Published in final edited form as:
Atherosclerosis. 2012 May ; 222(1): 135–137. doi:10.1016/j.atherosclerosis.2012.01.028.

The 9p21 genetic variant is additive to carotid intima media thickness and plaque in improving coronary heart disease risk prediction in white participants of the Atherosclerosis Risk in Communities (ARIC) Study

Vijay Nambi, MD PhD[*], **Eric Boerwinkle, PhD**[‡], **Kim Lawson, MS**[‡], **Ariel Brautbar, MD**[*], **Lloyd Chambless, PhD**[†], **Nora Franeschini, MD MPH**[††], **Kari E. North, PhD**[††], **Salim S Virani, MD**[*,€], **Aaron R Folsom, MD**[§], and **Christie M Ballantyne, MD**[*]

[*]Section of Atherosclerosis and Vascular Medicine, Department of Medicine, Baylor College of Medicine, and the Methodist DeBakey Heart and Vascular Center, the Methodist Hospital – Houston, TX

[‡]UT Houston School of Public Health – Houston, TX

[†]Collaborative Studies Coordinating Center and Epidemiology Department, University of North Carolina – Chapel Hill, NC

[††]Department of Epidemiology and Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC

[€]Michael E DeBakey Veterans Affairs Medical Center, Houston TX

[§]Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN

Abstract

OBJECTIVE—We evaluated whether the addition of carotid intima media thickness and plaque (CIMT-P), and, a single nucleotide polymorphism on chromosome 9p21 (9p21) together improve coronary heart disease (CHD) risk prediction in the ARIC study.

METHODS—Ten year CHD risk was estimated using the ARIC coronary risk score (ACRS) alone and in combination with CIMT-P and 9p21 individually and together in White participants (n=9338). Area under the receiver operating characteristic curve (AUC), model calibration, net reclassification index (NRI), integrated discrimination index (IDI) and number of individuals reclassified were estimated.

RESULTS—The AUC of the ACRS, ACRS+9p21, ACRS+CIMT-P and ACRS+CIMT-P+9p21 models were 0.748, 0.751, 0.763 and 0.766 respectively. The percentage of individuals reclassified, model calibration, NRI and IDI improved when CIMT-P and 9p21 were added to the ACRS only model (see manuscript).

Address for correspondence: Vijay Nambi, 6565 Fannin Street, M.S. A-601/ STE B160, Houston, Texas 77030, Phone: 713-798-7545, Fax: 713-798-7885, vnambi@bcm.tmc.edu.

CONCLUSION—Addition of 9p21 allele information to CIMT-P minimally improves CHD risk prediction in whites in the ARIC study.

Keywords

Carotid intima media thickness; Plaque; 9p21; Risk prediction; Coronary heart disease

Introduction

A single nucleotide polymorphism (SNP) in chromosome 9p21 (9p21) has been associated with coronary heart disease (CHD) in whites[1–3]. Data from the Atherosclerosis Risk In Communities (ARIC) study [4] have suggested that adding 9p21 to traditional risk factors (TRF) and similarly adding information on carotid intima media thickness (CIMT) and presence or absence of plaque to TRF[5] improves CHD risk prediction improves CHD risk prediction. Studies have however suggested that 9p21 is not associated with CIMT[6].

We therefore evaluated whether CIMT/ plaque information together with 9p21 further improves CHD risk prediction in the ARIC study.

Materials and Methods

The ARIC study is a population based study of cardiovascular disease incidence that recruited 15,792 middle aged individuals aged between 45 and 64 years of age in 1987–89 in four communities in the United States. A complete description of the study design, objectives and sampling strategy have been previously described[7]. After excluding non-whites [(n= 4314), because 9p21 is associated with CHD only in whites], individuals with prevalent CHD (n= 1050), those missing TRF covariate data (n= 356), missing CIMT +plaque information (n=606) or missing 9p21 data (n=386) there were 9,338 whites available for the current analysis.

For the TRF model we used the ARIC coronary risk score or ACRS [8]. The variables that constitute the ACRS are presented in table 1.

Details about genotyping and ultrasound measurements are provided in the supplement. We used the mean of the mean IMT of the far wall of the carotid arteries [distal 1 cm of the common carotid artery, the carotid artery bifurcation (1 cm proximal to the flow divider) and the proximal 1cm of the internal carotid artery] and the presence/ absence of plaque for our analysis. Missing CIMT values were imputed.

Incident Coronary Heart Disease Events

Incident CHD events included for our analysis included fatal CHD, definite or probable myocardial infarction (MI), silent MI between examinations indicated by electrocardiograms and coronary revascularization. Follow up for incident CHD was from baseline until December 31st 2004. Methods for ascertainment of incident CHD events in ARIC have been previously validated and published[9].

