 : 2.62	0.00 (1.01)	-2.64 : 3.36	0.78 (0.60-1.01)	0.062
3.959 ppm*	unknown	-0.04 (0.77)	-1.65 : 1.42	0.01 (1.02)	-2.11 : 9.13	0.93 (0.71-1.21)	0.570
3.974 ppm*	1,5-anhydrosorbitol	-0.10 (1.02)	-2.12 : 2.85	0.01 (1.00)	-2.85 : 2.98	1.02 (0.78-1.32)	0.897
3.981 ppm	Multiple metabolites	-0.16 (0.90)	-1.87 : 2.73	0.02 (1.01)	-2.46 : 5.23	0.86 (0.66-1.12)	0.259
4.044 ppm*	creatinine	0.38 (1.47)	-1.85 : 10.37	-0.05 (0.90)	-2.51 : 4.16	1.35 (1.04-1.76)	0.027
4.103 ppm*	lactate	0.20 (1.07)	-1.41 : 4.57	-0.02 (0.99)	-1.82 : 4.60	1.23 (0.96-1.57)	0.100
4.121 ppm*	proline	0.13 (0.93)	-2.01 : 2.20	-0.02 (1.01)	-2.20 : 4.39	0.97 (0.76-1.25)	0.831
4.167 ppm	phosphorylcholine	-0.02 (0.87)	-1.60 : 2.65	0.00 (1.02)	-2.68 : 6.10	0.82 (0.61-1.10)	0.179
4.223 ppm	3-phosphoglycerate	-0.01 (0.96)	-1.02 : 4.42	0.00 (1.00)	-1.20 : 4.84	0.98 (0.76-1.27)	0.894
4.239 ppm*	unknown	-0.12 (0.88)	-1.62 : 2.64	0.02 (1.02)	-2.00 : 4.14	0.92 (0.72-1.18)	0.519
4.503 ppm*	glucose	0.26 (1.14)	-1.52 : 5.96	-0.03 (0.97)	-2.54 : 4.61	1.23 (0.98-1.55)	0.074
5.180 ppm*	unknown	0.29 (1.42)	-2.20 : 7.36	-0.03 (0.94)	-2.20 : 5.14	1.27 (1.01-1.60)	0.046
5.230 ppm*	glucose	0.38 (1.47)	-1.79 : 9.75	-0.05 (0.90)	-1.98 : 7.97	1.23 (1.02-1.48)	0.035
5.299 ppm*	lipids (CH=CH) †	0.41 (1.65)	-0.61 : 7.82	-0.04 (0.91)	-0.76 : 7.82	1.37 (1.12-1.67)	0.002
6.890 ppm*	tyrosine	0.03 (0.90)	-1.74 : 2.89	-0.01 (1.02)	-2.38 : 5.14	0.85 (0.66-1.10)	0.212
7.186 ppm*	tyrosine	0.05 (1.01)	-1.50 : 2.95	-0.01 (1.00)	-2.15 : 4.98	0.91 (0.70-1.17)	0.444
8.451 ppm*	formate	0.02 (1.03)	-1.49 : 4.45	0.00 (1.00)	-1.30 : 9.41	0.90 (0.65-1.23)	0.495

Mean is expressed as area under the curve

*Detected and quantified in the confirmation study

†The term in parenthesis indicates the structural feature of the lipid measured by ^{1}H-NMR spectroscopy

Table S2 | Baseline characteristics of the traditional CHD risk factors in the primary study before removing participants with missing data.

	Cases (N=125)		**Sub-cohort (N=738)**[*]	
	N missing	**Mean±SD/%(N)**	**N missing**	**Mean±SD/%(N)**
Age (years)	0	50.49 ± 7.11	0	44.57 ± 8.23
Total cholesterol (mmol/L)	2	5.91 ± 0.99	13	5.33 ± 0.98
HDL cholesterol (mmol/L)	2	1.11 ± 0.28	13	1.33 ± 0.36
SBP (mmHg)	0	133.18 ± 17.83	1	121.23 ± 14.61
BMI (kg/m^2)	0	27.69 ± 4.45	0	25.48 ± 3.87
Men	0	95 (76.0%)	0	323 (43.8%)
Current smoking	2	69 (56.1%)	5	283 (38.6%)
Diabetes	0	6 (4.8%)	0	5 (0.7%)
Parental history of MI	14	58 (52.3%)	68	264 (39.4%)

HDL, high-density lipoprotein; *MI*, myocardial infarction; S*BP*, systolic blood pressure; *BMI*, body mass index
[*] Including 10 cases

Table S3 | The association between the metabolite score based on 16 ^{1}H-NMR signals and the metabolite score based on the 13 ^{1}H-NMR signals and incident CHD in the primary study.

	Univariate analysis[*]		**Adjusted for TRF**[†]	
	HR (95% CI)	**P-value**	**HR (95% CI)**	**P-value**
Metabolite score based on 16 ^{1}H-NMR signals	1.93 (1.50-2.48)	<0.001	1.58 (1.18-2.12)	0.002
Metabolite score based on 13 ^{1}H-NMR signals[‡]	1.91 (1.50-2.44	<0.001	1.50 (1.12-2.01)	0.007

HR, hazard ration*; TRF,* traditional risk factors
[*] Univariate HR were calculated per unit increase for the metabolite scores. Age in years was used as the time-scale variable.
[†] The TRF include total cholesterol, HDL cholesterol, SBP, BMI, gender, current smoking, self-reported diabetes and parental history of MI. Age in years was used as the time-scale variable.
[‡] This metabolite score is based on the 13 ^{1}H-NMR signals that were also available in the secondary study.

Table S4 | Reclassification of participants when the metabolite score was used in addition to a risk score based on traditional risk factors (*i.e.* age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes). For reclassification the following risk categories were used: 0-<5%, 5-<10%, 10-<20%, ≥20%.

	Model with TRF + metabolite score				
Model with TRF[*]	**<5%**	**5-<10%**	**10-<20%**	**≥20%**	**Total**
Incident cases					
<5%	39	3	1	0	43
5-<10%	5	13	3	1	22
10-<20%	0	2	4	0	6
≥20%	0	0	1	4	5
Total	44	18	9	5	76
Non-cases					
<5%	515	7	1	0	523
5-<10%	4	15	2	0	21
10-<20%	0	3	7	0	10
≥20%	0	0	0	0	0
Total	519	25	10	0	554

TRF, traditional risk factors

[*] The TRF include age in years, total cholesterol, HDL cholesterol, SBP, BMI, gender, current smoking, self-reported diabetes and parental history of MI.

Table S5 | Improvement in reclassification of incident CHD when using the metabolite score in addition to traditional risk factors (*i.e.* age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes). For calculating the NRI the following risk categories were used: 0-<5%, 5-<10%, 10-<20%, ≥20%.

	Reclassification				
	P Up	**P Down**	**NRI**	**Z-score**	**P-value**
Event	0.140	0.096	0.044	0.924	0.178
Non-event	0.018	0.013	-0.005	-1.593	0.944
Total	…	…	0.038	0.809	0.209

P Up, proportion of participants placed into a higher risk category; *P Down*, proportion of participants placed into a lower risk category; *NRI*, net reclassification index.

Table S6a | Comparing the predictive power of the single traditional risk factors, all traditional risk factors combined with and without the metabolite score in the primary case-cohort study using Harrell's *C* index.

	TRFs only	**TRF + MS**		
	C-index (95% CI)	**C-index (95% CI)**	**Diff (SE)**	**P-value**
Age	0.72 (0.66-0.77)	0.79 (0.74-0.84)	0.08 (0.02)	<0.001
Total cholesterol	0.66 (0.61-0.72)	0.77 (0.72-0.81)	0.10 (0.03)	<0.001
HDL cholesterol	0.67 (0.60-0.73)	0.74 (0.68-0.79)	0.07 (0.02)	<0.001
Systolic blood pressure	0.71 (0.65-0.77)	0.79 (0.74-0.84)	0.08 (0.02)	<0.001
Body mass index	0.64 (0.58-0.71)	0.74 (0.69-0.80)	0.10 (0.03)	<0.001
Male sex	0.66 (0.61-0.71)	0.76 (0.71-0.81)	0.10 (0.02)	<0.001
Current smoking	0.57 (0.51-0.62)	0.74 (0.69-0.80)	0.18 (0.03)	<0.001
Self-reported diabetes	0.51 (0.49-0.54)	0.76 (0.71-0.81)	0.25 (0.03)	<0.001
Parental history of MI	0.55 (0.49-0.60)	0.75 (0.70-0.80)	0.20 (0.03)	<0.001
All TRFs combined	*0.82 (0.78-0.87)*	*0.84 (0.80-0.87)*	*0.01 (0.01)*	*0.107*

TRFs, traditional risk factors; *MS*, metabolite score; *Diff*, Difference in C-indices; *SE*, standard error; *HDL*, high densitity lipoprotein; *MI*, myocardial infarction

Table S6b | Comparing the predictive power of the metabolite score combined with and without single traditional risk factors and all traditional risk factors combined in the primary case-cohort study using Harrell's Concordance index.

	C-index (95% CI)	**Diff (SE)**	**P-value**
Metabolite Score only	*0.75 (0.74-0.84)*	-	-
Age + MS	0.79 (0.74-0.84)	0.038 (0.022)	0.090
Total cholesterol + MS	0.77 (0.72-0.81)	0.011 (0.008)	0.194
HDL cholesterol +MS	0.74 (0.68-0.79)	0.017 (0.021)	0.412
Systolic blood pressure +MS	0.79 (0.74-0.84)	0.035 (0.019)	0.064
Body mass index +MS	0.74 (0.69-0.80)	0.011 (0.010)	0.308
Male sex +MS	0.76 (0.71-0.81)	0.007 (0.020)	0.720
Current smoking +MS	0.74 (0.69-0.80)	0.011 (0.015)	0.476
Self-reported diabetes +MS	0.76 (0.71-0.81)	0.002 (0.003)	0.453
Parental history of MI +MS	0.75 (0.70-0.80)	0.008 (0.008)	0.970
All TRFs combined	*0.84 (0.80-0.87)*	*0.081 (0.023)*	*<0.001*

Diff, Difference in C-indices between the metabolite score only and the traditional risk factor(s) and the metabolite score combined; *SE*, standard error; *MS*, metabolite score; *TRFs*, traditional risk factors

Table S7| Baseline characteristics of the traditional CHD risk factors in the secondary study before removing participants with missing data.

	Cases (N=174)		Controls (N=2170)*	
	N missing	**Mean±SD/%(N)**	**N missing**	**Mean±SD/%(N)**
Age (years)	0	60.98 (11.73)	0	47.70 (14.02)
Total cholesterol (mmol/L)	1	5.00 (1.07)	12	5.60 (1.06)
HDL cholesterol (mmol/L)	1	1.15 (0.32)	12	1.29 (0.37)
SBP (mmHg)	1	148.31 (22.91)	30	139.18 (19.75)
BMI (kg/m^2)	3	28.14 (4.43)	35	26.79 (4.61)
Men	0	108 (62.1%)	0	924 (42.6%)
Current smoking	1	62 (35.8%)	3	844 (38.9%)
Diabetes	0	25 (14.4%)	0	101 (4.7%)
Parental history of MI	69	54 (31.0%)	729	430 (19.9%)

HDL, high-density lipoprotein; *MI*, myocardial infarction; *SBP*, systolic blood pressure; *BMI*, body mass index

Table S8 | The association between the metabolite score and coronary heart disease in the total confirmation study (170 cases and 2157 controls) and after excluding statin users (n=299; 77 cases and 1946 controls left for analysis).

Metabolite score	**Crude OR (95% CI)**	***P-value***	**Adjusted OR (95%CI)***	***P-value***
Total population	2.72 (2.28-3.25)	2.0x10^{-28}	1.59 (1.19-2.13)	0.002
Non statin users	2.78 (2.16-3.57)	1.2x10^{-15}	2.02 (1.33-3.08)	0.001

OR, odds ratio; *CI,* confidence interval

Odds ratios were calculated per standard deviation increase in metabolite score

* The metabolite score was adjusted for age, total cholesterol, HDL cholesterol, systolic blood pressure, body mass index, sex, smoking, diabetes and parental history of myocardial infarction.

