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Background—Genome-wide association studies have identified gene variants associated with coronary artery disease risk; however, whether they affect disease progression is largely unknown. This study investigated associations between polymorphisms at 1p13.3 (rs599839), 1q41 (rs17465637), and 3q22.3 (rs9818870) and cardiovascular outcomes in healthy volunteers and in patients with established heart disease.

Methods and Results—Canterbury Healthy Volunteer study (HV) (n=1649), Coronary Disease Cohort Study (CDCS) (n=1797), and Post-Myocardial Infarction study (PMI) (n=906) participants (New Zealand), were genotyped for rs599839, rs9818870, and rs17465637. Associations between genotype and anthropometric characteristics, neurohormonal analysis, echocardiography, and clinical outcomes over medium-long-term follow-up (median HV, 5.9 years; CDCS, 3.7 years; PMI, 11.3 years) were tested. At 1p13.3, HV and CDCS participants carrying 1 or more rs599839 G allele had a lower prevalence of dyslipidemia ($P \leq 0.005$) or lower levels of low-density lipoprotein ($P=0.031$) and total ($P=0.004$) cholesterol and/or less history of myocardial infarction ($P \leq 0.04$) compared with AA participants. Moreover, CDCS and PMI AG/GG participants had better cardiac function as indicated by echocardiography ($P \leq 0.026$), and fewer CDCS AG/GG participants were readmitted for a non-ST-segment elevation MI ($P=0.012$) during follow-up. The polymorphism at 1q41 (rs17465637) was associated with better cardiovascular outcomes in the HV ($P=0.028$) and PMI ($P=0.008$) cohorts, and 3q22.3 (rs9818870) was a predictor of death/admission in the HV cohort ($P=0.045$).

Conclusions—These data suggest that coronary artery disease genomic risk variants at 1p13.3 and 1q41 are associated with subsequent clinical outcome in heart patients and confirm rs9818870 at 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease. **(*Circ Cardiovasc Genet*. 2011;4:636-646.)**

Key Words: coronary disease ■ genetic polymorphisms ■ genome wide association studies ■ cardiovascular diseases ■ outcomes

Coronary artery disease (CAD) has multifactorial origins, and although some families are particularly affected, no precise mode of inheritance has been identified. It is likely that this reflects the contribution of numerous genetic components, each conferring a small risk in cumulative interaction with environmental factors to substantively increase disease susceptibility.[1] In recent years, significant advances have been made in elucidating the genetic basis of CAD with the completion of several large genome-wide association studies (GWAS) that have looked at the DNA variation across the entire human genome. In a landmark study in 2007, the Wellcome Trust Case Control Consortium identified a region on chromosome 9 (9p21.3) that was the most strongly associated with the risk of developing CAD,[2] a finding that has since been replicated in several independent studies.[3–6] The 9p21.3 rs1333049 variant is common and may potentially be added to risk profiling in the future. Already it has been reported that adding the 9p21.3 genotype to the traditional risk score significantly improves CAD risk prediction in the community.[7]

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Clinical Perspective on p 646

The second most replicated region for risk of CAD is a locus at 1p13. A variant within this region, rs599839, is associated with less risk of both CAD and myocardial infarction (MI)[2,5,8–11]; lower levels of low-density lipoprotein (LDL), LDL triglycerides, and apoB; and an increased radius of LDL particles.[10–13] A genomic region at 1q41 has also been identified as a strong genetic predictor of CAD. Within 1q41, rs17465637, which lies within the melanoma-inhibitory family member gene (*MIA3*), has been identified as the strongest signal.[2,8,14,15]

Although early GWAS have been successful in identifying the chromosomal regions most significantly associated with CAD (including 9p21.3, 1p13.3, and 1q41), important regions with more modest effects or lower allele frequencies may have been missed. More recently, a second wave of studies has focused on these "smaller" signals, including a locus at 3q22.3 within *MRAS*.[14] Within 3q22.3, the most highly associated gene variant rs9818870 is located in the 3′ untranslated region of *MRAS* and is in proximity to a cluster of regulatory miRNA binding sites.[14]

In recent years, GWAS have generated a wealth of information on genetic susceptibility to CAD. Some regions are now predictive of incident CAD; however, whether these genetic risk regions influence disease progression in patients with established CAD remains largely unknown. Previously, we have reported that a common risk variant within the 9p21.3 risk region was associated with age of CAD onset but not subsequent mortality.[16] In the current study, we have investigated the association between risk variants at 1p13.3, 1q41, and 3q22.3 and cardiovascular outcomes in both individuals free of overt cardiovascular disease (CVD) at the time of recruitment and in patients with established CAD.

Methods

Study Participants

Canterbury Healthy Volunteers Study

Volunteers randomly selected from the Canterbury electoral roll and age and sex matched to existing patient cohorts were recruited into the Canterbury Healthy Volunteers study (HV) (n=1649). Participants were aged 20 to 108 years. Study participants were screened before recruitment using hospital Patient Management Systems (PMS) databases and had no personal history of overt CVD, including CAD, MI, or peripheral vascular disease. Participants completed a study questionnaire on their medical history, smoking status, alcohol consumption, and self-reported physical activity. Height, weight, waist, and hip measurements were taken. Blood pressure was recorded, and a blood sample was taken for neurohormone and genetic analyses. Median follow-up was 5.9 years (range, 0.1–8.7 years). Study participants were followed through PMS and New Zealand Health Information Services (NZHIS) databases. All admissions identified as cardiovascular were confirmed by clinical review at the time of discharge. Admissions that were determined to be noncardiac were excluded. The diagnosis at each hospital admission was defined using the ICD-10 (*International Classification of Diseases and Related Health Problems, 10th Revision*). The study was approved by the Upper South A Ethics Committee (Reference No. CTY/01/05/062), and each participant provided written, informed consent.

