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Cardiovascular Disease Risk Prediction With and Without Knowledge of Genetic Variation at Chromosome 9p21.3

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Background: Although genetic variation at chromosome 9p21.3 is associated with incident cardiovascular disease, it is unclear whether screening for this polymorphism improves risk prediction.

Objective: To determine whether knowledge of variation at chromosome 9p21.3 provides predictive information beyond that from other readily available risk factors.

Design: Prospective cohort study.

Setting: United States.

Patients: 22 129 female white health professionals participating in the Women's Genome Health Study, initially without any major chronic disease, who were prospectively followed over a median of 10.2 years for incident cardiovascular disease.

Measurements: Polymorphism at rs10757274 in chromosome 9p21.3 and additional cardiovascular disease risk factors (blood pressure, smoking status, diabetes, blood levels of cholesterol, high-sensitivity C-reactive protein, and family history of premature myocardial infarction).

Results: Polymorphism at rs10757274 was associated with an adjusted hazard ratio for incident cardiovascular disease of 1.25 (95% CI, 1.04 to 1.51) for the AG genotype and 1.32 (CI, 1.07 to 1.63) for the GG genotype. However, the addition of the genotype to a prediction model based on traditional risk factors, high-sensitivity C-reactive protein, and family history of premature myocardial infarction had no effect on model discrimination as measured by the c-index (0.807 to 0.809) and did not improve the Net Reclassification Improvement score (−0.2%; $P = 0.59$) or the Integrated Discrimination Improvement score (0.0; $P = 0.18$).

Limitation: Study participants were all white women.

Conclusion: In this large prospective cohort of white women, genetic variation in chromosome 9p21.3 was associated with incident cardiovascular disease but did not improve on the discrimination or classification of predicted risk achieved with traditional risk factors, high-sensitivity C-reactive protein, and family history of premature myocardial infarction.

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The success of risk prediction models for cardiovascular disease reflects an increasing understanding of the molecular basis of atherothrombosis. Aside from age, which integrates many biological activities and environmental exposures at once, other important components of risk prediction include plasma biomarkers for lipid metabolism, inflammation, thrombosis, and metabolic status (1). Current prediction models, however, cannot anticipate many cases of incident cardiovascular disease, motivating both the identification of new risk factors and the optimization of analytic methods for their use. Traditionally aggregated by the notion of family history, individual genetic variants may represent a class of risk factors with new prognostic information; they may be more removed from underlying disease processes than plasma risk factors or other clinical variables but may also be more general. For example, a recent exploration of the influence of lipid-associated variation on prediction of incident cardiovascular disease found residual predictive value for the genetic variation after adjustment for plasma lipid levels (2).

Genetic variation at the chromosome 9p21.3 region is a good candidate for adding more information to risk prediction. Variation at this locus has consistently been associated with coronary artery disease (3–5) and diabetes (6–9). In addition, the risk allele is carried by almost 75% of the white population and the lack of correlation between any of the disease-associated 9p21.3 genetic variants and major cardiovascular risk factors suggests novel influences on disease progression. To assess whether knowledge of variation at 9p21.3 improves global risk prediction, we examined the effect of adding genetic information from a single nucleotide polymorphism (SNP) in the 9p21.3 region to previously published prediction models for the WHS (Women's Health Study), which has a large, prospective cohort of initially healthy U.S. women who were followed over 10 years for incident cardiovascular events.

Methods

Study Population

Study participants were members of the WGHS (Women's Genome Health Study) (10), an ongoing pro-

See also:

Print

Editors' Notes 66
Editorial comment. 139
Related article. 73
Summary for Patients. I-36

Web-Only

Conversion of graphics into slides
Audio summary

Context

Although genetic variation at chromosome 9p21.3 is associated with cardiovascular disease, it is not known whether evaluating this polymorphism adds in a clinically meaningful way to the evaluation of cardiovascular risk using more conventional risk factors, such as family history of early cardiovascular disease, smoking, blood pressure, cholesterol level, and C-reactive protein level.

Contribution

This study evaluated 9p21.3 polymorphism and more conventional cardiovascular risk factors in 22 129 white, female health professionals followed for a median of 10 years and found that addition of the genetic information did not improve clinical classification of a woman's risk for cardiovascular disease.

Caution

The findings may not apply to men or to nonwhite women.

