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ORIGINAL ARTICLE

Genetic variation in the renin–angiotensin–aldosterone system is associated with cardiovascular risk factors and early mortality in established coronary heart disease

KL Ellis[1], BR Palmer[1], CM Frampton[1], RW Troughton[1], RN Doughty[2], GA Whalley[2,3], CJ Ellis[2], AP Pilbrow[1], L Skelton[1], TG Yandle[1], AM Richards[1] and VA Cameron[1]

This study examined renin–angiotensin–aldosterone (RAAS) system gene variants for associations with cardiovascular risk factors and outcomes in coronary heart disease. Coronary disease patients ($n = 1186$) were genotyped for 21 single-nucleotide polymorphisms (SNPs) within angiotensinogen (*AGT*), angiotensin-converting enzyme (*ACE*), angiotensin-II type-1 receptor (*AGTR1*) and aldosterone synthase (*CYP11B2*). Associations with all-cause mortality and cardiovascular readmissions were assessed over a median of 3.0 years. The *AGT* M235T 'T' allele was associated with a younger age of clinical coronary disease onset ($P = 0.006$), and the *AGT* rs2478545 minor allele was associated with lower circulating natriuretic peptides ($P = 0.0001$–$P = 0.001$) and E/E[1] ($P = 0.018$). Minor alleles of *AGT* SNPs rs1926723 and rs11122576 were associated with more frequent history of renal disease ($P \leqslant 0.04$) and type-2 diabetes ($P \leqslant 0.02$), higher body mass index ($P \leqslant 0.02$) and greater mortality ($P \leqslant 0.007$). *AGT* rs11568054 minor allele carriers had more frequent history of renal disease ($P = 0.04$) and higher plasma creatinine ($P = 0.033$). *AGT* rs6687360 minor allele carriers exhibited worse survival ($P = 0.02$). *ACE* rs4267385 was associated with older clinical coronary disease onset ($P = 0.008$) and hypertension ($P = 0.013$) onset, increased plasma creatinine ($P = 0.01$), yet greater mortality ($P = 0.044$). Less history of hypertension was observed with the *AGTR1* rs12685977 minor allele ($P = 0.039$). Genetic variation within the RAAS was associated with cardiovascular risk factors and accordingly poorer survival.

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INTRODUCTION

Global trends predict a steady rise in cardiovascular disease in the next decade, and by 2020 coronary heart disease is predicted to be the leading cause of death worldwide.[1] Although lifestyle is established as having an important role,[2] the influence of genetics on susceptibility to coronary heart disease has been demonstrated in many large candidate gene[3] investigations and more recently genome-wide association studies.[4] Cardiovascular disease likely arises as a result of complex interactions between multiple genetic and environmental factors each conferring a small cumulative increase in risk.

The renin–angiotensin–aldosterone (RAAS) system has a central role in the regulation of renal sodium and water absorption, blood pressure, the control of thirst, cardiac function and cellular growth,[5] and has been established as one of the most important systems in the pathogenesis of coronary heart disease. With cardiac injury and impaired cardiac output the RAAS is inappropriately activated leading to vasoconstriction and deleterious increases in peripheral vascular resistance, retention of sodium and water and damaging hypertrophic and fibrotic effects on the heart and vasculature. Drugs that target the RAAS cascade currently constitute a central pillar of cardiovascular therapeutics.[6]

In the classical RAAS, circulating renin metabolizes angiotensinogen (AGT), produced predominantly in the liver to form the decapeptide angiotensin-I (Ang-I). Inactive Ang-I is hydrolyzed by angiotensin-converting enzyme (ACE), removing the C-terminal dipeptide and leaving the biologically active and potent vascoconstrictor octapeptide Angiotensin-II (Ang-II (1–8)). Ang-II is then further processed by aminopeptidase A and N to produce angiotensin III and angiotensin IV.[7] Ang-II interacts with both the angiotensin-II type-1 and angiotensin-II type-2 G-protein-coupled receptors.[7]

Ang-II also acts at the adrenal zona glomerulosa to trigger the secretion of aldosterone. In heart failure Ang-II becomes the major aldosterone secretagogue as opposed to potassium and corticotropin in normal health.[8] Aldosterone is an independent risk factor for cardiovascular disease and stimulates both renal and extrarenal retention of sodium and water[9] and cardiac collagen synthesis and fibroblast proliferation via the activation of local mineralocorticoid receptors[10] leading to adverse remodeling. In severe heart failure, the pharmacological blockade of these receptors with mineralocorticoid antagonists such as spironolactone and eplerenone, has been shown to significantly reduce both morbidity and mortality in these patients.[11]

Numerous *RAAS* gene linkage and gene association studies have reported associations with cardiovascular diseases, including hypertension.[12] Within the *AGT* gene, the coding *M235T* and *T174M* polymorphisms have been particularly well studied, as have the *ACE* insertion/deletion and angiotensin-II type-1 receptor (*AGTR1*) A1166C gene variants.[12] Any pathophysiological association

[1]Christchurch Cardioendocrine Research Group, Department of Medicine, University of Otago-Christchurch, Christchurch, New Zealand; [2]Department of Medicine, Faculty of Medicine and Health Sciences, University of Auckland, Auckland, New Zealand and [3]Department of Medical Imaging, Unitec Institute of Technology, Auckland, New Zealand. Correspondence: Dr KL Ellis, Christchurch Cardioendocrine Research Group, Department of Medicine, University of Otago-Christchurch, PO Box 4345, Christchurch 8140, New Zealand.
E-mail: katrina.ellis@otago.ac.nz
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between these polymorphisms and CAD is plausibly via an increase in RAAS activity.