Coronary Disease Cohort Study

From July 2002, patients (n=1794) admitted to either Christchurch Hospital or Auckland City Hospital, New Zealand, were recruited into the study. Inclusion criteria were ischemic discomfort plus 1 or more of the following: ECG changes (ST-segment depression or elevation of at least 0.5 mm, T-wave inversion of at least 3 mm in at least 3 leads, or left bundle branch block), elevated levels of cardiac markers, a history of coronary disease, or ≥64 years of age in patients with diabetes mellitus or vascular disease. Patients were excluded from the study if they had a severe comorbidity that limited their life expectancy to <3 years. Within the Coronary Disease Cohort Study (CDCS) cohort, unstable angina accounted for 26.1% of all diagnoses at discharge, non-ST-segment elevation MI (NSTEMI) for 51.2%, and ST-segment elevation MI (STEMI) for 22.7%. Anthropometric and clinical characteristics were recorded at planned follow-up clinic visits at baseline, 4 months, and 12 months after admission. Clinical events were recorded from questionnaires, patient notes, and NZHIS and hospital PMS databases. Median follow-up was 3.7 years (range, 0.1–7.9 years). The study conformed to the principles outlined in the Declaration of Helsinki and Title 45, US Code of Federal Regulations, Part 46, and was approved by the New Zealand Multi-region Ethics Committee (Reference No. CTY/02/02/018). Each participating patient provided written, informed consent.

Post-Myocardial Infarction Study

From November 1994, patients were recruited into the Christchurch Post-Myocardial Infarction (PMI) study (n=905). Acute MI (including STEMI and NSTEMI) was defined by the presence of typical cardiac ischemic symptoms, ischemic change on the ECG in 2 or more contiguous leads, and peak elevation of plasma creatine kinase to at least twice normal (400 U/L). Although there were no specific exclusion criteria, the inclusion criteria included age <80 years, absence of cardiogenic shock, and survival for at least 24 hours after MI. Although this was not an inclusion criterion, all patients were positive for troponin-T. Patients were followed over a median 11.3 years (range, 0–15.9 years). Subsequent cardiovascular events were determined by clinical review at the time of discharge and were recorded from PMS and NZHIS databases. The study was approved by the Canterbury Ethics Committee (Reference No. CTY/94/08/783), and each participating patient provided written, informed consent.

Neurohormone Measurement and Echocardiography

Neurohormone Measurement

For the CDCS cohort, baseline samples and measurements were obtained between 5 and 56 days from the date of index admission. In PMI study participants, blood samples were taken between 24 and 96 hours after the onset of symptoms in the morning (between 0700 and 1300 hours) from an indwelling intravenous cannula placed at least 30 minutes before sampling and with the patient resting quietly while semirecumbent. For each study, blood was collected into chilled EDTA tubes and stored on ice. Plasma was separated within 20 minutes by centrifugation and stored at −80°C. Plasma was assayed for B-type natriuretic peptide[17] and N-terminal pro-B-type natriuretic peptide.[18]

Echocardiography

Transthoracic echocardiography was performed for CDCS and PMI patients using a GE Vivid 3 ultrasound system (GE Medical Systems; Waukesha, WI) at Christchurch Hospital and an ATL HDI 5000 (Philips Healthcare; Andover, MA) at Auckland City Hospital. The standardized imaging protocol included apical 4- and 2-chamber views according to the American Society of Echocardiography.[19] Parasternal and short- and long-axis views (averaged over 4 cycles) were obtained for M-mode measurements of left ventricular (LV) dimensions. Biplane diastolic and systolic volumes and ejection fraction were calculated by planimetry according to the Simpson method.

Table 1. Canterbury Healthy Volunteers, Coronary Disease Cohort Study, and Post-Myocardial Infarction Study Baseline Cohort Characteristics

	Healthy Volunteers Cohort (n=1649)	Coronary Disease Cohort Study (n=1797)	Post-Myocardial Infarction Study (n=906)
Age, y	62.8±10.8	66.5±12.3	62.4±10.7
Male sex	66.3	70.8	78.5
Ethnicity	. . .	. . .	. . .
European	89.0	83.0	87.6
Maori/Pacific Island	2.1	7.0	2.2
BMI, kg/m^2	26.5±4.2	27.7±6.1	26.6±4.1
Current smoker	6.8	6.6	29.8
Systolic blood pressure, mm Hg	133.6±20.1	128.9±21.9	116.5±15.7
Diastolic blood pressure, mm Hg	79.8±11.3	74.9±12.1	66.7±9.7
Hypertension	26.9	52.4	37.7
Hyperlipidemia	22.8	54.4	35.2
Previous MI	0	29.9	18.3
Type 2 diabetes	4.8	16.7	12.7
Family history of CVD	. . .	48.9	48.1
Plasma NT-proBNP, pmol/L	16.4 (1.0–575.4)	75.9 (0.9–2238.7)	113.7 (8.2–1096.6)
LVEF	. . .	57.0±12.4	47.5±12.5
Plasma creatinine	. . .	0.10 (0.02–0.10)	0.08 (0.001–0.45)

Data are presented as mean±SD, %, or geometric mean and range. BMI indicates body mass index; CVD, cardiovascular disease; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

DNA Extraction and Genotyping Assays

DNA was extracted from whole blood as previously described.[20] Participants were genotyped for rs599839, rs9818870, and rs17465637 in 5-μL reaction volumes using allele-specific TaqMan genotyping probes (Applied Biosystems; Carlsbad, CA) and 100 ng of genomic DNA. Twenty-five percent of samples were reassayed and were 99% concordant with the original genotype calls. Samples for which concordance was not obtained were excluded from the study.

Statistical Analyses

For each single-nucelotide polymorphism (SNP), we identified the genetic model (dominant, additive, or recessive) based on the model that showed the strongest and most consistent associations with baseline patient characteristics and cardiovascular outcomes across all 3 cohorts. As a result, associations between rs599839, rs9818870, and rs17465637 and baseline patient characteristics were reported for a dominant genetic model. For genotype associations with continuous variables, regression coefficients (b) and 95% CIs were determined, and for discrete variables, odds ratios (ORs) and 95% CIs were reported. Hormone data exhibited a skewed distribution and were log-transformed before analysis. The primary end point was the composite end point death and/or readmission to the hospital for a CVD event. The CVD diagnoses combined into this composite outcome measure included unstable angina, STEMI, NSTEMI, heart failure, ischemic stroke, and hemorrhagic stroke. Secondary associations between genotype and readmission for NSTEMI (the most common readmission event type) were explored, as was the association with cardiovascular event rate.