—The Editors

spective genetic evaluation study being conducted among initially healthy U.S. women who enrolled in the WHS, a trial of aspirin and vitamin E for the primary prevention of cardiovascular disease and cancer in women 45 years or older (11). Beginning in 1992, the WHS recruited female health professionals in the United States who had no major chronic disease at baseline, including cancer and cardiovascular disease, and followed them prospectively for incident myocardial infarction, stroke, coronary revascularization, and cardiovascular death. The institutional review board of the Brigham and Women's Hospital, Boston, Massachusetts, approved the study.

Among the WHS participants, 28 345 provided blood samples that were stored in liquid nitrogen until the time of analysis, as well as consent for ongoing analyses linking blood-derived observations with baseline risk factor profiles and incident disease events. Of these women, 23 226 had standard risk factor information available and were genotyped for the rs10757274 polymorphism. To reduce the potential for population stratification to affect our results, we included only the 22 129 (95.3%) white women.

Risk Factor Ascertainment

Baseline information on age, diabetes, smoking status, parental history of myocardial infarction before 60 years of age, blood pressure, and hypertension treatment were collected at study initiation. Plasma biomarkers were analyzed in a core laboratory facility, certified by the National Heart, Lung, and Blood Institute/Centers for Disease Control and Prevention Lipid Standardization Program, for total cholesterol, high-density lipoprotein cholesterol, apolipoprotein B-100, apolipoprotein A-I, high-sensitivity C-reactive protein, lipoprotein(a), and hemoglobin A_{1c}.

We determined genotypes for rs10757274 in the WGHS participants by using an oligonucleotide ligation procedure that combined polymerase chain reaction amplification of target sequences from 3 ng of genomic DNA with subsequent allele-specific oligonucleotide ligation (12). The ligation products of the 2 alleles were separated by hybridization to product-specific oligonucleotides, each coupled to spectrally distinct Luminex100 xMAP microspheres (Luminex, Austin, Texas). The captured products were fluorescently labeled with streptavidin R–phycoerythrin (Prozyme, San Leandro, California), sorted on the basis of microsphere spectrum, and detected by a Luminex100 instrument (12).

Table 1. **Baseline Characteristics**

Characteristic	rs10757274 Genotype			*P* Value for Trend
	AA (*n* = 5793)	AG (*n* = 10 952)	GG (*n* = 5384)	
Median age (25th, 75th percentile), *y*	53 (48, 59)	52 (48, 58)	52 (48, 59)	0.095
Median systolic blood pressure (25th, 75th percentile), *mm Hg*	125 (115, 135)	125 (115, 135)	125 (115, 135)	0.77
Median total cholesterol level (25th, 75th percentile)				0.81
mmol/L	5.38 (4.76, 6.10)	5.38 (4.73, 6.08)	5.38 (4.76, 6.08)	
mg/dL	208 (184, 236)	208 (183, 235)	208 (184, 235)	
Median high-density lipoprotein cholesterol level (25th, 75th percentile)				0.166
mmol/L	1.35 (1.12, 1.62)	1.34 (1.12, 1.61)	1.33 (1.10, 1.61)	
mg/dL	52.2 (43.4, 62.8)	51.9 (43.3, 62.3)	51.6 (42.7, 62.4)	
Median high-sensitivity C-reactive protein level (25th, 75th percentile), *mg/L*	19.9 (7.9, 43.0)	20.2 (8.1, 43.6)	20.0 (7.7, 43.1)	0.98*
Current smoker, *n (%)*†	663 (11.4)	1221 (11.1)	656 (12.2)	0.24
Antihypertensive use, *n (%)*†	722 (12.5)	1324 (12.1)	643 (11.9)	0.40
Family history of myocardial infarction, *n (%)*†	677 (11.7)	1431 (13.1)	751 (13.9)	<0.001
History of diabetes, *n (%)*†	142 (2.5)	258 (2.4)	166 (3.1)	0.039
Median hemoglobin A_{1c} level (25th, 75th percentile), %‡	7.0 (6.1, 8.9)	6.9 (5.8, 8.3)	7.0 (5.9, 8.2)	0.26

* Natural logarithm used for linear regression trend test.
† Patients answering "yes."
‡ Diabetic patients only.