Whether genetic variation within this system has a role in disease progression is unknown. We have screened polymorphisms in genes encoding *AGT, ACE, AGTR1* and *CYP11B2,* selected either because of previous associations with CAD risk or as key tagging single-nucleotide polymorphisms (SNPs) in these genes, for association with cardiovascular risk factors and outcomes in a prospective cohort of patients with established coronary heart disease.

METHODS

Coronary disease cohort

Patients ($n = 1186$) were recruited into the study according to the following inclusion criteria; ischemic discomfort plus one or more of electrocardiogram changes (ST segment depression or elevation of at least 0.5 mm, T-wave inversion of at least 3 mm in at least three leads, or left bundle branch block), elevated levels of cardiac markers, a history of coronary disease, or were aged at least 65 years in patients with diabetes or vascular disease. Patients were excluded from the study if they had a severe comorbidity that limited their life expectancy to less than 3 years. Plasma was collected at a baseline clinic, 5–56 days from the date of index admission. Blood was collected into chilled EDTA tubes and stored on ice. Plasma was separated within 20 min by centrifugation and stored at $-80\,^{\circ}C$. Plasma was assayed for atrial natriuretic peptide (ANP),[13] N-terminal ANP (NT-proANP),[14] B-type natriuretic peptide (BNP),[15] N-terminal B-type natriuretic peptide (NT-proBNP),[16] C-type natriuretic pepetide (CNP)[17] and cyclic guanosine monophosphate (cGMP).[18] Transthoracic echocardiography was performed using a GE Vivid 3 ultrasound system (GE Medical Systems, Waukesha, WI, USA) at Christchurch Hospital and an ATL HDI 5000 (Philips Healthcare, Andover, MA, USA) at Auckland City Hospital. The standardized imaging protocol included apical 4- and 2-chamber views according to the American Society of Echocardiography.[19]

Patients were followed for a median 3.0 years (0.1–3.0 years). Diagnoses at each readmission were defined using the International Statistical Classification of Diseases and Health Related Problems 10th Revision (ICD-10). Outcome data were obtained from National Health Information Systems and hospital Patient Management Systems databases and cardiovascular admissions were determined at discharge by clinical review. 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.

DNA extraction and genotyping

Extraction of genomic DNA was performed as described previously.[20] For *AGT*, patients were screened for *M235T* (rs699), *T174M* (rs4762) and six tag SNPs (rs2478539, rs6687360, rs11568054, rs11122576, rs1926723 and rs2478545). The *ACE* intron 16 I/D (rs4646994) and three *ACE* tag SNPs (rs4329, rs4353 and rs4267385) were assayed, as was the *AGTR1* A1166C polymorphism and seven *AGTR1* tag SNPs (rs2933249, rs931490, rs1492103, rs718858, rs1492103, rs718858, rs1492099, rs12721331 and rs12695877). Study participants were additionally genotyped for the *CYP11B2* C-344T polymorphism. For each gene, tag SNPs were selected from HapMap data for the CEU population (Utah residents with ancestry from northern and western Europe) (Supplementary Figures 1–3).

The *ACE* intron 16 I/D (rs4646994) assay was performed as previously described.[20] *ACE* rs4267385 was genotyped using a PCR-RFLP assay consisting of the amplification primers: forward 5′-CCT TGT GTG CCC TCT CCT AA-3′ and reverse 5′-ATG GAT GGA TGG ATG GAT GT-3′. PCR amplification included an initial denaturation of 4 min at 94 °C, 30 cycles of 60 sec denaturation at 94 °C, 1 min annealing at 60 °C and 1 min extension at 72 °C and final extension of 7 min at 72 °C. The resulting 219 bp amplimer was digested with *NspI* (New England Biolabs, Beverly, MA, USA) and visualized on large format 2% agarose, $0.5 \times$ TBE gel with ethidium bromide staining. The T allele yielded digestion products of 42 and 177 bp. All other genotyping was performed using 100 ng of DNA and either SNPlex (Applied Biosystems, Mulgrave, Victoria, Australia) or TaqMan genotyping assays (Applied Biosystems) according to the manufacturers instructions.

