0021-972X/07/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 92(6):2363–2369

doi: 10.1210/jc.2006-0071

Alanine to Serine Polymorphism at Position 986 of the Calcium-Sensing Receptor Associated with Coronary Heart Disease, Myocardial Infarction, All-Cause, and Cardiovascular Mortality

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Background: Disorders of calcium homeostasis have been implicated in atherosclerosis. The calcium-sensing receptor (CASR) is crucial to the regulation of calcium metabolism. An alanine (A) to serine (S) polymorphism at codon 986 (A986S) of the CASR gene has been associated with higher calcium and osteoporosis; the association with coronary artery disease (CAD) has not been studied.

Methods and Results: We investigated this polymorphism in individuals with CAD (n = 2561), including survivors of myocardial infarction (MI) (n = 1358) compared to 698 controls without angiographic CAD. Compared to AA homozygotes, the prevalence of CAD [multivariate odds ratio 1.25; 95% confidence interval (CI) 1.02–1.54] and previous MI (multivariate odds ratio 1.33; 95% CI 1.06–1.68) was increased in carriers of at least one S-allele. With each S-allele, the prevalence of CAD and MI increased 1.22-fold (95% CI 1.02–1.47) and 1.30-fold (95% CI 1.06–1.60), respectively. Fully adjusted hazard ratios for total and cardiovascular mortality per one S-allele were 1.24 (95% CI 1.05–1.46) and 1.38 (95% CI 1.13–1.67), respectively. In carriers of at least one S-allele, the adjusted hazard ratios for all-cause and cardiovascular death were 1.25 (95% CI 1.04–1.51) and 1.48 (95% CI 1.18–1.86), respectively. These associations were independent of cardiovascular risk factors, calcium and phosphate. The S-allele was associated with higher calcium ($P < 0.001$) and PTH ($P < 0.02$), and lower phosphate ($P < 0.003$) in CAD patients and controls.

Conclusion: Serine at position 986 of CASR may be an independent genetic predictor of angiographic CAD, previous MI, and cardiovascular mortality. **(*J Clin Endocrinol Metab* 92: 2363–2369, 2007)**

VASCULAR CALCIUM DEPOSITS have reflected the severity of coronary atherosclerosis and predicted future cardiovascular events (1, 2). Disorders of calcium homeostasis like primary hyperparathyroidism may increase the risk of death from cardiovascular causes (3). Bone loss has been linked to vascular calcification and atherosclerosis, (4–6) independent of age (7, 8) and other cardiovascular risk factors.

The calcium-sensing receptor (CASR) is a G protein-coupled receptor consisting of a hydrophilic extracellular domain, seven hydrophobic transmembrane domains, and a cytosolic carboxyl-terminal tail (9). The major function of the CASR is to sense extracellular calcium levels and elicit adaptive intracellular reactions, mainly in the parathyroid gland and kidney, directed to maintain calcium homeostasis in the body (10, 11).

In the chief cells of the parathyroid gland, stimulation of the CASR by calcium results in decreased release of PTH (12, 13). In renal tubular epithelia, high concentrations of plasma calcium enhance the urinary excretion of calcium, magnesium, and water via the CASR (14). Furthermore, the CASR is expressed in tissues other than the parathyroid and kidney, including C cells of the thyroid gland, brain, pituitary, intestine, bone marrow, and skin, but its precise role in these tissues has not been established so far.

Gain of function mutations of the CASR may lead to autosomal dominant hypocalcemia and may be implicated in the pathogenesis of Bartter syndrome (15, 16). Heterozygous and homozygous inactivating mutations are responsible for dominant familial benign hypocalciuric hypercalcemia and recessive neonatal severe hyperparathyroidism, respectively (13, 14, 17).

A common alanine (A) to serine (S) polymorphism of amino acid 986 (CASR-PM) (rs1801725) has been described (18, 19). In studies of healthy women, slight increases in calcium concentrations, not associated with symptoms, have been found in carriers of the more rare S-allele (19–21). The S-allele was also associated with low bone mineral density in

First Published Online March 20, 2007

Abbreviations: BMI, Body mass index; CAD, coronary artery disease; CASR, calcium-sensing receptor; CASR-PM, alanine (A) to serine (S) polymorphism at amino acid 986 of the CASR; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; LDL-C, low-density lipoprotein cholesterol; LURIC, Ludwigshafen Risk and Cardiovascular Health; MI, myocardial infarction; NSTEMI, non-STEMI; OR, odds ratio; STEMI, ST elevation MI.

adolescent girls (21), but not in postmenopausal women (22–24).