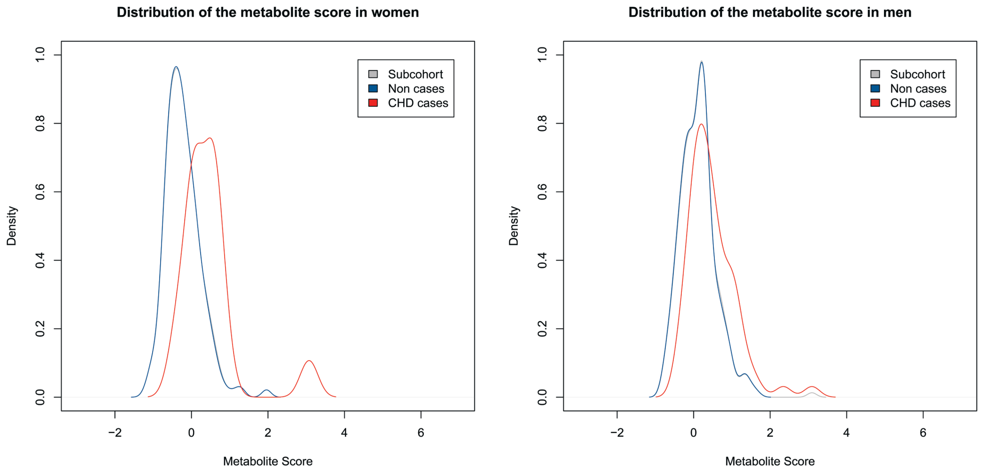

Figure S1 | Kernel density plots showing the distribution of the metabolite score in the sub-cohort and in the cases of the primary case-cohort study.

Supplement II

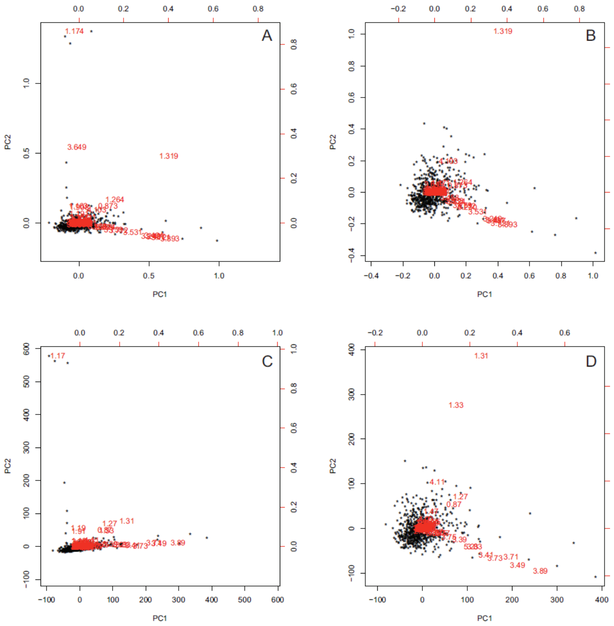

Figure S1 | Results from the principal component analysis on the 98 ^{1}H-NMR peaks (a/b) and on the 401 ^{1}H-NMR bins (c/d). The black dots represent the participants and the red numbers represent the peaks or the bins. There are three individuals with aberrant values for the peak located at 1.174 ppm representing ethanol (a). After removing this peak, there were some individuals with high values for peaks representing glucose (b). A bin located at 1.17 was aberrant for the same three individuals (c). After removing this bin, there were six individuals with high values for bins located near peaks representing glucose (d). The participants with high values for peaks or bins representing glucose also had high glucose levels according to a conventional method to determine glucose.

Supplement III

Material and methods

Statistical analysis for LASSO GRSs

Not all GWAS-identified SNPs may contribute to the risk of future CHD in our population-based prospective study. Therefore, we selected a sub-set of the most informative SNPs from all GWAS identified SNPs using least absolute shrinkage and selection operator (LASSO) regression. With LASSO regression it is possible to perform variable selection and shrinkage at the same time, which makes it an effective method for finding prediction rules based in high dimensional data.[1-3] The penalty parameter, which determines the number of variables selected for the model, was set by the 10-fold cross-validated partial likelihood. With 10-fold cross-validation we have addressed the issue of internal validation.[4] The resulting SNPs and their weights, based on the coefficients resulting from 10-fold cross-validated LASSO regression, were then used to construct the LASSO GRS. This approach was used to select subsets of informative SNPs from the 29 CHD SNPs (CHD LASSO GRS) and all 179 SNPs (LASSO GRS). For this analysis the R package penalized (version 0.9-32), was used.

References

1. Goeman JJ. L1 penalized estimation in the cox proportional hazards model. Biom.J. 2010;52:70-84
2. Tibshirani R. Regression shrinkage and selection via the lasso. J.R.Statist.Soc.B.1996;58:267-288
3. Tibshirani R. The lasso method for variable selection in the cox model. Stat.Med. 1997;16:385-395

Supplementary results

Construction the LASSO GRSs and their associations with incident CHD

By performing 10-fold cross-validated LASSO regression on the 29 CHD SNPs, a subset of 3 SNPs (see supplementary table S7) was selected as the optimal predictor for incident CHD in our data-set, namely rs10757278, rs11556924 and rs646776 (see supplementary table S7 for locus and nearby genes). When performing LASSO regression on all 179 SNPs, a subset of 14 SNPs was selected (see supplementary table S7). Three SNPs (rs10757278, rs11556924 and rs646776) were also selected for the CHD LASSO GRS, the remaining 11 SNPs were: rs2925979, rs6882076, rs2954029, rs6987702, rs10889352, rs2972146, rs514230, rs8050136, rs181362, rs925946 and rs2000999 (for locus and nearby genes see table S7).

Supplementary Tables

Supplementary table S1 can be found online at:

http://circgenetics.ahajournals.org/content/5/2/202/suppl/DC1

Supplementary table S1 | Candidate SNPs (based on the association between CHD and intermediate risk factors from previously published GWAS) for genotyping in the CAREMA study.

Table S2 | Success rates (in the total population, and in the cases and the sub-cohort separately) and the Hardy-Weinberg equilibrium (only in sub-cohort) for every genotyped SNP in the CAREMA cohort.

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	**N miss**	**Success rate**	**N miss**	**Success rate**	**N miss**	**Success rate**	**A1/A2**	**GENO**	**Obs/Exp**	***P-value***	**Remarks**
SNPs selected for their association with coronary artery diseases											
RS46522	8	1.00	7	1.00	1	1.00	C/T	403/1014/559	0.51/0.50	0.160	
RS216172	12	1.00	8	1.00	5	0.99	G/C	242/892/841	0.45/0.45	0.843	
RS579459	26	0.99	16	0.99	11	0.98	C/T	113/689/1165	0.35/0.36	0.412	
RS599839	na	na	na	na	na	na	na	na	na	na	No PCR product
RS646776	23	0.99	21	0.99	3	1.00	G/A	86/665/1211	0.34/0.34	0.687	
RS964184	31	0.99	28	0.99	5	0.99	C/G	41/469/1445	0.24/0.24	0.708	
RS974819	8	1.00	7	1.00	1	1.00	A/G	189/860/927	0.44/0.43	0.638	
RS1122608	24	0.99	21	0.99	4	0.99	T/G	104/730/1128	0.37/0.36	0.352	
RS1412444	14	0.99	11	0.99	3	1.00	A/G	237/849/886	0.43/0.45	0.130	
RS1746048	91	0.96	72	0.96	23	0.97	T/C	40/437/1434	0.23/0.23	0.328	
RS2505083	13	0.99	9	1.00	5	0.99	C/T	326/958/690	0.49/0.48	0.852	
RS2895811	9	1.00	7	1.00	2	1.00	C/T	333/952/691	0.48/0.48	0.889	
RS3825807	11	1.00	10	0.99	1	1.00	C/T	354/942/677	0.48/0.49	0.405	
RS4380028	10	1.00	9	1.00	1	1.00	A/G	317/910/747	0.46/0.48	0.156	
RS4773144	8	1.00	7	1.00	1	1.00	G/A	392/992/592	0.50/0.49	0.555	
RS6725887	127	0.95	100	0.95	34	0.95	G/A	34/400/1449	0.21/0.22	0.290	
RS6922269	64	0.97	40	0.98	28	0.96	A/G	140/733/1070	0.38/0.39	0.347	
RS9818870	45	0.98	40	0.98	6	0.99	T/C	49/536/1358	0.28/0.27	0.740	
RS9982601	71	0.97	54	0.97	17	0.97	T/C	28/460/1441	0.24/0.23	0.238	
RS10757278	103	0.96	82	0.96	22	0.97	G/A	450/938/513	0.49/0.50	0.614	
RS10953541	10	1.00	9	1.00	1	1.00	T/C	123/718/1133	0.36/0.37	0.503	
RS11206510	38	0.99	27	0.99	12	0.98	C/T	77/572/1307	0.29/0.30	0.155	
RS11556924	10	1.00	9	1.00	1	1.00	T/C	313/935/726	0.47/0.48	0.672	
RS12190287	10	1.00	8	1.00	2	1.00	G/C	272/881/822	0.45/0.46	0.144	
RS12413409	11	1.00	10	0.99	1	1.00	A/G	17/297/1659	0.15/0.15	0.377	
RS12526453	63	0.98	41	0.98	27	0.96	G/C	237/841/864	0.43/0.45	0.142	

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	N miss	Success rate	N miss	Success rate	N miss	Success rate	A1/A2	GENO	Obs/Exp	*P-value*	Remarks
RS12936587	10	1.00	8	1.00	2	1.00	A/G	372/981/622	0.50/0.49	0.681	
RS17114036	24	0.99	16	0.99	9	0.99	G/A	21/303/1643	0.15/0.16	0.119	
RS17465637	35	0.99	29	0.99	8	0.99	A/C	157/753/1044	0.39/0.40	0.210	
RS17609940	13	0.99	9	1.00	5	0.99	C/G	61/613/1300	0.31/0.30	0.298	
SNPs selected for their association with blood pressure											
RS381815	16	0.99	13	0.99	3	1.00	T/C	144/741/1085	0.38/0.39	0.267	
RS1004467	15	0.99	13	0.99	3	1.00	C/T	17/319/1634	0.16/0.16	0.680	
RS1378942	8	1.00	8	1.00	0	1.00	G/T	210/854/911	0.43/0.44	0.643	
RS1530440	10	1.00	9	1.00	1	1.00	T/C	67/581/1326	0.29/0.30	0.705	
RS2681472	7	1.00	7	1.00	0	1.00	C/T	51/516/1409	0.26/0.26	0.670	
RS3184504	7	1.00	7	1.00	0	1.00	C/T	466/998/512	0.51/0.50	0.653	
RS9815354	12	1.00	11	0.99	1	1.00	A/G	58/563/1351	0.29/0.29	1.000	
RS11014166	14	0.99	12	0.99	3	1.00	T/A	228/872/871	0.44/0.45	0.687	
RS11191548	9	1.00	8	1.00	1	1.00	C/T	17/295/1663	0.15/0.15	0.304	
RS12946454	7	1.00	7	1.00	0	1.00	T/A	136/759/1081	0.38/0.39	0.861	
RS16948048	7	1.00	7	1.00	0	1.00	G/A	296/922/758	0.47/0.47	0.568	
RS16998073	9	1.00	8	1.00	1	1.00	T/A	173/811/991	0.41/0.41	0.704	
RS17367504	10	1.00	10	0.99	0	1.00	G/A	39/503/1431	0.25/0.25	0.590	
SNPs selected for their association with diabetes type 2											
RS5215	74	0.97	58	0.97	16	0.98	G/A	269/894/762	0.46/0.47	0.807	
RS5219	154	0.94	125	0.94	36	0.95	A/G	248/848/762	0.46/0.46	0.616	
RS864745	70	0.97	58	0.97	13	0.98	G/A	455/933/537	0.48/0.50	0.201	
RS1111875	65	0.97	47	0.98	20	0.97	T/C	332/902/702	0.47/0.48	0.157	
RS2237892	100	0.96	78	0.96	26	0.96	T/C	9/212/1684	0.11/0.11	0.415	
RS2237897	85	0.97	61	0.97	30	0.96	T/C	8/141/1773	0.07/0.08	0.012	
RS4402960	32	0.99	28	0.99	6	0.99	A/C	198/845/912	0.43/0.43	0.917	
RS4506565	74	0.97	63	0.97	13	0.98	T/A	197/901/822	0.47/0.45	0.032	
RS4607103	57	0.98	36	0.98	25	0.96	T/C	130/714/1103	0.37/0.38	0.334	
RS4689388	8	1.00	7	1.00	1	1.00	G/A	325/963/688	0.49/0.48	0.710	