Statistical analyses

Associations between genotype and baseline patient characteristics were tested using an additive genetic model, except for those SNPs where the minor genotype occurred at a frequency of $<10\%$. For these variants a dominant genetic model was used. Hormone data exhibited a skewed distribution and was log transformed. Before undertaking statistical analyses to investigate the association between SNPs and baseline characteristics, associations between genotype and ethnicity were assessed. For SNPs where a significant difference in genotype frequency was observed, subsequent analyses were performed on each ethnic subgroup separately as well as on the cohort as a whole. Ethnicity, as well as other factors (including age, gender and body mass index (BMI)) were adjusted for in univariate analyses where there is evidence to suggest that these could be potential confounding factors. Ethnicity was self-declared and was based on grandparent information. Patients were predominantly of European ancestry (83.3%). The majority of study participants of Other ethnicity were of Maori/Pacific Island, Asian and Unknown ancestry. For continuous variables, associations with baseline patient characteristics were assessed using ANOVA with a linear contrast, with the results summarized as the regression coefficient (b) and 95% confidence intervals. Odds ratios and 95% confidence intervals are reported for discrete variables. The primary endpoint was death; however, secondary associations between genotype and readmission for non ST-elevation MI (the most common cause of cardiovascular disease readmission) were explored. Univariate associations between each variant and outcome was assessed using Kaplan–Meier survival analyses and the log-rank test statistic of linear trend. Cox-proportional hazard analysis was performed to establish independent associations with outcome by adjusting for established predictors of increased risk; age of disease onset, gender, ethnicity, history of myocardial infarction, history of hypertension, β-blocker treatment, plasma creatinine, left ventricular ejection fraction and NT-proBNP. Cox-proportional hazards modeling was performed if the association between SNP and outcome was $P<0.1$ in Kaplan–Meier analyses. The power of this study to assess the relationship between genotype and cardiovascular outcomes was estimated assuming 80% power and two-tailed $\alpha = 0.05$. Adopting a conservative approach, this resulted in power to detect a hazard ratio of $\leqslant 1.79$. Analyses were performed using PASW version 18.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Coronary disease cohort patient characteristics

Patient characteristics of the Coronary Disease Cohort Study are shown in Table 1. Genotype frequencies of each RAAS polymorphism are shown in Table 2. Throughout 3-year follow-up, cumulative mortality was 12.5% ($n = 147$) and 20.3% ($n = 239$) of patients were readmitted for non ST-elevation MI.

AGT polymorphisms

AGT rs2478545 minor allele carriers ($n = 373$) had lower levels of circulating ANP ($P = 0.00017$; $b = -1.16$; 95% CI $= -1.25$ to -1.07), NT-proANP ($P = 0.00012$; $b = -1.15$; 95% CI $= -1.23$ to -1.07), BNP ($P = 0.00025$; $b = -1.23$; 95% CI $= -1.37$ to -1.10), NT-proBNP ($P = 0.00049$; $b = -1.24$; 95% CI $= -1.39$ to -1.10), CNP ($P = 0.001$; $b = -1.10$; 95% CI $= -1.17$ to -1.04) and cGMP ($P = 0.013$; $b = -1.09$; 95% CI $= -1.16$ to -1.02) after adjustment for potential confounders age, gender, ethnicity and BMI. The minor allele of rs2478545 was associated with a lower adjusted E/E^1 ($P = 0.018$; $b = -1.01$; 95% CI $= -1.84$ to -0.18).

Minor alleles of rs1926723 and rs11122576 were associated with a greater prevalence of type-2 diabetes (rs1926723, $P = 0.020$, OR $= 1.59$, 95% CI $= 1.07$ to 2.35; rs11122576, $P = 0.018$, OR $= 1.60$, 95% CI $= 1.09$ to 2.37) and a larger BMI (rs1926723, $P = 0.008$, $b = 1.07$, 95% CI $= 0.28$ to 1.86; rs11122576, $P = 0.021$, $b = 0.93$, 95% CI $= 0.14$ to 1.73) (Table 3). The same genotypes were also associated with a more frequent history of renal disease (rs1926723, $P = 0.042$, OR $= 1.63$; 95% CI $= 1.02$ to 2.62; rs11122576, $P = 0.023$; OR $= 1.72$; 95% CI $= 1.08$ to 2.75). To confirm that this association did not result from overrepresentation of the minor allele among at-risk ethnic groups, the major ethnic subgroups were analyzed separately. For both SNPs, trends

Table 1. CDCS baseline patient characteristics

	n	
Age (years)[a]	1180	66.9 ± 12.5
Gender (M)	1182	70.6%
Ethnicity	1168	
European	973	83.3%
Other/unknown ethnicity	195	16.7%
BMI (kg m^{-2})[a]	1159	27.4 ± 4.8
Current smoker	1173	7.2%
Hypertension	1169	53.4%
Previous MI	1167	31.6%
Type-2 diabetes	1174	16.4%
Family history of CAD	1153	46.7%
β-Blocker-treated	1176	86.5%
ACE inhibitor-treated	1176	57.1%
Plasma NT-pro BNP (pmol l^{-1})[b]	1165	77.6(0.93–2187.8)
Left ventricular ejection fraction[a]	1054	56.8 ± 12.8
Plasma creatinine (mmol l^{-1})[b]	1161	0.1(0.02–0.95)
Diagnosis at discharge	1162	
Non ST-elevation MI	583	50.2%
ST-elevation MI	236	20.3%
Unstable angina	343	29.5%

Abbreviations: ACE, angiotensin-converting enzyme; BMI, body mass index; MI, myocardial infraction; CAD, coronary artery disease; NT-pro BNP, N-terminal brain natriuretic peptide. [a]Arithmetic mean ± s.d. [b]Geometric mean and range.