Statistical Methods

Please refer to supplement for detailed statistical methods. Briefly, individuals were classified into low, low-intermediate, intermediate-high and high risk (0–5%, 5–10%, 10–20% and >20% predicted 10 year CHD risk respectively) groups using ACRS to form the TRF model.

Information about the 9p21 genotype and CIMT/plaque were added to the TRF model individually and together resulting in the following models: i. TRF –only (ACRS based); ii. TRF+9p21 iii. TRF+CIMT+plaque; and iv. TRF+9p21 +CIMT +plaque models. The number of individuals reclassified to a higher or lower risk group by adding 9p21 and CIMT +plaque alone or together to the TRF model was described.

To evaluate the model performance in risk prediction we performed tests to evaluate model discrimination and calibration: We then described the area under the receiver operator characteristic curve (AUC), net reclassification index (NRI), clinical NRI (i.e. NRI in the intermediate risk groups [5–20% 10-year predicted CHD risk)], integrated discrimination index (IDI) and % reclassified for 10-year risk using methods that accounted for censoring[1011]. Using bootstraps we calculated the 95% confidence intervals when one model was compared with another. Model calibration was tested using the Grønnesby-Borgan goodness of fit test statistic[12].

Experimental Results

Over a mean follow up of 14.7 years there were 1,231 incident CHD events (74 CHD deaths, 612 myocardial infarctions, and 545 coronary revascularizations). The baseline characteristics of the study cohort are described in supplemental tables1a and1b. The baseline mean CIMT was 0.71 mm and the distribution of genotypes GG, AG, AA, of the rs10757274 variant was 24%, 50% and 26% respectively; 9p21 was associated with a hazards ratio of 1.21 (95% CI 1.12,1.31) after adjusting for ACRS+C-IMT+plaque (Supplemental Table 2).

Addition of 9p21 or CIMT+plaque individually to the TRF model significantly improved the AUC with the improvement being greater for the addition of CIMT+plaque; the largest impovement was obtained when both CIMT/plaque and 9p21 were added to the TRF model (Table 1).

Adding 9p21 to the TRF model reclassified 8.9% while adding CIMT+plaque reclassified 24.7% and adding both 9p21 information and CIMT+plaque reclassified 25.9% (Table 2, Supplemental Table 3) of the individuals. No individualwas reclassified from the high to low risk group or vice versa for any of the model comparisons.

When the NRI and clinical NRI were examined, the addition of 9p21 improved only the clinical NRI while the addition of CIMT+plaque improved both the NRI and clinical NRI. However, the addition of CIMT+plaque and 9p21 provided the largest increases in NRI and clinical NRI (Table 2). The results of the integrated discrimination index (IDI) were similar (Table 2).

When model fit was examined, both the model adding CIMT+plaque and the model adding CIMT+plaque+9p21to TRF showed improved fit while the model adding 9p21 to TRF did not improve model fit (Table 2).

Finally we evaluated the addition of CIMT+plaque to a model that included TRF+9p21 and similarly added 9p21 to a model that included TRF+CIMT+plaque. Both of these significantly improved the AUC (Table 1). When 9p21 was added to a TRF+C1IMT+plaque model, 8.6% of the individuals were reclassified and the resulting NRI and clinical NRI were 1.5% and 6.2% respectively. On the other hand, addition of CIMT+plaque to a base model that included TRF and 9p21 resulted in larger improvements with 25.2% being reclassified and a resulting NRI of 10.3% and clinical NRI of 29.8%.

Discussion

Traditional risk factors form the basis of any CHD risk prediction score used clinically[8]. However, there is significant room for improvement in CHD risk prediction. Several additional markers including biomarkers, imaging markers and genetic markers have been and will continue to be evaluated for their ability to improve CHD risk prediction. Although several efforts have examined the combination of multiple biomarkers[13] or genotypes [14] none have combined these biomarkers/ genetic markers of higher risk with imaging markers. We now report that a genetic marker (9p21 genotype) and an imaging marker (CIMT and plaque presence) (that we have previously shown to individually improve CHD risk prediction[4, 5]) in combination improved CHD risk prediction beyond that offered by each marker alone. Although several studies have documented the association between the 9p21 variant and CHD,[1, 2] no association between 9p21 and CIMT has been shown[6]. In the ARIC study, 9p21 was associated with body mass index but with no other TRF.[4] The utility of 9p21 in CHD risk prediction has been tested, but yielded mixed results. Two studies, one that included only men[2] and another that included only women,[15] did not show significant improvements in CHD risk prediction with the addition of 9p21. However, in the ARIC study, which included men and women, and had a greater number of events than these other studies, a modest, yet significant improvement was noted[4].