Table 2. **Association of 9p21.3 Genotype With Cardiovascular Outcomes**

Condition	rs10757274 Genotype			rs10757274 G Allele*
	AA (*n* = 5793)	AG (*n* = 10 952)	GG (*n* = 5384)	
Total cardiovascular disease				
Patients, *n*	158	362	195	–
Hazard ratio (95% CI)				
Crude	1.0	1.22 (1.01–1.46)	1.33 (1.08–1.65)	1.15 (1.04–1.28)
Age-adjusted	1.0	1.25 (1.03–1.50)	1.38 (1.12–1.70)	1.17 (1.06–1.30)
Adjusted for ATP III covariates†	1.0	1.25 (1.04–1.51)	1.32 (1.07–1.63)	1.15 (1.03–1.27)
Coronary heart disease‡				
Patients, *n*	102	241	126	–
Hazard ratio (95% CI)				
Crude	1.0	1.25 (0.99–1.58)	1.33 (1.03–1.73)	1.15 (1.01–1.31)
Age-adjusted	1.0	1.28 (1.02–1.61)	1.36 (1.05–1.77)	1.16 (1.02–1.32)
Adjusted for ATP III covariates†	1.0	1.28 (1.02–1.62)	1.30 (1.00–1.69)	1.13 (1.00–1.29)
Myocardial infarction				
Patients, *n*	42	103	51	–
Hazard ratio (95% CI)				
Crude	1.0	1.30 (0.91–1.86)	1.31 (0.87–1.97)	1.14 (0.93–1.38)
Age-adjusted	1.0	1.33 (0.93–1.91)	1.34 (0.89–2.02)	1.15 (0.95–1.40)
Adjusted for ATP III covariates†	1.0	1.33 (0.93–1.91)	1.28 (0.85–1.92)	1.12 (0.92–1.36)
Stroke				
Patients, *n*	50	123	72	–
Hazard ratio (95% CI)				
Crude	1.0	1.30 (0.94–1.81)	1.55 (1.08–2.23)	1.24 (1.04–1.48)
Age-adjusted	1.0	1.34 (0.97–1.86)	1.61 (1.12–2.31)	1.26 (1.06–1.50)
Adjusted for ATP III covariates†	1.0	1.33 (0.96–1.85)	1.54 (1.07–2.21)	1.23 (1.04–1.47)

ATP III = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.
* This model assumes that each copy of the G allele contributes equally to cardiovascular risk.
† The ATP III covariates include the natural logarithm of age, systolic blood pressure, total and high-density lipoprotein cholesterol, and smoking status; antihypertensive use; and history of diabetes.
‡ Comprising myocardial infarction, coronary revascularization, and deaths from coronary heart disease.

Outcome Ascertainment

Study participants were followed through March 2004 for total cardiovascular disease, which comprised incident myocardial infarction, ischemic stroke, coronary revascularization, and cardiovascular deaths. An end-points committee adjudicated events by using medical record review. Morbidity data were available on nearly all the women through 8 years of follow-up.