Table 2. Genotype frequencies of RAAS polymorphisms in the CDCS cohort

	n	*AA*, n *(%)*	*Aa*, n *(%)*	*aa*, n *(%)*
AGT				
rs1926723 (A/G)	987	801 (81.2)	163 (16.5)	23 (2.3)
rs2478545 (C/T)	991	618 (62.4)	331 (33.4)	42 (4.2)
rs2478539 (A/C)	812	349 (43.0)	328 (40.4)	135 (16.6)
rs6687360 (C/T)	938	383 (40.8)	422 (45.0)	133 (14.2)
rs11568054 (C/T)	943	833 (88.3)	104 (11.0)	6 (0.6)
rs11122576 (A/G)	994	808 (81.3)	173 (17.4)	13 (1.3)
rs699 (M235T)	1164	399 (34.3)	541 (46.5)	224 (19.2)
rs4762 (T174M)	1165	906 (77.8)	248 (21.3)	11 (0.9)
ACE				
rs4646994 (ACE I/D)	936	207 (22.1)	424 (45.3)	305 (32.6)
rs4267385 (C/T)	914	194 (21.2)	463 (50.7)	257 (28.1)
rs4329 (A/G)	927	277 (29.9)	454 (49.0)	196 (21.1)
rs4353 (A/G)	929	261 (28.1)	460 (49.5)	208 (22.4)
AGTR1				
rs2933249 (C/T)	986	679 (68.9)	279 (28.3)	28 (2.8)
rs12695877 (T/C)	991	427 (43.1)	446 (45.0)	118 (11.9)
rs931490 (A/G)	941	653 (69.4)	261 (27.7)	27 (2.9)
rs1492103 (T/C)	903	637 (70.5)	244 (27.0)	22 (2.4)
rs718858 (C/T)	988	670 (67.8)	289 (29.3)	29 (2.9)
rs1492099 (G/A)	986	739 (74.9)	228 (23.1)	19 (1.9)
rs12721331 (T/C)	900	785 (87.2)	110 (12.2)	5 (0.6)
rs5186 (A1166C)	1185	628 (53.0)	458 (38.6)	99 (8.4)
CYP11B2				
rs1799998 (C-344T)	1142	354 (31.0)	555 (48.6)	233 (20.4)

Where 'A' represents the major allele and 'a' the minor allele for each SNP. SNPs italicized and in bold were selected based on previous associations reported in the literature. All other SNPs are 'tag' SNPs and were identified from HapMap data for the CEU population.

in the same direction were observed with renal disease and type-2 diabetes in both the European ($P = 0.151$–0.227) and combined other/unknown ($P = 0.073$–0.121) patient groups. Patients carrying the 'T' allele of rs11568054 demonstrated a more frequent history of renal disease ($P = 0.04$; OR = 1.82; 95% CI = 1.03 to 3.21) and higher levels of plasma creatinine ($P = 0.033$; $b = 1.08$; 95% CI = 1.01 to 1.16).

The *AGT 235 'TT'* 'high-risk' genotype was associated with a 2.9-year younger age of clinical coronary disease onset ($P = 0.006$; $b = -1.41$; 95% CI = −2.42 to −0.41) (Table 3). As the minor allele of M235T, was less frequent in patients of European ancestry when compared with those of other/unknown ethnicity, the analyses for these SNPs were run on both patient groups separately. The association between the *235 'T'* allele and a younger age of clinical coronary disease onset was present in the other/unknown ethnic groups only ($P < 0.043$).

Kaplan–Meier survival analysis revealed that in addition to a higher BMI, and more type-2 diabetes and renal disease, *AGT* rs11122576 (log-rank $P = 0.045$) and rs1926723 (log-rank $P = 0.049$) minor allele carriers experienced greater mortality (Figure 1). The *AGT* rs6687360 minor allele trended towards worse survival (log-rank $P = 0.055$, Figure 1). For each variant, the association with increased mortality was significant when adjusting for ethnicity and other established predictors of increased risk ($P \leqslant 0.020$, Table 4).

ACE polymorphisms

The minor allele of *ACE* rs4267385 was associated with an older age of onset of clinical coronary disease ($P = 0.008$; $b = 1.58$; 95% CI = 0.43 to 2.72) and hypertension onset ($P = 0.016$; $b = 2.15$; 95% CI = 041 to 3.88) and higher plasma creatinine ($P = 0.01$; $b = 1.04$; 95% CI = 1.01 to 1.07) (Table 3). Despite this, *ACE* rs4267385 was additionally associated with increased mortality in both univariate (log-rank $P = 0.002$, Figure 1) and multivariate ($P = 0.044$, Table 4) survival analyses.

Patients carrying the 'high-risk' *ACE* I/D 'D' allele, were more likely to be readmitted for non ST-elevation MI, the most common cause of CVD readmission in the cohort overall (log-rank $P = 0.038$, Figure 2). However, in multivariate analyses that adjusted for established predictors of increased risk, *ACE* I/D genotype was not independently prognostic ($P = 0.131$, Supplementary Table 1). No differences in age, gender, ethnicity or pharmacotherapy were observed with *ACE* genotype.