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	N miss	Success rate	N miss	Success rate	N miss	Success rate	A1/A2	GENO	Obs/Exp	*P-value*	Remarks
RS4712524	94	0.96	70	0.96	26	0.96	G/A	198/804/911	0.42/0.43	0.313	
RS6931514	92	0.96	71	0.96	23	0.97	G/A	141/762/1009	0.40/0.40	0.908	
RS7578597	52	0.98	41	0.98	13	0.98	G/A	25/384/1533	0.20/0.20	0.820	
RS7961581	93	0.96	75	0.96	21	0.97	C/T	180/779/949	0.41/0.42	0.274	
RS8050136	112	0.96	96	0.95	22	0.97	A/C	280/811/796	0.43/0.46	0.002	
RS10811661	49	0.98	32	0.98	21	0.97	C/T	69/606/1276	0.31/0.31	0.826	
RS10923931	111	0.96	90	0.95	25	0.96	A/C	21/309/1563	0.16/0.17	0.217	
RS10946398	63	0.98	47	0.98	18	0.97	C/A	197/825/914	0.43/0.43	0.598	
RS12779790	119	0.95	93	0.95	31	0.95	G/A	60/555/1275	0.29/0.29	1.000	
RS13266634	54	0.98	35	0.98	23	0.97	T/C	177/845/926	0.43/0.43	0.457	
SNPs selected for their association with blood lipid levels											
RS328	94	0.96	73	0.96	23	0.97	G/C	14/397/1499	0.21/0.20	0.027	
RS693	51	0.98	32	0.98	23	0.97	T/C	457/958/536	0.49/0.50	0.468	
RS7679	124	0.95	106	0.95	23	0.97	C/T	87/564/1226	0.30/0.32	0.040	
RS12916	21	0.99	10	0.99	12	0.98	C/T	370/966/637	0.49/0.49	0.927	
RS157580	77	0.97	66	0.97	14	0.98	G/A	273/896/748	0.47/0.47	0.884	
RS173539	na	na	na	na	na	na	na	na	na	na	No PCR product
RS174547	56	0.98	35	0.98	25	0.96	G/A	205/870/873	0.45/0.44	0.608	
RS174570	157	0.94	121	0.94	42	0.94	T/C	50/405/1407	0.22/0.23	0.003	
RS181362	25	0.99	16	0.99	10	0.99	T/C	101/639/1227	0.32/0.34	0.140	
RS439401	105	0.96	71	0.96	41	0.94	T/C	244/828/840	0.43/0.45	0.076	
RS442177	26	0.99	18	0.99	9	0.99	G/T	343/924/698	0.47/0.48	0.225	
RS471364	93	0.96	68	0.97	30	0.96	G/A	30/387/1498	0.20/0.21	0.375	
RS492602	14	0.99	12	0.99	2	1.00	G/A	389/954/628	0.48/0.49	0.437	
RS514230	25	0.99	16	0.99	10	0.99	A/T	446/989/532	0.50/0.50	0.752	
RS515135	na	na	na	na	na	na	na	Na	na	na	Abnormal clusterplot
RS581080	14	0.99	9	1.00	6	0.99	C/G	64/614/1296	0.31/0.31	0.461	
RS599839	na	na	na	na	na	na	na	na	na	na	No PCR product

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	N miss	Success rate	N miss	Success rate	N miss	Success rate	A1/A2	GENO	Obs/Exp	*P-value*	Remarks
RS605066	100	0.96	80	0.96	23	0.97	C/T	376/849/678	0.45/0.49	<0.001	Out of HWE
RS629301	15	0.99	11	0.99	4	0.99	C/A	86/664/1222	0.34/0.33	0.787	
RS645040	26	0.99	17	0.99	10	0.99	G/T	84/656/1226	0.33/0.33	0.786	
RS646776	23	0.99	21	0.99	3	1.00	G/A	86/665/1211	0.34/0.34	0.687	
RS737337	110	0.96	85	0.96	28	0.96	C/T	16/288/1594	0.15/0.15	0.456	
RS780093	68	0.97	53	0.97	15	0.98	T/C	331/917/682	0.48/0.48	0.451	
RS838880	11	1.00	10	0.99	1	1.00	G/A	193/820/960	0.42/0.42	0.367	
RS964184	31	0.99	28	0.99	5	0.99	C/G	41/469/1445	0.24/0.24	0.708	
RS1042034	11	1.00	10	0.99	1	1.00	G/A	102/679/1192	0.34/0.35	0.697	
RS1084651 (RS1652507)	12	1.00	8	1.00	5	0.99	C/T	44/491/1440	0.25/0.25	0.787	
RS1167998	39	0.98	31	0.98	11	0.98	G/T	213/905/834	0.46/0.45	0.174	
RS1260326	65	0.97	43	0.98	28	0.96	T/C	356/931/653	0.48/0.49	0.457	
RS1367117	506	0.80	406	0.80	113	0.83	T/C	191/448/938	0.28/0.39	<0.001	Success- rate <90%
RS1495741	10	1.00	9	1.00	1	1.00	G/A	85/714/1175	0.36/0.35	0.080	
RS1501908	73	0.97	65	0.97	10	0.99	C/G	257/888/773	0.46/0.46	0.961	
RS1532085	30	0.99	25	0.99	6	0.99	A/G	281/921/756	0.47/0.47	1.000	
RS1532624	70	0.97	50	0.97	22	0.97	A/C	354/937/642	0.48/0.49	0.710	
RS1564348	50	0.98	35	0.98	16	0.98	C/T	52/470/1426	0.24/0.25	0.086	
RS1689800	17	0.99	13	0.99	5	0.99	G/A	296/887/787	0.45/0.47	0.076	
RS1800562	11	1.00	10	0.99	1	1.00	A/G	9/231/1733	0.12/0.12	0.571	
RS1800588	21	0.99	18	0.99	4	0.99	T/C	101/698/1166	0.36/0.35	0.848	
RS1800961	70	0.97	51	0.97	21	0.97	T/C	2/98/1832	0.05/0.05	0.388	
RS1883025	101	0.96	73	0.96	33	0.95	A/G	125/686/1099	0.36/0.37	0.216	
RS2000999	8	1.00	7	1.00	1	1.00	A/G	79/632/1265	0.32/0.32	1.000	
RS2068888	15	0.99	10	0.99	6	0.99	A/G	411/958/604	0.49/0.50	0.388	
RS2072183	24	0.99	16	0.99	9	0.99	G/C	101/711/1155	0.36/0.36	0.569	
RS2075650	65	0.97	49	0.98	18	0.97	G/A	28/483/1423	0.25/0.24	0.072	
RS2081687	11	1.00	10	0.99	1	1.00	T/C	252/880/841	0.45/0.46	0.374	

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	N miss	Success rate	N miss	Success rate	N miss	Success rate	A1/A2	GENO	Obs/Exp	*P-value*	Remarks
RS2083637	49	0.98	32	0.98	21	0.97	G/A	124/779/1048	0.40/0.39	0.200	
RS2228671	28	0.99	26	0.99	4	0.99	T/C	31/398/1528	0.20/0.21	0.383	
RS2240466	51	0.98	33	0.98	22	0.97	A/G	21/364/1565	0.19/0.19	1.000	
RS2241213 (RS2338104)	93	0.96	76	0.96	21	0.97	A/G	436/917/554	0.48/0.50	0.130	
RS2247056	na	na	na	na	na	na	na	Na	na	Na	No PCR product
RS2255141	10	1.00	9	1.00	1	1.00	T/C	152/858/964	0.43/0.42	0.045	
RS2271293	115	0.95	96	0.95	24	0.96	A/G	35/434/1418	0.23/0.23	0.766	
RS2277862	8	1.00	7	1.00	1	1.00	T/C	30/530/1416	0.27/0.25	0.013	
RS2290159	15	0.99	11	0.99	5	0.99	C/G	84/617/1271	0.31/0.32	0.397	
RS2293889	24	0.99	16	0.99	9	0.99	A/C	328/940/699	0.48/0.48	0.708	
RS2304130	27	0.99	24	0.99	4	0.99	G/A	18/306/1635	0.16/0.16	0.393	
RS2412710	11	1.00	10	0.99	1	1.00	A/G	0/91/1882	0.05/0.05	0.624	
RS2479409	16	0.99	11	0.99	7	0.99	G/A	273/867/832	0.44/0.46	0.056	
RS2642442	187	0.93	150	0.92	42	0.94	C/T	537/528/768	0.29/0.49	<0.001	Out of HWE
RS2650000	88	0.97	71	0.96	21	0.97	A/C	242/816/854	0.43/0.45	0.032	
RS2652834	29	0.99	21	0.99	10	0.99	A/G	73/646/1243	0.33/0.32	0.362	
RS2737229	8	1.00	7	1.00	1	1.00	G/T	212/861/903	0.44/0.44	0.759	
RS2814944	25	0.99	17	0.99	9	0.99	T/C	43/504/1419	0.26/0.26	0.930	
RS2814982	10	1.00	9	1.00	1	1.00	A/G	17/373/1584	0.19/0.18	0.394	
RS2902940	24	0.99	16	0.99	9	0.99	G/A	197/796/974	0.40/0.42	0.069	
RS2923084	24	0.99	16	0.99	9	0.99	G/A	75/601/1291	0.31/0.31	0.610	
RS2925979	24	0.99	16	0.99	9	0.99	T/C	153/856/958	0.44/0.42	0.045	
RS2929282	11	1.00	10	0.99	1	1.00	A/T	8/187/1778	0.09/0.10	0.241	
RS2954029	57	0.98	37	0.98	24	0.96	T/A	424/1002/520	0.51/0.50	0.159	
RS2967605	184	0.93	150	0.92	42	0.94	T/C	147/504/1182	0.28/0.34	<0.001	Out of HWE
RS2972146	12	1.00	8	1.00	5	0.99	G/T	265/877/833	0.44/0.46	0.155	
RS3177928	17	0.99	11	0.99	7	0.99	A/G	34/276/1662	0.14/0.16	<0.001	Out of HWE
RS3757354	11	1.00	10	0.99	1	1.00	T/C	85/659/1229	0.33/0.33	0.839	
RS3764261	26	0.99	17	0.99	10	0.99	A/C	193/848/925	0.43/0.43	1.000	