Given the epidemiological coincidence of chronic disorders of calcium metabolism such as osteoporosis on the one hand and atherosclerosis on the other hand, we hypothesized that the CASR-PM might not only impact on systemic calcium concentrations or bone mineral density but also on the development of coronary artery disease (CAD). Therefore, we investigated whether the CASR-PM was associated with angiographic CAD, previous myocardial infarction (MI), all-cause mortality, or mortality from cardiovascular causes in a large cohort of individuals who had previously undergone coronary angiography.

Patients and Methods

Study design and participants

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study includes consecutive white patients hospitalized for coronary angiography between June 1997 and May 2001. A detailed description of LURIC has been published (25). The ethics review committee at the "Landesärztekammer Rheinland-Pfalz" (Mainz, Germany) approved the study. Written informed consent was obtained from each of the participants. Clinical indications for angiography were chest pain or noninvasive tests consistent with myocardial ischemia. To limit clinical heterogeneity, individuals suffering from acute illness other than acute coronary syndromes, chronic noncardiac diseases, and a history of malignancy within the five past years were excluded.

CAD has been defined angiographically using the maximum luminal narrowing estimated by visual analysis. CAD was defined as the presence of a visible luminal narrowing (≥20% stenosis) in at least one of 15 coronary segments according to a classification of the American Heart Association (26). Individuals with stenoses less than 20% were considered as not having CAD. To examine the impact of other definitions of CAD on the current analysis, we provisionally used the presence of one stenosis ≥50% (n = 2158) as a criterion. MI was defined as evidence for any MI [acute, previous, ST elevation MI (STEMI), or non-ST elevation MI (NSTEMI)]. Acute MI was defined as a MI that had occurred within the 4 wk before enrollment into LURIC. A previous MI was diagnosed if a MI had been survived for more than 1 month before enrollment into LURIC. A definite STEMI was diagnosed if typical electrocardiogram changes were present along with prolonged chest pain refractory to sublingual nitrates and/or enzyme or troponin T elevations. NSTEMI was diagnosed if symptoms and/or enzyme criteria, but not the electrocardiogram criteria for STEMI, were met. Previous MI was also graded as definite if a hospitalization with a discharge diagnosis of MI was documented.

Of the 3259 individuals in whom CASR genotypes were available, 698 (21.4%) had no angiographic CAD, 1532 (47.0%) had stable CAD, while upon recruitment 624 (19.1%), 114 (3.5%), and 291 (8.9%) presented with unstable angina, NSTEMI (troponin T >0.1 μg/liter), or STEMI (troponin T >0.1 μg/liter).

Diabetes mellitus was diagnosed according to the criteria of the American Diabetes Association (27). Furthermore, individuals with a history of diabetes, or treatment with oral antidiabetics or insulin were considered diabetic. Hypertension was defined as a systolic and/or diastolic blood pressure ≥140 and/or ≥90 mm Hg, or a significant history of hypertension. Individuals with either high-density lipoprotein cholesterol (HDL-C) less than 40 mg/dl or low-density lipoprotein cholesterol (LDL-C) more than 160 mg/dl, or triglycerides more than 200 mg/dl were considered dyslipidemic.

Information on vital status was obtained from local person registries. No patients were lost to follow-up. Of the 3259 subjects included in this examination, 501 deaths (15.4%) had occurred during a median observation time of 5.45 yr. Death certificates were obtained for 486 of the decedents (97%) and were missing for 15 decedents (3%) who were included in the total mortality analysis but excluded from the cardiovascular mortality analysis. Cardiovascular death included the following categories: sudden death, fatal MI, death due to congestive heart failure, death immediately after intervention to treat CAD, fatal stroke, and other causes of death due to CAD. All clinical assessments were made blinded to the knowledge of CASR genotype. Two experienced physicians adjudicated independently the causes of death. In any case of disagreement, one of the principal investigators of LURIC (W.M.) made the final assignment.

Laboratory procedures

Fasting blood samples were obtained by venipuncture in the early morning. Blood glucose, cholesterol, triglycerides, creatinine, calcium, and inorganic phosphate were measured by standard laboratory procedures, as described previously (25). Albumin-adjusted calcium was obtained as: calcium (mmol/liter) − 0.02 · [albumin (g/liter) − 40]. Electrochemiluminescence on an Elecsys 2010 (Roche Diagnostics, Mannheim, Germany) measured intact PTH. LDL-C and HDL-C were measured after separating lipoproteins with a combined ultracentrifugation-precipitation method (25, 28). The creatinine clearance was derived according to Cockcroft and Gault (29) as: C_{CR} (ml/min) = [140 − age (yr)] · body weight (kg)/[72 · creatinine (mg/dl)]; or C_{CR} (ml/min) = 0.85 [140 − age (years)] · body weight (kg)/[72 · creatinine (mg/dl)] in men and women, respectively.

