Serum Uric Acid Levels, but Not rs7442295 Polymorphism of SCL2A9 Gene, Predict Mortality in Clinically Stable Coronary Artery Disease

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Abstract: Serum uric acid (SUA) has been associated with cardiovascular disease, but up to now whether SUA is an independent cardiovascular risk factor or merely a disease-related epiphenomenon remains still controversial. within the framework of the Verona Heart Study, we prospectively followed 703 subjects with angiographically demonstrated and clinically stable coronary artery disease between May 1996 and March 2007. At baseline, SUA levels were measured in all the patients. Genotype data of SCL2A9 rs7442295 polymorphism, which has been associated with SUA by genome-wide association studies, were available for 686 subjects (97.6%). After a median follow-up of 57 months, 116 patients (16.5%) had died, 83 (11.8%) because of cardiovascular causes. Patients with hyperuricemia, defined by SUA levels above the 75th percentile (≥0.41 mmol/L), had an increased total and cardiovascular mortality rate than those with SUA below this threshold level (23.3% vs 14.1%, $P = 0.048$ and 19.4% vs 9.2%, $P = 0.001$, respectively, by Kaplan-Meier with Log-Rank test). These associations were

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confirmed by Cox regression after adjustment for sex, age, other predictors of mortality, coronary revascularization, and drug therapies at discharge (hazard ratio for total mortality 1.87 [1.05-3.34], $P = 0.033$; hazard ratio for cardiovascular mortality 2.09 [1.03-4.25], $P = 0.041$). Although associated with SUA levels, rs7442295 polymorphism did not predict total or cardiovascular mortality. our data support that SUA may be a prognostic cardiovascular biomarker, predicting total and cardiovascular mortality in the setting of secondary prevention of coronary artery disease. On the other hand, SCL2A9 gene polymorphism, notwithstanding a clear influence on SUA levels, was not associated with mortality. (Curr Probl Cardiol 2021;46:100798.)

Introduction

Serum uric acid (SUA) is the final breakdown product of purine metabolism. The degradation of purine nucleotides is regulated mainly by the xanthine oxidoreductase enzyme, which converts hypoxanthine to xanthine and finally xanthine to UA.[1] SUA plasma levels are influenced by several factors, like dietary purine intake, alterations of purine metabolism, reduced intestinal secretion, and impaired renal function, as well as drug therapies, like diuretics and UA-lowering agents.[2]

UA has been hypothesized to play a role in cardiovascular disease (CVD) since the late 19th century, when an association of SUA with hypertension and renal disease was described.[3] Subsequently, several studies proposed a link between SUA and a wide variety of cardiovascular conditions, from metabolic syndrome to atherosclerotic diseases, including coronary artery disease (CAD), one of the most common CVD which is burdened with substantial morbidity and mortality.[4] However, the clinical importance of these associations remains controversial so far and is still debated if UA is an independent risk factor of CAD[5,6] or merely a disease-related epiphenomenon.[7] Reflecting xanthine oxidase activity, which is characterized by peroxidation and production of reactive oxygen species, SUA is considered as an indirect marker of oxidative stress.[8] It is worthy to note that UA appears like a Janus-faced molecule with both prooxidant and antioxidant activities. High levels of SUA promote oxygenation of low-density lipoprotein cholesterol, enhance lipid peroxidation and stimulate vascular smooth muscle cells proliferation, thereby suggesting a harmful role of UA fostering atherosclerosis

development and progression.[9] On the other hand, UA has also some significant antioxidant activities and cytoprotective actions,[10] in particular in the setting of neurodegenerative diseases[11,12] but even at vascular and/or endothelial level.[13]

The primary aim of this study was to explore whether SUA levels may predict total and cardiovascular mortality in subjects with angiographically demonstrated and clinically stable CAD. Moreover, we investigated genotype data of rs7442295 polymorphism in SCL2A9 gene, which codifies for a urate transporter playing a role in the urate reabsorption by renal proximal tubules and whose genetic variants have been consistently associated with SUA by genome-wide association studies.[14-16]

Material and Methods

Study Population

This observational study was performed within the framework of the Verona Heart Study (VHS), a regional survey that assessed new CAD risk factors in subjects with angiographic documentation of the state of their coronary vessels.[17,18] All the subjects in the VHS are required to have no history of any acute illness, including acute coronary syndrome, in the month preceding the enrolment. Subjects with severe renal failure (estimated glomerular filtration rate <30 mL/min) and those with severe hepatic impairment (clinically defined diagnosis of liver cirrhosis) were also excluded from this study. A total of 703 CAD patients of both sexes with available baseline evaluation of SUA were included in the present study. All these subjects had angiographically−documented CAD, with at least one of the major epicardial coronary arteries (left anterior descending coronary, circumflex, and right) affected with 1 or more significant stenoses (≥50% lumen reduction). All the CAD patients were newly diagnosed at time of enrolment (ie, at the time of coronary angiography). The angiograms were assessed in a blinded manner by 2 cardiologists who were unaware that the patients were to be included in this study.