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	**N miss**	**Success rate**	**N miss**	**Success rate**	**N miss**	**Success rate**	**A1/A2**	**GENO**	**Obs/Exp**	***P-value***	**Remarks**
RS3846662	364	0.86	281	0.86	97	0.85	G/A	355/842/505	0.49/0.50	0.922	Success- rate <90%
RS3905000	137	0.95	114	0.94	30	0.96	T/C	41/376/1452	0.20/0.22	0.007	
RS4129767	13	0.99	8	1.00	6	0.99	A/G	472/971/532	0.49/0.50	0.499	
RS4148008	24	0.99	16	0.99	9	0.99	C/G	189/810/968	0.41/0.42	0.310	
RS4420638	128	0.95	107	0.95	24	0.96	G/A	0/604/1272	0.32/0.27	<0.001	Out of HWE
RS4660293	57	0.98	46	0.98	12	0.98	C/T	119/729/1089	0.38/0.37	0.903	
RS4731702	59	0.98	45	0.98	15	0.98	T/C	461/929/548	0.48/0.50	0.084	
RS4765127	18	0.99	13	0.99	6	0.99	T/G	180/887/903	0.45/0.43	0.077	
RS4846914	119	0.95	85	0.96	42	0.94	G/A	323/928/647	0.49/0.49	0.777	
RS4939883	175	0.93	139	0.93	43	0.94	T/C	53/495/1296	0.27/0.27	0.495	
RS5756931	247	0.90	199	0.90	90	0.87	G/A	257/844/683	0.47/0.47	0.920	Success- rate <90%
RS6029526	29	0.99	18	0.99	12	0.98	A/T	435/951/579	0.48/0.50	0.238	
RS6450176	21	0.99	13	0.99	10	0.99	T/C	151/732/1087	0.37/0.39	0.081	
RS6511720	62	0.98	39	0.98	28	0.96	T/G	39/384/1521	0.20/0.21	0.017	
RS6544713	89	0.97	78	0.96	14	0.98	T/C	172/796/937	0.42/0.42	0.870	
RS6754295	83	0.97	73	0.96	12	0.98	G/T	118/698/1094	0.37/0.37	0.621	
RS6756629	22	0.99	20	0.99	3	1.00	T/C	5/242/1716	0.12/0.12	0.345	
RS6882076	12	1.00	11	0.99	1	1.00	A/G	266/911/795	0.46/0.46	0.846	
RS6987702	19	0.99	18	0.99	1	1.00	C/T	155/793/1017	0.40/0.40	1.000	
RS7134375	9	1.00	7	1.00	2	1.00	A/C	359/926/691	0.47/0.49	0.116	
RS7255436	14	0.99	11	0.99	3	1.00	C/A	490/934/548	0.47/0.50	0.022	
RS7395662	49	0.98	39	0.98	12	0.98	A/G	345/899/700	0.46/0.48	0.060	
RS7515577	8	1.00	7	1.00	1	1.00	G/T	100/702/1174	0.36/0.35	0.750	
RS7557067	75	0.97	55	0.97	23	0.97	G/A	119/706/1103	0.37/0.37	0.667	
RS7570971	9	1.00	7	1.00	2	1.00	A/C	364/903/709	0.46/0.48	0.011	
RS7819412	69	0.97	42	0.98	31	0.95	T/C	466/972/503	0.50/0.50	0.964	
RS7941030	8	1.00	7	1.00	1	1.00	C/T	292/915/769	0.46/0.47	0.474	
RS8017377	8	1.00	7	1.00	1	1.00	A/G	418/986/572	0.50/0.50	0.892	

	CAREMA N=2548		Subcohort N=1983		Cases N=667			HWE in subcohort			
SNP	N miss	Success rate	N miss	Success rate	N miss	Success rate	A1/A2	GENO	Obs/Exp	*P-value*	Remarks
RS9488822	12	1.00	9	1.00	3	1.00	A/T	212/881/881	0.45/0.44	0.722	
RS9686661	10	1.00	9	1.00	1	1.00	T/C	74/609/1291	0.31/0.31	0.828	
RS9987289	15	0.99	12	0.99	4	0.99	A/G	8/283/1680	0.14/0.14	0.337	
RS10195252	na	na	na	na	na	na	na	na	Na	na	Abnormal cluster plot
RS10401969	22	0.99	20	0.99	3	1.00	G/A	9/257/1697	0.13/0.13	1.000	
RS10468017	61	0.98	46	0.98	17	0.97	T/C	159/746/1032	0.39/0.40	0.139	
RS10889352	31	0.99	26	0.99	7	0.99	G/A	205/901/851	0.46/0.45	0.155	
RS10903129	110	0.96	89	0.96	24	0.96	A/G	398/902/594	0.48/0.49	0.104	
RS11206510	38	0.99	27	0.99	12	0.98	C/T	77/572/1307	0.29/0.30	0.155	
RS12272004	83	0.97	52	0.97	38	0.94	A/C	9/235/1687	0.12/0.12	0.711	
RS12670798	23	0.99	20	0.99	4	0.99	C/T	108/687/1168	0.35/0.35	0.610	
RS12678919	66	0.97	51	0.97	17	0.97	G/A	15/392/1525	0.20/0.19	0.062	
RS12740374	76	0.97	59	0.97	17	0.97	A/C	85/649/1190	0.34/0.34	0.838	
RS17216525	73	0.97	64	0.97	11	0.98	T/C	14/291/1614	0.15/0.15	0.765	
SNP selected for their association with anthropomorphic traits											
RS6265	146	0.94	121	0.94	32	0.95	A/G	81/610/1171	0.33/0.33	0.888	
RS29939	96	0.96	71	0.96	28	0.96	A/G	186/816/910	0.43/0.43	0.873	
RS925946	12	1.00	11	0.99	1	1.00	T/G	184/743/1045	0.38/0.40	0.003	
RS987237	10	1.00	9	1.00	1	1.00	G/A	69/604/1301	0.31/0.31	1.000	
RS1121980	na	na	na	na	na	na	na	na	na	na	No PCR product
RS1260326	65	0.97	43	0.98	28	0.96	T/C	356/931/653	0.48/0.49	0.457	
RS1558902	8	1.00	8	1.00	0	1.00	A/T	313/852/810	0.43/0.47	<0.001	Out of HWE
RS2568958	72	0.97	54	0.97	20	0.97	G/A	297/922/710	0.48/0.48	0.962	
RS2605100	14	0.99	12	0.99	3	1.00	A/G	193/774/1004	0.39/0.42	0.017	
RS2844479	na	na	na	na	na	na	Na	na	na	Na	No PCR product
RS3764261	26	0.99	17	0.99	10	0.99	A/C	193/848/925	0.43/0.43	1.000	
RS6499640	93	0.96	68	0.97	29	0.96	G/A	341/956/618	0.50/0.49	0.401	
RS6548238	97	0.96	77	0.96	24	0.96	T/C	51/554/1301	0.29/0.28	0.422	
RS7498665	71	0.97	54	0.97	17	0.97	G/A	235/926/768	0.48/0.46	0.094	

	CAREMA N=2548		Subcohort N=1983		Cases N=667		HWE in subcohort				
SNP	**N miss**	**Success rate**	**N miss**	**Success rate**	**N miss**	**Success rate**	**A1/A2**	**GENO**	**Obs/Exp**	***P-value***	**Remarks**
RS7561317	190	0.93	148	0.93	49	0.93	A/G	51/540/1244	0.29/0.29	0.466	
RS7647305	121	0.95	86	0.96	41	0.94	A/G	66/606/1225	0.32/0.31	0.421	
RS7826222	na	na	na	na	na	na	na	na	na	na	No PCR product
RS8050136	112	0.96	96	0.95	22	0.97	A/C	280/811/796	0.43/0.46	0.002	
RS10146997	7	1.00	7	1.00	0	1.00	G/A	79/602/1295	0.30/0.31	0.385	
RS10838738	127	0.95	99	0.95	33	0.95	C/T	180/836/868	0.44/0.43	0.313	
RS10913469	187	0.93	150	0.92	45	0.93	G/A	80/539/1214	0.29/0.31	0.049	
RS10938397	130	0.95	105	0.95	31	0.95	G/A	384/862/632	0.46/0.49	0.005	
RS11084753	151	0.94	120	0.94	36	0.95	A/G	197/813/853	0.44/0.44	0.874	
RS12970134	91	0.96	72	0.96	25	0.96	A/G	125/731/1055	0.38/0.38	0.952	

HWE, Hardy-Weinberg equilibrium; ***SNP***, single nucleotide polymorphism; ***N miss,*** number of missing genotypes; ***A1/A2,*** minor allele/major allele; ***GENO***, genotype distribution; ***Obs/Exp,*** observed/expected

Table S3 | Associations between the different genetic risk scores and future CHD in subjects with complete genotype information.

Genetic Risk Scores	N SNPs	Cases (N=272)	Sub-cohort (N=878)	Coronary heart disease		
				HR (95% CI)	HR std† (95% CI)	*P*
Overall GRS	179	185.4 ± 8.4	182.5 ± 9.4	1.02 (1.01-1.04)	1.25 (1.06-1.46)	0.006
Adjusted for TRF*				1.01 (1.00-1.03)	1.11 (0.96-1.28)	0.152
Risk Factor GRS	153	157.0 ± 8.1	155.3 ± 8.4	1.02 (1.01-1.04)	1.22 (1.05-1.42)	0.011
Adjusted for TRF*				1.01 (0.99-1.03)	1.08 (0.94-1.24)	0.303
CHD GRS	29	31.1 ± 3.2	30.7 ± 3.5	1.05 (1.01-1.09)	1.79 (1.03-1.35)	0.020
Adjusted for TRF*				1.03 (0.97-1.09)	1.12 (0.93-1.36)	0.285
Weighted CHD GRS	29	11.4 ± 1.3	11.1 ± 1.3	1.23 (1.10-1.37)	1.32 (1.13-1.53)	<0.001
Adjusted for TRF*				1.14 (1.01-1.29)	1.19 (1.02-1.40)	0.032
LASSO CHD GRS	3	1.80 ± 0.70	1.63 ± 0.73	1.57 (1.28-1.94)	1.39 (1.20-1.62)	<0.001
Adjusted for TRF*				1.39 (1.13-1.71)	1.19 (1.02-1.40)	0.002
LASSO GRS	14	4.1 ± 1.0	3.7 ± 1.0	1.64 (1.36-1.99)	1.65 (1.36-2.00)	<0.001
Adjusted for TRF*				1.53 (1.24-1.90)	1.54 (1.24-1.91)	<0.001

* Traditional risk factors include sex, current smoking (yes/no), systolic blood pressure,
total cholesterol, HDL cholesterol, self-reported diabetes (yes/no), body mass index and parental history of MI parents with MI, one parent with MI, both parents with MI). Age was used as the time scale variable

† Increase in hazard ratio per standard deviation

HR, hazard ratio; *std,* standardized; *SNPs*, single nucleotide polymorphisms; *CHD*, coronary heart disease; TRF, traditional risk factors; *GRS,* genetic risk score; *LASSO,* least absolute shrinkage and selection operator

Table S4 | Weights for the SNPs selected for their association with CHD. The weights are based on the log odds ratio as published in Schunker et al. 2011 or Peden et al 2011.