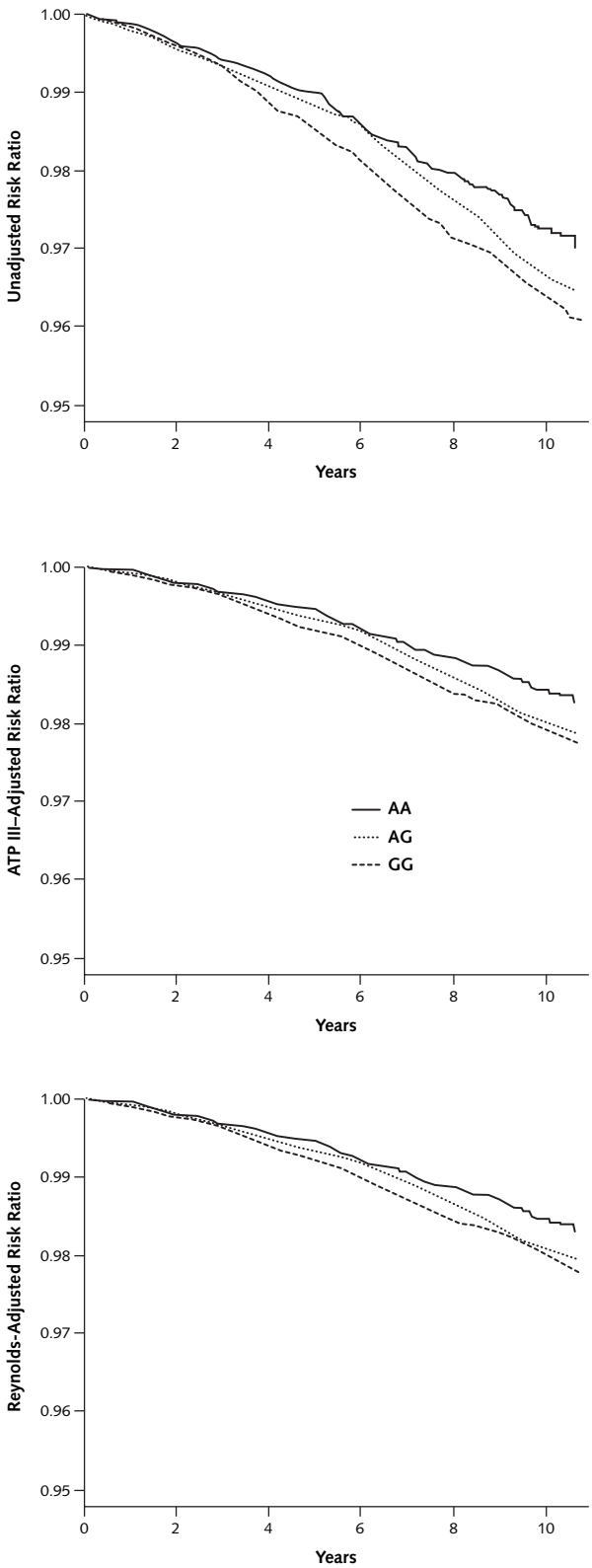
Risk Prediction Models

To assess the effect of variation at rs10757274 on global cardiovascular disease risk prediction, we considered covariates from 2 nongenetic risk prediction models. The first included the covariates from the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III) risk score, as well as history of diabetes (noted as a high-risk equivalent) (13). The second used the covariates from the Reynolds Risk Score (1), a model that includes additional biomarker information, as well as data on family history. To provide direct comparability and the highest level of internal validity, we elected on an a priori basis to model all covariates, including rs10757274, in the same base population rather than use β-coefficients for the nongenetic covariates derived from previously published models. In so doing, we avoided the potential for bias that might occur by modeling the effect of the genetic data within the test cohort but using estimates of effect for the other covariates from a second, unrelated cohort.

Statistical Analysis

We used Cox proportional hazard models to generate crude and adjusted hazard ratios across genotypes and to test for trend. We used models with separate effects for each genotype in all analyses and generated adjusted survival curves by stratifying Cox proportional hazard models by genotype. We also used Cox models to generate estimates of predicted risk with and without genotype information, which we then assessed for accuracy. We saw no evidence of departures from proportionality in any of the models used. Our primary measure of discrimination was the Harrell c-index (14), a generalization of the area under the receiver-operating characteristic curve that allows for censored data. The c-index assesses the ability of the risk score to rank women who develop incident cardiovascular disease higher than women who do not. We assessed general calibration across deciles of predicted risk by using the Hosmer–Lemeshow goodness-of-fit test (15) to compare

Figure. **Kaplan–Meier survival curves for cardiovascular disease, by rs10757274 genotype.**



ATP III = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.

the average predicted risk with the Kaplan–Meier risk estimate within each decile and considered a chi-square value of 20 or higher ($P < 0.01$) to be poor calibration (16).

We assessed risk reclassification (1, 17) by sorting the predicted 10-year risk for each model into 4 categories (<5%, 5% to <10%, 10% to <20%, and ≥20%). We then compared the assigned categories for a pair of models. For each pair, we calculated the proportion of participants who were reclassified by the comparison model versus the reference model; we considered reclassification to be correct if the Kaplan–Meier risk estimate for the reclassified group was closer to the comparison category than the reference. We computed the Hosmer–Lemeshow statistic for the reclassification tables (18), which assesses agreement between the Kaplan–Meier risk estimate and predicted risk within the reclassified categories. We also computed the Net Reclassification Improvement (19), which compares the shifts in reclassified categories by observed outcome, and the Integrated Discrimination Improvement (19), which directly compares the average difference in predicted risk for women who go on to develop cardiovascular disease with women who do not for the 2 models, on the women who were not censored before 8 years.