CIMT and plaque are associated with both TRF and CHD, and, improve CHD risk prediction when added to TRF[5]. We hypothesized that given the lack of association between 9p21 and CIMT, their ability to improve CHD risk prediction will be additive to each other and indeed found this to be true. We used the ACRS as the TRF model given that ACRS is the best CHD prediction model in ARIC. Use of Framingham risk score (i.e. the TRF model recommended by ATP III) will likely have resulted in weaker TRF associations and greater improvements in risk prediction by the novel risk factors as seen in our previous analyses[4, 5].

Although, our analysis suggests that adding both 9p21 and CIMT+plaque decreases the number of individuals in the intermediate risk groups by ~10% and overall improves risk prediction the clinical impact/ relevance is likely to be minimal as the addition of CIMT +plaque accounted for most of the improvement. However, our analysis provides a proof of concept that the addition of genetic markers to imaging markers may further improve the ability of each marker to predict CHD risk. In the future as the field of genomics further advances, additional genetic risk scores are likely to be described and when combined with biomarker and imaging markers may yield better risk prediction tools.

Limitations

Our data cannot be generalized to all races. Specifically, our results pertain only to whites as 9p21 has been associated with CHD only in whites and not in African Americans (Franceschini et al submitted). Our risk models used data from the baseline ARIC visit only. Therapies and risk factors will likely have changed during the follow up time period. However, this is true for any risk prediction model/effort.

Conclusion

We used one genetic marker (a SNP in 9p21) and one imaging marker (CIMT + assessment of presence or absence of plaque), both of which have previously been shown to be associated with CHD and improve CHD risk prediction, in combination and now show that the combination of these two markers further marginally improves CHD risk prediction in whites.

Highlights

- CIMT/ plaque and 9p21 SNP are known to be associated with CHD
- CIMT/ plaque and 9p21 SNP can improve CHD risk prediction
- We now show that combining CIMT/plaque, 9p21 further marginally improves CHD risk prediction
- Improvements in CHD risk prediction conferred by adding CIMT/plaque were greater than 9p21

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. The authors thank the staff and participants of the ARIC study for their important contributions.

Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

DR Nambi is supported by grant K23 HL096893 from the National Institutes of Health/National Heart, Lung, and Blood Institute.

He has research collaboration with GE

References

1. McPherson R, Pertsemlidis A, Kavaslar N, Stewart A, Roberts R, Cox DR, Hinds DA, Pennacchio LA, Tybjaerg-Hansen A, Folsom AR, Boerwinkle E, Hobbs HH, Cohen JC. A common allele on chromosome 9 associated with coronary heart disease. Science. 2007; 316:1488–1491. [PubMed: 17478681]
2. Talmud PJ, Cooper JA, Palmen J, Lovering R, Drenos F, Hingorani AD, Humphries SE. Chromosome 9p21.3 coronary heart disease locus genotype and prospective risk of CHD in healthy middle-aged men. Clin Chem. 2008; 54:467–474. [PubMed: 18250146]
3. Schunkert H, Gotz A, Braund P, McGinnis R, Tregouet DA, Mangino M, Linsel-Nitschke P, Cambien F, Hengstenberg C, Stark K, Blankenberg S, Tiret L, Ducimetiere P, Keniry A, Ghori MJ, Schreiber S, El Mokhtari NE, Hall AS, Dixon RJ, Goodall AH, Liptau H, Pollard H, Schwarz DF, Hothorn LA, Wichmann HE, Konig IR, Fischer M, Meisinger C, Ouwehand W, Deloukas P, Thompson JR, Erdmann J, Ziegler A, Samani NJ. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation. 2008; 117:1675–1684. [PubMed: 18362232]
4. Brautbar A, Ballantyne CM, Lawson K, Nambi V, Chambless L, Folsom AR, Willerson JT, Boerwinkle E. Impact of adding a single allele in the 9p21 locus to traditional risk factors on reclassification of coronary heart disease risk and implications for lipid-modifying therapy in the Atherosclerosis Risk in Communities study. Circ Cardiovasc Genet. 2009; 2:279–285. [PubMed: 20031596]
5. Nambi V, Chambless L, Folsom AR, He M, Hu Y, Mosley T, Volcik K, Boerwinkle E, Ballantyne CM. Carotid intima-media thickness and presence or absence of plaque improves prediction of coronary heart disease risk: the ARIC (Atherosclerosis Risk In Communities) study. J Am Coll Cardiol. 2010; 55:1600–1607. [PubMed: 20378078]