AGTR1 polymorphisms

Less frequent history of hypertension was observed for *AGTR1* rs12695877 minor allele carriers ($P = 0.039$; OR = 0.82; 95% CI = 0.68 to 0.99) (Table 3). Univariate outcome analyses identified a significant association between *AGTR1* rs2933249 (log-rank $P = 0.03$), rs1492103 (log-rank $P = 0.027$) and readmission for non ST-elevation MI (Figure 2). However, when adjusting for established predictors of increased risk, no association between *AGTR1* genotype and readmission for non ST-elevation MI was observed ($P = 0.083$–0.087, Supplementary Table 1). There were no associations between *AGTR1* genotype, gender or pharmacotherapy. Minor alleles of rs2933249, rs931490, rs1492103 and rs1492099 occurred at a lower frequency in European patients when compared with individuals of other/unknown ethnicity ($P < 0.05$; European minor allele frequency = 0.176–0.241, other/unknown minor allele frequency = 0.268–0.328). *AGTR1* SNP rs718858 occurred at an increased frequency in participants of European descent ($P = 0.030$; European minor allele frequency = 0.376, other/unknown minor allele frequency = 0.307).

Aldosterone synthase CYP11B2 C-344T

Genotype frequencies for *CYP11B2* C-344T were as follows, CC = 31.0% ($n = 354$), CT = 48.6% ($n = 555$) and TT = 20.4% ($n = 233$). There were no associations between *CYP11B2 C-344T* and baseline patient characteristics or cardiovascular outcomes,

Table 3. Significant associations between polymorphisms and CDCS baseline patient characteristics

AGT	*ANP (pmol l^{-1})*	*NT-proANP (nmol l^{-1})*	*BNP (pmol l^{-1})*	*NT-proBNP (pmol l^{-1})*	*CNP (pmol l^{-1})*	*cGMP (pmol l^{-1})*	*E/E'*[1]
rs2478545							
CC	38.7 (36.7–40.7)*	1.1 (1.1–1.2)*	20.3 (18.8–21.8)*	85.9 (79.1–93.2)*	1.2 (1.2–1.3)*	6.9 (6.6–7.2)***	12.7 ± 7.6***
CT/TT	32.6 (30.4–34.8)	1.0 (0.9–1.0)	15.8 (14.4–17.4)	65.4 (58.5–73.2)	1.1 (1.1–1.2)	6.3 (6.0–6.7)	11.4 ± 4.9
	History of renal disease	*Type-2 diabetes*	*BMI (kg m^{-2})*				
rs1926723							
AA	9.7%***	16.1%***	27.3 ± 4.8**				
AG/GG	14.9%	23.4%	28.3 ± 5.4				
rs11122576							
AA	9.6%***	16.0%***	27.3 ± 4.8***				
AG/GG	15.5%	23.4%	28.2 ± 5.4				
	History of renal disease	*Creatinine (mmol/l)*					
rs11568054							
CC	9.2%***	0.09(0.09–0.10)***					
CT/TT	15.6%	0.10(0.09–0.10)					
	Age of coronary disease onset (years)						
M235T							
MM	68.2 ± 12.0						
MT	67.1 ± 12.8**						
TT	65.3 ± 12.7						
ACE	*Age of coronary disease onset (years)*	*Age of hypertension (years)*	*Creatinine (mmol/l)*				
rs4267385							
CC	66.9 ± 12.0	49.4 ± 14.9	0.09(0.09–0.10)				
CT	67.5 ± 12.9**	53.3 ± 13.5***	0.10(0.09–0.10)**				
TT	69.9 ± 11.5	53.9 ± 12.5	0.10(0.09–0.11)				
AGTR1	*History of hypertension*						
rs12695877							
CC	58.5%						
CT	50.6%***						
TT	51.3%						

Abbreviations: ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic pepetide; cGMP, cyclic guanosine monophosphate; NT-proANP, N-terminal ANP. Data are presented as arithmetic mean ± s.d., geometric mean (95% CI), or as percentages unless otherwise indicated. Significance *$P < 0.001$,**$P < 0.01$,***$P < 0.05$.

and no difference in genotype frequency with gender, ethnicity, comorbidity or pharmacotherapy.

DISCUSSION

Inappropriate RAAS activation is a major pathophysiological culprit in cardiovascular disease. To our knowledge we have for the first time reported a broad screen of RAAS polymorphisms in a prospective study of patients with established coronary heart disease. Our data suggest a novel association between a genetic variant in *AGT* (rs2478545) and significantly lower levels of circulating natriuretic peptides. Two other *AGT* SNPs (rs1926723 and rs11122576) were consistently associated with a larger BMI, type-2 diabetes, renal disease and accordingly poorer survival. Variants within *ACE* and *AGTR1* may be weakly associated with increased mortality and/or history of hypertension.