Role of the Funding Source

This study was supported by grants from the National Heart, Lung, and Blood Institute and National Cancer Institue, National Institutes of Health; the Donald W. Reynolds Foundation; and the Leducq Foundation. Additional support for DNA extraction, reagents, and data analysis was provided by Roche Diagnostics and Amgen. Genotyping of the 9p21.3 variant was performed by Celera. The funding sources had no role in the design, conduct, or reporting of this study or the decision to submit the manuscript for publication.

RESULTS

Of the 22 129 white women genotyped for this analysis, 5793 (26.2%) had no risk (G) alleles at rs10757374, 10 952 (49.5%) had 1 risk allele, and 5384 (24.3%) had 2 risk alleles. The number of risk alleles had a significant association with family history of premature myocardial infarction ($P < 0.001$ for trend) and a modest association with a history of diabetes, driven by the higher proportion of diabetic patients among those with 2 risk alleles ($P = 0.04$ for trend) (**Table 1**). We observed no such associations for the other standard cardiovascular disease risk factors or for the newer biomarkers. In addition, we found no association between genotype and other lipid measures, including lipoprotein(a), apolipoprotein A-I, and apolipoprotein B-100 (data not shown).

The crude association between rs10757274 genotype and total cardiovascular disease was not affected by adjustment for age or additional risk factors (**Table 2**), and the cardiovascular hazard associated with an increasing number of risk alleles was consistent across all components of the

Table 3. **Comparison of Cardiovascular Risk Prediction Models With and Without 9p21.3 Genotype***

Predictor	ATP III Covariates	ATP III Covariates Plus Genotype	Reynolds Risk Score Covariates	Reynolds Risk Score Covariates Plus Genotype
β-coefficient ± SE (*P* value)†				
Age‡	4.092 ± 0.287 (<0.001)	4.108 ± 0.287 (<0.001)	0.074 ± 0.005 (<0.001)	0.074 ± 0.005 (<0.001)
Systolic blood pressure§	3.578 ± 0.381 (<0.001)	3.569 ± 0.381 (<0.001)	3.653 ± 0.353 (<0.001)	3.648 ± 0.353 (<0.001)
Total cholesterol§	1.174 ± 0.198 (<0.001)	1.173 ± 0.198 (<0.001)	0.997 ± 0.200 (<0.001)	0.996 ± 0.200 (<0.001)
High-density lipoprotein cholesterol§	−1.114 ± 0.143 (<0.001)	−1.117 ± 0.143 (<0.001)	−0.978 ± 0.145 (<0.001)	−0.979 ± 0.145 (<0.001)
Current smoker	0.888 ± 0.091 (<0.001)	0.887 ± 0.091 (<0.001)	0.880 ± 0.092 (<0.001)	0.876 ± 0.092 (<0.001)
Use of antihypertensives	0.243 ± 0.093 (0.009)	0.243 ± 0.093 (0.009)	–	–
History of diabetes	1.340 ± 0.110 (<0.001)	1.335 ± 0.110 (<0.001)	–	–
Hemoglobin A_{1c}‖	–	–	0.163 ± 0.014 (<0.001)	0.163 ± 0.014 (<0.001)
High-sensitivity C-reactive protein¶	–	–	0.181 ± 0.036 (<0.001)	0.180 ± 0.036 (<0.001)
Family history of myocardial infarction	–	–	0.423 ± 0.100 (<0.001)	0.415 ± 0.101 (<0.001)
rs10757274 genotype AG	–	0.223 ± 0.095 (0.019)	–	0.222 ± 0.095 (0.020)
rs10757274 genotype GG	–	0.280 ± 0.107 (0.009)	–	0.274 ± 0.107 (0.011)
Harrell c-index ± SE**	0.803 ± 0.019	0.805 ± 0.019	0.807 ± 0.019	0.809 ± 0.019
Hosmer–Lemeshow chi-square (*P* value)††	6.24 (0.62)	5.96 (0.65)	7.75 (0.46)	7.43 (0.49)

ATP III = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.
* Dashes indicate variables not included in model.
† β-coefficients from Cox proportional hazards model for variables shown.
‡ The natural logarithm was used for the ATP III models only.
§ The natural logarithm was used for all models.
‖ Diabetic patients only.
¶ The natural logarithm was used for the Reynolds Risk Score covariates models only.
** The Harrell c-index measures model discrimination.
†† The Hosmer–Lemeshow chi-square measures the calibration of the model; chi-square >20 suggests lack of calibration.