		Subcohort		**Cases**				
SNP	**RA**	**N**	**RAF**	**N**	**RAF**	**OR**	**Weighting factor**[*]	***Source***
RS10757278[1]	G	1832	0.49	623	0.55	1.29	1.00	Schunkert *et al.* (2011)
RS10953541	C	1902	0.76	641	0.75	1.08	0.30	Peden *et al.* (2011)
RS11206510	T	1881	0.81	632	0.81	1.08	0.30	Schunkert *et al.* (2011)
RS1122608	G	1887	0.76	640	0.76	1.14	0.51	Schunkert *et al.* (2011)
RS11556924	C	1902	0.60	641	0.64	1.09	0.34	Schunkert *et al.* (2011)
RS12190287	C	1901	0.64	641	0.65	1.08	0.30	Schunkert *et al.* (2011)
RS12413409	G	1901	0.92	641	0.90	1.12	0.45	Schunkert *et al.* (2011)
RS12526453	C	1865	0.66	616	0.65	1.10	0.37	Schunkert *et al.* (2011)
RS12936587	G	1900	0.56	641	0.58	1.07	0.27	Schunkert *et al.* (2011)
RS1412444	A	1900	0.33	639	0.35	1.09	0.34	Peden *et al.* (2011)
RS17114036	A	1890	0.91	635	0.92	1.17	0.62	Schunkert *et al.* (2011)
RS1746048	C	1838	0.86	622	0.87	1.09	0.34	Schunkert *et al.* (2011)
RS17465637	C	1880	0.73	636	0.75	1.14	0.51	Schunkert *et al.* (2011)
RS17609940	G	1897	0.81	637	0.81	1.07	0.27	Schunkert *et al.* (2011)
RS216172	G	1898	0.35	637	0.35	1.07	0.27	Schunkert *et al.* (2011)
RS2505083[2]	C	1897	0.41	637	0.43	1.05	0.19	Schunkert *et al.* (2011)
RS2895811	C	1901	0.41	641	0.41	1.07	0.27	Schunkert *et al.* (2011)
RS3825807	T	1901	0.58	641	0.57	1.08	0.30	Schunkert *et al.* (2011)
RS4380028	G	1902	0.61	641	0.6	1.07	0.27	Peden *et al.* (2011)
RS46522	T	1901	0.54	642	0.55	1.06	0.23	Schunkert *et al.* (2011)
RS4773144	G	1901	0.45	642	0.46	1.07	0.27	Schunkert *et al.* (2011)
RS579459	C	1890	0.23	633	0.23	1.10	0.37	Schunkert *et al.* (2011)
RS646776	A	1887	0.79	641	0.82	1.11	0.41	Schunkert *et al.* (2011)
RS6725887	G	1811	0.13	611	0.13	1.14	0.51	Schunkert *et al.* (2011)
RS6922269	A	1866	0.26	615	0.27	1.06	0.23	Schunkert *et al.* (2011)
RS964184	C	1880	0.14	639	0.14	1.13	0.48	Schunkert *et al.* (2011)
RS974819	A	1901	0.31	642	0.31	1.07	0.27	Peden *et al.* (2011)
RS9818870	T	1869	0.17	638	0.16	1.12	0.45	Schunkert *et al.* (2011)
RS9982601	T	1859	0.13	626	0.15	1.18	0.65	Schunkert *et al.* (2011)

[1] Instead of rs10757278, rs4977574 was selected for replication in Schunkert et al.

[2] Instead of rs2505083, rs7920682 was selected for replication in Schunkert et al.

[*] The weighting factor is determined by dividing the log odds ratio of a SNP by the log odds ratio of rs10757278 (the SNP with the highest log odds ratio).

Table S5 | The association between the 179 successfully genotyped SNPs and incident CHD in the CAREMA study.

		Subcohort		**Cases**		**CHD**		**CHD adjusted***	
SNP	**RA**	**N**	**RAF**	**N**	**RAF**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
SNPs selected for their association with coronary artery diseases									
RS10757278	G	1832	0.49	623	0.55	**1.34 (1.17-1.53)**	**<0.001**	**1.29 (1.05-1.59)**	**0.015**
RS10953541	C	1902	0.76	641	0.75	**0.98 (0.84-1.14)**	**0.779**	**0.87 (0.77-0.99)**	**0.028**
RS11206510	T	1881	0.81	632	0.81	0.99 (0.83-1.17)	0.861	1.02 (0.88-1.17)	0.840
RS1122608	G	1887	0.76	640	0.76	1.01 (0.87-1.18)	0.869	1.01 (0.89-1.15)	0.919
RS11556924	C	1902	0.60	641	0.64	**1.16 (1.01-1.32)**	**0.033**	1.23 (0.93-1.63)	0.155
RS12190287	C	1901	0.64	641	0.65	1.05 (0.92-1.21)	0.451	1.06 (0.94-1.20)	0.323
RS12413409	G	1901	0.92	641	0.90	0.88 (0.69-1.14)	0.334	0.86 (0.71-1.04)	0.124
RS12526453	C	1865	0.66	616	0.65	0.92 (0.80-1.06)	0.255	1.00 (0.89-1.13)	0.991
RS12936587	G	1900	0.56	641	0.58	1.08 (0.95-1.24)	0.247	1.04 (0.93-1.17)	0.505
RS1412444	A	1900	0.33	639	0.35	1.07 (0.93-1.24)	0.325	1.15 (0.77-1.73)	0.489
RS17114036	A	1890	0.91	635	0.92	1.06 (0.85-1.33)	0.598	0.99 (0.80-1.23)	0.924
RS1746048	C	1838	0.86	622	0.87	1.01 (0.82-1.23)	0.960	1.05 (0.88-1.25)	0.568
RS17465637	C	1880	0.73	636	0.75	1.14 (0.99-1.32)	0.064	1.04 (0.92-1.17)	0.535
RS17609940	G	1897	0.81	637	0.81	0.99 (0.83-1.17)	0.864	0.88 (0.63-1.24)	0.459
RS216172	G	1898	0.35	637	0.35	1.02 (0.88-1.17)	0.809	1.09 (0.93-1.28)	0.286
RS2505083	C	1897	0.41	637	0.43	1.07 (0.93-1.23)	0.322	1.00 (0.89-1.12)	0.942
RS2895811	C	1901	0.41	641	0.41	0.99 (0.87-1.14)	0.905	0.99 (0.89-1.11)	0.864
RS3825807	T	1901	0.58	641	0.57	1.00 (0.87-1.14)	0.956	1.04 (0.93-1.17)	0.447
RS4380028	G	1902	0.61	641	0.60	0.99 (0.87-1.13)	0.890	1.06 (0.95-1.18)	0.337
RS46522	T	1901	0.54	642	0.55	1.10 (0.96-1.26)	0.168	1.07 (0.94-1.21)	0.327
RS4773144	G	1901	0.45	642	0.46	0.99 (0.87-1.13)	0.846	0.99 (0.88-1.11)	0.818
RS579459	C	1890	0.23	633	0.23	0.97 (0.84-1.13)	0.727	0.99 (0.87-1.12)	0.821
RS646776	A	1887	0.79	641	0.82	**1.27 (1.09-1.47)**	**0.002**	**1.25 (1.07-1.45)**	**0.004**
RS6725887	G	1811	0.13	611	0.13	1.05 (0.85-1.30)	0.637	1.08 (0.90-1.29)	0.395
RS6922269	A	1866	0.26	615	0.27	1.06 (0.91-1.24)	0.431	1.07 (0.92-1.25)	0.386
RS964184	C	1880	0.14	639	0.14	0.96 (0.8-1.170)	0.704	0.80 (0.56-1.14)	0.215
RS974819	A	1901	0.31	642	0.31	1.02 (0.88-1.18)	0.812	0.96 (0.85-1.09)	0.552
RS9818870	T	1869	0.17	638	0.16	0.94 (0.79-1.11)	0.452	0.92 (0.79-1.07)	0.285
RS9982601	T	1859	0.13	626	0.15	1.19 (0.96-1.49)	0.114	1.16 (0.98-1.37)	0.092
SNPs selected for their association with blood pressure									
RS1004467	T	1894	0.91	639	0.90	0.89 (0.70-1.14)	0.347	**0.83 (0.69-0.99)**	**0.038**
RS11014166	A	1895	0.66	639	0.68	1.05 (0.91-1.20)	0.526	1.14 (1.00-1.30)	0.051
RS11191548	T	1899	0.92	641	0.91	0.92 (0.72-1.18)	0.523	0.90 (0.75-1.09)	0.282
RS12946454	T	1900	0.26	642	0.24	0.91 (0.79-1.06)	0.218	0.83 (0.68-1.03)	0.084
RS1378942	G	1899	0.32	642	0.34	1.11 (0.96-1.28)	0.163	1.00 (0.88-1.12)	0.937
RS1530440	C	1898	0.18	641	0.18	1.05 (0.88-1.25)	0.622	0.89 (0.77-1.03)	0.112
RS16948048	G	1900	0.38	642	0.39	1.06 (0.93-1.22)	0.390	1.09 (0.97-1.23)	0.132
RS16998073	T	1899	0.29	641	0.31	1.06 (0.91-1.23)	0.460	1.08 (0.95-1.22)	0.250
RS17367504	A	1897	0.15	642	0.15	1.03 (0.85-1.26)	0.735	1.15 (0.98-1.36)	0.082
RS2681472	T	1900	0.84	642	0.84	0.93 (0.77-1.13)	0.464	**0.85 (0.73-0.99)**	**0.038**
RS3184504	T	1900	0.51	642	0.50	0.98 (0.86-1.12)	0.731	0.92 (0.81-1.06)	0.241
RS381815	T	1894	0.26	639	0.28	1.16 (0.99-1.36)	0.075	**1.22 (1.08-1.38)**	**0.002**

		Subcohort		**Cases**		**CHD**		**CHD adjusted***	
SNP	**RA**	**N**	**RAF**	**N**	**RAF**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
RS9815354	A	1896	0.17	641	0.17	0.99 (0.83-1.17)	0.865	1.06 (0.92-1.22)	0.437
SNPs selected for their association with diabetes type II									
RS10811661	T	1874	0.81	622	0.80	0.94 (0.79-1.12)	0.491	0.91 (0.76-1.08)	0.284
RS10923931	A	1821	0.09	620	0.10	1.16 (0.90-1.50)	0.253	1.14 (0.81-1.59)	0.455
RS10946398	C	1861	0.31	624	0.31	1.01 (0.88-1.17)	0.867	1.06 (0.94-1.20)	0.316
RS1111875	C	1861	0.60	622	0.61	1.03 (0.89-1.18)	0.726	1.11 (0.81-1.53)	0.512
RS12779790	G	1818	0.18	613	0.19	1.04 (0.87-1.25)	0.655	1.08 (0.92-1.25)	0.350
RS13266634	C	1871	0.69	620	0.69	1.02 (0.88-1.18)	0.838	0.99 (0.87-1.12)	0.881
RS2237892	C	1831	0.94	619	0.94	0.94 (0.70-1.27)	0.703	0.80 (0.59-1.09)	0.153
RS2237897	C	1848	0.96	613	0.96	0.98 (0.69-1.39)	0.913	0.89 (0.54-1.45)	0.635
RS4402960	A	1880	0.32	638	0.34	1.12 (0.97-1.30)	0.124	1.11 (0.98-1.25)	0.109
RS4506565	T	1847	0.34	631	0.35	1.01 (0.88-1.17)	0.853	0.94 (0.72-1.22)	0.637
RS4607103	C	1870	0.75	618	0.77	1.12 (0.97-1.30)	0.126	1.09 (0.96-1.24)	0.181
RS4689388	A	1900	0.59	641	0.60	1.07 (0.94-1.23)	0.311	1.15 (0.89-1.49)	0.291
RS4712524	G	1838	0.31	617	0.32	1.04 (0.89-1.20)	0.643	1.09 (0.97-1.24)	0.152
RS5215	G	1856	0.37	627	0.36	0.93 (0.81-1.06)	0.270	1.03 (0.92-1.16)	0.639
RS5219	A	1789	0.36	608	0.36	0.94 (0.82-1.09)	0.417	1.06 (0.95-1.20)	0.305
RS6931514	G	1843	0.27	621	0.28	1.02 (0.88-1.19)	0.801	1.09 (0.96-1.23)	0.174
RS7578597	A	1869	0.89	632	0.89	1.03 (0.83-1.27)	0.806	1.05 (0.87-1.26)	0.608
RS7961581	C	1836	0.30	623	0.31	1.08 (0.93-1.26)	0.295	1.04 (0.91-1.19)	0.541
RS8050136	A	1815	0.36	622	0.40	**1.19 (1.04-1.38)**	**0.014**	**1.20 (1.07-1.34)**	**0.002**
RS864745	T	1857	0.52	631	0.50	0.91 (0.79-1.04)	0.159	0.90 (0.80-1.02)	0.090
SNPs selected for their association with blood lipid levels									
RS10401969	A	1888	0.93	641	0.92	0.85 (0.64-1.14)	0.279	0.76 (0.44-1.33)	0.338
RS1042034	A	1901	0.78	641	0.75	0.88 (0.74-1.04)	0.123	**0.83 (0.73-0.94)**	**0.005**
RS10468017	C	1863	0.72	625	0.70	0.95 (0.81-1.10)	0.460	1.00 (0.88-1.13)	0.965
RS1084651	T	1898	0.85	637	0.85	0.88 (0.72-1.08)	0.234	0.92 (0.77-1.08)	0.302
RS10889352	A	1882	0.66	637	0.68	1.13 (0.98-1.30)	0.082	1.07 (0.95-1.21)	0.249
RS10903129	G	1820	0.55	620	0.53	0.96 (0.84-1.10)	0.594	1.05 (0.93-1.17)	0.437
RS11206510	T	1881	0.81	632	0.81	0.99 (0.83-1.17)	0.861	1.02 (0.88-1.17)	0.840
RS1167998	T	1877	0.66	633	0.68	1.11 (0.97-1.28)	0.138	1.07 (0.95-1.21)	0.256
RS12272004	A	1855	0.07	606	0.06	0.85 (0.66-1.09)	0.193	**0.77 (0.60-0.99)**	**0.043**
RS1260326	T	1863	0.42	615	0.44	1.07 (0.93-1.23)	0.343	0.91 (0.72-1.15)	0.426
RS12670798	C	1888	0.23	640	0.24	1.01 (0.86-1.17)	0.949	0.91 (0.80-1.04)	0.173
RS12678919	A	1857	0.89	625	0.90	1.19 (0.96-1.46)	0.107	1.02 (0.84-1.24)	0.831
RS12740374	C	1854	0.79	626	0.82	**1.26 (1.08-1.47)**	**0.003**	**1.24 (1.07-1.43)**	**0.004**
RS12916	C	1896	0.43	632	0.41	0.96 (0.84-1.10)	0.562	0.96 (0.85-1.09)	0.528
RS1495741	G	1902	0.23	641	0.24	1.05 (0.89-1.24)	0.534	1.09 (0.95-1.24)	0.233
RS1501908	G	1845	0.63	634	0.66	1.10 (0.96-1.26)	0.194	1.11 (0.99-1.25)	0.085
RS1532085	G	1883	0.62	638	0.60	0.93 (0.81-1.06)	0.276	0.95 (0.84-1.06)	0.355
RS1532624	C	1860	0.57	620	0.57	1.00 (0.88-1.15)	0.973	0.94 (0.83-1.05)	0.270
RS1564348	T	1873	0.85	628	0.86	1.05 (0.88-1.25)	0.624	1.13 (0.97-1.32)	0.123
RS157580	A	1844	0.62	630	0.63	1.01 (0.88-1.17)	0.842	0.99 (0.88-1.12)	0.837
RS1689800	G	1893	0.37	637	0.37	1.00 (0.87-1.15)	0.998	0.93 (0.83-1.04)	0.225