Analysis of the CASR-PM

Genomic DNA was prepared from EDTA anticoagulated peripheral blood using a common salting-out procedure. The G/T (A986S) polymorphism (rs1801725) was genotyped by PCR and *Hinf* I restriction fragment-length analysis using primer pair 5′-agc ttt gat gag cct cag aag *g*ac-3′ and 5′-cac tgc agc ggg agt aat ggc-3′. The 5′primer sequence was mismatched (base in *italic*) to generate an allele-specific restriction enzyme site in the amplicon. To confirm genotype assignment, two scientists on two separate occasions analyzed independently the restriction fragments. The results were scored blinded as to case-control status.

Statistics

Continuous variables were compared between groups by univariate ANOVA. χ^2 Testing or logistic regression analysis examined associations between categorical variables. To examine the relationship of the CASR-PM on mortality, we calculated hazard ratios (HRs) and 95% confidence intervals (CIs) by Cox proportional hazards regression. The time to death random variable was defined as the time period between enrollment and death or the time to the last follow-up (October 1, 2004) for the censored subjects.

Models were adjusted for age, gender, type 2 diabetes, body mass index (BMI), smoking, hypertension, dyslipidemia, calcium, and phosphate, as indicated in Tables 1 and 2. In models assuming a codominant (additive) effect of the S-allele of the CASR-PM, the genotypes AA, AS, and SS were coded as 0, 1, and 2, respectively, and genotypes were either treated as interval-scaled or categorical variables, the AA genotype being considered as the reference category in the latter case. When assuming a dominant effect, genotype AA was coded as 0, and AS and SS combined as 1. When assuming a recessive effect, genotypes AA and AS were coded as 0, and SS as 1. To examine the relationship of the CASR-PM with total mortality and mortality from cardiovascular causes, we calculated HRs and 95% CIs using the Cox proportional hazards model. Multivariable adjustment was performed as indicated. The criterion for statistical significance was $P < 0.05$. The SPSS statistical package (version 11.5 for Windows; SPSS Inc., Chicago, IL) was used.

Results

Study participants

We included 3259 subjects in the current analysis. Compared with the control group without CAD, patients with angiographic CAD were significantly older; current or past smoking, diabetes mellitus, and hypertension were more prevalent. CAD patients had higher systolic blood pressure, higher fasting glucose, higher triglycerides, and lower HDL-C. Crude LDL-C concentrations did not significantly differ between the two groups, due to the fact that 57% of

TABLE 1. ORs for angiographic CAD or previous MI according to the CASR genotype

	Model 1		Model 2		Model 3		Model 4	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Angiographic CAD								
AA	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
AS[a]	1.20 (0.99–1.46)	0.064	1.21 (0.99–1.49)	0.065	1.23 (1.00–1.52)	0.056	1.25 (1.01–1.54)	**0.041**
SS[a]	1.40 (0.76–2.55)	0.281	1.38 (0.74–2.58)	0.315	1.30 (0.68–2.50)	0.428	1.33 (0.70–2.54)	0.384
AA *vs.* AS *vs.* SS[b]	1.20 (1.01–1.42)	**0.038**	1.20 (1.01–1.43)	**0.043**	1.20 (1.00–1.45)	**0.049**	1.22 (1.02–1.47)	**0.034**
AA *vs.* AS or SS[a]	1.22 (1.01–1.47)	**0.043**	1.23 (1.00–1.50)	**0.045**	1.23 (1.01–1.51)	**0.045**	1.25 (1.02–1.54)	**0.031**
AA or AS *vs.* SS[a]	1.33 (0.73–2.43)	0.356	1.31 (0.70–2.45)	0.396	1.23 (0.64–2.34)	0.529	1.25 (0.66–2.39)	0.492
MI								
AA	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
AS[a]	1.24 (1.00–1.53)	**0.049**	1.23 (0.98–1.54)	0.074	1.27 (1.00–1.62)	**0.047**	1.31 (1.03–1.66)	**0.029**
SS[a]	1.69 (0.89–3.18)	0.107	1.70 (0.87–3.32)	0.119	1.57 (0.77–3.19)	0.212	1.68 (0.83–3.39)	0.151
AA *vs.* AS *vs.* SS[b]	1.26 (1.05–1.51)	**0.015**	1.25 (1.03–1.52)	**0.023**	1.27 (1.04–1.56)	**0.022**	1.30 (1.06–1.60)	**0.012**
AA *vs.* AS or SS[a]	1.27 (1.03–1.56)	**0.023**	1.26 (1.02–1.58)	**0.036**	1.30 (1.03–1.64)	**0.027**	1.33 (1.06–1.68)	**0.015**
AA or AS *vs.* SS[a]	1.59 (0.85–3.00)	0.149	1.61 (0.83–3.13)	0.161	1.50 (0.75–3.03)	0.254	1.56 (0.77–3.14)	0.217