The association between each polymorphism and outcome was assessed using Kaplan-Meier survival analyses and the log-rank test statistic. If significant associations were observed in univariate Kaplan-Meier survival analyses, Cox proportional hazards multivariate analysis was performed to test for independent genotype associations. To increase precision by reducing the residual variation and increasing the power to detect SNP associations, these analyses adjusted for established predictors of increased risk. Where the data allowed, the same covariates were included for each outcome, cohort, and SNP to ensure consistency among models. The established predictors of risk included in multivariate analyses were as follows:

- CDCS and PMI cohorts: age of disease onset, sex, ethnicity, history of MI, history of hypertension, history of high cholesterol, history of type 2 diabetes, history of heart failure, β-blocker treatment, smoking status, body mass index, estimated glomerular filtration rate, LV ejection fraction, and N-terminal pro-B-type natriuretic peptide.
- HV cohort: age, sex, ethnicity, hypertension, high cholesterol, and smoking status.

Interestingly, LV ejection fraction was not a significant predictor of outcome in multivariate survival analysis. However, when entered into a model on its own, LV ejection fraction was a significant predictor of death/CVD readmission in both the CDCS (OR, 0.99; 95% CI, 0.98–0.99; $P=1.0\times10^{-8}$) and the PMI (OR, 0.99; 95% CI, 0.98–1.00; $P=0.002$) cohorts, suggesting that it is not independent of or as strong a prognostic marker as other established predictors of increased risk (eg, N-terminal pro-B-type natriuretic peptide).

Diagnoses at each readmission were defined using the ICD-10. Outcome data were obtained from NZHIS and hospital PMS databases. The power of this study to assess the relationship between genotype and cardiovascular outcomes was estimated assuming 80% power and 2-tailed $\alpha=0.05$. Adopting a conservative approach, this resulted in power to detect a hazard ratio of $\leq$1.54 in the HV cohort, $\leq$1.47 in the CDCS cohort, and $\leq$1.90 in the PMI cohort as being statistically significant. Analyses were performed using PASW version 18.0 (SPSS Inc; Chicago, IL) statistical software.

Results

Baseline Cohort Characteristics and Genotype Frequencies

Baseline characteristics of each cohort are shown in Table 1 and genotype frequencies in Table 2. There was a significant

Table 2. Genotype Frequencies of rs599839, rs17465637, and rs9818870

	rs599839				rs17465637				rs9818870			
	n	AA	AG	GG	n	CC	AC	AA	n	CC	CT	TT
HV	1633	1016 (62.2)	525 (32.1)	92 (5.6)	1636	832 (50.9)	669 (40.9)	135 (8.2)	1649	1209 (73.3)	400 (14.3)	40 (2.4)
CDCS	1774	1110 (62.6)	562 (31.7)	102 (5.7)	1794	1001 (55.8)	650 (36.2)	143 (8.0)	1784	1265 (70.9)	472 (26.5)	47 (2.6)
PMI	858	549 (64.0)	271 (31.6)	38 (4.4)	901	512 (56.8)	326 (36.2)	63 (3.4)	905	646 (71.4)	236 (26.1)	23 (2.5)

Data are presented as n (%). CDCS indicates Coronary Disease Cohort Study; HV, Canterbury Healthy Volunteer study; PMI, Post-Myocardial Infarction study.

difference in 1q41 (rs17465637) genotype frequency among study groups, with the CC genotype more prevalent in the CDCS cohort (P=0.005) and the PMI cohort (P=0.002) compared with the HV cohort. These associations remained significant when individuals of European ancestry were analyzed separately ($P \leq 0.014$). There was no difference in genotype frequencies across cohorts for 1p13.3 (rs599839) (P=0263–0.901) or 3q22.2 (rs9818870) (P=0.134–0.338).

Associations Between Genotype and Baseline Patient Characteristics

rs599839 A/G (1p13.3)

Within the HV cohort, study participants carrying 1 or more 1p13.3 rs599839 G allele had a lower prevalence of high cholesterol (OR, 0.60; 95% CI, 0.46–0.78; P=0.0001) and family history of MI (OR, 0.81; 95% CI, 0.66–0.99; P=0.043) than those carrying the AA genotype (Table 3). These findings were robustly replicated in CDCS participants (OR, 0.75; 95% CI, 0.62–0.92; P=0.005 for high cholesterol, and OR, 0.78; 95% CI, 0.63–0.97; P=0.026 for history of MI). Furthermore, when adjusted for age, sex, and ethnicity, CDCS AG/GG allele carriers had less adverse remodeling, having, on average, a greater LV ejection fraction (b=1.61; 95% CI, 0.37–2.86; P=0.011) and a smaller LV internal dimension in diastole (b=−1.00; 95% CI, −1.71 to −0.29; P=0.006) and systole (b=−1.08; 95% CI, −1.94 to −0.23; P=0.013) and LV end-diastolic (b=−5.29; 95% CI, −9.66 to −0.92; P=0.018) and end-systolic (b=−4.53; 95% CI, −8.00 to −1.07; P=0.010) volumes at the baseline visit (Table 4). The genotype-related differences in these indicators of cardiac structure and function remained significant at both 4 months and 12 months postrecruitment into the study (P=0.001–0.042) (data not shown).

Consistent with these findings, PMI patients carrying the AG/GG genotypes had reduced total cholesterol (b=−0.32; 95% CI, −0.55 to −0.11; P=0.004) and LDL cholesterol (b=−0.20, 95% CI, −0.39 to −0.02; P=0.031) and significantly smaller LV internal dimension in systole (b=−0.18; 95% CI, −0.31 to −0.05; P=0.008) and diastole (b=−0.14; 95% CI, −0.26 to −0.02; P=0.024) and LV end-diastolic (b=−8.83; 95% CI, −16.11 to −1.55; P=0.018) and end-systolic (b=−7.37; 95% CI, −12.92 to −1.81; P=0.009) volumes compared with those carrying the AA genotype after adjustment for age, sex, and ethnicity (Table 5). In addition, PMI AG/GG carriers were, on average, older when recruited into the study (b=1.58; 95% CI, 0.06–3.11; P=0.042). There were no significant differences in 1p13.3 genotype frequency with sex, ethnicity, or pharmacotherapy in the CDCS and PMI cohorts; however, HV participants carrying the minor 'G' allele were more likely to be treated with a statin (P=0.0002).

rs17465637 A/C (1q41)

Although there were no consistent associations between 1q41 (rs17465637) genotype and baseline patient characteristics across the cohorts, univariate analyses revealed that PMI 1q41 A allele carriers had higher measured systolic blood pressure (b=2.56; 95% CI, 0.32–4.80; P=0.025), and fewer such carriers had dyslipidemia (OR, 0.71; 95% CI, 0.53–0.95; P=0.021) (Table 5). There were no associations between 1q41 and baseline cohort characteristics in the HV or CDCS cohorts (Tables 3 and 4). No difference in 1q41 genotype frequency with sex or ethnicity were observed; however, in the PMI cohort, 1q41 'A' allele carriers were less likely to be treated with a lipid-lowering drug (P=0.024).