primary study end point. Specifically, polymorphism at rs10757274 was associated with an adjusted hazard ratio (HR) with incident cardiovascular disease of 1.25 (95% CI, 1.04 to 1.51) for the AG genotype and 1.32 (CI, 1.07 to 1.63) for the GG genotype. We observed similar associations with less power for coronary heart disease (AG, 1.28 [CI, 1.02 to 1.62]; GG, 1.30 [CI, 1.00 to 1.69]), myocardial infarction (AG, 1.33 [CI, 0.93 to 1.91]; GG, 1.28 [CI, 0.85 to 1.92]), and stroke (AG, 1.33 [CI, 0.96 to 1.85]; GG, 1.54 [CI, 1.07 to 2.21]). The stratified mean survival curves (**Figure**) show attenuation in the absolute risk from the use of the mean covariate values in the adjusted models, but no change in the relative risk between groups.

Knowledge of genotype had little effect on the coefficients for the other risk factors in either the ATP III or Reynolds Risk Score covariates (**Table 3**). **Table 3** also shows that the addition of genotype information to either the ATP III covariates or the Reynolds Risk Score covariates had no effect on discrimination (c-index, 0.803 to 0.805 and 0.807 to 0.809, respectively). All models were calibrated as measured by the Hosmer–Lemeshow chi-square test.

We found a modest improvement in reclassification when we added genotype to the ATP III covariates (**Table 4**), with an additional 606 (2.7%) of the women reclassified. Of those, 526 (86.9%) were reclassified correctly, corresponding to a Net Reclassification Improvement of 2.7% ($P = 0.02$) and an Integrated Discrimination Improvement of 0.001 ($P = 0.11$). Both the original ATP III covariates and the ATP III covariates with the genotype information remained calibrated within the reclassification table (reclassification Hosmer–Lemeshow chi-square, 17.9 and 14.9, respectively).

By contrast, when we added genotype information to the Reynolds Risk Score covariates, 585 (2.6%) of the women were reclassified. However, of those, only 214 (36.6%) were reclassified correctly, corresponding to a Net Reclassification Improvement score of −0.2% ($P = 0.59$) and an Integrated Discrimination Improvement score of 0.0 ($P = 0.18$). Both the original Reynolds Risk Score covariates and the Reynolds Risk Score covariates with the genotype information remained calibrated within the reclassification table (reclassification Hosmer–Lemeshow chi-square, 12.6 and 12.7, respectively). We observed almost identical results in analyses that added genotype information to the published Reynolds Risk Score, rather than the Reynolds Risk Score covariates.

DISCUSSION

By using recently developed prediction models and performance measures, we examined the incremental contribution of 9p21.3 variation to cardiovascular risk among more than 22 000 initially healthy white participants in the Women's Genome Health Study. Similar to a previous report examining this same variation among white men (20), we find a strong and significant association of 9p21.3 variation with cardiovascular disease in white women. However, in our data, knowledge of this genetic variation only marginally improved the classification of risk prediction in a model based on ATP III covariates and did not improve classification in a model that included family his-

Table 4. **Effect of Adding Genotype to Traditional Risk Factors: Reclassification of Participants Between Predicted 10-Year Cardiovascular Disease Risk Categories**

Covariates*	Covariates* Plus Genotype				Reclassified Correctly/ Reclassified, %/%
	<5%	5% to <10%	10% to <20%	≥20%	
ATP III					
<5%					1.1/1.1
Patients, *n (% reclassified)*	18 609	205 (1.1)	–	–	
Kaplan–Meier estimate, %	1.5	8.0	–	–	
5% to <10%					12.0/12.0
Patients, *n (% reclassified)*	181 (8.2)	1933	83 (3.8)	–	
Kaplan–Meier estimate, %	4.9	8.0	19.3	–	
10% to <20%					3.8/13.7
Patients, *n (% reclassified)*	–	80 (9.9)	697	31 (3.8)	
Kaplan–Meier estimate, %	–	10.9	12.9	23.6	
≥20%					8.4/8.4
Patients, *n (% reclassified)*	–	–	26 (8.4)	284	
Kaplan–Meier estimate, %	–	–	15.0	31.0	
Reynolds Risk Score					
<5%					0.0/1.0
Patients, *n (% reclassified)*	18 527	188 (1)	–	–	
Kaplan–Meier estimate, %	1.5	2.7	–	–	
5% to <10%					8.3/11.6
Patients, *n (% reclassified)*	183 (8.3)	1960	75 (3.4)	–	
Kaplan–Meier estimate, %	1.4	7.7	8.3	–	
10% to <20%					3.5/13.2
Patients, *n (% reclassified)*	–	85 (9.7)	761	31 (3.5)	
Kaplan–Meier estimate, %	–	10.6	15.2	21.4	
≥20%					0/7.2
Patients, *n (% reclassified)*	–	–	23 (7.2)	296	
Kaplan–Meier estimate, %	–	–	31.5	30.4	

