Association Between 9p21 Genomic Markers and Heart Disease: A Meta-analysis

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A Meta-analysis

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Context Associations between chromosome 9p21 single-nucleotide polymorphisms (SNPs) and heart disease have been reported and replicated. If testing improves risk assessments using traditional factors, it may provide opportunities to improve public health.

Objectives To perform a targeted systematic review of published literature for effect size, heterogeneity, publication bias, and strength of evidence and to consider whether testing might provide clinical utility.

Data Sources Electronic search via HuGE Navigator through January 2009 and review of reference lists from included articles.

Study Selection English-language articles that tested for 9p21 SNPs with coronary heart/artery disease or myocardial infarction as primary outcomes. Included articles also provided race, numbers of participants, and data to compute an odds ratio (OR). Articles were excluded if reporting only intermediate outcomes (eg, atherosclerosis) or if all participants had existing disease. Twenty-five articles were initially identified and 16 were included. A follow-up search identified 6 additional articles.

Data Extraction Independent extraction was performed by 2 reviewers and consensus was reached. Credibility of evidence was assessed using published Venice criteria.

Data Synthesis Forty-seven distinct data sets from the 22 articles were analyzed, including 35 872 cases and 95 837 controls. The summary OR for heart disease among individuals with 2 vs 1 at-risk alleles was 1.25 (95% confidence interval [CI], 1.21-1.29), with low to moderate heterogeneity. Age at disease diagnosis was a significant covariate, with ORs of 1.35 (95% CI, 1.30-1.40) for age 55 years or younger and 1.21 (95% CI, 1.16-1.25) for age 75 years or younger. For a 65-year-old man, the 10-year heart disease risk for 2 vs 1 at-risk alleles would be 13.2% vs 11%. For a 40-year-old woman, the 10-year heart disease risk for 2 vs 1 at-risk alleles would be 2.4% vs 2.0%. Nearly identical but inverse results were found when comparing 1 vs 0 at-risk alleles. Three studies showed net reclassification indexes ranging from –0.1% to 4.8%.

Conclusion We found a statistically significant association between 9p21 SNPs and heart disease that varied by age at disease onset, but the magnitude of the association was small.

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PREVENTING AND MANAGING CARdiovascular disease (CVD) presents a challenge for health care and public health.[1,2] Nonmodifiable risk factors include increasing age, male sex, and heredity. Modifiable risk factors include smoking, hypertension, dyslipidemia, obesity, physical inactivity, and diabetes.[3-5] Among men, the annual rate of initial CVD events increases from 3 to 74 per 1000 from ages 35 to 44 years to ages 85 to 94 years, respectively. Similar increases occur among women a decade later.[6] Biomarkers (eg, C-reactive protein) have been combined with traditional risk factors to predict CVD events,[7] and molecular markers hold further promise. In 2007, genome-wide association studies on CVD identified a series of associated single-nucleotide polymorphisms (SNPs) in an intergenic region of chromosome 9p21, near the *CDKN2A* (NM_000077) and *CDKN2B* (NM_004936) genes.[8,9]

Currently, no comprehensive compilation of the 9p21 literature uses formal methods to estimate the strength of the association with heart disease (eg, effect size, heterogeneity, publication bias, credibility of cumulative evidence) and examine clinical utility. This analysis is part of a targeted systematic review on existing cardiogenomic panels that included 28 genes in addition to the 9p21 SNPs. That review was commissioned by the Evaluation of Genomic Applica-

See also p 631.

CME available online at www.jamaarchivescme.com and questions on p 675.

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tions in Practice and Prevention (EGAPP) initiative and overseen by the EGAPP Working Group (EWG).[10]

METHODS

The review team included an experienced consultant to the EWG (G.E.P.) contracted by the Office of Public Health Genomics (OPHG) at the Centers for Disease Control and Prevention, Atlanta, Georgia, an OPHG researcher (S.M.), and a clinical geneticist (L.A.B.). Standard methods for evaluating clinical validity included systematic literature searches, preset inclusion/exclusion criteria, data abstraction, meta-analysis, and grading of studies and cumulative evidence.[11-13] Expert guidance on literature searches and analytic methods was provided by the Technical Expert Panel. Electronic searches used the HuGE Navigator (version 1.3)[14,15] with search term (9p21[Text+Mesh]). Previous validations had cross-checked HuGE Navigator and PubMed[16] search results for selected gene-disease associations and found the HuGE Navigator searches equally sensitive but more specific (data available from the author on request). Limiting key questions and truncating search strategies are 2 common methods in rapid reviews[17] that we chose to use. The Web site of the laboratory offer-