		Subcohort		**Cases**		**CHD**		**CHD adjusted***	
SNP	**RA**	**N**	**RAF**	**N**	**RAF**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
RS17216525	C	1846	0.92	633	0.91	1.02 (0.80-1.29)	0.901	0.97 (0.79-1.18)	0.729
RS174547	G	1871	0.33	618	0.33	1.05 (0.90-1.21)	0.555	1.04 (0.62-1.72)	0.896
RS174570	C	1793	0.86	602	0.86	0.95 (0.78-1.16)	0.591	1.02 (0.86-1.20)	0.832
RS1800562	G	1901	0.94	641	0.94	1.03 (0.79-1.34)	0.822	**0.77 (0.62-0.96)**	**0.020**
RS1800588	T	1890	0.23	640	0.22	0.97 (0.82-1.13)	0.675	1.00 (0.87-1.16)	0.961
RS1800961	T	1857	0.03	621	0.03	1.15 (0.72-1.85)	0.558	0.80 (0.30-2.14)	0.649
RS181362	T	1890	0.21	634	0.24	1.17 (0.99-1.40)	0.071	**1.24 (1.09-1.41)**	**0.001**
RS1883025	A	1836	0.25	609	0.25	1.02 (0.88-1.20)	0.766	0.91 (0.79-1.03)	0.140
RS2000999	A	1901	0.20	642	0.24	**1.32 (1.08-1.60)**	**0.006**	**1.21 (1.02-1.43)**	**0.027**
RS2068888	G	1896	0.55	637	0.54	0.96 (0.84-1.10)	0.568	**0.86 (0.75-0.99)**	**0.034**
RS2072183	G	1890	0.23	635	0.22	1.01 (0.86-1.19)	0.900	0.96 (0.84-1.11)	0.582
RS2075650	G	1859	0.14	624	0.15	1.01 (0.83-1.24)	0.893	1.00 (0.85-1.18)	1.000
RS2081687	T	1901	0.35	641	0.37	1.10 (0.96-1.27)	0.176	**1.13 (1.01-1.27)**	**0.036**
RS2083637	A	1874	0.74	622	0.75	1.01 (0.86-1.18)	0.933	0.90 (0.68-1.18)	0.433
RS2228671	C	1883	0.88	640	0.89	**1.22 (1.01-1.46)**	**0.037**	1.16 (0.97-1.38)	0.101
RS2240466	G	1873	0.90	621	0.89	0.91 (0.72-1.15)	0.412	0.95 (0.73-1.22)	0.670
RS2255141	T	1902	0.30	641	0.32	1.06 (0.91-1.23)	0.450	1.12 (0.85-1.47)	0.425
RS2271293	A	1817	0.87	620	0.87	1.02 (0.84-1.24)	0.837	0.93 (0.75-1.15)	0.503
RS2277862	C	1901	0.85	642	0.83	0.90 (0.74-1.10)	0.317	0.97 (0.83-1.13)	0.697
RS2290159	G	1895	0.80	637	0.80	0.93 (0.78-1.11)	0.440	0.88 (0.76-1.02)	0.078
RS2293889	A	1890	0.41	635	0.41	1.01 (0.89-1.16)	0.853	1.00 (0.89-1.11)	0.958
RS2304130	A	1886	0.91	640	0.91	1.01 (0.80-1.27)	0.933	0.93 (0.76-1.12)	0.426
rs2241213	G	1834	0.47	623	0.45	0.95 (0.83-1.08)	0.444	0.99 (0.86-1.13)	0.860
RS2412710	A	1901	0.02	641	0.03	1.08 (0.68-1.70)	0.758	0.82 (0.58-1.16)	0.251
RS2479409	G	1895	0.36	635	0.36	0.99 (0.86-1.13)	0.865	0.98 (0.88-1.10)	0.728
RS2650000	A	1839	0.34	623	0.34	1.01 (0.88-1.17)	0.858	1.00 (0.89-1.13)	0.981
RS2652834	A	1886	0.20	633	0.19	0.98 (0.83-1.16)	0.776	0.93 (0.80-1.08)	0.346
RS2737229	T	1901	0.67	642	0.68	1.00 (0.87-1.15)	0.979	0.92 (0.79-1.09)	0.343
RS2814944	T	1890	0.15	635	0.13	0.91 (0.76-1.09)	0.307	0.93 (0.77-1.11)	0.400
RS2814982	G	1902	0.90	641	0.90	0.95 (0.75-1.19)	0.643	0.98 (0.81-1.19)	0.870
RS2902940	A	1890	0.70	635	0.71	1.04 (0.90-1.19)	0.607	1.06 (0.61-1.85)	0.828
RS2923084	G	1890	0.19	635	0.18	0.93 (0.79-1.09)	0.351	0.97 (0.84-1.12)	0.707
RS2925979	T	1890	0.30	635	0.33	1.23 (1.05-1.44)	0.012	1.13 (0.93-1.38)	0.217
RS2929282	A	1901	0.05	641	0.05	0.99 (0.74-1.33)	0.952	1.01 (0.79-1.30)	0.927
RS2954029	A	1870	0.52	619	0.54	1.07 (0.94-1.23)	0.310	1.05 (0.94-1.18)	0.388
RS2972146	T	1898	0.64	637	0.67	1.19 (1.05-1.36)	0.009	1.06 (0.94-1.19)	0.353
RS328	C	1835	0.89	620	0.90	1.17 (0.95-1.44)	0.146	1.03 (0.84-1.26)	0.770
RS3757354	C	1898	0.79	642	0.80	1.05 (0.89-1.23)	0.551	1.00 (0.86-1.17)	0.994
RS3764261	C	1889	0.69	634	0.70	1.09 (0.95-1.25)	0.215	1.06 (0.94-1.20)	0.327
RS3905000	T	1798	0.12	614	0.10	0.87 (0.72-1.06)	0.161	0.86 (0.54-1.39)	0.544
RS4129767	G	1898	0.51	637	0.52	1.01 (0.89-1.16)	0.840	0.97 (0.86-1.09)	0.575
RS4148008	C	1890	0.30	635	0.32	1.13 (0.97-1.31)	0.113	0.97 (0.85-1.10)	0.583
rs6544713	T	1832	0.30	630	0.30	1.06 (0.91-1.23)	0.475	0.96 (0.85-1.08)	0.491
RS439401	C	1838	0.66	602	0.65	0.99 (0.86-1.13)	0.827	0.98 (0.87-1.10)	0.678