Models: 1, unadjusted; 2, adjusted for age and gender; 3, in addition, adjusted for type 2 diabetes, BMI, smoking, hypertension, and dyslipidemia; and 4, in addition, adjusted for calcium and phosphate. *Boldface* indicates *P* values below 0.05. 1.0[reference] represents the reference category for the calculation of OR and HR.

[a] Genotypes treated as categorical variables and compared to the reference category.

[b] Genotypes treated as interval-scaled variables.

CAD patients were treated with lipid-lowering drugs compared with 18% of controls. However, when we accounted for the use of lipid-lowering drugs, age, and gender, LDL-C was significantly higher in CAD patients (adjusted mean 118 mg/dl) than in controls (adjusted mean 113 mg/dl). BMI and diastolic blood pressure were similar between patients and controls (Table 3).

Basically, the same applied to the subgroup of 1358 CAD patients who had suffered and survived an MI, with the following exceptions: the prevalence of hypertension was not different from controls, there was no significant difference in systolic blood pressure, diastolic blood pressure was slightly lower, and there was a tendency toward lower LDL-C in the MI patients than in controls (Table 3). The latter findings may relate to the fact that 27% of the patients with previous MI were studied within 14 d of the event.

The CASR-PM associated with CAD and previous MI

Genotypes were in Hardy-Weinberg equilibrium in both CAD patients and controls. Compared with controls, the genotypes AS and SS occurred more frequently in CAD patients (statistically not significant by χ^2 testing) and in patients with previous MI ($P = 0.05$ by χ^2 testing; Table 4).

Compared with AA homozygotes, the prevalence of CAD was increased in carriers of the AS genotype. Depending on the statistical model, odds ratio (OR) ranged between 1.20 and 1.25, significance levels between 0.041 and 0.065 (Table 1). The prevalence of CAD was even higher in SS homozygotes, but ORs did not reach statistical significance. Treating the CASR genotype as an interval-scaled variable suggested that each S-allele increased the OR of CAD by approximately 1.2-fold (*P* values between 0.034 and 0.049). Using the wild-type genotype AA as the reference, the combined AS and SS

TABLE 2. HRs for death according to the CASR genotype

	Model 1		Model 2		Model 3		Model 4	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Total mortality								
AA	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
AS[a]	1.16 (0.96–1.41)	0.134	1.18 (0.97–1.44)	0.091	1.18 (0.97–1.43)	0.100	1.23 (1.01–1.49)	**0.043**
SS[a]	1.49 (0.90–2.45)	0.121	1.51 (0.92–2.50)	0.107	1.48 (0.89–2.44)	0.130	1.58 (0.95–2.62)	0.075
AA *vs.* AS *vs.* SS[b]	1.18 (1.00–1.39)	**0.045**	1.20 (1.02–1.41)	**0.029**	1.19 (1.01–1.40)	**0.035**	1.24 (1.05–1.46)	**0.011**
AA *vs.* AS or SS[a]	1.22 (1.01–1.47)	0.077	1.21 (1.00–1.50)	**0.047**	1.20 (1.00–1.45)	0.055	1.25 (1.04–1.51)	**0.020**
AA or AS *vs.* SS[a]	1.43 (0.87–2.35)	0.163	1.44 (0.88–2.37)	0.150	1.41 (0.85–2.32)	0.179	1.39 (0.90–2.45)	0.120
Cardiovascular mortality								
AA	1.0[reference]		1.0[reference]		1.0[reference]		1.0[reference]	
AS[a]	1.41 (0.12–1.77)	**0.003**	1.43 (1.14–1.80)	**0.002**	1.43 (1.14–1.80)	**0.002**	1.50 (1.18–1.88)	**0.001**
SS[a]	1.33 (0.69–2.60)	0.398	1.35 (0.69–2.63)	0.379	1.29 (0.66–2.52)	0.452	1.28 (0.71–2.71)	0.342
AA *vs.* AS *vs.* SS[b]	1.32 (1.09–1.60)	**0.005**	1.34 (1.10–1.62)	**0.003**	1.33 (1.09–1.61)	**0.004**	1.38 (1.13–1.67)	**0.001**
AA *vs.* AS or SS[a]	1.40 (1.12–1.75)	**0.003**	1.43 (1.14–1.78)	**0.002**	1.42 (1.14–1.78)	**0.002**	1.48 (1.18–1.86)	**0.001**
AA or AS *vs.* SS[a]	1.20 (0.62–2.33)	0.587	1.21 (0.62–2.35)	0.573	1.16 (0.60–2.25)	0.664	1.22 (0.63–2.37)	0.561