Figure 1. Literature Search Results

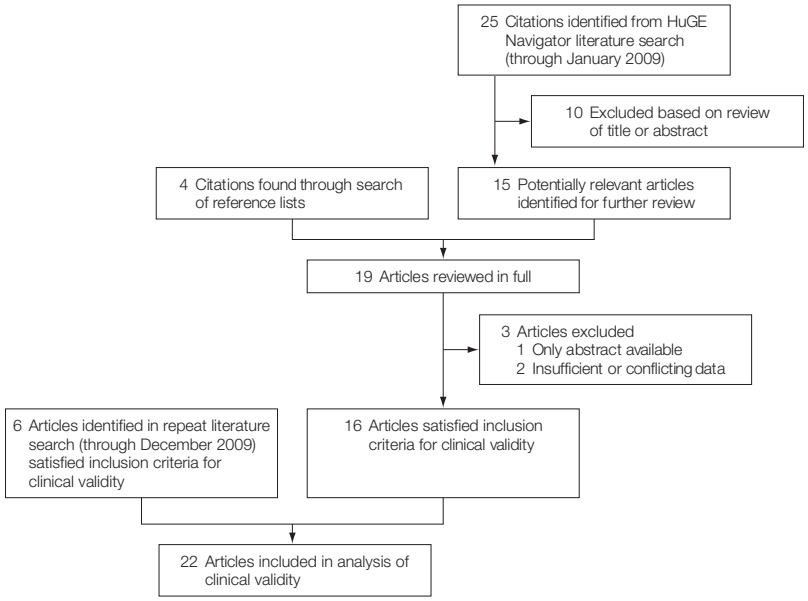


Table. Summary of Evidence for 9p21 SNPs and Selected Cardiovascular Outcomes

Source and Data Set	Study Type	Cases/ Controls	SNP[a]	OR High[b]	OR Low[b]	Race	Primary Outcome	Age, Mean, y[c]	Age Cutoff, y[d]
Abdullah et al,[47] 2008 Cleveland, Ohio	Case-control	310/560	rs10757274	1.78	0.56	White	MI/CAD	40	48[e]
Anderson et al,[24] 2008 Utah	Case-control	999/1111	rs10757274	1.26	0.81	White	CAD	51	62[e]
Assimes et al,[23] 2008 Older	Case-control	943/675	rs10757274	1.36	0.77	White	CAD	62	75[e]
Younger	Case-control	253/359	rs10757274	1.60	0.96	White	CAD	45	55[e]
Broadbent et al,[20] 2008 Germany	Case-control	325/571	rs2891168	1.26	0.79	White	CAD	NR	65
Italy	Case-control	436/524	rs2891168	1.26	0.79	White	CAD	NR	65
Sweden	Case-control	480/519	rs2891168	1.36	0.74	White	CAD	NR	65
United Kingdom	Case-control	3010/2829	rs2891168	1.28	0.78	White	CAD	NR	65
Dehghan et al,[48] 2008 Rotterdam	Cohort	412/5835	rs10757274	0.99	1.16	White	MI	70	82[e]
Ding et al,[18] 2009 China	Case-control	510/554	rs10757278	1.42	0.78	Asian	MI/CAD	NR	NR
Helgadottir et al,[8] 2007 Atlanta, Georgia	Case-control	576/1257	rs2383207	1.21	0.82	White	MI	NR	NR
Durham, North Carolina	Case-control	1118/709	rs2383207	1.41	1.04	White	MI	NR	NR
Iceland A	Case-control	1608/6720	rs2383207	1.16	0.78	White	MI	63	75
Iceland B	Case-control	636/3532	rs2383207	1.27	0.80	White	MI	63	75
Philadelphia, Pennsylvania	Case-control	557/482	rs2383207	1.42	0.75	White	MI	NR	NR
Hinohara et al,[49] 2008 Japan	Case-control	604/1151	rs1333049	1.33	0.77	Asian	CAD	NR	NR
Korea	Case-control	679/706	rs1333049	1.22	0.87	Asian	CAD	NR	NR

(continued)

Table. Summary of Evidence for 9p21 SNPs and Selected Cardiovascular Outcomes (continued)

Source and Data Set	Study Type	Cases/ Controls	SNP[a]	OR High[b]	OR Low[b]	Race	Primary Outcome	Age, Mean, y[c]	Age Cutoff, y[d]
Hiura et al,[50] 2008 Japan	Case-control	586/2432	rs1333049	1.24	0.93	Asian	MI	61	79
Lemmens et al,[19] 2009 Belgium	Case-control	914/809	rs10757278	1.18	0.65	White	CAD	NR	NR
McPherson et al,[9] 2007 US ARIC	Cohort	1037/7743	rs10757274	1.10	0.81	White	CHD	54	63[e]
CCHS	Case-control	1525/9053	rs10757274	1.11	0.82	White	CHD	NR	NR
Dallas DHS	Case-control	154/527	rs10757274	1.04	0.56	White	CHD	57	80[e]
Ottawa, Ontario 1	Case-control	322/312	rs10757274	1.61	0.58	White	CHD	49	60
Ottawa, Ontario 2	Case-control	304/326	rs10757274	1.55	0.76	White	CHD	47	60
Ottawa, Ontario 3	Case-control	647/847	rs10757274	1.21	0.67	White	CHD	50	62[e]
Newton-Cheh et al,[53] 2009 Boston, Massachusetts	Case-control	492/1460	rs10757274	1.21	0.83	White	SCD	NR	NR
Paynter et al,[43] 2009 ATP III	Cohort	196/21 933	rs10757274	1.01	0.77	White	MI	NR	NR
Peng et al,[54] 2009 China	Case-control	520/560	rs1333049	1.45	0.67	Asian	MI	NR	NR
Samani et al,[51] 2007 Germany	Case-control	844/1605	rs1333049	1.22	0.68	White	MI	51	60
England	Case-control	1924/2936	rs1333049	1.29	0.68	White	MI	56	66
Samani et al,[46] 2009 AMC-PAS	Case-control	744/1299	rs1333049	1.21	0.83	White	CAD/MI	NR	50
ECTM	Case-control	1146/1102	rs1333049	1.15	0.87	White	MI	NR	64
EPIC-Norfolk	Case-control	1081/2175	rs1333049	1.09	0.91	White	CAD	NR	90
LURIC	Case-control	2038/1334	rs1333049	1.25	0.80	White	CAD	NR	NR
MORGAM	Case-control	1418/1433	rs1333049	1.21	0.83	White	CAD/MI	NR	NR
Schunkert et al,[42] 2008 AtheroGene	Case-control	370/345	rs1333049	1.27	0.76	White	CAD	NR	75
GerMIFS II	Case-control	685/878	rs1333049	1.37	0.90	White	MI	51	65
LMDS	Case-control	483/442	rs1333049	1.40	0.78	White	CAD	61	78[e]
MONICA/KORA	Case-control	567/1003	rs1333049	1.44	0.79	White	MI	51	60
PopGen	Case-control	1070/999	rs1333049	1.49	0.83	White	CAD	48	55
PRIME	Case-control	525/520	rs1333049	1.20	0.74	White	CAD	NR	69
UK MI	Case-control	756/727	rs1333049	1.15	0.79	White	MI	NR	65
Shen et al,[21] 2008 Italy	Case-control	416/308	rs1075724	1.25	0.80	White	MI	60	75[e]
Shen et al,[22] 2008 South Korea	Case-control	611/294	rs10757274	1.29	0.78	Asian	CAD	64	79[e]
Talmud et al,[25] 2008 NPHS II	Cohort	264/2430	rs10757274	1.11	0.67	White	CHD	64	79
Zhang et al,[55] 2009 China	Case-control	417/430	rs10757274	1.17	0.61	Asian	MI	NR	NR
Zhou et al,[52] 2008 China	Case-control	1360/1360	rs2383207	1.38	0.99	Asian	CHD	61	76[e]

Abbreviations: CAD, coronary artery disease; CHD, coronary heart disease; MI, myocardial infarction; NR, not reported; OR, odds ratio; SCD, sudden cardiac death; SNP, single-nucleotide polymorphism.
[a] Table is restricted to 5 SNPs (rs1333049, rs10757274, rs2383207, rs2891168, and rs10757278) that cover all studies/data sets.
[b] OR high is the odds of disease among those with 2 at-risk alleles vs those with 1 at-risk allele (reference category). OR low is the odds of disease among those with 0 at-risk alleles vs those with 1 at-risk allele (reference category).
[c] The reported mean age at diagnosis for cases.
[d] The reported or estimated age cutoff level for diagnosis. If only the mean and standard deviation were provided, the cutoff level was estimated to be mean age + 1.5 × SD.
[e] Estimated.

ing 9p21 testing was reviewed for references or gray data. Reference lists of included articles were hand searched. Two team members (G.E.P., S.M.) reviewed included articles on clinical validity and extracted the raw data and demographic information into spreadsheets; discrepancies were resolved through discussion.

Included articles were published in English; contained primary outcomes of coronary heart disease (CHD), myocardial infarction (MI), or coronary artery disease (CAD); tested for 9p21 SNPs; reported race and numbers of affected and unaffected participants; and provided the odds ratio (OR) with confidence intervals (CIs) or data suffi-

cient to compute it. If more than 1 outcome was reported, the best-described phenotype was chosen (eg, MI rather than CAD). The authors' definition of phenotype was not an exclusion criterion. Excluded articles reported only on stroke, intermediate outcomes (eg, atherosclerosis), or subgroups (eg, patients with diabetes). Several included articles reported consortium results with multiple independent populations. These populations were listed as separate data sets.