rs9818870 C/T (3q22.3)

In the CDCS cohort, associations were found between the 3q22.3 rs9818870 T allele and a greater age at the time of recruitment to the cohort (b=1.94; 95% CI, 0.69–3.19; P=0.002) and higher high-density lipoprotein cholesterol (b=0.10; 95% CI, 0.02–0.18; P=0.019) (Table 4). In the PMI cohort, the 3q22.3 T allele was associated with greater total cholesterol (b=0.28; 95% CI, 0.05–0.50; P=0.016) (Table 5). There were no associations between 3q22.3 genotype and HV and PMI baseline characteristics (Tables 3 and 5). No significant difference in pharmacotherapy was observed in the HV and PMI cohorts; however, in the CDCS cohort, fewer T allele carriers were treated with a statin (P=0.008). The T allele was more common in HV male than female participants (P=0.009) and occurred at a lower frequency in CDCS participants of Maori/Pacific Island ancestry (P=0.039).

Associations Between Genotype and Cardiovascular Outcomes

Throughout follow-up, the number of participants experiencing a CVD event for each cohort were as follows: HV cohort, 6.2% (n=100) died and 12.8% (n=207) were admitted for a CVD event, 0.8% (n=13) for unstable angina, 2.6% (n=42) for NSTEMI, 0.8% (n=13) for STEMI, 1.6% (n=26) for heart failure, 1.4% (n=22) for ischemic stroke, and 0.5% (n=8) for hemorrhagic stroke; CDCS cohort, 16.2% (n=347) died and 61.5% (n=1314) were readmitted for a CVD event, 17.1% (n=377) for unstable angina, 22.0% (n=470) for NSTEMI, 5.5% (n=117) for STEMI, 16.1% (n=345) for heart failure, 3.3% (n=70) for ischemic stroke, and 0.9% (n=19) for hemorrhagic stroke; and PMI cohort, 16.2% (n=327) died and 76.0% (n=687) were readmitted for a

Table 3. Association Between rs599839, rs17465637, rs9818870 and HV Baseline Patient Characteristics

	rs599839			rs17465637			rs9818870		
	AA	AG/GG	*P*	CC	AC/AA	*P*	CC	CT/TT	*P*
Age, y	64.5±10.2	65.1±10.7	0.442	65.1±10.4	64.4±10.3	0.150	64.7±10.4	64.6±10.5	0.865
Male/female sex, n	693/306	406/109	0.448	554/262	545/239	0.484	787/393	319/115	0.009*
Ethnicity									
European	892 (62.4)	538 (37.6)	0.716	730 (51.0)	702 (49.0)	0.783	1058 (73.3)	386 (26.7)	0.238
Maori/Pacific Island	16 (64.0)	9 (36.0)		11 (47.8)	12 (52.2)		22 (91.7)	2 (8.3)	
Asian	15 (100)	0 (0)		5 (35.7)	9 (64.3)		14 (93.3)	1 (6.7)	
Other/unknown	74 (57.8)	54 (42.2)		69 (53.5)	60 (46.5)		85 (65.9)	44 (34.1)	
Systolic blood pressure, mm Hg	134.3±19.2	134.4±20.9	0.913	134.8±19.1	133.9±20.6	0.354	134.3±19.5	134.5±20.9	0.804
Diastolic blood pressure, mm Hg	78.8±10.8	79.0±11.8	0.729	78.9±10.6	78.9±11.8	0.949	78.8±11.1	79.2±11.4	0.552
Medical/social history, %									
Hypertension (Y)	30.1	28.2	0.407	29.2	29.3	0.984	29.4	29.3	0.950
Dyslipidemia (Y)	24.2	16.0	0.0001*	20.4	21.8	0.497	21.5	20.1	0.572
Family history of MI (Y)	45.0	39.8	0.043*	44.3	42.5	0.469	42.5	44.8	0.417
Biochemistry									
NT-proBNP, pmol/L†‡	17.7 (16.8–18.7)	19.4 (18.1–20.7)	0.124	19.1 (18.0–20.2)	17.6 (16.6–18.7)	0.252	18.6 (17.7–19.5)	17.6 (16.2–19.2)	0.514
Drug treatment, %									
β-blocker (Y)	3.1	3.5	0.671	3.2	3.2	0.997	3.2	3.2	0.998
ACE inhibitor (Y)	12.4	10.2	0.188	11.2	11.3	0.962	11.4	11.5	0.932
Statin (Y)	8.8	4.0	0.0002*	7.4	6.8	0.644	7.8	5.1	0.057
Diuretic (Y)	10.2	10.5	0.815	10.5	10.0	0.758	10.5	9.5	0.528

Data are presented as mean±SD or n (%), unless otherwise indicated. ACE indicates angiotensin-converting enzyme. Other abbreviations as in Tables 1 and 2. Y indicates yes.

*Statistical significance $P<0.05$.

†Data are presented as geometric mean and 95% CI.

‡Analysis adjusted for age, sex, and ethnicity.

Table 4. Association Between rs599839, rs17465637, rs9818870 and CDCS Baseline Patient Characteristics