Models: 1, unadjusted; 2, adjusted for age and gender; 3, in addition, adjusted for type 2 diabetes, BMI, smoking, hypertension, and dyslipidemia; and 4, in addition, adjusted for calcium and phosphate. *Boldface* indicates *P* values below 0.05. 1.0[reference] represents the reference category for the calculation of OR and HR.

[a] Genotypes treated as categorical variables and compared to the reference category.

[b] Genotypes treated as interval-scaled variables.

TABLE 3. Baseline characteristics and risk factors in patients with angiographically proven CAD and previous MI as compared to controls without CAD

	No CAD	CAD	*P* value[a]	CAD and MI	*P* value[a]
No. of males	364	1916		1071	
No. of females	334	645		287	
Mean male age ± SD (yr)	55 ± 12	63 ± 10		63 ± 10	
Mean female age ± SD (yr)	62 ± 10	66 ± 10	<0.001[b]	65 ± 11	<0.001[b]
Mean male BMI ± SD (kg/m^2)	27 ± 4	28 ± 4		27 ± 4	
Mean female BMI ± SD (kg/m^2)	27 ± 5	27 ± 5	NS	27 ± 5	NS
Smoker (former and current)					
No. of males (%)	240 (66)	1501 (78)		873 (82)	
No. of females (%)	100 (30)	253 (39)	<0.001	146 (51)	<0.001
Diabetes mellitus					
No. of males (%)	63 (18)	660 (34)		378 (35)	
No. of females (%)	65 (20)	253 (39)	<0.001	120 (42)	<0.001
Systemic hypertension					
No. of males (%)	212 (58)	1412 (74)		742 (69)	
No. of females (%)	229 (69)	518 (80)	0.001	220 (77)	NS
Mean male systolic blood pressure ± SD (mm Hg)	135 ± 21	142 ± 23		138 ± 23	
Mean female systolic blood pressure ± SD (mm Hg)	138 ± 23	143 ± 25	0.007[c]	139 ± 27	NS[c]
Mean male diastolic blood pressure ± SD (mm Hg)	81 ± 12	82 ± 11		80 ± 11	
Mean female diastolic blood pressure ± SD (mm Hg)	79 ± 11	80 ± 12	NS[c]	78 ± 11	0.02[c]
Mean male fasting blood glucose ± SD (mg/dl)	106 ± 30	115 ± 34		114 ± 33	
Mean female fasting blood glucose ± SD (mg/dl)	104 ± 24	119 ± 44	<0.001	120 ± 45	<0.001
Mean male LDL-C SD (mg/dl)	117 ± 31	113 ± 33		110 ± 32	
Mean female LDL-C SD (mg/dl)	124 ± 32	124 ± 39	0.002[d]	118 ± 39	NS[d]
Mean male HDL-C SD (mg/dl)	40 ± 11	36 ± 9		35 ± 9	
Mean female HDL-C SD (mg/dl)	46 ± 12	42 ± 11	<0.001[d]	40 ± 11	<0.001[d]
Median (25th and 75th percentile) male triglycerides (mg/dl)	145 (103–212)	150 (112–201)		150 (112–198)	
Median (25th and 75th percentile) female triglycerides (mg/dl)	129 (92–177)	152 (115–206)	<0.013[d]	155 (116–218)	NS[d]

NS, Not significant.

[a] ANOVA or logistic regression, respectively, adjusted for age and gender.

[b] ANOVA adjusted for gender only.

[c] ANOVA additionally adjusted for use of lipid-lowering drugs, β blockers, angiotensin-converting enzyme inhibitors, AT1 receptor antagonists, calcium channel blockers, and diuretics.