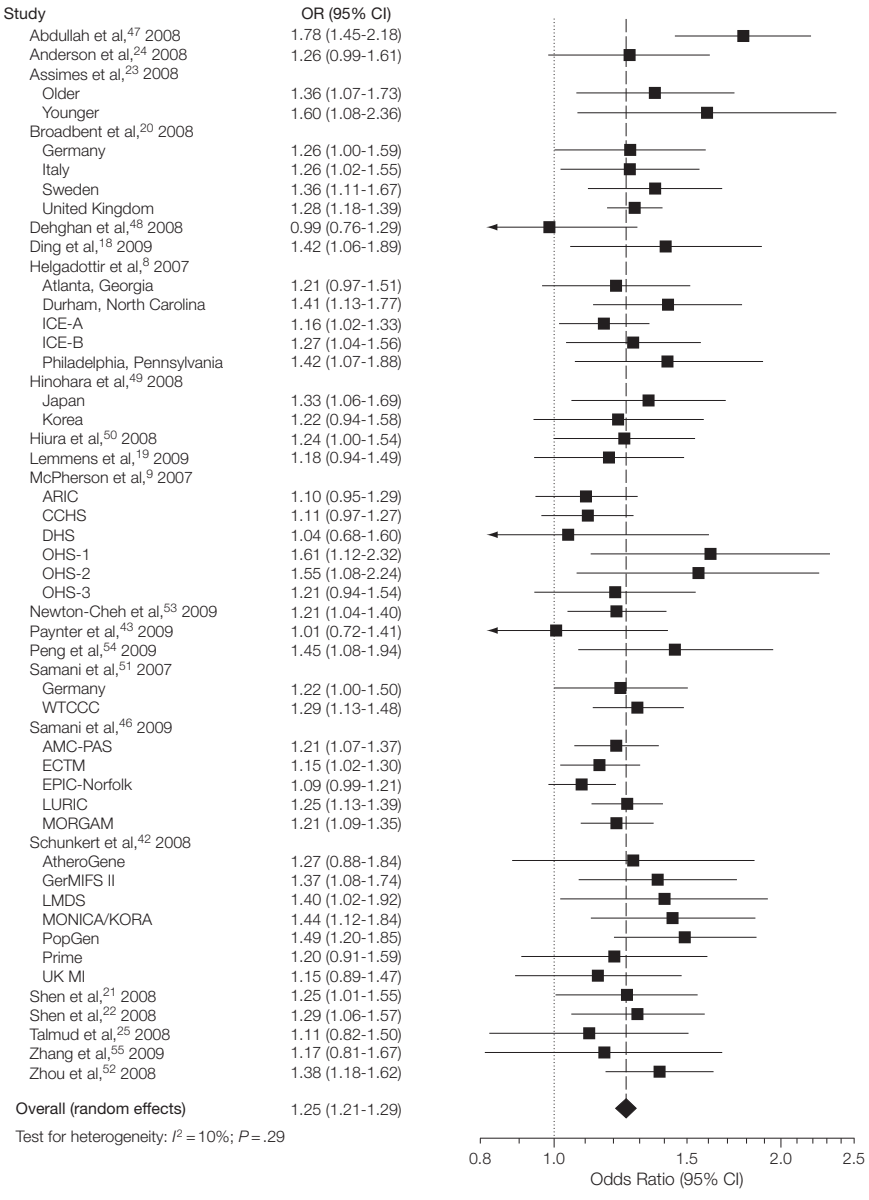
Not all researchers use the same 9p21 SNPs, and most articles reported results for multiple SNPs (uniquely identified by their rs number). We extracted data for all SNPs used by at least 2 of the 22 included articles, but we report herein 3 common SNPs (rs1333049, rs10757274, and rs2383207) that were included in all but 3 articles.[18-20] These SNPs are in high linkage disequilibrium ($D' = 1.00$; $r^2 > 0.85$).[8,21-25] The remaining 3 articles used 2 additional SNPs, rs2891168[20] and rs10757278.[18,19] These were also in high linkage disequilibrium.[19] One additional SNP (rs2383206) was reported in at least 2 included articles. When possible, we restricted results in each data set to a single race. Information about age at diagnosis and race/ethnicity was also collected.

As previously reported,[26,27] individuals with 1 at-risk SNP allele (heterozygotes) were designated as the reference group. Heterozygotes comprise about 50% of the white population. For each data set, the observed genotype frequencies in controls were compared with expected frequencies based on Hardy-Weinberg equilibrium (χ^2 test with 2 degrees of freedom). All P values are 2-sided at the $P = .05$ level.

Summary ORs and corresponding 95% CIs were derived (by reanalysis when possible) and summarized using random-effects modeling weighted by each data set's total variance (Comprehensive Meta-Analysis, version 2, Biostat Inc, Englewood, New Jersey).[28] Subgroup differences were compared using the Q test for heterogeneity for each covariate separately. A fixed-effects meta-regression was performed for the OR vs age at diagnosis.[28] Studies that did not report a value for any covariate were excluded. Publication bias was examined by performing a cumulative effects analysis.[28] Wider ranges of these summary ORs indicate potential for publication bias.

Quality of individual studies (levels 1-4) and overall quality of evidence for clinical validity (convincing, adequate, or inadequate) were evaluated with EWG methods,[11] consistent with other grading systems.[29,30] Assessment of cumulative evidence used a consensus evaluation guideline (Venice criteria) specific to gene-disease association studies[12] and focused on amount of evidence, replication of evidence, and protection from

Figure 2. 9p21 Single-Nucleotide Polymorphism Markers and Heart Disease, Comparing Individuals With 2 At-Risk Alleles vs Those With 1 At-Risk Allele



The odds ratios (ORs) and 95% confidence intervals (CIs) are shown for the 37 data sets comparing individuals with 2 at-risk alleles vs those with 1 at-risk allele. The consensus (dashed vertical line) and no effect (dotted vertical line) are also shown. Heterogeneity is low.

Figure 3. Meta-regression of 9p21 Single-Nucleotide Polymorphism Markers and Heart Disease, Comparing Individuals With 2 At-Risk Alleles vs Those With 1 At-Risk Allele

The upper age cutoff for the occurrence (eg, myocardial infarction at age ≤50 years) is plotted vs the odds ratio on a logarithmic scale. Larger symbols indicate more precise estimates (standard errors of the log odds ratios of <0.1, 0.1-0.14, 0.15-0.19, and ≥0.20). Although 47 data sets are included, only the 33 data sets reporting upper age cutoff levels were used in the regression.

bias. Cumulative evidence was strong for 3 "A" grades, moderate for "B" grades but no "C" grades, and weak if it received at least 1 "C" grade.

The net reclassification index (NRI)[31] was used as an intermediate measure of potential clinical utility. To compute the NRI, the cases (events) and controls (nonevents) in a population were cross-classified by risk assessment using traditional factors with and without 9p21 SNP results. The proportions of all cases reclassified into higher (correct) or lower (incorrect) risk categories were then computed and added together. A similar computation was made for controls. The sum of the 2 proportions (expressed as a percentage) is the NRI, with positive results indicating improved risk prediction.

RESULTS

Twenty-five citations were identified through the original literature search through January 2009; none were meta-analyses. Ten citations were excluded: 2 related to other diseases,[32,33] 3 addressed subgroups of the population,[34-36] 3 addressed intermediate outcomes,[37-39] and 2 addressed stroke.[40,41] The remaining 15 articles underwent a full review along with 4 additional citations identified through hand-searching reference lists.[20,25,42,43] Three of these 19 articles were excluded: 2 due to inadequate or inconsistent data[44,45] and 1 had availability of only an electronic abstract at that time.[46] The 16 remaining articles were included.[8,9,20-25,42,43,47-52] In December 2009, the search was repeated using the same methods, and 6 additional articles satisfied the inclusion criteria.[18,19,46,53-55] The analysis is based on data sets from all 22. In 1 article,[46] 9 data sets were reported; only 5 were included. The remaining 4 data sets were excluded because at least some data from these sets were in published studies already included in our analysis (written communication, Nilesh J. Samani, MD, January 15, 2010). FIGURE 1 provides a summary of the literature review.