ATP III = Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.
* The ATP III covariates includes the natural logarithm of age, systolic blood pressure, total and high-density lipoprotein cholesterol, and smoking status; antihypertensive use; and history of diabetes. Reynolds Risk Score covariates include age, smoking status, family history of myocardial infarction, hemoglobin A_{1c} levels (diabetic patients only), and the natural logarithm of systolic blood pressure and total and high-density lipoprotein.

tory and C-reactive protein (Reynolds Risk Score covariates). In both settings, the addition of genotype information had no appreciable effect on the c-index.

The rs10757274 SNP is one of a cluster of tightly linked SNPs at 9p21.3 that are associated with coronary artery disease (3–5), myocardial infarction (3, 6), and stroke (21); abdominal aortic aneurysm (6); and intracranial aneurysm (6). The linkage disequilibrium block spanning these SNPs extends 28 kb from rs10757274 toward the telomere about 55.7 kb from the 3′ end of the *CDKN2B* transcript, although weaker linkage disequilibrium continues through the *CDKN2A/CDKN2B* gene region. The major transcript of a third gene, *MTAP*, corresponds with an entirely separate linkage disequilibrium block still further toward the telomere, but an alternatively spliced variant may be transcribed into the linkage disequilibrium block containing *CDKN2A/CDKN2B*. Toward the centromere, the linkage disequilibrium block that includes the associations with the arterial diseases extends 28.3 kb bases from rs10757274 and is juxtaposed with a smaller linkage disequilibrium block of about 6.9 kb that includes an SNP (rs10822661) associated with diabetes (6–9).

Although the mechanism of association between the rs10757274 genotype and cardiovascular disease remains unclear, the magnitude of association observed in our study of women is consistent with reported associations for 9p21.3 SNPs (5). Our data also extend previous work that demonstrated significant association with incident stroke (21). Because we could not find correlations between polymorphisms in rs10757274 and standard lipids, apolipoprotein B-100, apolipoprotein A-I, lipoprotein(a), or high-sensitivity C-reactive protein, our data provide further evidence that the effect of this genetic variation is unlikely to reflect genetic differences in lipid levels or biomarkers of hemostasis and inflammation.

Despite high prevalence of the risk allele and consistent association of rs10757274 with vascular events, inclusion of genotype information did not improve global risk prediction; instead, the addition of genotype information to a model that included C-reactive protein and family history of cardiovascular disease worsened classification. Thus, although knowledge of variation in chromosome 9p21.3 may yield important pathophysiologic insights into atherothrombosis, it seems unlikely that screening for SNPs in this region will be clinical useful in daily practice.

Improved cardiovascular risk prediction has clear potential for public health but has been difficult to achieve, and the potential role of genetic information is just begin-

ning to be explored. Whether a precisely measured gene panel will prove superior to knowledge of family history of cardiovascular disease is uncertain, although combination scores have had promising effects on prediction (2). As shown in this paper, and as expected from statistical work by Pepe and colleagues (22) and Cook (17), single polymorphisms alone, even common ones with consistent modest associations, are unlikely to improve prediction.

Our study benefits from a large sample size and extensive prospective follow-up. However, because of the small numbers of nonwhite participants, our results are limited to white women. Further studies are needed in other populations, both of the association between the rs10757274 genotype and cardiovascular disease and the resultant effect on risk prediction. Our study also evaluated only variation at chromosome 9p21.3 and thus does not exclude the possibility that multigene panels might correctly reclassify a larger proportion of persons. Our study did not examine the additional effect of genotype relative to the previously published risk scores, but rather examined the additional effect after refitting all covariates in the same population. Although this comparison potentially limits the generalizability of our results, it preserves their validity.

In conclusion, in a large prospective cohort of white women, we confirmed the association of 9p21.3 variation with total cardiovascular disease, coronary heart disease, myocardial infarction, and stroke. However, adding 9p21.3 genetic variation did not improve discrimination or classification of the predicted risk achieved with traditional risk factors, C-reactive protein, and family history of cardiovascular disease.