[d] ANOVA additionally adjusted for use of lipid-lowering drugs.

genotypes also significantly increased the probability of angiographic CAD. Compared with both the AA and AS genotype combined, the SS genotype was associated with ORs for CAD between 1.23 and 1.33 that were, however, not significantly different from unity because of the low prevalence of the SS genotype (see above). These associations were hardly affected by the inclusion of age and gender, conventional cardiovascular risk factors, and calcium and phosphate as covariables (Table 1, models 2–4). Considering individuals with at least one stenosis ≥50% as having CAD (n = 2158) and individuals with stenoses less than 20% as controls (n = 698) resulted in slight increases in significance levels due to decreasing statistical power, but none of the aforementioned ORs materially changed (data not shown). Basically, identical results were obtained when we analyzed the association between the CASR genotype and previous MI using individuals without angiographic CAD as controls (Table 1).

TABLE 4. Prevalence of CASR genotypes in patients with angiographically proven CAD and previous MI

	No CAD (n = 698)	CAD (n = 2561)	CAD and MI (n = 1358)
AA	517 (74)	1799 (70)	940 (69)
AS	168 (24)	699 (27)	378 (28)
SS	13 (2)	63 (3)	40 (3)
P value[a]		0.128	0.05

Data are expressed as number (percent).

[a] χ^2 Test comparing either the CAD or the CAD and MI group with the no CAD group.

The CASR-PM associated with all-cause and cardiovascular mortality

Of the 3259 subjects included in this examination, 501 deaths (15.4%) had occurred during a median observation time of 5.45 yr. Compared with AA homozygotes, HRs for death from all causes were increased in AS heterozygotes (~1.20) and SS homozygotes (~1.50). In the fully adjusted model, the HR for death in AS heterozygotes was significantly different ($P = 0.043$) from the reference category (AA homozygotes). In the combined genotypes AS or SS, the HR for all-cause mortality ranged between 1.20 and 1.25, and reached statistical significance in the model adjusted for age and gender ($P = 0.047$) and in the fully adjusted model ($P = 0.020$). Treating the CASR genotype as an interval-scaled variable suggested that each S-allele increased the HR for death from any cause by approximately 1.2-fold (*P* values between 0.011 and 0.045; Table 2).

Because death certificates were not available from 15 deceased persons, the analysis for cardiovascular mortality included a total of 3244 individuals. Of these, 337 (10.4%) died of cardiovascular causes. The CASR-PM had no influence on death from noncardiovascular causes. In carriers of the AS

genotype, HRs for death from cardiovascular causes were higher than those for death from any cause (*P* value between 0.001 and 0.003). The same applies to the combined genotypes AS or SS, the HR for cardiovascular mortality ranging between 1.40 and 1.48 and *P* values between 0.001 and 0.003. When the CASR genotype was used as an interval-scaled variable, each S-allele increased the HR for death from cardiovascular causes by approximately 1.3-fold (*P* values between 0.001 and 0.005; Table 2).

The CASR-PM, calcium, and phosphate

To analyze the association of calcium, phosphate, and PTH with the CASR genotype, we used ANOVA in which the genotype groups (defined by the presence or the absence of an S-allele) and the CAD status were included as main effects; statistical adjustments were made for age and gender, calculated creatinine clearance, and cardiovascular risk factors (BMI, diabetes mellitus, hypertension, and smoking) in a first, second, and third step, respectively.

Compared with AA homozygotes, carriers of the S-allele had significantly higher calcium ($P < 0.001$ in unadjusted and adjusted models), albumin-adjusted calcium ($P < 0.001$), and PTH ($P < 0.02$), and lower phosphate ($P < 0.003$) (Fig. 1), independent of the absence or presence of angiographic CAD. The CAD patients had a slightly lower average calcium concentration than the controls, but the difference was not statistically significant ($P = 0.268$) and almost disappeared when albumin-adjusted calcium concentrations were considered ($P = 0.869$). There was a tendency toward higher PTH in CAD patients ($P = 0.067$), but this difference disappeared after including creatinine clearance into the general linear model ($P = 0.611$). Phosphate was significantly lower in CAD patients than in controls ($P = 0.045$), but the overall relative difference was less than 2%.