The *AGT* gene is located on chromosome 1 at position 1q42, spans over 11 kb, and contains five exons and over 215 known polymorphisms. Among these, the *M235T* and *T174M* polymorphisms have been particularly well studied. Several meta-analyses have reported small positive associations between both the *235T* and *T174* alleles and hypertension,[12] with blood pressure and arterial stiffness,[21] and with increased heart failure mortality.[22] *AGT* 235 'TT' homozygotes have also been shown to have approximately 20% higher concentrations of plasma AGT.[23] Consistent with these findings, in the current study, participants with the *AGT* 235 'TT' genotype were younger at the time of recruitment by 2.9 years. Although it has been demonstrated that M235T is non-functional, this variant is in tight linkage disequilibrium with a variant in the proximal promoter of the *AGT* gene.[24] This variant encodes an adenine instead of a guanine six nucleotides upstream from the site of transcription (-6A/G), and has been shown to affect the interaction between at least one *trans*-acting nuclear factor and the promoter of the *AGT* gene.[23] This is likely to explain the increased levels of plasma AGT levels in T235 homozygotes compared with M235 homozygotes.

In the current study, an intriguing association was observed between the minor allele of the *AGT* tag SNP rs2478545 and circulating levels of natriuretic peptides. The mutually antagonistic actions of the natriuretic peptide system and the RAAS have been well established. Cardiac natriuretic peptides directly reduce both renin and aldosterone secretion, and their beneficial effects on the cardiovascular system depend on their ability in part to antagonize the RAAS at multiple levels.[25] Interestingly, in this

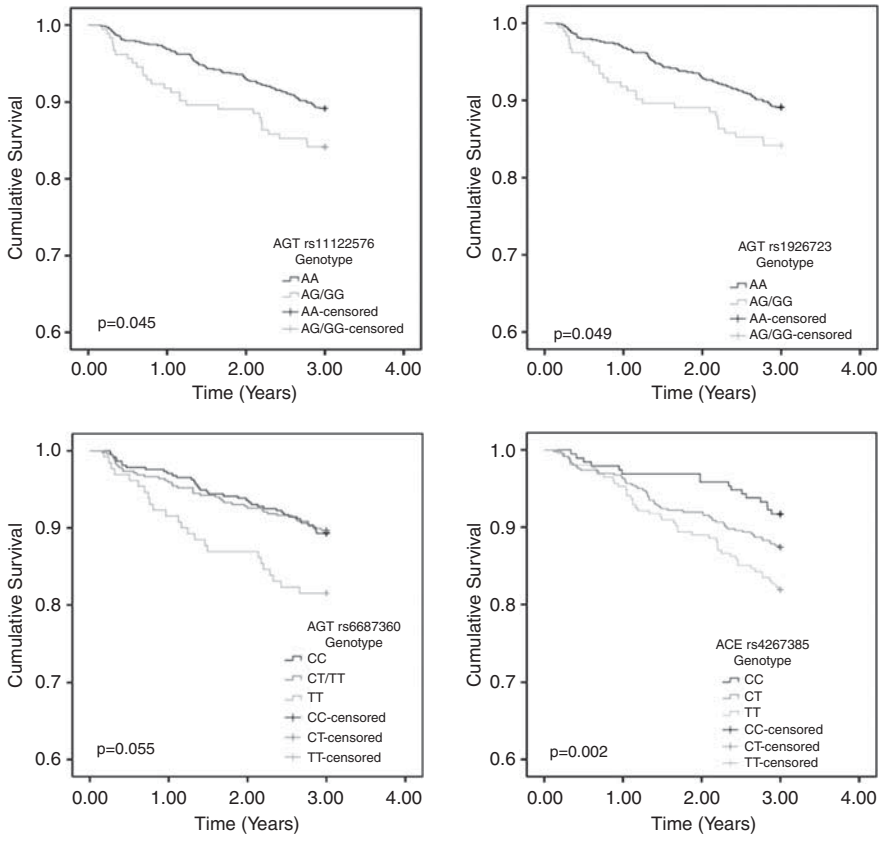

Figure 1. Association between *AGT* rs11122576, *AGT* rs1926723, *AGT* rs6687360 and *ACE* rs4267385 and mortality.

Table 4. Cox-proportional analysis of factors predictive of death and AGT rs11122576, rs1926723 and rs6687360 genotype