Forty-seven distinct data sets were analyzed; most were case-control studies. Observed genotype frequencies were available from 33 data sets (70%), and all but one[23] satisfied Hardy-Weinberg criteria (eTable 1; available at http://www.jama.com). Consensus genotype frequencies in controls were 27% (range, 22%-36%), 50% (range, 47%-57%), and 23% (range, 17%-30%) for 0, 1, and 2 at-risk alleles, respectively. To demonstrate that the choice of SNP is relatively unimportant, we compared the ORs using 4 SNPs that the first 2 articles[24,47] listed in the TABLE had in common (eFigure 1). Although the average ORs are different between the 2 articles (1.78 and 1.26), they are very similar within (eg, 4 SNPs[47] provide nearly identical ORs of 1.78, 1.67, 1.75, and 1.72). This is expected, given the high linkage disequilibrium, and justifies reporting on a single SNP per data set.

Comparison of Individuals Having 2 At-Risk Alleles With Those Having 1 At-Risk Allele

Using a random-effects model, the summary OR was 1.25 (95% CI, 1.21-1.29, $P<.001$). Heterogeneity was low ($Q=51$; $I^2=10\%$; $P=.29$). FIGURE 2 presents these data. The ORs were stratified by race, 9p21 SNP tested, heart disease outcome, and age cutoff at diagnosis for cases. No differences in ORs were found between Asians (8 data sets) and whites (39 data sets) (ORs, 1.32 and 1.24, respectively; $P=.17$); the SNPs used (18 data sets with rs1333049, 17 with rs10757274, 6 with rs2383207, 4 with rs2891168, and 2 with rs10757278) (ORs, 1.23, 1.24, 1.28, 1.29, and 1.27, respectively; $P=.75$); or the outcomes of CAD (19 data sets), MI (17 data sets), or CHD (9 data sets) (ORs, 1.27, 1.23, and 1.21, respectively; $P=.45$).

Finally, we evaluated age at CAD diagnosis. Some articles reported only on early onset disease[47,51] or included data sets restricted to early onset disease.[9,23,42,46,47] Others enrolled cases in wider age ranges.[25,48] Some provided an upper age cutoff (eg, MI occurring by age 50 years) while others provided mean age (and standard deviation). Missing age cutoffs were estimated using information from 11 data sets that provided both (Table).[8,9,25,42,50,51] Fourteen data sets not reporting this covariate were excluded.* A meta-regression performed on the remaining 33 ORs vs the upper age cutoff level showed a significant association between higher ORs at earlier ages of disease onset ($P<.001$; slope and intercept of −0.00558 and 0.60881) (FIGURE 3). The regressed OR was 1.35 (95% CI, 1.30-1.40) for an upper age cutoff of 55 years and 1.21 (95% CI, 1.16-1.25) for 75 years; remaining heterogeneity was reduced ($Q=29$; $I^2=0\%$; $P=.58$). Consistent results were found using the mean age at onset (eFigure 2). One way to estimate the OR for all adults is to use the 14 data sets with upper age cutoff levels of greater than 70 years. In this group, the summary OR was 1.19 (95% CI, 1.13-1.25; $P<.001$), with low heterogeneity ($Q=13$; $I^2=1\%$; $P=.44$).

Comparison of Individuals Having No At-Risk Alleles With Those Having 1 At-Risk Allele

The summary OR was 0.80 (95% CI, 0.77-0.82; $P<.001$), with moderate

*References 8, 9, 18, 19, 43, 46, 49, 53, 54.

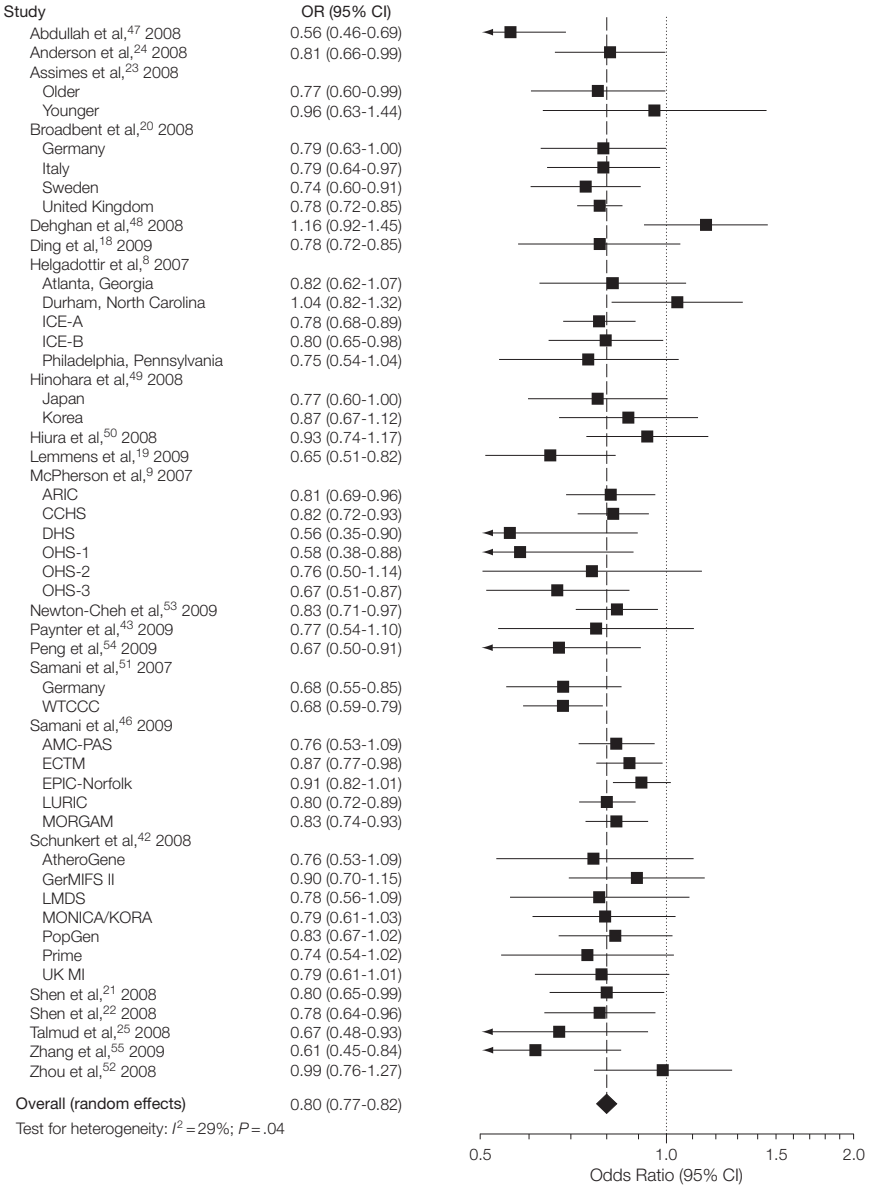
heterogeneity (Q=65; I^2=29%; P=.04) (FIGURE 4). No differences in ORs were found between Asians and whites (ORs, 0.81 and 0.79, respectively; P=.79); the SNP used (ORs, 0.82, 0.77, 0.84, 0.78, and 0.70; P=.22); or the outcomes of CAD, CHD, and MI (ORs, 0.79, 0.79, and 0.81; P=.87). Meta-regression again showed a significant slope (P=.001), with lower ORs associated with earlier ages of onset (slope and intercept of 0.00500 and –0.57854) (FIGURE 5). The regressed OR was 0.74 (95% CI, 0.71-0.77) for an upper age cutoff of 55 years and 0.82 (95% CI, 0.79-0.85) for 75 years; heterogeneity was lower after fitting the model (Q=40; I^2=22%; P=.39). Results were similar when mean age was used (eFigure 2). When restricted to the 14 data sets with an upper age cutoff levels of 70 years or higher, the summary OR was 0.83 (95% CI, 0.78-0.89; $P<.001$). However, heterogeneity was moderate (Q=21; I^2=37%; P=.08). With the most discrepant finding removed (OR, 1.16),[48] this summary OR became 0.82 (95% CI, 0.78-0.87) and heterogeneity was reduced (Q=13; I^2=7%; P=.37).