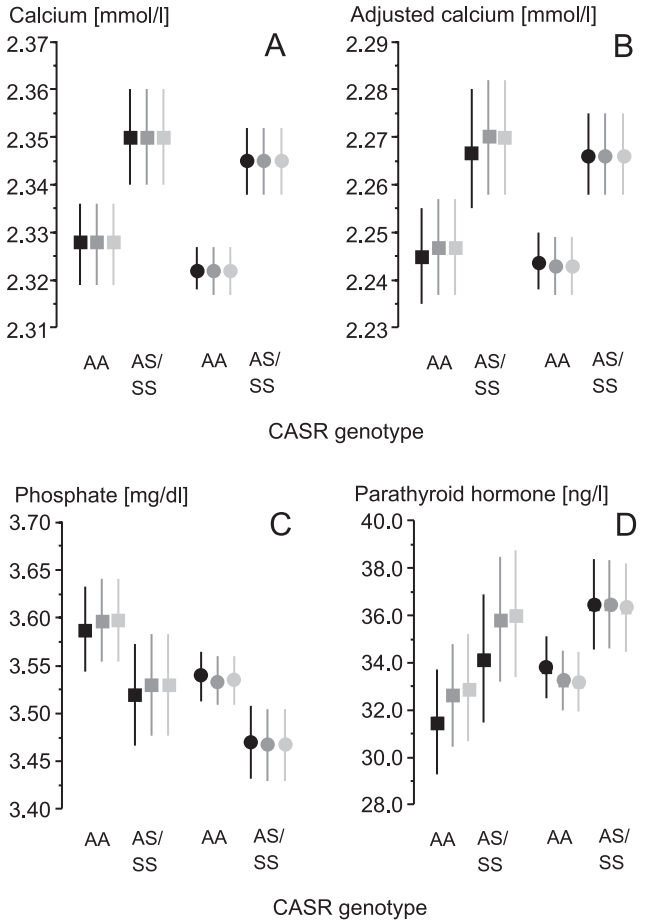

FIG. 1. Calcium (A), adjusted calcium (B), phosphate (C), and PTH (D) by CASR genotypes. *Squares* indicate individuals in whom CAD had been excluded by angiography. *Circles* designate individuals with angiographically proven CAD. Estimated marginal means (±95% CIs) of a linear model (ANOVA), including the CASR genotype and CAD status as main effects (*black*). Estimated marginal means adjusted for the calculated creatinine clearance (*dark gray*). Estimated marginal means in addition adjusted for age, gender, smoking (never, former, current), BMI, diabetes mellitus, and hypertension (*light gray*). The S-allele (genotypes AS and SS combined) of the CASR was associated with significantly higher calcium ($P < 0.001$ in unadjusted and adjusted models), albumin-adjusted calcium ($P < 0.001$ in unadjusted and adjusted models), and PTH ($P < 0.02$ in unadjusted and adjusted models), and with lower phosphate ($P < 0.003$ in unadjusted and adjusted models), independent of the absence or presence of angiographic CAD. The differences in calcium and corrected calcium between CAD patients and controls were not statistically significant. There was a tendency toward higher PTH in CAD patients ($P = 0.067$), but this difference disappeared after including creatinine clearance into the general linear model ($P = 0.611$). Phosphate was lower in CAD patients than in controls ($P = 0.045$).

Discussion

This study shows that a common genetic polymorphism of the CASR is associated with angiographic CAD, previous MI, and cardiovascular mortality independent of the well-established cardiovascular risk factors. Consistent with previous studies of patients with stable and unstable coronary disease (30, 31), total mortality largely reflected death from MI and sudden death in this study. Thus, the association of the CASR-PM with total mortality was weaker than with cardiovascular mortality, but it was still significant in some of the statistical models.

Mechanistically, these associations might be mediated by the effects of the CASR-PM on calcium homeostasis, vascular calcification, or the concentration of PTH. Homozygous and heterozygous loss of function mutations of the CASR have been implicated in neonatal severe hyperparathyroidism and familial benign hypocalciuric hypercalcemia, respectively (11, 13, 14). Neonatal severe hyperparathyroidism is characterized by marked hypercalcemia, parathyroid hyperplasia, and bone abnormalities. In humans, parathyroidectomy apparently cures the skeletal abnormalities, suggesting that the skeletal phenotype is mainly due to elevated PTH. In familial benign hypocalciuric hypercalcemia, calcium levels are only moderately elevated, and PTH is inappropriately normal or slightly elevated. Similar to mutations responsible for benign hypocalciuric hypercalcemia, the S-allele at position 986 of the CASR obviously impairs the sensitivity of the renal tubule to ambient calcium, reduces renal calcium elimination, and enhances the excretion of phosphate. Furthermore, the S-allele is likely to increase the set point of the parathyroid gland for calcium ions and increase PTH concentrations. Consistently, the S-allele was associated with high-calcium and low-phosphate concentrations in the current and previous studies (19, 20). In addition, we were able

to demonstrate that the S-allele also significantly increased the concentrations of circulating PTH, as expected. Cole *et al.* (19) did not notice this, probably because their study of 163 women was underpowered to detect the small difference between AA homozygotes and carriers of the S-allele.

