

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. *N Engl J Med* 2008;358:1240-9.

Polymorphisms Associated with Cholesterol and Risk of Cardiovascular Events

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ONLINE SUPPLEMENTARY APPENDIX

RISK RECLASSIFICATION BY GENOTYPE SCORE

We evaluated the ability of genotype score to reclassify risk across National Cholesterol Education Program Adult Treatment Panel III risk categories using two separate approaches suggested by Cook et al. and Pencina et al. (see references in main text). Individuals in the Adult Treatment Panel III intermediate risk category constituted 9% of the study sample (370 of 4232 subjects), 26% (95) were reclassified into a higher or lower risk category in the risk model with genotype score compared to one without (**Supplementary Table 1**). When we used the ‘net reclassification index’ to account for the correct movement in categories – upward for individuals who subsequently developed cardiovascular disease and downward for individuals free of incident cardiovascular disease, there was a significant improvement in risk classification by a model that incorporated genotype score compared to one that did not ($P=0.01$, **Supplementary Table 2**).

Supplementary Table 1. Comparison of Observed and Predicted Risks for Cardiovascular Disease With and Without Genotype Score*

Model Without Genotype Score 10-Year Risk (%)	Model With Genotype Score 10-Year Risk (10%)			% Reclassified
	0 to ≤10%	>10 to ≤20%	>20%	
0 to ≤10%				
Total, n	3671	68	0	-
% [†]	98.0	1.8	0	1.8
Observed 10-year risk (%) [‡]	2.9	17.6	0	-
>10 to ≤20%				
Total, n	61	275	34	-
%	16.5	74.3	9.2	25.7
Observed 10-year risk (%)	8.2	14.5	14.7	-
>20%				
Total, n	0	23	100	-
%	0	18.7	81.3	18.7
Observed 10-year risk (%)	0	0	26.0	-

*Comparison is for the fully-adjusted model without and with genotype score (Models 3a and 3b as shown in Supplementary Table 1)

[†]Percent classified in each stratum in the model with genotype score

[‡]Observed proportion of individuals who developed cardiovascular disease at 10 years of follow-up

Supplementary Table 2. Reclassification Among Individuals Who Experience a Cardiovascular Disease Event and Those Who Do Not Experience a Cardiovascular Disease Event on Follow-Up

Model Without Genotype Score Frequency (Row per cent)	Model With Genotype Score			Total
	0 to \leq 10%	>10 to \leq 20%	>20%	
Participants who experience a cardiovascular disease event				
0 to \leq 10%	105 (89.7)	12 (10.3)	0 (0.0)	117
>10 to \leq 20%	5 (10.0)	40 (80.0)	5 (10.0)	50
>20%	0 (0.0)	0 (0.0)	26 (100.0)	26
Total	110	52	31	193
Participants who do not experience a cardiovascular disease event				
0 to \leq 10%	3566 (98.5)	56 (1.5)	0 (0.0)	3622
>10 to \leq 20%	56 (17.5)	235 (73.4)	29 (9.1)	320
>20%	0 (0.0)	23 (23.7)	74 (76.3)	97
Total	3622	314	103	4039

INCREMENTAL PREDICTIVE VALUE OF SNPS IN MULTIVARIABLE MODELS

To explore alternate approaches to modeling the impact of lipid-modulating SNPs on cardiovascular risk, we fitted three pairs of nested multivariable Cox proportional hazards regression models with or without entering all nine validated lipid-modulating SNPs. The SNPs were incorporated into models either as a set or as a continuous genotype score (see the main text for a description of the calculation of the genotype score).

We used a likelihood-ratio test to determine the P value for the addition of the SNPs, either as a set or as a genotype score, to a model without them. In each case, the $-2 \log$ likelihood from the larger model (clinical covariates and SNPs) was subtracted from that of the smaller model (clinical covariates only).