Variable	*AGT rs11122576*			*AGT rs1926723*			*AGT rs6687360*			*ACE rs4267385*		
	Risk ratio	*95% CI*	P	*Risk ratio*	*95% CI*	P	*Risk ratio*	*95% CI*	P	*Risk ratio*	*95% CI*	P
Age	1.06	1.03–1.08	<**0.001***	1.06	1.03–1.08	<**0.001***	1.05	1.02–1.08	<**0.001***	1.06	1.03–1.09	<**0.001***
Gender	0.82	0.50–1.37	0.452	0.80	0.49–1.33	0.397	0.73	0.43–1.22	0.227	1.27	0.79–2.06	0.323
Ethnicity	—	—	**0.036***	—	—	**0.038***	—	—	0.139	—	—	0.467
Maori/Pacific vs European	2.10	1.02–4.32		2.07	1.00–4.27		1.72	0.81–3.65		1.16	0.36–3.75	
Asian vs European	2.12	0.67–7.19		2.19	0.67–7.20		1.90	0.57–6.34		4.59	0.56–37.9	
Other/ unknown vs European	0.38	0.11–1.28		0.38	0.11–1.27		0.42	0.13–1.42		0.74	0.29–1.88	
History of myocardial infarction	1.65	1.05–2.61	**0.031***	1.63	1.03–2.57	**0.036***	1.59	0.99–2.57	0.057	1.67	1.07–2.62	**0.025***
History of hypertension	1.22	0.76–1.96	0.406	1.22	0.76–1.95	0.412	1.15	0.70–1.90	0.585	0.83	0.53–1.29	0.406
B-blocker treatment	0.88	0.45–1.70	0.692	0.87	0.45–1.69	0.680	1.19	0.62–2.27	**0.607**	0.50	0.30–0.84	**0.009***
Creatinine ($mmol\,l^{-1}$)	3.53	1.59–7.85	**0.002***	3.56	1.60–7.92	**0.002***	3.87	1.68–8.93	**0.001***	2.29	0.88–5.96	0.090
LVEF	0.98	0.97–1.00	**0.044***	0.98	0.96–1.00	**0.039***	0.98	0.96–1.00	**0.038***	1.00	0.98–1.02	0.924
NT-proBNP ($pmol\,l^{-1}$)	4.14	2.00–8.58	<**0.001***	4.01	1.93–8.31	<**0.001***	5.28	2.47–11.28	<**0.001***	6.37	2.93–13.82	<**0.001***
Genotype	2.01	1.21–3.32	**0.007***	2.05	1.24–3.39	**0.005***	1.48	1.07–2.05	**0.020***	1.37	1.01–1.86	**0.044***

Abbreviation: LVEF, left ventricular ejection fraction. *Significance $P<0.05$. Bold values indicated statistically significance values $P<0.05$.

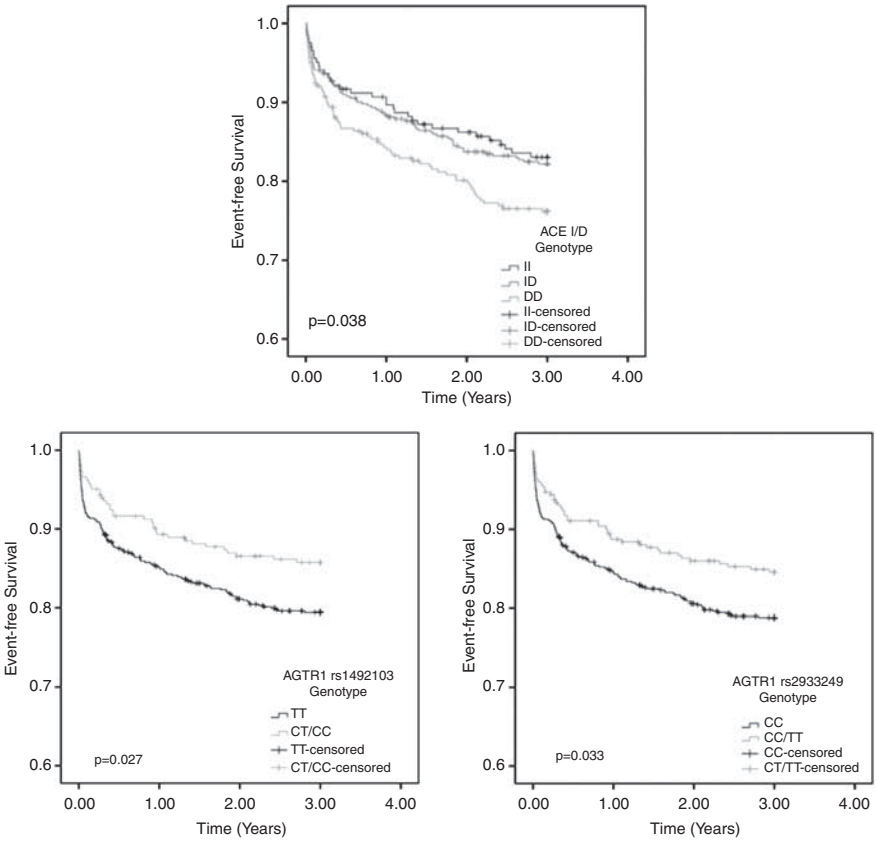

Figure 2. Association between *ACE* I/D, *AGTR1* rs1492103 and *AGTR1* rs2933249 and readmission for non ST-elevation MI.

current study the association between rs2478545 and lower natriuretic peptides also translated into a lower E/E[1], indicating less ventricular diastolic dysfunction. Whether this suggests that the rs2478545 minor allele is associated with less cardiac stress via a natriuretic peptide-independent mechanism, accompanied by lower natriuretic peptide levels, or whether the level of natriuretic peptides is inappropriately low for given cardiac damage, is difficult to determine. However, if the lower natriuretic peptides were providing less compensatory cardioprotection, then it might be expected that an association with worse outcomes would also have been observed.

In the present study, two *AGT* variants (rs1926723 and rs11122576), were associated with increased BMI, type-2 diabetes and renal disease. Links between the RAAS and both the initiation and progression of these diseases has been demonstrated, and clinical trials have demonstrated that RAAS inhibition reduces the incidence of vascular complications in diabetes patients.[26] Minor alleles of both rs1926723 and rs11122576 were also associated with greater mortality over 3-year follow-up. These associations with cardiovascular outcomes occurred despite the wide use of medications that interrupt the RAAS over follow-up, potentially indicating incomplete inhibition of this system.