	rs599839			rs17465637			rs9818870		
	AA	AG/GG	*P*	CC	AC/AA	*P*	CC	CT/TT	*P*
Age, y	67.0±12.4	67.6±11.7	0.332	66.7±12.3	67.9±12.0	0.050	66.7±12.2	68.6±12.2	0.002*
Male/female sex, n	776/324	477/183	0.439	702/289	565/224	0.753	898/358	359/155	0.487
Ethnicity									
European	903 (61)	572 (39)	0.069	840 (56.3)	653 (43.3)	0.148	1032 (69.5)	452 (30.5)	0.039*
Maori/Pacific Island	75 (81)	18 (19)		56 (57.7)	41 (42.3)		83 (84.7)	15 (15.3)	
Asian	27 (63)	16 (37)		18 (42.9)	24 (57.1)		35 (83.3)	7 (16.7)	
Other/unknown	79 (66)	40 (34)		61 (51.3)	58 (48.7)		85 (73.3)	31 (26.7)	
Systolic blood pressure, mm Hg	129.1±22.0	129.4±21.4	0.780	128.4±21.3	130.0±22.1	0.118	129.4±21.8	128.7±21.5	0.516
Diastolic blood pressure, mm Hg	74.7±12.0	74.8±12.2	0.814	74.9±12.0	74.4±12.1	0.423	74.8±12.1	74.5±11.9	0.574
Medical/social history, %									
Hypertension (Y)	52.2	53.8	0.510	52.4	53.9	0.543	52.3	54.6	0.368
Dyslipidemia (Y)	58.7	51.7	0.005*	56.3	55.6	0.769	56.0	56.3	0.926
MI (Y)	32.9	27.8	0.026*	31.5	30.3	0.594	29.9	33.4	0.147
Biochemistry									
Total cholesterol, mmol/L	4.9±1.2	4.9±1.2	0.490	4.9±1.2	4.9±1.2	0.929	4.9±1.2	4.9±1.2	0.936
LDL cholesterol, mmol/L	3.0±1.0	2.9±1.1	0.410	3.0±1.1	3.0±1.0	0.537	3.0±1.0	3.0±1.1	0.972
HDL cholesterol, mmol/L	1.2±0.6	1.3±0.8	0.062	1.3±0.8	1.2±0.6	0.376	1.2±0.6	1.3±0.8	0.019*
NT-proBNP, pmol/L†‡	79.2 (73.4–85.3)	76.3 (69.3–84.2)	0.059	76.6 (70.8–82.8)	81.0 (74.0–88.5)	0.794	75.4 (70.1–81.1)	83.3 (75.2–92.3)	0.951
Echocardiography‡									
LVEF, %	56.4±12.6	57.8±11.5	0.011*	56.6±12.4	57.4±12.1	0.090	56.8±12.2	57.6±12.0	0.199
LVIDs, mm	37.0±8.9	36.1±7.8	0.013*	37.0±8.8	36.1±8.0	0.013*	36.6±8.2	36.3±8.8	0.496
LVIDd, mm	54.1±7.4	53.2±7.0	0.006*	54.1±7.4	53.3±7.0	0.022*	53.7±7.1	53.6±7.3	0.954
LVESV, mL	50.9±31.8	46.7±29.2	0.010*	49.6±31.8	48.5±28.9	0.501	49.3±29.4	48.0±31.9	0.410
LVEDV, mL	112.9±40.4	107.8±40.0	0.018*	111.3±40.9	110.1±38.5	0.591	111.2±38.7	108.8±41.2	0.369
Drug treatment, %									
β-blocker (Y)	86.7	85.2	0.373	86.4	86.0	0.775	85.7	87.7	0.275
ACE inhibitor (Y)	54.2	56.9	0.287	55.4	55.7	0.875	55.2	55.9	0.777
Statin (Y)	87.4	84.6	0.100	85.9	86.7	0.627	88.0	83.2	0.008*
Diuretic (Y)	26.5	27.7	0.602	27.2	27.3	0.954	26.9	27.6	0.762

Data are presented as mean±SD or n (%), unless otherwise indicated. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; LVIDd, left ventricular internal dimension end diastole; LVIDs, left ventricular internal dimension end systole; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume. Other abbreviations as in Tables 1 through 3. Y indicates yes.

*Statistical significance $P<0.05$.

†Geometric mean and 95% CI.

‡Analysis adjusted for age, sex, and ethnicity.

Table 5. Association Between rs599839, rs17465637, rs9818870 and PMI Baseline Patient Characteristics

	rs599839			rs17465637			rs9818870		
	AA	AG/GG	*P*	CC	AC/AA	*P*	CC	CT/TT	*P*
Age, y	61.6±10.4	63.1±9.8	0.042*	62.2±10.5	62.2±10.3	0.986	62.5±10.7	61.6±9.9	0.266
Male/female sex, n	396/101	221/57	0.952	372/96	279/69	0.810	474/114	178/53	0.256
Ethnicity									
European	350 (64.1)	196 (35.9)	0.852	318 (56.8)	242 (43.2)	0.513	395 (70.0)	169 (30.0)	0.362
Maori/Pacific Island	10 (83.3)	2 (16.7)		7 (58.3)	5 (41.7)		11 (91.7)	1 (8.3)	
Asian	31 (64.6)	17 (35.4)		27 (51.9)	25 (48.1)		41 (78.8)	11 (21.2)	
Other/unknown	7 (53.8)	6 (46.2)		7 (53.8)	6 (46.2)		7 (53.8)	6 (46.2)	
Systolic blood pressure, mm Hg	117.0±15.5	117.5±16.2	0.636	116.0±15.7	118.6±16.1	0.025*	117.1±16.0	116.9±15.7	0.863
Diastolic blood pressure, mm Hg	66.7±10.1	67.3±10.0	0.407	66.7±9.6	67.1±10.5	0.586	66.7±10.1	67.1±9.8	0.653
Medical/social history, %									
Hypertension (Y)	37.0	40.3	0.370	38.0	38.2	0.957	38.9	36.4	0.494
Dyslipidemia (Y)	39.4	34.2	0.147	39.7	31.9	0.021*	36.9	35.1	0.623
MI (Y)	17.9	16.2	0.544	16.2	18.4	0.420	17.3	16.9	0.874
Biochemistry									
Total cholesterol, mmol/L	6.0±1.3	5.7±1.1	0.004*	5.9±1.2	5.9±1.3	0.691	5.8±1.2	6.1±1.2	0.016*
LDL cholesterol, mmol/L	4.0±1.1	3.8±1.0	0.031*	3.9±1.0	3.9±1.1	0.503	3.9±1.1	4.0±1.1	0.192
NT-proBNP, pmol/L†‡	107.2 (100.2–114.8)	115.6 (105.6–126.3)	0.944	111.6 (103.9–120.1)	110.5 (102.1–119.7)	0.959	110.6 (103.8–117.9)	113.6 (102.9–125.3)	0.384
Echocardiography‡									
LVEF, %	41.5±10.5	42.3±10.5	0.369	40.5±11.0	42.7±10.1	0.428	41.6±10.7	41.4±10.3	0.385
LVIDS, mm	38.3±7.8	37.1±6.8	0.008*	38.4±7.6	37.5±7.6	0.479	38.4±7.8	37.2±7.1	0.338
LVIDD, mm	53.2±6.8	52.4±6.2	0.024*	53.3±6.6	53.8±6.8	0.226	53.3±6.7	52.6±6.4	0.779
LVESV, mL	67.0±36.2	61.4±27.1	0.009*	67.4±35.0	63.8±32.4	0.284	67.5±35.5	62.0±28.5	0.374
LVEDV, mL	139.8±41.2	134.5±36.8	0.018*	139.8±39.8	137.0±40.8	0.501	139.9±40.8	136.0±38.9	0.757
Drug treatment,%									
β-blocker	69.6	72.4	0.471	73.9	67.7	0.084	70.9	73.0	0.638
ACE inhibitor	39.0	41.5	0.557	42.1	39.6	0.525	41.1	40.8	1.000
Lipid lowering	47.9	46.8	0.764	49.4	41.4	0.024*	46.1	46.8	0.864