The first pair of models compared age and sex as covariates (Model 1a) with age, sex, and the SNPs (Model 1b). The second pair compared age, sex, LDL cholesterol, HDL cholesterol, and log triglycerides as covariates (Model 2a) with these clinical covariates plus the SNPs (Model 2b). The third pair compared a full set of 14 available covariates [age, sex, family history of myocardial infarction, LDL cholesterol, HDL cholesterol, triglycerides, systolic blood pressure, diastolic blood pressure, diabetes mellitus, body mass index, cigarette smoking, C-reactive protein, lipid lowering therapy, and anti-hypertensive treatment (Model 3a)] with a full set of risk factors plus the SNPs (Model 3b). In all of these analyses, the lipid-related SNPs were significantly associated with cardiovascular events (see **Supplementary Table 4**).

Supplementary Table 3. Prediction of First Cardiovascular Event in Models With and Without 9 Validated Lipid Polymorphisms

Model	P for Difference Between 'a' and 'b' Models			
	SNPs modeled as a set*		SNPs modeled as genotype score [†]	
	Change in χ^2	P	Change in χ^2	P
Model 1a: Age, sex	30.1	0.0004	19.5	0.00001
Model 1b: Age, sex, SNPs				
Model 2a: Age, sex, LDL cholesterol, HDL cholesterol, triglycerides	22.3	0.002	13.0	0.0003
Model 2b: Age, sex, LDL cholesterol, HDL cholesterol, triglycerides, SNPs				
Model 3a: Age, sex, parental or sibling history of myocardial infarction, LDL cholesterol, HDL cholesterol, log triglycerides, systolic blood pressure, diastolic blood pressure, body mass index, diabetes mellitus, cigarette smoking, log C-reactive protein, lipid lowering therapy, and anti-hypertensive treatment	23.6	0.005	13.6	0.0002
Model 3b: Age, sex, parental or sibling history of myocardial infarction, LDL cholesterol, HDL cholesterol, log triglycerides, systolic blood pressure, diastolic blood pressure, body mass index, diabetes mellitus, cigarette smoking, log C-reactive protein, lipid lowering therapy, anti-hypertensive treatment, and SNPs				

*The difference between models 1a and 1b; 2a and 2b; and 3a and 3b with the addition of 9 SNPs in aggregate was evaluated using the difference of -2log likelihoods, referenced to a chi square distribution with 9 degrees of freedom.

[†]The difference between model pairs with the addition of a single composite genotype score was determined as a chi square distribution with 1 degree of freedom.

SECONDARY ANALYSES

We considered individually the nonfatal myocardial infarction or death due to coronary heart disease and ischemic stroke outcomes. Genotype score was associated with incident myocardial infarction or death due to coronary heart disease (multivariable-adjusted hazard ratio 1.17, 95% confidence interval 1.07 – 1.29, $P=0.001$). Genotype score was also associated with incident ischemic stroke (multivariable-adjusted hazard ratio 1.13, 95% confidence interval 1.00 – 1.27, $P=0.047$).

To explore whether specific SNPs that comprised the score appeared to drive the association with cardiovascular disease, we used backward elimination (retention threshold $P<0.05$) in a model that included the full set of clinical covariates and 9 SNPs (Model 3). Five SNPs were retained as predictors of incident cardiovascular disease events: *LDLR* rs1529729 ($P=0.02$), *PCSK9* rs11591147 ($P=0.04$), *LIPC* rs1800588 ($P=0.005$), *LPL* rs328 ($P=0.049$), and *APOE* rs4420638 ($P=0.04$). A score composed of just these 5 SNPs was also associated with incident cardiovascular disease (multivariable-adjusted $P=0.0001$).

We evaluated the relative contributions of SNPs related to LDL cholesterol and those related to HDL cholesterol by constructing separate LDL and HDL cholesterol genotype scores. Both the LDL cholesterol genotype score (composed of 5 SNPs, score range from 0-10) and the HDL cholesterol genotype score (composed of 4 SNPs, score range from 0-8) were independently related to incident cardiovascular disease events in fully-adjusted models (HR 1.11, 95% CI 1.01-1.23, $P=0.03$ for LDL cholesterol genotype score; HR 1.22, 95% CI 1.08-1.37, $P=0.001$ for HDL cholesterol genotype score).

We tested for but did not observe a statistical interaction between genotype score and each of the following variables: age, gender, cigarette smoking, family history of myocardial infarction, and body mass index (each interaction $P > 0.05$).