Several included articles reported the effect size as an allele-specific OR,[8,9,21,22,46,49-51,53] where an equivalent "dose" of risk was conferred per allele. That would imply that the reciprocal of our low OR should be similar to the high OR (eg, 1/0.80=1.25; the high OR is 1.25).

Assessment of Evidence for 9p21 and Heart Disease

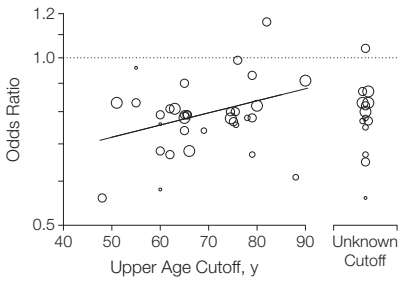
Three cohort studies (Table) were deemed level 1 designs and the remaining case-control studies were level 2.[11] Based on this and internal validity assessments, the overall quality of evidence for clinical validity was convincing to adequate. After accounting for age at onset, the Venice grading[12] was A for amount of evidence (>1000 cases/controls with the least common genotype) and A for replication (low heterogeneity after meta-regression and for the 12 data sets with later ages of onset). Bias required examining phenotype definition, genotyping, population stratification, and selective reporting using the predefined "typical" effect size category (ORs, 1.15-1.80).[12] The use of widely agreed-on definitions for CAD, CHD, and MI (eg, CHD defined as requiring coronary artery bypass graft surgery) in the data sets made the likelihood of phenotype bias low or none. Nearly all data sets were tested using commercial or well-described genotyping platforms, making the likelihood of genotyping bias low. Most case-control studies matched on race/ethnicity, and, where possible, we restricted results to a single race. We assigned the likelihood of stratification bias to be low, resulting in an overall protection from bias grade of A.

Figure 4. 9p21 Single-Nucleotide Polymorphism Markers and Heart Disease, Comparing Individuals With No At-Risk Alleles vs Those With 1 At-Risk Allele



The odds ratios (ORs) and 95% confidence intervals (CIs) are shown for the 37 data sets comparing individuals with no at-risk alleles vs those with 1 at-risk allele. The consensus (dashed vertical line) and no effect (dotted vertical line) are also shown. Heterogeneity is moderate.

Figure 5. Meta-regression of 9p21 Single-Nucleotide Polymorphism Markers and Heart Disease, Comparing Individuals With No At-Risk Alleles vs Those With 1 At-Risk Allele



The upper age cutoff for the occurrence (eg, myocardial infarction at age ≤50 years) is plotted vs the odds ratio on a logarithmic scale. Larger symbols indicate more precise estimates (standard errors of the log odds ratios of <0.1, 0.1-0.14, 0.15-0.19, and ≥0.20). Although 47 data sets are included, only the 33 data sets reporting upper age cutoff levels were used in the regression.

Data sets restricted to early onset heart disease are likely to be smaller in size (since these events are uncommon) and have a larger effect size (Figure 3). This might appear to be positive publication bias. To avoid confounding, each of the high ORs (Table) was divided by the expected OR given that data set's age at onset (Figure 3). These age-adjusted ORs (median, 1.000) could then be evaluated for publication bias. The cumulative effect size analysis indicated a trivial 4% change from the most precise to the least precise cumulative estimates (0.96 for the 6 largest data sets to 1.00 for all 33 data sets).

The OR associated with an individual's 9p21 test result can be used to modify heart disease risk based on traditional factors. For example, a 65-year-old man with no other traditional risk factors would have a 10-year heart disease risk of 11%,[56] while a 40-year-old woman with no other risk factors would have a 10-year risk of 2%. If both were to have 2 at-risk 9p21 alleles, risk estimates would increase to about 13.2% (11×1.2) and 2.4% (2×1.2), respectively, compared with an individual with 1 at-risk allele. Having no at-risk alleles would result in reduced risk estimates of 9.2% and 1.7%, respectively, compared with an individual with 1 at-risk allele. Comparing no at-risk alleles with 2 at-risk alleles could modify risk estimates by 1.44-fold.

Net Reclassification

Three studies that included 1721 cases and 34 797 controls also provided information to compute the NRI.[25,43,57] Four NRI estimates were made because 1 study[43] reported 2 risk algorithms. For the 4 data sets, the proportions of cases reclassified were 0.5%, 0.7%, 2.5%, and –0.1% (P=.65, .79, .03, and .87), respectively; proportions of controls reclassified were 0.3%, 4.2%, –0.1%, and 0% (P=.36, <.001, .52, and .87), respectively. The corresponding NRIs were 0.8%, 4.9%, 2.5%, and –0.2% (P=.51, .09, .03, and .96), respectively. Details are given in eTable 2.

COMMENT

Based on EWG and Venice criteria, evidence for the association between heart disease and the 9p21 SNP markers has strong credibility. The 9p21 markers have been identified through genome-wide association studies that were not hypothesis-driven[8,9,51] and appear independent of traditional risk factors or family history.[24,57] The pathophysiological mechanism is not yet understood.[24,25,57] Risk alleles are more strongly associated with heart disease events in younger persons (OR, 1.35) than with heart disease in general (OR, 1.21). One company offering 9p21 SNP testing and interpretation uses an OR of 1.3 in its clinical reports for "MI at any age" and 1.6 for "MI at a younger age."[27]

Most data sets are limited to white populations, usually of European descent, so the results for other racial/ethnic groups might differ. In 8 studies among Asians, however, the association appears similar. We did not include data for black populations, but limited evidence suggests a smaller effect.[9,23] Rather than adjusting ORs for age/sex, we based our OR on raw data when possible, because adjusted ORs appear to be close to raw estimates. For example, 1 article[43] reported per-allele hazard ratios that were 1.14 when unadjusted, 1.15 after adjustment for age, and 1.12 after adjustment for age, blood pressure, lipid measurements, smoking status, diabetes, and antihypertensive use. These differences were not statistically significant. Finally, we removed 2 articles from the meta-analysis for which we could not generate reliable ORs. Both were small (202 cases of CAD/MI[44] and 118 individuals with MI/coronary insufficiency)[45] and inclusion would be unlikely to influence the results reported using larger sample sets.