Data are presented as mean±SD or n (%), unless otherwise indicated. Abbreviations as in Tables 1 through 4. Y indicates yes.
*Statistical significance $P<0.05$.
†Geometric mean and 95% CI.
‡Analysis adjusted for age, sex, and ethnicity.

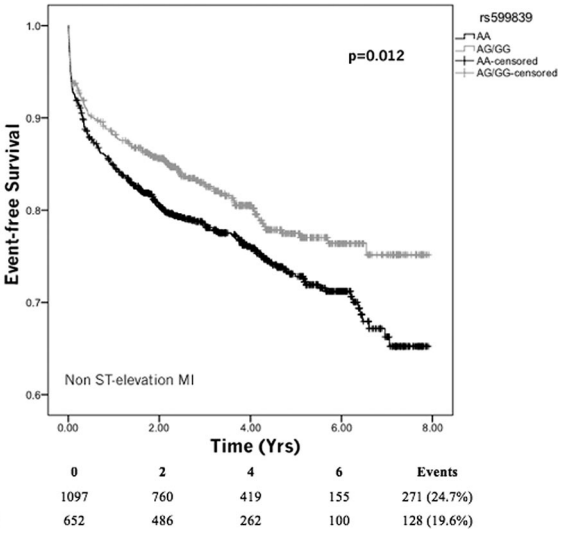

Figure 1. Association between SNP rs599839 and readmission for non-ST-segment elevation MI in the Coronary Disease Cohort Study cohort. MI indicates myocardial infarction.

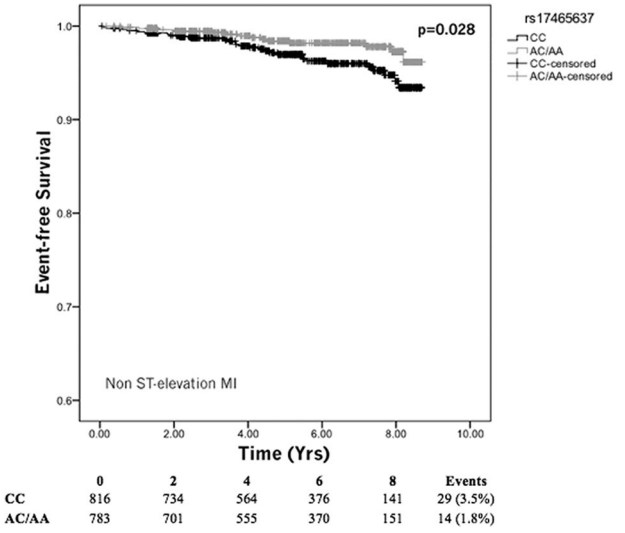

Figure 2. Association between SNP rs17465637 and death/CVD readmission in Post-Myocardial Infarction study patients. CVD indicates cardiovascular disease.

CVD event, 25.9% (n=234) for unstable angina, 16.3% (n=147) for NSTEMI, 29.4% (n=266) for STEMI, 20.6% (n=186) for heart failure, 5.3% (n=480) for ischemic stroke, and 1.4% (n=13) for hemorrhagic stroke.

The average number of CVD admissions for HV participants was 0.19 (range, 0–13 admissions). CDCS patients were readmitted an average of 2.09 times (range, 0–30 readmissions), and PMI patients were readmitted an average of 2.57 times (range, 0–40 readmissions) throughout the follow-up.

rs599839 A/G (1p13.3)

Within the CDCS cohort, the 1p13.3 rs599839 'G' allele was associated with a reduced risk of readmission for NSTEMI (log-rank P=0.012) (Figure 1) and a lower CVD readmission event rate (AA, 0.55 events/person per year; AG/GG, 0.49 events/person per year; P=0.003). The association with NSTEMI was independent of established predictors of increased risk (OR, 0.75; 95% CI, 0.57–0.99; P=0.044) (online-only Data Supplement Table I). There were no associations between 1p13.3 genotype and cardiovascular outcomes in the HV and PMI cohorts.

rs17465637 A/C (1q41)

Within the PMI cohort, 1q41 was associated with survival/readmission, with the A allele conferring a protective effect (log-rank P=0.008) (Figure 2). The association with outcome was independent of established risk predictors (OR, 0.72; 95% CI, 0.54–0.96; P=0.024) (online-only Data Supplement Table II). Consistent with this, in the HV cohort, rs17464637 was associated with admission for NSTEMI (log-rank P=0.028) (Figure 3). Fewer study participants carrying the AC/AA genotypes were admitted for NSTEMI throughout follow-up than those carrying the CC genotype. There were no associations between 1q41 and cardiovascular outcomes in the CDCS cohort.

rs9818870 C/T (3q22.3)

In the HV cohort, a significant association between 3q22.2 and survival/admission was observed (log-rank P=0.045) (Figure 4). Those participants carrying 1 or more T allele were more likely to have died or experienced a CVD event than those with the CC genotype. Cox proportional hazards analysis revealed that the association between 3q22.3 and survival/admission was independent of established predictors of increased risk (OR, 1.30; 95% CI, 1.01–1.70; P=0.043) (online-only Data Supplement Table III). There were no significant associations between 3q22.3 and cardiovascular outcomes in either the CDCS or the PMI patient cohorts.