It is an open question whether or not the changes in the concentrations of calcium and phosphate directly translate into accelerated atherosclerosis, more aggressive calcification of plaques, or noncoronary cardiac pathology. Calcium deposition is a salient feature of complex atherosclerotic lesions, and high circulating calcium concentrations might facilitate the formation of calcium deposits in coronary plaques. However, a negative correlation between serum calcium and CAD has been reported, whereas phosphate levels increased in parallel with the severity of angiographic changes (32). Furthermore, neither dietary nor supplemental intakes of calcium were appreciably associated with CAD risk among 50,000 men in the Health Professionals Follow-up study (33).

In our study, the association of the CASR-PM with CAD, MI, and mortality (all-cause and cardiovascular) remained statistically significant after adjusting for calcium and phosphate concentrations. Thus, high concentrations of PTH may in part account for the vascular effects of the S-allele of the CASR. In fact, PTH has been associated with metabolic risk factors and vascular pathologies. Primary hyperparathyroidism and hypophosphatemia appear to coincide with insulin resistance and diabetes mellitus (34–36). Removal of parathyroid adenomas in patients with primary hyperparathyroidism improves glucose tolerance, hypophosphatemia, (37) and abnormalities in lipoprotein metabolism (38). Consistently, PTH induces insulin resistance (39) and dyslipidemia (40) in chronic renal failure.

Parathyroid hormone may affect signaling pathways in endothelial cells (41, 42) and modify the tone of coronary arteries (43). In primary hyperparathyroidism, cardiac structure is abnormal, and mortality is increased (44–46). Further PTH has adverse effects on myocardial cells (47–49), and may represent a cofactor in cardiac fibrosis (50) and arteriolar wall thickening (51).

Some limitations pertain to our study. The control individuals were recruited at a tertiary referral center, underwent coronary angiography, and may, therefore, not be representative for a random population sample. However, this may also be regarded as a strength of the study. The prevalence of clinically asymptomatic coronary atherosclerosis has been very high at or older than 50 yr of age (52). Therefore, angiography based recruitment of controls rules out that individuals with significant, yet clinically unapparent, CAD are inadvertently allocated to the control group. Furthermore, the major cardiovascular risk factors occur at a similar frequency in our controls compared with the general population. The prevalence of hypertension is close to that found in a random probability sample from Germany (53). Prima vista, diabetes mellitus appears 2–3 times more frequently than in the German population (54). However, this is most likely due to the fact we did not rely on self-reports. Rather, we measured fasting glucose and performed an oral glucose challenge in individuals not previously known to have diabetes mellitus (55). Based on clinical history or fasting glucose measurements, the National Health and Nutrition Examination Surveys (NHANES) 1999–2000 reports prevalence rates of diabetes mellitus of 9.2% and 19.3% in adults 40–59 yr or older than 60 yr, respectively (56). In the current study, 12.1% of the controls had diabetes mellitus according to this criterion, while another 5.6% were detected by elevated post-challenge glucose only.

The validity of genetic association studies using single nucleotide polymorphisms has recently been questioned (57). Frequently, publications reporting positive results were followed and disproved by negative studies. One reason for a false-positive result could be population stratification. Although we restricted recruitment to Caucasians, we can currently not exclude that our cohort includes a subgroup with different ancestry and with a higher risk of CAD or MI, so that a chance allele frequency difference between this subgroup and the remaining cohort would appear associated with CAD or MI.

Of course, the present study is the first one to investigate the importance of the CASR-PM to vascular disease; hence it requires replication. Furthermore, the CASR-PM should be examined in relation to coronary calcium deposition as determined by noninvasive imaging methods like ultrafast multislice tomography. Finally, it would be interesting to see whether the CASR is expressed in cells involved in arterial calcification and atherosclerosis such as monocyte-derived macrophages.

In summary, we found a statistically significant association between the CASR polymorphism and CAD, MI, all-cause, and cardiovascular mortality. The results should be interpreted cautiously until replicated by other studies.

Acknowledgments

We thank Andrea Pfister and Manuela Gutenkunst (Freiburg), Elisabeth Winter and Sabine Kern (Graz) for technical assistance, and the LURIC study team and the laboratory staff at the Ludwigshafen General Hospital and the Universities of Freiburg and Ulm, Germany. We also thank the German registration offices and local public health departments for their assistance.

Received January 12, 2006. Accepted March 12, 2007.

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Conflict of Interest and Financial Disclosure Statement: Roche Diagnostics (Mannheim, Germany) provided unrestricted financial support and the assay kits for the determination of PTH free of charge but did not assume any other role in the conduct of the study. All authors have nothing to disclose.

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