		Subcohort		**Cases**		**CHD**		**CHD adjusted***	
SNP	**RA**	**N**	**RAF**	**N**	**RAF**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
RS442177	T	1888	0.59	635	0.59	1.03 (0.90-1.18)	0.622	1.11 (0.99-1.24)	0.086
RS4660293	C	1861	0.25	633	0.25	0.97 (0.83-1.13)	0.686	**0.82 (0.72-0.94)**	**0.003**
RS471364	G	1841	0.12	612	0.13	1.08 (0.87-1.34)	0.464	1.08 (0.91-1.28)	0.369
RS4731702	C	1863	0.52	631	0.52	1.01 (0.88-1.15)	0.944	1.01 (0.91-1.13)	0.802
RS4765127	G	1893	0.68	637	0.70	1.04 (0.89-1.20)	0.648	1.02 (0.88-1.17)	0.832
RS4846914	G	1825	0.41	602	0.43	1.08 (0.94-1.25)	0.256	0.98 (0.86-1.11)	0.755
RS492602	G	1899	0.44	640	0.45	1.05 (0.92-1.19)	0.517	1.04 (0.93-1.16)	0.483
RS4939883	C	1777	0.84	602	0.86	1.15 (0.97-1.36)	0.106	**1.20 (1.04-1.40)**	**0.015**
RS514230	T	1890	0.52	634	0.54	1.14 (1.00-1.31)	0.051	1.19 (0.94-1.51)	0.151
RS581080	C	1897	0.19	636	0.19	1.01 (0.85-1.19)	0.938	0.97 (0.84-1.12)	0.639
RS6029526	A	1888	0.47	632	0.46	0.97 (0.85-1.11)	0.690	1.01 (0.90-1.13)	0.925
RS629301	A	1897	0.79	639	0.82	**1.25 (1.07-1.45)**	**0.004**	**1.23 (1.06-1.42)**	**0.005**
RS6450176	T	1893	0.26	633	0.26	0.98 (0.85-1.14)	0.789	**0.86 (0.76-0.98)**	**0.019**
RS645040	T	1890	0.79	634	0.79	0.98 (0.83-1.16)	0.808	0.91 (0.80-1.05)	0.195
RS646776	A	1887	0.79	641	0.82	**1.27 (1.09-1.47)**	**0.002**	**1.25 (1.07-1.45)**	**0.004**
RS6511720	G	1867	0.12	615	0.11	0.80 (0.67-0.95)	0.013	0.85 (0.71-1.01)	0.062
RS6544713	T	1832	0.30	630	0.30	1.06 (0.91-1.23)	0.475	0.96 (0.85-1.08)	0.491
RS6754295	T	1839	0.76	632	0.74	0.92 (0.78-1.08)	0.294	**0.85 (0.75-0.97)**	**0.016**
RS6756629	C	1888	0.94	641	0.93	0.97 (0.74-1.27)	0.817	0.81 (0.65-1.01)	0.057
RS6882076	G	1900	0.63	641	0.66	1.11 (0.97-1.27)	0.138	**1.13 (1.00-1.27)**	**0.044**
RS693	T	1874	0.48	620	0.48	1.01 (0.89-1.16)	0.860	0.93 (0.83-1.04)	0.191
RS6987702	C	1890	0.72	641	0.75	**1.16 (1.01-1.34)**	**0.038**	**1.19 (1.04-1.34)**	**0.008**
RS7134375	C	1901	0.59	641	0.60	1.01 (0.88-1.15)	0.912	**0.88 (0.79-0.98)**	**0.019**
RS7255436	C	1897	0.49	640	0.51	1.05 (0.93-1.20)	0.439	1.10 (0.89-1.36)	0.375
RS737337	C	1826	0.08	616	0.10	1.12 (0.87-1.45)	0.393	1.11 (0.92-1.33)	0.275
RS7395662	G	1871	0.59	632	0.58	0.89 (0.77-1.02)	0.091	0.88 (0.56-1.38)	0.574
RS7515577	T	1901	0.77	642	0.78	1.05 (0.90-1.23)	0.569	1.03 (0.90-1.18)	0.691
RS7557067	A	1853	0.76	620	0.74	0.92 (0.79-1.08)	0.329	**0.87 (0.76-0.99)**	**0.029**
RS7570971	A	1901	0.41	641	0.44	1.09 (0.95-1.24)	0.217	**1.14 (1.02-1.27)**	**0.019**
RS7679	C	1807	0.20	622	0.19	1.02 (0.85-1.21)	0.869	1.04 (0.90-1.20)	0.595
RS780093	T	1860	0.41	628	0.44	1.11 (0.96-1.27)	1.530	0.95 (0.85-1.07)	0.426
RS7819412	C	1866	0.51	612	0.49	0.93 (0.81-1.06)	0.271	0.93 (0.82-1.04)	0.194
RS7941030	C	1901	0.38	642	0.39	1.03 (0.89-1.18)	0.725	0.96 (0.86-1.07)	0.463
RS8017377	A	1901	0.46	642	0.46	0.99 (0.86-1.13)	0.855	0.92 (0.82-1.03)	0.158
RS838880	A	1901	0.69	641	0.71	1.03 (0.90-1.19)	0.643	1.01 (0.89-1.14)	0.890
RS9488822	T	1899	0.67	640	0.67	1.00 (0.87-1.15)	0.990	1.09 (0.97-1.23)	0.149
RS964184	C	1880	0.14	639	0.14	0.96 (0.80-1.17)	0.704	0.80 (0.56-1.14)	0.215
RS9686661	T	1902	0.19	641	0.20	1.08 (0.91-1.29)	0.378	1.15 (1.00-1.32)	0.050
RS9987289	A	1896	0.07	639	0.07	0.88 (0.69-1.11)	0.274	**0.68 (0.53-0.88)**	**0.003**
SNPs selected for their association with anthropomorphic related traits									
RS10146997	G	1900	0.19	642	0.21	1.11 (0.93-1.32)	0.262	1.07 (0.93-1.23)	0.341
RS10838738	C	1813	0.32	612	0.32	1.05 (0.91-1.22)	0.506	1.02 (0.90-1.16)	0.720
RS10913469	G	1764	0.19	599	0.21	1.19 (0.98-1.44)	0.081	**1.29 (1.10-1.51)**	**0.001**
RS10938397	G	1810	0.43	615	0.42	0.94 (0.83-1.08)	0.371	0.96 (0.86-1.07)	0.478

		Subcohort		Cases		CHD		CHD adjusted*	
SNP	**RA**	**N**	**RAF**	**N**	**RAF**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
RS11084753	G	1794	0.68	609	0.67	0.94 (0.81-1.09)	0.389	**0.81 (0.70-0.94)**	**0.004**
RS1260326	T	1863	0.42	615	0.44	1.07 (0.93-1.23)	0.343	0.91 (0.72-1.15)	0.426
RS12970134	A	1839	0.26	621	0.28	1.10 (0.94-1.29)	0.239	1.01 (0.89-1.15)	0.833
RS2568958	A	1854	0.61	622	0.61	0.96 (0.84-1.11)	0.572	0.95 (0.83-1.10)	0.495
RS2605100	G	1895	0.70	639	0.71	1.04 (0.90-1.19)	0.616	1.10 (0.98-1.24)	0.115
rs29939	G	1837	0.69	615	0.68	0.91 (0.78-1.06)	0.212	**0.79 (0.66-0.93)**	**0.006**
RS3764261	C	1889	0.69	634	0.70	1.09 (0.95-1.25)	0.215	1.06 (0.94-1.20)	0.327
RS6265	G	1792	0.79	612	0.78	0.91 (0.76-1.08)	0.267	0.99 (0.86-1.12)	0.825
RS6499640	G	1841	0.57	614	0.57	1.03 (0.89-1.18)	0.728	1.04 (0.93-1.16)	0.551
RS6548238	C	1831	0.83	618	0.82	0.96 (0.80-1.16)	0.694	0.91 (0.79-1.06)	0.225
RS7498665	G	1859	0.36	626	0.38	1.06 (0.92-1.22)	0.455	1.04 (0.90-1.21)	0.562
RS7561317	G	1767	0.83	596	0.82	0.98 (0.82-1.18)	0.858	0.93 (0.80-1.09)	0.368
RS7647305	G	1824	0.81	602	0.80	1.01 (0.85-1.20)	0.902	0.97 (0.84-1.12)	0.671
RS8050136	A	1815	0.36	622	0.40	**1.19 (1.04-1.38)**	**0.014**	**1.20 (1.07-1.34)**	**0.002**
RS925946	T	1896	0.28	641	0.30	1.14 (0.98-1.32)	0.091	**1.29 (1.10-1.51)**	**0.002**
RS987237	G	1899	0.19	641	0.18	0.95 (0.81-1.12)	0.561	0.96 (0.83-1.12)	0.613

SNP, single nucleotide polymorphism; BP, base pair; RA, risk allele; RAF, risk allele frequency; CHD, coronary heart disease; HR, hazard ratio; CI, confidence interval; P, P-value; MI, myocardial infarction; CAD, coronary artery disease
* Adjusted for sex, current smoking, systolic blood pressure, total cholesterol, HDL cholesterol, body mass index, diabetes and family history of MI, age was used as the time scale variable

Table S6a | Reclassification of subjects when a Genetic Risk Score (GRS) composed of all 179 SNPs was used in addition to traditional risk factors (age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes).

Model without Overall GRS	**Model with Overall GRS**				**Total**
	<5%	**5-<10%**	**10-<20%**	**≥20%**	
Incident cases					
<5%	191	4	0	0	195
5-<10%	1	126	2	0	129
10-<20%	0	4	76	0	80
≥20%	0	0	0	30	30
Total	192	134	78	30	434
Non-cases					
<5%	1755	7	0	0	1762
5-<10%	11	157	4	0	172
10-<20%	0	3	61	0	64
≥20%	0	0	0	14	14
Total	1766	167	65	14	2012

Table S6b | Reclassification of subjects when a Genetic Risk Score (GRS) composed of the 153 CHD risk factor SNPs was used in addition to traditional risk factors (age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self- reported diabetes).

Model without Risk Factor GRS	Model with Risk Factor GRS				
	<5%	5-<10%	10-<20%	≥20%	Total
Incident cases					
<5%	193	2	0	0	195
5-<10%	1	126	2	0	129
10-<20%	0	1	79	0	80
≥20%	0	0	0	30	30
Total	194	129	81	30	434
Non-cases					
<5%	1759	3	0	0	1762
5-<10%	2	169	1	0	172
10-<20%	0	1	63	0	64
≥20%	0	0	0	14	14
Total	1761	173	64	14	2012

Table S6c | Reclassification of subjects when a Genetic Risk Score (GRS) composed of the 29 SNPs previously associated with CHD was used in addition to traditional risk factors (age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes).

Model without CHD GRS	Model with CHD GRS				
	<5%	5-<10%	10-<20%	≥20%	Total
Incident cases					
<5%	190	5	0	0	195
5-<10%	4	116	9	0	129
10-<20%	0	4	73	3	80
≥20%	0	0	4	26	30
Total	194	125	86	29	434
Non-cases					
<5%	1751	11	0	0	1762
5-<10%	18	150	4	0	172
10-<20%	0	5	56	3	64
≥20%	0	0	1	13	14
Total	1769	166	61	16	2012

Table S6d | Reclassification of subjects when a Genetic Risk Score (GRS) composed of the 29 weighted CHD risk SNPs was used in addition to traditional risk factors (age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes).

Model without weighted CHD GRS	Model with weighted CHD GRS				
	<5%	5-<10%	10-<20%	≥20%	Total
Incident cases					
<5%	183	12	0	0	195
5-<10%	3	114	12	0	129
10-<20%	0	9	68	3	80
≥20%	0	0	4	26	30
Total	186	135	84	29	434
Non-cases					
<5%	1748	14	0	0	1762
5-<10%	22	144	6	0	172
10-<20%	0	8	51	5	64
≥20%	0	0	1	13	14
Total	1770	166	58	18	2012

Table S6e | Reclassification of subjects when a weighted Genetic Risk Score (GRS) composed of 3 SNPs selected using LASSO regression on the 29 SNPs previously associated to CHD was used in addition to traditional risk factors (age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes).

Model without weighted CHD GRS	Model with weighted CHD GRS				
	<5%	5-<10%	10-<20%	≥20%	Total
Incident cases					
<5%	180	15	0	0	195
5-<10%	11	104	14	0	129
10-<20%	0	10	65	5	80
≥20%	0	0	5	25	30
Total	191	129	84	30	434
Non-cases					
<5%	1743	19	0	0	1762
5-<10%	29	134	9	0	172
10-<20%	0	10	52	2	64
≥20%	0	0	2	12	14
Total	1772	163	63	14	2012

Table S6f | Reclassification of subjects when a weighted Genetic Risk Score (GRS) composed of 14 SNPs selected using LASSO regression on all 179 SNPs was used in addition to traditional risk factors (age, sex, current smoking, total cholesterol, HDL cholesterol, BMI, parental history of MI and self-reported diabetes).

Model without LASSO GRS	Model with LASSO GRS				
	<5%	**5-<10%**	**10-<20%**	**≥20%**	**Total**
Incident cases					
<5%	173	22	0	0	195
5-<10%	15	83	31	0	129
10-<20%	1	17	53	9	80
≥20%	0	0	4	26	30
Total	189	122	88	35	434
Non-cases					
<5%	1730	32	0	0	1762
5-<10%	47	113	12	0	172
10-<20%	0	17	42	5	64
≥20%	0	0	4	10	14
Total	1777	162	58	15	2012

Table S8 | Information about the subsets of SNPs, their coefficients and the appropriate weighting factors. The subsets of SNPs were selected using 10-fold cross-validated LASSO regression on only the 29 SNPs previously associated with CHD and all 179 SNPs.