Discussion

In recent years, GWAS have made significant advances in identifying chromosome loci associated with the genetic risk of CAD. However, once CAD is established, whether these variants are associated with greater disease progression and poorer outcomes remains largely unknown. The present data

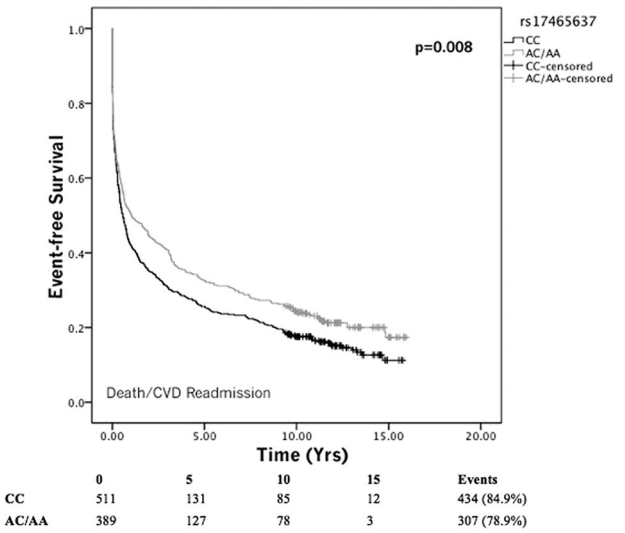

Figure 3. Association between SNP rs17465637 and hospital admission for non-ST-segment elevation MI in the Healthy Volunteer cohort. Abbreviation as in Figure 1.

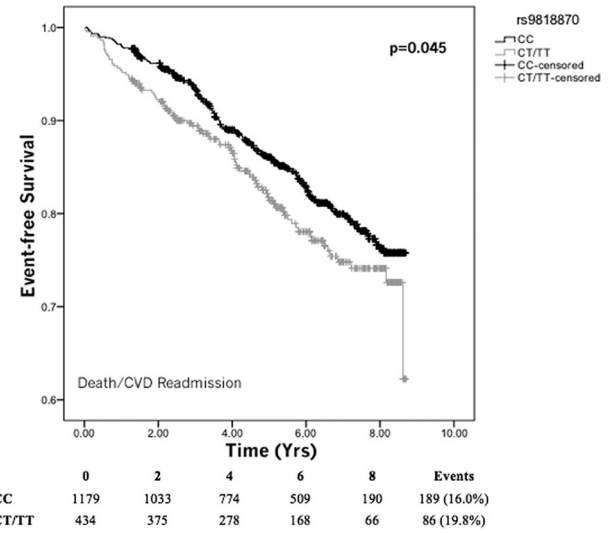

Figure 4. Association between SNP rs9818870 and death/CVD admission in the Healthy Volunteer cohort. Abbreviation as in Figure 2.

suggest that gene variants within the genomic risk loci at 1p13.3, 1q41, and 3q22.3 previously associated in GWAS with the risk of developing CAD may also be associated with subsequent cardiovascular outcomes, including mortality and hospital admission for CVD events in established heart disease. In addition, we confirmed previously diagnosed associations between 1p13.3 and cholesterol and between 3q22.3 and increased risk of CAD in a prospective study of individuals without previous heart disease. These findings suggest that some genomic risk variants not only may be important risk factors for CAD development, but also may influence disease progression once CAD is established.

The 1p13.3 variant rs599839, located in a large 97-kb block of fragmented disequilibrium, has been associated with a significant reduction in CAD and MI risk in several studies, and a 41% reduction of CAD risk has been reported when comparing GG with AA homozygotes.[2,5,8–10] In the current study, we confirmed previous associations among the rs599839 G allele, lipid levels, and MI in both patients with CAD and healthy controls.[2,5,8–13] We also observed significant differences in cardiac function with the 1p13.3 genotype, with less LV remodeling observed in patients carrying the rs599839 G allele. Moreover, in the CDCS cohort, G allele carriers were less likely to experience a subsequent NSTEMI during follow-up, independent of established predictors of risk, and had a lower CVD event readmission rate over a median of 3.7 years follow-up.

The genomic region at 1p13.3 contains 4 genes: proline/serine-rich coiled protein 1 (*PSRC1*), cadherin EGF LAG sevenpass G-type receptor 2 (*CELSR2*), myosin-binding protein H-like (*MYBHL*), and sortilin 1 (*SORT1*). Although 4 genes are located within the 1p13.3 locus, *SORT1* has emerged as the most likely candidate causal gene. *SORT1* is a transmembrane protein receptor that binds a variety of ligands and is involved in the endocytosis and degradation of lipoprotein lipase, a rate-limiting enzyme for the hydrolysis of triglycerides in lipoproteins.[21] More recently, sortilin has been connected to the endocytosis of apoA-V-containing chylomicrons.[22] Because LDL plays a causal role in the development of CVD, it has been hypothesized that increased sortilin expression in G allele carriers leads to greater LDL tissue uptake, which results in reduced circulating LDL levels and, subsequently, a lower CAD risk.[23] However, because most studies have demonstrated only a modest reduction in LDL cholesterol in GG homozygotes, it is likely that this does not fully explain the significant reductions in CAD risk that have been observed.

Chromosome 1q41 (rs17465637) has been associated with CAD and MI in multiple GWAS, with the more frequent C allele increasing CAD risk by ≈14%.[5,8,14,15] In the current study, an independent association was observed with survival/readmission in the PMI cohort, and significantly fewer HV participants carrying AC/CC experienced an NSTEMI throughout follow-up compared with CC carriers. The variant rs17465637 lies in intron 4 of the melanoma inhibitory activity family member 3 gene (*MIA3*). *MIA3* has a number of important biological functions and is linked to collagen processing and collagen VII secretion, factors that are critically involved in the response to cardiovascular injury.[24]

Early GWAS tended to focus on just a few chromosomal regions (including 9p21.3 and 1p13) with the strongest signals. The genomic risk locus at 3q22.3 was identified in a GWAS for MI using a less stringent cutoff for association.[14] Within this genomic locus, SNP rs9818870 was identified as the lead SNP, with the C allele conferring an increased risk of 15% for CAD.

In the current study, we observed an association between 3q22.3 and death/admission for a CVD event in the HV cohort. Although participants within this study were free of overt CVD at the time of recruitment, carriers of the 3q22.3 rs9818870 T allele were more likely to have died or experienced a CVD event during the follow-up period. These participants also experienced a greater CVD readmission rate than AA individuals.