A test has clinical utility when its results lead to a measurable improvement in health outcomes. Intervention trials to establish the clinical utility of adding 9p21 testing were lacking.[58-60] There is an expectation that genetic susceptibility information could increase motivation for long-term lifestyle changes (eg, improvement in risk-reducing behaviors, treatment adherence).[61,62] However, measuring behavioral change is challenging. Communicating genetic information to patients has resulted in encouraging reports of short-term positive effects[63,64] and has not shown reduced adherence or fatalistic thinking.[64,65]

Achievement of more accurate risk by adding 9p21 to traditional risk factors could be considered an intermediate measure of clinical utility. The computed NRIs ranged from a 0.2% decrease to a 4.9% improvement. The study showing the largest NRI[25] achieved most of the risk reclassification because of reduced risk in individuals without events and, therefore, would have little chance of improving outcomes. The study reporting 2 traditional risk factor models[43] showed that adding 9p21 testing to the Adult Treatment Panel III model showed significant improvement (NRI, 2.5%), while adding testing to the Reynolds Risk Score (RRS) showed no improvement (NRI, –0.2%). One main difference between the 2 models was inclusion of a family history of myocardial infarction in the RRS. None of the 3 studies modified treatment protocols based on addition of 9p21 or exam-

ined long-term health or behavioral outcomes. How best to analyze the effect of adding genomic markers to traditional risk factors (eg, area under the curve, NRI, integrated discrimination improvement) remains an active research area.[31,66-68]

The incidence of MI can be reduced by drugs that lower cholesterol/low-density lipoproteins and blood pressure in individuals with risk factors.[69] Improved risk assessment might influence decision making about effective interventions and behavioral change. However, only 37% of US physicians reported regular use of a heart disease risk score.[70] A systematic review found preliminary evidence that CHD risk scores may translate into modest benefits (eg, increased drug treatment, short-term blood pressure reduction) without clinical harms.[71] However, the need for higher-quality evidence on long-term outcomes, and for replication of the results in different clinical settings, was emphasized.[71] Other complicating factors may be patient adherence to lipid-lowering medications (about half reach target lipid levels and one-quarter continue long-term drug treatment)[72] and access to medical care and medications. Therefore, the clinical utility of adding 9p21 markers to traditional risk factors cannot be assumed, even if risk assessment is improved. One proposal suggests that heart disease may be more effectively prevented by implementing an inexpensive, standardized, multidrug intervention (ie, the polypill) in all persons aged 55 years or older, regardless of individual risk levels.[69]

In summary, showing that a genetic test has clinical validity does not necessarily lead to improved health. Clinical trials need to demonstrate that use of the test is associated with changes in physician management decisions, patient motivation and long-term behavioral changes, improved health outcomes, and/or reduced costs to the health care system. Using genomic tests to improve existing risk models would likely require the inclusion of many markers like 9p21.[73,74] Such risk assessments may be more accurate but may not result in more appropriate treatments until the underlying mechanisms are known. Uncovering these mechanisms may provide insights into new or improved treatments and prevention activities.

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Study concept and design: Palomaki, Bradley.
Acquisition of data: Palomaki, Melillo.
Analysis and interpretation of data: Palomaki, Melillo.
Drafting of the manuscript: Palomaki, Melillo, Bradley.
Critical revision of the manuscript for important intellectual content: Palomaki, Melillo, Bradley.
Statistical analysis: Palomaki, Melillo.
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Administrative, technical, or material support: Palomaki, Melillo, Bradley.
Study supervision: Palomaki.
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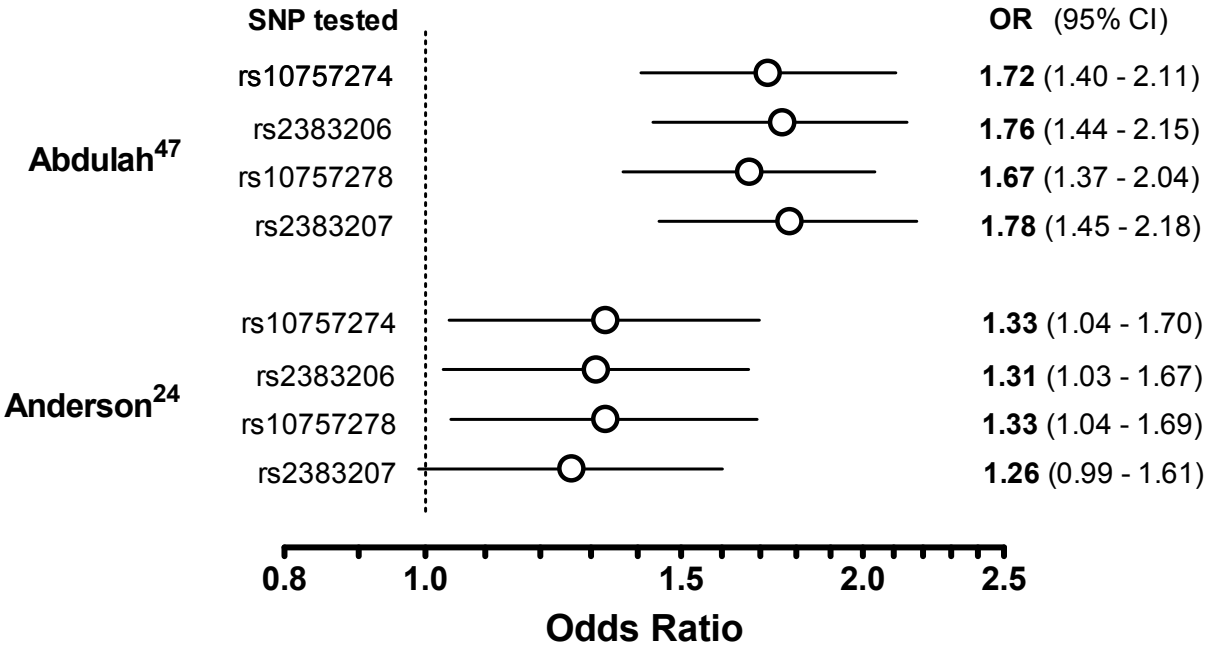
Supplementary Online Content

Palomaki GE, Melillo S, Bradley LA. Association Between 9p21 Genomic Markers and Heart Disease: A Meta-analysis. *JAMA*. 2010;303(7):648-656.