From Brigham and Women's Hospital, Boston, Massachusetts, and Celera, Alameda, California.

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Reproducible Research Statement: *Study protocol and statistical code:* Available from Dr. Paynter (e-mail, npaynter@partners.org). *Data set:* Not available.

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Current author addresses and author contributions are available at www.annals.org.

References

1. **Ridker PM, Buring JE, Rifai N, Cook NR.** Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. JAMA. 2007;297:611-9. [PMID: 17299196]

2. **Kathiresan S, Melander O, Anevski D, Guiducci C, Burtt NP, Roos C, et al.** Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med. 2008;358:1240-9. [PMID: 18354102]

3. **Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottir A, et al.** A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science. 2007;316:1491-3. [PMID: 17478679]

4. **McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al.** A common allele on chromosome 9 associated with coronary heart disease. Science. 2007;316:1488-91. [PMID: 17478681]

5. **Schunkert H, Götz A, Braund P, McGinnis R, Tregouet DA, Mangino M, et al.Cardiogenics Consortium.** Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation. 2008;117:1675-84. [PMID: 18362232]

6. **Helgadottir A, Thorleifsson G, Magnusson KP, Grétarsdottir S, Steinthorsdottir V, Manolescu A, et al.** The same sequence variant on 9p21 associates with myocardial infarction, abdominal aortic aneurysm and intracranial aneurysm. Nat Genet. 2008;40:217-24. [PMID: 18176561]

7. **Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research.** Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007;316:1331-6. [PMID: 17463246]

8. **Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al.** A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007;316:1341-5. [PMID: 17463248]

9. **Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, et al. Wellcome Trust Case Control Consortium (WTCCC).** Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007;316:1336-41. [PMID: 17463249]

10. **Ridker PM, Chasman DI, Zee RY, Parker A, Rose L, Cook NR, et al. Women's Genome Health Study Working Group.** Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. Clin Chem. 2008;54:249-55. [PMID: 18070814]

11. **Ridker PM, Cook NR, Lee IM, Gordon D, Gaziano JM, Manson JE, et al.** A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med. 2005;352:1293-304. [PMID: 15753114]

12. **Shiffman D, O'Meara ES, Bare LA, Rowland CM, Louie JZ, Arellano AR, et al.** Association of gene variants with incident myocardial infarction in the Cardiovascular Health Study. Arterioscler Thromb Vasc Biol. 2008;28:173-9. [PMID: 17975119]

13. **National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).** Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002;106:3143-421. [PMID: 12485966]

14. **Harrell FE Jr, Lee KL, Mark DB.** Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15:361-87. [PMID: 8668867]

15. **Lemeshow S, Hosmer DW Jr.** A review of goodness of fit statistics for use in the development of logistic regression models. Am J Epidemiol. 1982;115:92-106. [PMID: 7055134]

16. **D'Agostino RB Sr, Grundy S, Sullivan LM, Wilson P. CHD Risk Prediction Group.** Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA. 2001;286:180-7. [PMID: 11448281]

17. **Cook NR.** Use and misuse of the receiver operating characteristic curve in risk prediction. Circulation. 2007;115:928-35. [PMID: 17309939]

18. **Cook NR.** Statistical evaluation of prognostic versus diagnostic models: beyond the ROC curve. Clin Chem. 2008;54:17-23. [PMID: 18024533]

19. **Pencina MJ, Larson MG, D'Agostino RB.** Choice of time scale and its effect on significance of predictors in longitudinal studies. Stat Med. 2007;26:1343-59. [PMID: 16955538]

20. **Talmud PJ, Cooper JA, Palmen J, Lovering R, Drenos F, Hingorani AD, et al.** Chromosome 9p21.3 coronary heart disease locus genotype and prospective risk of CHD in healthy middle-aged men. Clin Chem. 2008;54:467-74. [PMID: 18250146]

21. **Matarin M, Brown WM, Singleton A, Hardy JA, Meschia JF. ISGS investigators.** Whole genome analyses suggest ischemic stroke and heart disease share an association with polymorphisms on chromosome 9p21 [Letter]. Stroke. 2008; 39:1586-9. [PMID: 18340101]

22. **Pepe MS, Janes H, Longton G, Leisenring W, Newcomb P.** Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am J Epidemiol. 2004;159:882-90. [PMID: 15105181]

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