6. Samani NJ, Raitakari OT, Sipila K, Tobin MD, Schunkert H, Juonala M, Braund PS, Erdmann J, Viikari J, Moilanen L, Taittonen L, Jula A, Jokinen E, Laitinen T, Hutri-Kahonen N, Nieminen MS, Kesaniemi YA, Hall AS, Hulkkonen J, Kahonen M, Lehtimaki T. Coronary artery disease-associated locus on chromosome 9p21 and early markers of atherosclerosis. Arterioscler Thromb Vasc Biol. 2008; 28:1679–1683. [PubMed: 18599798]
7. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. Am J Epidemiol. 1989; 129:687–702. [PubMed: 2646917]
8. Chambless LE, Folsom AR, Sharrett AR, Sorlie P, Couper D, Szklo M, Nieto FJ. Coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC) study. J Clin Epidemiol. 2003; 56:880–890. [PubMed: 14505774]
9. White AD, Folsom AR, Chambless LE, Sharret AR, Yang K, Conwill D, Higgins M, Williams OD, Tyroler HA, The AI. Community surveillance of coronary heart disease in the Atherosclerosis Risk in Communities (ARIC) Study: Methods and initial two years' experience. Journal of Clinical Epidemiology. 1996; 49:223. [PubMed: 8606324]
10. Chambless LE, Diao G. Estimation of time-dependent area under the ROC curve for long-term risk prediction. Stat Med. 2006; 25:3474–3486. [PubMed: 16220486]
11. Chambless LE, Cummiskey CP, Cui G. Several methods to assess improvement in risk prediction models: extension to survival analysis. Stat Med. 2011; 30:22–38. [PubMed: 20827726]
12. May S, Hosmer DW. A simplified method of calculating an overall goodness-of-fit test for the Cox proportional hazards model. Lifetime Data Anal. 1998; 4:109–120. [PubMed: 9658770]
13. Wang TJ, Gona P, Larson MG, Tofler GH, Levy D, Newton-Cheh C, Jacques PF, Rifai N, Selhub J, Robins SJ, Benjamin EJ, D'Agostino RB, Vasan RS. Multiple biomarkers for the prediction of first major cardiovascular events and death. N Engl J Med. 2006; 355:2631–2639. [PubMed: 17182988]
14. Morrison AC, Bare LA, Chambless LE, Ellis SG, Malloy M, Kane JP, Pankow JS, Devlin JJ, Willerson JT, Boerwinkle E. Prediction of coronary heart disease risk using a genetic risk score: the Atherosclerosis Risk in Communities study. Am J Epidemiol. 2007; 166:28–35. [PubMed: 17443022]
15. Paynter NP, Chasman DI, Buring JE, Shiffman D, Cook NR, Ridker PM. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. Ann Intern Med. 2009; 150:65–72. [PubMed: 19153409]

Table 1

Area under the receiver operator characteristics curve and its differences when models adding information related to the 9p21 allele, CIMT and plaque are compared to the ARIC coronary risk score alone in the prediction of CHD incidence

Model	AUC	Difference in AUC	95% Confidence interval
ACRS	0.748		Ref
ACRS + 9p21	0.751	0.003	0.001, 0.007
ACRS + CIMT + plaque	0.763	0.015	0.008, 0.021
ACRS + CIMT + plaque +9p21	0.766	0.018	0.011, 0.025
ACRS + 9p21 vs. ACRS + CIMT+plaque + 9p21		0.014	0.008, 0.021
ACRS + CIMT + plaque vs. ACRS + CIMT+plaque + 9p21		0.003	0.001, 0.007

ACRS: ARIC coronary risk score, includes age and its quadratic form (age^2), gender, systolic blood pressure, antihypertensive medication use, total cholesterol, high-density lipoprotein cholesterol (HDL-C), diabetes, and smoking status. Diabetes was defined as self reported diabetes or a fasting serum glucose >126 mg/dL or use of blood sugar lower (anti-diabetes) medications or a non-fasting glucose >200 mg/dL.

CIMT : carotid intima media thickness

Table 2

Statistical Parameters for Coronary Heart Disease Risk Prediction with the addition of 9p21 allele, CIMT and plaque to traditional risk factors: The ARIC study

	ACRS only	**ACRS+9p21 allele**	**ACRS+ CIMT+plaque (*)**	**ACRS+CIMT+plaque + 9p21allele (*)**
% reclassified (#)		8.9 (n=828)	24.7% (n=2305)	25.9% (n=2416)
G-B goodness of fit test (p value)	15.75 (p=0.07)	17.6 (p=0.04)	14.48 (p=0.11)	15.27 (p=0.08)
NRI (%) (#)		−0.5	8.3	9.8
Clinical NRI (%) (#)		4.1	25.01	28.6
IDI (#)		0.002	0.013	0.016

(#)Comparison of various models to the ARS only model

(*)Comparison of ARS+CIMT+plaque+9p21 allele to ARS+CIMT+plaque: % reclassified 8.6% (n=803), NRI 1.5%, clinical NRI 6.2%, IDI 0.002,

G-B: Grønnesby Borgan test of model calibration; NRI: Net reclassification index; clinical NRI: NRI in the intermediate (5–20% risk groups); IDI: Integrated discrimination index