SNP	Locus	Nearby genes	Coefficient	weighting factor*	Previously associated with:
LASSO regression on the 29 CHD SNPs					
RS10757278	9p21.3	CDKN2A, CDKN2B	0.160	1.000	CHD
RS11556924	7q32.2	ZC3HC1	0.038	0.238	CAD
RS646776	1p13.3	CELSR2, PSRC1, SORT1	0.041	0.256	MI
LASSO regression on all 179 SNPs					
rs10757278	9p21.3	CDKN2A, CDKN2B	0.159	1.000	CHD
rs2925979	16q23.2	CMIP	0.066	0.414	HDL
rs6882076	5q33.3	TIMD4	0.006	0.039	TC
rs2954029	8q24.13	TRIB1	0.001	0.007	TG
rs6987702	8q24.13	TRIB1	0.011	0.067	TC
rs10889352	1p31.1	DOCK7	0.023	0.142	TC
rs2972146	2q36.3	IRS1	0.050	0.316	HDL-C
rs11556924	7q32.2	ZC3HC1	0.031	0.198	CAD
rs514230	1q42.3	IRF2BP2	0.021	0.135	TC
rs8050136	16q12.2	FTO	0.063	0.394	DM2, DMI
rs181362	22q11.21	UBE2L3	0.015	0.096	HDL
rs646776	1p13.3	CELSR2, PSRC1, SORT1	0.065	0.406	MI, LDL-C
rs925946	11p14.1	BDNF	0.022	0.136	BMI
rs2000999	16q22.2	HPR	0.128	0.804	TC

* The weighting factor is set to 1 for the SNP with the highest coefficient

Supplement IV

Table S1 | Association between baseline δ-5 desaturase activity and incident coronary heart disease according to rs174547 genotypes

rs174547 (# cases)	Quintile of δ-5 desaturase activity[1]					*P* value for trend[2]
	First	Second	Third	Fourth	Fifth	
AA (n = 205)	1[3]	0.61 (0.27-1.41)	0.35 (0.15-0.79)	0.36 (0.16-0.80)	0.25 (0.11-0.54)	<0.0001
	1[4]	0.60 (0.25-1.43)	0.35 (0.15-0.83)	0.48 (0.21-1.11)	0.35 (0.15-0.81)	0.022
	1[5]	0.63 (0.26-1.53)	0.40 (0.17-0.97)	0.55 (0.24-1.27)	0.44 (0.19-1.04)	0.087
AG/GG (n = 276)	1[3]	0.69 (0.46-1.03)	0.62 (0.38-1.00)	0.54 (0.31-0.93)	1.15 (0.57-2.33)	0.027[6]
	1[4]	0.62 (0.39-0.98)	0.71 (0.42-1.21)	0.72 (0.40-1.30)	1.64 (0.76-3.53)	0.463
	1[5]	0.63 (0.40-1.02)	0.75 (0.44-1.27)	0.78 (0.43-1.41)	1.86 (0.84-4.12)	0.649

[1] δ-5 desaturase activity was assessed by the ratio of C20:4n-6 to C20:3n-6 in plasma cholesteryl esters.

[2] From models with desaturase activity included as a continuous variable.

[3] Model was adjusted for age, sex, systolic blood pressure, hypertensive medication use, current smoking, and diabetes.

[4] Model was adjusted for age, sex, systolic blood pressure, hypertensive medication use, current smoking, diabetes, total cholesterol, and high-density lipoprotein cholesterol.

[5] Model was adjusted for age, sex, systolic blood pressure, hypertensive medication use, current smoking, diabetes, total cholesterol, high-density lipoprotein cholesterol, and percentages of C22:6n-3 (DHA) in plasma cholesteryl esters.

[6] Hazard ratio per unit desaturase activity (95% confidence interval) = 0.89 (0.80-0.99).

Table S2 | Association of rs174547 with incident coronary heart disease (CHD) risk

	rs174547 genotype			rs174547 G allele[1]
	AA	*AG*	*GG*	
Incident CHD, n	234	259	56	-
Model 1[2]	1.0	1.00 (0.80-1.26)	0.96 (0.67-1.37)	0.99 (0.84-1.16)
Model 2[3]	1.0	1.15 (0.88-1.49)	0.91 (0.60-1.39)	1.02 (0.85-1.22)

[1] The model assumes that each copy of the G allele contributes equally to coronary heart disease risk.

[2] Model 1 was adjusted for age and sex.

[3] Model 2 was adjusted for age, sex, systolic blood pressure, hypertensive medication use, current smoking, diabetes, total cholesterol, and high-density lipoprotein cholesterol.

Table S3 | Association between baseline n-6 PUFA in plasma cholesteryl esters (precursors and products of δ5- or δ6-desaturase) and incident coronary heart disease (CHD)

	Quintile of C18:2n-6 (linoleic acid)					*P* value for trend[1]
	First	Second	Third	Fourth	Fifth	
Incident CHD, n	127	125	102	97	86	
Model 1[2]	1	1.11 (0.80-1.54)	1.02 (0.72-1.44)	1.10 (0.78-1.56)	0.80 (0.57-1.13)	0.247
Model 2[3]	1	1.16 (0.81-1.66)	1.27 (0.88-1.83)	1.30 (0.89-1.89)	0.91 (0.62-1.32)	0.861
Model 3[4]	1	1.09 (0.74-1.60)	1.34 (0.91-1.96)	1.38 (0.92-2.09)	1.01 (0.68-1.48)	0.395
Model 4[5]	1	1.09 (0.74-1.61)	1.34 (0.91-1.96)	1.39 (0.92-2.09)	1.01 (0.69-1.49)	0.389

	Quintile of C18:3n-6					*P* value for trend[1]
	First	Second	Third	Fourth	Fifth	
Incident CHD, n	93	109	100	107	128	
Model 1[2]	1	1.05 (0.74-1.50)	0.89 (0.62-1.28)	0.92 (0.64-1.32)	1.00 (0.71-1.41)	0.706
Model 2[3]	1	1.12 (0.76-1.64)	0.90 (0.61-1.33)	1.02 (0.69-1.50)	0.93 (0.64-1.37)	0.867
Model 3[4]	1	1.13 (0.75-1.72)	0.90 (0.58-1.38)	1.01 (0.66-1.53)	1.05 (0.70-1.57)	0.605
Model 4[5]	1	1.14 (0.75-1.73)	0.90 (0.58-1.39)	1.02 (0.67-1.56)	1.06 (0.70-1.61)	0.536

	Quintile of C20:3n-6					*P* value for trend[1]
	First	Second	Third	Fourth	Fifth	
Incident CHD, n	88	80	107	125	137	
Model 1[2]	1	0.98 (0.68-1.43)	1.14 (0.80-1.64)	1.29 (0.91-1.82)	1.39 (0.99-1.96)	0.024
Model 2[3]	1	0.91 (0.61-1.34)	1.06 (0.73-1.55)	1.14 (0.78-1.66)	1.43 (1.00-2.05)	0.011
Model 3[4]	1	0.94 (0.61-1.45)	1.13 (0.75-1.69)	1.00 (0.67-1.50)	1.11 (0.75-1.67)	0.494
Model 4[5]	1	0.95 (0.62-1.47)	1.15 (0.76-1.73)	1.03 (0.67-1.57)	1.14 (0.75-1.74)	0.420

	Quintile of C20:4n-6 (arachidonic acid)					*P* value for trend[1]
	First	Second	Third	Fourth	Fifth	
Incident CHD, n	127	107	120	79	104	
Model 1[2]	1	0.89 (0.63-1.24)	0.96 (0.70-1.34)	0.65 (0.45-0.92)	0.76 (0.54-1.06)	0.031
Model 2[3]	1	0.86 (0.60-1.22)	0.92 (0.65-1.31)	0.64 (0.43-0.94)	0.77 (0.53-1.10)	0.088
Model 3[4]	1	1.06 (0.72-1.56)	1.09 (0.74-1.60)	0.79 (0.51-1.21)	0.86 (0.58-1.28)	0.163
Model 4[5]	1	1.04 (0.70-1.55)	1.07 (0.73-1.58)	0.76 (0.48-1.20)	0.83 (0.53-1.28)	0.150

[1] From models with fatty acids included as continuous variables.

[2] Model 1 is adjusted for age and sex.

[3] Model 2 is adjusted for age, sex, systolic blood pressure, hypertensive medication use, current smoking, and diabetes.

[4] Model 3 is adjusted for all covariates in model 2, total cholesterol, and high-density lipoprotein cholesterol.

[5] Model 4 is adjusted for all covariates in model 3 and baseline n-3 PUFA in plasma cholesteryl esters.

Table S4 | Association of baseline C20:5n-3 (EPA) and C22:6n-3 (DHA) in plasma cholesteryl esters with incident coronary heart disease (CHD)

	Quintile of C20:5n-3 (EPA)					*P* value for trend[2]
	First (0.00)[1]	Second (0.28)	Third (0.38)	Fourth (0.52)	Fifth (0.83)	
Incident CHD, n	96	100	104	114	123	
Model 1[3]	1	0.81 (0.57-1.16)	0.88 (0.62-1.26)	0.82 (0.57-1.16)	0.82 (0.58-1.17)	0.348
Model 2[4]	1	0.86 (0.59-1.24)	0.91 (0.62-1.33)	0.88 (0.61-1.27)	0.76 (0.52-1.11)	0.243
Model 3[5]	1	0.90 (0.60-1.34)	0.90 (0.59-1.39)	0.79 (0.52-1.20)	0.89 (0.58-1.35)	0.733
Model 4[6]	1	0.90 (0.60-1.35)	0.90 (0.59-1.39)	0.80 (0.52-1.22)	0.89 (0.58-1.35)	0.724
	Quintile of C22:6n-3 (DHA)					*P* value for trend[2]
	First (0.00)[1]	Second (0.20)	Third (0.27)	Fourth (0.34)	Fifth (0.46)	
Incident CHD, n	89	139	93	112	104	
Model 1[3]	1	1.45 (1.03-2.05)	0.87 (0.60-1.25)	1.07 (0.74-1.53)	0.93 (0.65-1.34)	0.286
Model 2[4]	1	1.49 (1.05-2.13)	0.84 (0.58-1.24)	1.13 (0.77-1.67)	0.86 (0.58-1.29)	0.268
Model 3[5]	1	0.96 (0.65-1.43)	0.61 (0.41-0.92)	0.89 (0.59-1.34)	0.65 (0.42-0.99)	0.049
Model 4[6]	1	0.95 (0.64-1.40)	0.57 (0.38-0.87)	0.82 (0.53-1.26)	0.59 (0.37-0.93)	0.027

[1] Median percentages of EPA and DHA in each quintile are listed between brackets.
[2] From models with fatty acids included as continuous variables.
[3] Model 1 is adjusted for age and sex.
[4] Model 2 is adjusted for age, sex, systolic blood pressure, hypertensive medication use, current smoking, and diabetes.
[5] Model 3 is adjusted for all covariates in model 2, total cholesterol, and high-density lipoprotein cholesterol.
[6] Model 4 is adjusted for all covariates in model 3 and baseline n-6 PUFA in plasma cholesteryl esters.

Supplementary Note S1

Analysis of intermediate factors of coronary heart disease (CHD)

To explore whether δ-5 desaturase activity might exert its protective effect against CHD through known intermediate risk (or protective) factors of CHD (total and HDL cholesterol levels, systolic blood pressure, diabetes mellitus, EPA and DHA), we regressed these factors against δ-5 desaturase activity with adjustment for age and sex. It seems some of these intermediate factors (HDL cholesterol levels, EPA and DHA) might be involved in the protective effects of δ-5 desaturase activity against CHD risk (See Table S5).

Table S5 | Association between intermediate risk (protective) factors for coronary heart disease and δ-5 desaturase activity at baseline in a random subcohort (n = 1263).[1]

Covariates	*B*	*P*
Total cholesterol (mmol/L)[2]	-0.021 ± 0.011	0.076
HDL cholesterol (mmol/L)[2]	0.020 ± 0.003	< 0.0001
Systolic blood pressure (mmHg)[2]	-0.29 ± 0.17	0.078
EPA (%)[2]	0.044 ± 0.004	< 0.0001
DHA (%)[2]	0.023 ± 0.002	< 0.0001
Diabetes mellitus (Yes/no)[3]	-0.020 ± 0.12	0.86

[1] δ-5 desaturase activity was assessed by the ratio of C20:4n-6 to C20:3n-6 in plasma cholesteryl esters.

[2] *B* and *P* are β regression coefficients ± SE and corresponding p values obtained by using a regression model adjusted for age and sex.

[3] *B* and *P* are β regression coefficient ± SE and corresponding p value obtained by using a logistic model adjusted for age and sex.