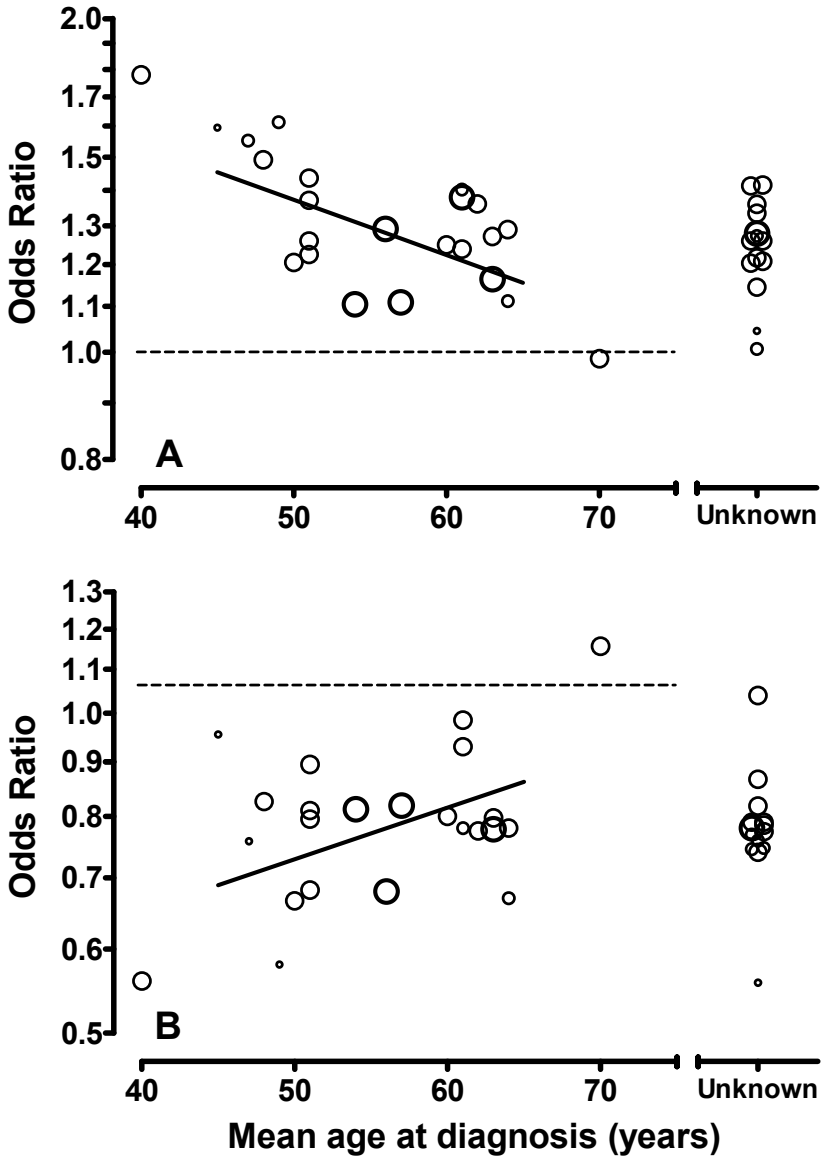
This supplementary material has been provided by the authors to give readers additional information about their work.

eFigure 1. Odds Ratios From Two Studies, Each Testing the Same Four 9p21 SNPs



Each study found nearly identical ORs (open circles, with lines indicating 95% CIs) for each of the four SNPs tested, but the two studies differ substantially on the effect size. This heterogeneity is likely due to differences in the study population and/or study design.

eFigure 2. Meta-regression of 9p21 SNP Markers and Heart Disease



The mean age for the occurrence of heart disease is plotted versus the odds ratio on a logarithmic scale. The relationship is shown separately for comparison of individuals with two at-risk alleles (Supplemental Figure 2A) and no at-risk alleles (Figure 2B), versus the referent category. Larger symbols indicate more precise estimates (standard errors of the log odds ratios of < 0.1, 0.1 to 0.14, 0.15 to 0.19 and >0.20). Although 37 datasets are included in the figure, only the 23 datasets reporting upper age cut-off levels were used in the regression analysis.

eTable 1. Genotype Frequencies (or Odds Ratios) for Included Studies, Along With Additional Study Information

Study	Reported	Odds Ratio		Heart Disease				No Heart Disease				HWE	
				At-Risk Alleles				At-Risk Alleles					
		High	Low	0	1	2	All	0	1	2	All	X^2	*P*
Abdullah	Odds ratio only	1.78	0.56	(allele-specific OR)			310				560		
Anderson	Odds ratio only	1.26	0.81				999				1111		
Assimes (older)	C/C - genotypes			193	448	302	943	183	329	163	675	0.40	0.82
Assimes (younger)	C/C - genotypes			51	129	73	253	84	203	72	359	6.27	0.04
Broadbent (Germany)	Odds ratio only	1.26	0.79	(allele-specific OR)			325				571		
Broadbent (Italy)	Odds ratio only	1.26	0.79	(allele-specific OR)			436				524		
Broadbent (Sweden)	Odds ratio only	1.36	0.74	(allele-specific OR)			480				519		
Broadbent (UK)	Odds ratio only	1.28	0.78	(allele-specific OR)			3010				2829		
Dehghan	Cohort - genotypes			133	197	82	412	1699	2909	1227	5835	0.08	0.96
Ding	C/C - genotypes			114	233	163	510	164	261	129	554	1.61	0.45
Helgadottir (Atlanta)	C/C - genotypes			100	270	206	576	273	603	381	1257	1.41	0.50
Helgadottir (Durham)	C/C - genotypes			230	535	353	1118	156	377	176	709	2.93	0.23
Helgadottir (ICE-a)	C/C - genotypes			389	811	408	1608	2022	3280	1418	6720	1.69	0.43
Helgadottir (ICE-b)	C/C - genotypes			146	319	171	636	1016	1770	746	3532	0.23	0.89
Helgadottir (Philadelphia)	C/C - genotypes			86	274	197	557	105	250	127	482	0.75	0.69
Hinohara (Japanese)	C/C - genotypes			114	312	178	604	286	606	259	1151	3.30	0.19
Hinohara (Korean)	C/C - genotypes			158	335	186	679	192	353	161	706	0.00	1.00
Hiura	C/C - genotypes			137	279	170	586	636	1204	592	2432	0.22	0.90
Lemmens	C/C - genotypes			176	461	277	914	227	386	196	809	1.59	0.45
McPherson (ARIC)	C/C - genotypes			230	525	282	1037	2063	3822	1858	7743	1.13	0.57
McPherson (CCHS)	C/C - genotypes			393	792	340	1525	2752	4543	1758	9053	2.29	0.32
McPherson (DHS)	C/C - genotypes			27	85	42	154	147	258	122	527	0.18	0.91
McPherson (OHS-1)	C/C - genotypes			49	148	125	322	85	149	78	312	0.61	0.74
McPherson (OHS-2)	C/C - genotypes			56	140	108	304	85	161	80	326	0.05	0.98
McPherson (OHS-3)	C/C - genotypes			121	333	193	647	228	418	201	847	0.12	0.94
Newton-Cheh	Odds ratio only	1.21	0.83	(allele-specific OR)			492				1460		
Paynter	Cohort - genotypes			42	103	51	196	5751	10849	5333	21933	2.35	0.31
Peng	C/C - genotypes			99	265	156	520	159	285	116	560	1.71	0.43

(continued on next page)

eTable 1. Genotype Frequencies (or Odds Ratios) for Included Studies, Along With Additional Study Information (continued)

Study	Reported	Odds Ratio		Heart Disease				No Heart Disease					
				At-Risk Alleles				At-Risk Alleles				HWE	
		High	Low	0	1	2	All	0	1	2	All	X^2	*P*
Samani 07 (German)	C/C - genotypes			158	453	233	844	425	831	349	1605	2.30	0.32
Samani 07 (WTCCC)	C/C - genotypes			378	960	586	1924	829	1431	676	2936	1.49	0.47
Samani 09 (AMC-PAS)	Odds ratio only	1.21	0.83	(allele-specific OR)			744				1299		
Samani 09 (ECTIM)	Odds ratio only	1.15	0.87	(allele-specific OR)			1146				1102		
Samani 09 (EPIC-Norfolk)	Odds ratio only	1.09	0.91	(allele-specific OR)			1081				2175		
Samani 09 (LURIC)	Odds ratio only	1.25	0.80	(allele-specific OR)			1038				1334		
Samani 09 (MORGAM)	Odds ratio only	1.21	0.83	(allele-specific OR)			1418				1433		
Schunkert (AtheroGene)	C/C - genotypes			79	193	98	370	96	178	71	342	0.48	0.79
Schunkert (GerMIFS II)	C/C - genotypes			149	330	206	685	226	448	204	878	0.39	0.82
Schunkert (LMD)	C/C - genotypes			90	252	141	483	109	238	95	442	2.69	0.26
Schunkert (MONICA/KORA)	C/C - genotypes			115	284	168	567	266	522	215	1003	1.90	0.39
Schunkert (PopGen)	C/C - genotypes			246	512	312	1070	292	502	205	999	0.16	0.92
Schunkert (PRIME)	Cohort - genotypes			93	261	171	525	123	257	261	641	0.06	0.97
Schunkert (UK MI)	C/C - genotypes			174	381	201	756	207	356	164	727	0.00	1.00
Shen (Italy)	Odds ratio only	1.25	0.80	(allele-specific OR)			416				308		
Shen (Korea)	Odds ratio only	1.29	0.78	(allele-specific OR)			611				294		
Talmud	Cohort - genotypes			53	138	73	264	680	1186	564	2430	1.14	0.57
Zhang	C/C - genotypes			103	220	94	417	154	202	74	430	0.31	0.86
Zhou	C/C - genotypes			138	520	702	1360	163	605	592	1360	1.23	0.54
All							**35872**				**95837**		