The lead SNP rs9818870 within 3q22.3 is located in the 3′ untranslated region of *MRAS* and is near a cluster of miRNA-binding sites. *MRAS* is approximately 33 kb in length and contains 5 exons. Studies in mice have demonstrated that M-ras is involved in tumor necrosis factor-α-stimulated LFA-1 activation in splenocytes, suggesting that M-ras could play a role in the atherosclerosis process through adhesion signaling.[25] The M-ras protein is broadly expressed in tissues and occurs at high levels in the cardiovascular system, particularly in the heart.

The main limitation of the current study is the potential for type 1 error because of multiple comparisons. However, this was not a screening study of newly identified SNPs; we were in the main testing SNPs that have previously been robustly associated with the risk of CVD and its associated disorders in large international cohorts. In addition to confirming many of these previous findings, this study tested the next logical phase in establishing the risk of these SNPs on cardiovascular outcomes in both healthy controls and patients with established coronary heart disease.

The independent association between both 1p13.3 (rs599839) and 1q41 (rs17465637) with cardiovascular outcomes in patients with coronary heart disease suggests that

these risk loci may influence disease progression once CAD is established. For SNP rs599839, the association with outcome may be explained in part through protection against adverse LV remodeling. The association between rs9818870 and the primary end point of death/admission for a CVD event in the HV cohort confirms 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease. Further work is required to elucidate the mechanisms by which these regions contribute to the development of CAD.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The genomic risk loci at 1p13.3, 1q41, and 3q22.3 have been strongly associated with the risk of developing coronary heart disease in many large genome-wide association and cohort studies. This study investigated whether these important coronary artery disease risk variants may also contribute to disease progression and poorer outcomes in patients with established cardiovascular disease. Whether these risk loci also increase the risk of subsequently experiencing a cardiovascular event in individuals with no known overt cardiovascular disease at the time of recruitment also was assessed. The findings from this study support previous associations between 1p13.3 and myocardial infarction and lipid levels. Extending these findings, we observed in patients with coronary heart disease an association between 1p13.3 and readmission for non ST-segment elevation MI and between 1q41 and the composite end point of death/cardiovascular disease readmission. The coronary artery disease risk locus at 3q22.3 was associated with subsequent survival/admission for cardiovascular disease event in individuals who were free of overt coronary artery disease at the time of study inclusion. These findings provide further evidence for an important role of 1p13.3, 1q41, and 3q22.3 in coronary artery disease. Furthermore, this study suggests that variants at 1p13.3 and 1q41 are independently associated with clinical outcomes in patients with established coronary heart disease and confirms 3q22.3 as a predictor of cardiovascular risk in individuals free of overt heart disease.

SUPPLEMENTAL MATERIAL

Supplementary Table 1 Cox Proportional Hazards analysis of candidate predictive factors (including rs599839 genotype) for non ST-elevation MI in the CDCS cohort

Variable	Risk Ratio	95% CI	*p*
Age	1.02	1.01-1.03	**0.007***
Gender	0.93	0.65-1.34	0.709
Ethnicity	-	-	**0.039***
Maori/Pacific Island vs. European	1.27	0.74-2.16	
Asian vs. European	2.55	1.32-4.91	
Other/Unknown vs. European	1.02	0.62-1.68	
History of Myocardial Infarction	1.50	1.12-2.00	**0.006***
History of Hypertension	1.16	0.88-1.52	0.299
History of High Cholesterol	0.89	0.68-1.18	0.418
History of type 2 diabetes	1.16	0.84-1.60	0.370
History of Heart Failure	1.52	1.04-2.21	**0.030***
Smoking Status	-	-	0.570
Current Smoker vs Never Smoked	1.19	0.68-2.09	
Ex-Smoker vs Never Smoked	1.16	0.87-1.53	
B-blocker treatment	1.01	0.70-1.45	0.962
Waist/Hip ratio	0.23	0.03-2.24	0.229
eGFR (MDRD)	1.00	0.99-1.00	0.477
LVEF	1.00	0.99-1.02	0.456
NT-proBNP (pmol/L)	2.06	1.40-3.03	**<0.001***
rs599839	0.75	0.57-0.99	**0.044***

Hormone data were log transformed before analysis

* Statistical significance $p<0.05$

Supplementary Table 2 Cox Proportional Hazards analysis of candidate predictive factors (including rs17465637 genotype) for survival/readmission in the PMI cohort

Variable	Risk Ratio	95% CI	*p*
Age	1.00	0.98-1.02	0.922
Gender	1.00	0.67-1.49	0.995
Ethnicity	-	-	0.122
Maori/Pacific Island vs. European	1.41	0.46-4.23	
Asian vs. European	0.46	0.22-0.96	
Other/Unknown vs. European	0.47	0.11-1.93	
History of Myocardial Infarction	1.05	0.75-1.47	0.768
History of Hypertension	1.12	0.83-1.52	0.465
History of High Cholesterol	0.95	0.70-1.29	0.754
History of type 2 diabetes	1.01	0.65-1.56	0.980
History of Heart Failure	0.90	0.61-1.34	0.603
Smoking Status	-	-	0.381
Current Smoker vs Never Smoked	1.27	0.91-1.78	
Ex-Smoker vs Never Smoked	1.14	0.79-1.65	
B-blocker treatment	0.73	0.51-1.04	0.083
Body Mass Index	0.99	0.95-1.03	0.504
eGFR (MDRD)	1.00	0.99-1.00	0.383
LVEF	0.99	0.98-1.01	0.214
NT-proBNP (pmol/L)	1.19	0.72-1.96	0.497
rs17465637	0.72	0.54-0.96	**0.024***

Hormone data were log transformed before analysis

* Statistical significance $p<0.05$

Supplementary Table 3 Cox Proportional Hazards analysis of candidate predictive factors (including rs9818870 genotype) for survival/CVD admission in the Healthy Volunteer cohort

Variable	Risk Ratio	95% CI	*p*
Age	1.08	1.06-1.10	**<0.001***
Gender	0.64	0.49-0.85	**0.002***
Ethnicity	-	-	0.788
Maori/Pacific Island vs. European	1.64	0.61-4.47	
Asian vs. European	1.23	0.30-4.99	
Other/Unknown vs. European	1.04	0.66-1.65	
History of Hypertension	1.58	1.23-2.02	<0.001
History of High Cholesterol	1.11	0.83-1.48	0.499
Smoking Status			0.811
Current Smoker vs Never Smoked	1.13	0.66-1.94	
Ex-Smoker vs Never Smoked	1.08	0.83-1.41	
rs9818870	1.31	1.01-1.70	**0.043***

* Statistical significance $p<0.05$