Within the *ACE* gene, the D allele of the common insertion/deletion (I/D) polymorphism in intron 16 has been shown to predict approximately half the interindividual variability in both serum and tissue levels of ACE,[27] and DD homozygotes have been identified at higher frequencies in hypertensive men than their mildly hypertensive or normotensive counterparts.[28] The ACE D allele has proven to be a consistent marker of initial risk[20,29] and a predictor of clinical outcome after MI in prospective studies of at-risk groups.[20] Supporting these findings, in the current study the *ACE* D allele was associated with an increased risk of experiencing a subsequent non ST-elevation MI. However, as statistical significance was lost when adjusting for several established risk factors any association is likely to be modest. Previous associations of the *ACE* D allele with elevated levels of ACE in human plasma[27] and myocardium[30] provides a mechanistic basis for the polymorphism's relationship with heart disease, given the central role of the RAAS in the regulation of blood pressure, sodium balance and cardiac remodeling following heart injury. However, as ACE I/D is located within intron 6 of the *ACE* gene, this polymorphism is unlikely to directly effect ACE activity. Instead, other alleles in LD located within or nearby the *ACE* gene are thought to explain the observed associations between ACE and cardiovascular disease. In this study, we also identified an association between the minor allele of the tag SNP rs4267385 and a 3 year older age of onset of clinical coronary disease and onset of hypertension, but also a greater mortality independent of age and other prognostic markers. These apparently conflicting findings require further validation.

Located on chromosome 3q21-25, *AGTR1* is >55 kb in length. Of the more than 278 polymorphisms identified, the 3′UTR A1166C variant has been the most widely investigated. The *AGTR1* A1166C 'C' allele has been shown in case–control studies to occur at greater frequency in hypertensive patients than in normotensive subjects.[31] Within the general population, both clinic systolic and diastolic blood pressures were determined to be on average 11.3 mm Hg and 4.2 mm Hg lower in CC homozygotes when compared with AC heterozygotes and AA homozygote; however, ambulatory blood pressure was similar across genotypes.[32] It has been hypothesized that this gene variant can activate the RAAS as it is located in a *cis* regulatory site, recognized by the specific microRNA, miR155.[33] Although strong associations have been reported in the literature between the A1166C variant and CVD

characteristics in multiple populations,[34] we observed no association with baseline patient characteristics or cardiovascular outcomes in coronary disease cohort study participants. In contrast, the *AGTR1* tag SNP rs12695877 was associated with less history of hypertension, and both rs111492103 and rs2933249 minor allele carriers were less likely to have been readmitted for a non ST-elevation MI during the follow-up period.

The *CYP11B2 C-344T* polymorphism located in the promoter region of the aldosterone synthase gene involves a C/T substitution in a putative binding site for the steroidogenic transcription factor SF-1.[35] The *CYP11B2 -344T* allele has been associated in some but not all studies with an increased risk of hypertension,[36] coronary disease[37] and poorer event-free survival in African-Americans with heart failure.[38] However, in the current study no association between C-344T and either baseline patient characteristics or cardiovascular outcomes were observed. This lack of association could reflect the widespread use of beta-blockers, ACE inhibitors and angiotensin-II receptor blockers in these coronary disease patients.

We have performed a broad screen of genetic variation within the RAAS hormone system for association with cardiovascular risk factors and outcomes in patients with established coronary heart disease. Associations were observed between the minor allele of *AGT* rs2478545 and lower levels of plasma natriuretic peptides, and between the *AGT* M235T 'T' allele and an earlier age of clinical coronary disease onset. Patients carrying the minor alleles of *AGT* rs1926723 and rs11122576 had more history of renal disease and type-2 diabetes, and increased mortality throughout follow-up. *AGT* rs6687360 and *ACE* rs4267385 were independently prognostic of all-cause mortality, and *AGTR1* rs12685977 minor allele carriers had less frequent history of hypertension. These observed associations with cardiovascular risk factors and outcomes provides further evidence that genetic variation within this important hormone cascade has an integral role in the development and progression of cardiovascular disease.

What is known about this topic

- The RAAS has a central role in the regulation of renal sodium and water absorption, blood pressure, the control of thirst, cardiac function and cellular growth, and has been established as one of the most important systems in the pathogenesis of coronary heart disease.
- Numerous *RAAS* gene linkage and gene association studies have reported associations with cardiovascular diseases, including hypertension.

What this study adds

- To our knowledge, we have the first time reported a broad screen of RAAS polymorphisms in a prospective study of patients with established coronary heart disease.
- We have identified a novel association between a genetic variant in *AGT* (rs2478545) and significantly lower levels of circulating natriuretic peptides.
- Two other *AGT* SNPs (rs1926723 and rs11122576) were consistently associated with a larger BMI, type-2 diabetes, renal disease and accordingly poorer survival.
- Variants within *ACE* and *AGTR1* may be weakly associated with increased mortality and/or history of hypertension.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Journal of Human Hypertension website (http://www.nature.com/jhh)