eTable 2. Computation of the Net Reclassification Indices (NRI) in Three Published Studies

				ACRS and 9p21 results												
															All Data	
	10 Y Risk	<5%		5-10%		10-20%		>20%			Total		Agreed upon			
		4460		157		0		0			4617	N		cases	89.9%
	<5%	71		5		0		0			76	events		cont	92.1%
		1.60%		3.30%		0.00%		0.00%			1.65%	%			
A													Incorrected moved		
C		188		2429		146		0			2763	N		cases down	4.8%
R	5-10%	5		194		17		0			216	events		cont up	3.8%
S		2.70%		8.00%		11.40%		0.00%			7.82%	%			
													Correctly moved		
A		0		160		1741		91			1992	N		cases up	5.3%
l	10-20%	0		18		258		19			295	events		cont down	4.1%
o		0.00%		11.50%		14.80%		21.20%			14.81%	%			
n													Improvement		
e		0		0		66		560			626	N		cases	0.5%
	>20%	0		0		14		168			182	events		cont	0.3%
		0.00%		0.00%		20.50%		30.00%			29.07%	%		net	0.8%
		4648		2746		1953		651		9998	9998		Checks	cases	100.0%
	Total	76		217		289		187		769	769			cont	100.0%
		1.64%		7.90%		14.80%		28.73%		7.69%	7.69%				
															P-value
		Data from Brautbar A, et al. *Circ Cardiovasc Genet*. 2009;2:279-285										Z net		0.66	0.51
												Z cases		0.45	0.65
												Z cont		0.92	0.36

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eTable 2. Computation of the Net Reclassification Indices (NRI) in Three Published Studies (continued)

				CRF + rs10757274												
															All Data	
	10 Y Risk	**<5%**		**5-10%**		**10-20%**		**>20%**			**Total**		Agreed upon			
		362		117		0		0			479	**N**			cases	78.5%
	<5%	14		9		0		0			23	**events**			cont	78.0%
		3.80%		8.10%		0.00%		0.00%			4.80%	**%**				
C													Incorrected moved			
R		90		894		71		0			1055	**N**			cases down	10.4%
F	**5-10%**	1		75		9		0			85	**events**			cont up	8.9%
		1.20%		8.40%		12.10%		0.00%			8.06%	**%**				
A													Correctly moved			
l		0		216		701		55			972	**N**			cases up	11.1%
o	**10-20%**	0		24		96		13			133	**events**			cont down	13.1%
n		0.00%		11.10%		13.70%		24.00%			13.68%	**%**				
e													Improvement			
		0		0		36		128			164	**N**			cases	0.7%
	>20%	0		0		4		34			38	**events**			cont	4.2%
		0.00%		0.00%		12.20%		26.30%			23.17%	**%**			net	4.9%
		452		1227		808		183		2670	2670		Checks		cases	100.0%
	Total	15		108		109		47		279	279				cont	100.0%
		3.32%		8.80%		13.49%		25.68%		10.45%	10.45%					
																***P*-value**
		Data from Talmud et al. *Clin Chem*. 2008;54:467											**Z net**		1.69	0.091
													Z cases		0.26	0.79
													Z cont		4.66	<0.001

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eTable 2. Computation of the Net Reclassification Indices (NRI) in Three Published Studies (continued)

				ATPIII and 9p21 genotype												
															All Data	
	10 Y Risk	<5%		5-10%		10-20%		>20%			Total			Agreed upon		
		18609		205		0		0			18814	N			cases	90.9%
	<5%	279		16		0		0			295	events			controls	97.5%
A		1.50%		8.00%		0.00%		0.00%			1.57%	%				
T														Incorrected moved		
P		181		1933		83		0			2197	N			cases down	3.3%
	5-10%	9		155		16		0			180	events			cont up	1.3%
I		4.90%		8.00%		19.30%		0.00%			8.19%	%				
I														Correctly moved		
I		0		80		697		31			808	N			cases up	5.8%
	10-20%	0		9		90		7			106	events			cont down	1.2%
A		0.00%		10.90%		12.90%		23.60%			13.12%	%				
I														Improvement		
o		0		0		26		284			310	N			cases	2.5%
n	>20%	0		0		4		88			92	events			controls	-0.1%
e		0.00%		0.00%		15.00%		31.00%			29.68%	%			net	2.5%
		18790		2218		806		315		22129	22129			Checks	cases	100.0%
	Total	288		180		110		95		673	673				cont	100.0%
		1.53%		8.12%		13.65%		30.16%		3.04%	3.04%					
																P-value
		Data from Paynter NP et al. *Ann Intern Med.* 2009												Z net	2.11	0.035
														Z cases	2.18	0.029
														Z cont	-0.65	0.52

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eTable 2. Computation of the Net Reclassification Indices (NRI) in Three Published Studies (continued)

				Reynolds Risk Score and 9p21 genotype										
													All Data	
	10 Y Risk	**<5%**		**5-10%**		**10-20%**		**>20%**		**Total**		Agreed upon		
		18527		188		0		0		18715	**N**		cases	94.5%
	<5%	278		5		0		0		283	**events**		cont	97.4%
		1.50%		2.70%		0.00%		0.00%		1.51%	**%**			
R												Incorrected moved		
R		183		1960		75		0		2218	**N**		cases down	2.8%
S	**5-10%**	3		151		6		0		160	**events**		cont up	1.3%
		1.40%		7.70%		8.30%		0.00%		7.21%	**%**			
A												Correctly moved		
l		0		85		761		31		877	**N**		cases up	2.7%
o	**10-20%**	0		9		116		7		132	**events**		cont down	1.3%
n		0.00%		10.60%		15.20%		21.40%		15.05%	**%**			
e												Improvement		
		0		0		23		296		319	**N**		cases	-0.1%
	>20%	0		0		7		90		97	**events**		cont	0.0%
		0.00%		0.00%		31.50%		30.40%		30.41%	**%**		net	-0.2%
		18710		2233		859		327		22129		Checks	cases	100.0%
	Total	281		165		129		97		672			cont	100.0%
		1.50%		7.39%		15.02%		29.66%		3.04%				
														P-value
		Data from Paynter NP et al., Ann Intern Med 2009										**Z net**	-0.18	0.86
												Z cases	-0.16	0.87
												Z cont	-0.17	0.87