All participants came from the same geographical area of northern Italy. At the time of blood sampling, a complete clinical history was collected, as well as data about drug therapies. The study complies with the Declaration of Helsinki and was approved by the ethics committee of our institution (Azienda Ospedaliera Universitaria Integrata, Verona). A written informed consent was obtained from all the participants.

Assessment of Outcome in Follow−Up Analysis

Subjects were followed until death or until March 31, 2007. Study subjects' status was determined by searching in the National Population Register and by an ambulatory or telephone survey. Certification and date of death were obtained from the National Population Register. The causes of death were obtained from death certificates kept at the Italian Institute of Statistics. Death from cardiovascular causes was defined as death caused by ischemic heart disease, heart failure, peripheral vascular disease, or cerebrovascular disease.

Laboratory Testing

Samples of venous blood were drawn from each subject, after an overnight fast, at the time of enrolment before coronary angiography. Serum lipids, as well as other CAD risk factors, including high−sensitivity C−reactive protein (hs-CRP), were determined as previously described.[17,19] SUA was tested by enzymatic-colorimetric assay with Roche/Hitachi 902 analyser.

SCL2A9 Gene rs7442295 Polymorphism Genotyping

DNA was extracted from peripheral-blood lymphocytes by the phenol−chloroform method. Genotyping was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Polymerase chain reaction primers were designed by Sequenom Mass-Array-Assay-Design program (Sequenom, San Diego, CA). Genotype data were available for 686 out of 703 subjects (97.6%).

Statistical Analyses

All calculations were performed using the IBM SPSS 23.0 (IBM Inc, Armonk, NY) statistical package. Distributions of continuous variables in groups were expressed as mean ± standard deviations. Skewed variables, like hs-CRP, were logarithmically transformed, and geometric means with 95% confidence intervals were reported. Quantitative data were assessed using the Student t−test or by ANOVA, with polynomial contrast for linear trend when indicated. Qualitative data were analysed with use of the $\chi 2$ test or $\chi 2$ for linear trend when indicated.

Survival was assessed by using the Kaplan−Meier method (Log−Rank statistic) and Cox regression models. Kaplan−Meier curves

were used for survival plots stratifying the CAD population on the basis of either SUA quartile distribution or threshold level at 75th percentile, that is, 0.41 mmol/L (6.9 mg/dL) defining hyperuricemia. Multivariate Cox proportional hazards for both total and cardiovascular mortality were performed considering the SUA 75° percentile value as threshold and including in the different models sex, age, all the predictors of mortality at univariate analyses at baseline, as well as some drug therapies at discharge. Final models were obtained with backward stepwise logistic regression models, with $P > 0.10$ as the critical value for excluding variables in the model. Hazard ratios and 95% confidence intervals are reported with 2−tailed probability values.

A value of $P < 0.05$ was considered statistically significant.

Results

After a follow-up with a median period of 57 months, 116 patients (16.5%) had died, 83 of them (11.8%) of cardiovascular causes.

SUA levels were significantly higher in patients who died (0.37 ± 0.10 mmol/L, $P = 0.04$) or died because of cardiovascular causes (0.38 ± 0.11 mmol/L, $P < 0.001$) compared to the survivors (0.35 ± 0.08 mmol/L). Stratifying the study population according to SUA quartile distribution, as shown by Kaplan-Meier survival curves (Fig 1), the highest quartile had increased total (Fig 1A) and cardiovascular mortality rate (Fig 1B). Therefore, we addressed our interest to the analysis of this subgroup of subjects with hyperuricemia, defined by SUA levels above the threshold level of the 75th percentile (≥0.41 mmol/L). Table 1 shows the clinical and laboratory characteristics of the study population divided on the basis of this threshold level of 0.41 mmol/L. Subjects with hyperuricemia were more often male, had a higher prevalence of arterial hypertension, increased levels of creatinine, triglycerides, total and LDL, as well as lower plasma concentration of HDL cholesterol and a worse left ventricular ejection fraction. As expected, allopurinol was more frequently prescribed to subjects with hyperuricemia, while no difference was found for the most common cardiovascular drugs (Table 1).

Subjects with hyperuricemia had an increased total and cardiovascular mortality rate than those with SUA <0.41 mmol/L (Table 1) and this result was confirmed by Kaplan-Meier survival curves (Fig 2A-B). High levels of SUA were associated with an about 2-fold increased risk of total and cardiovascular mortality (Table 2) and this associations remained significant after adjustment for sex, age, coronary revascularization procedure, and all the other predictors of mortality at univariate analyses, that

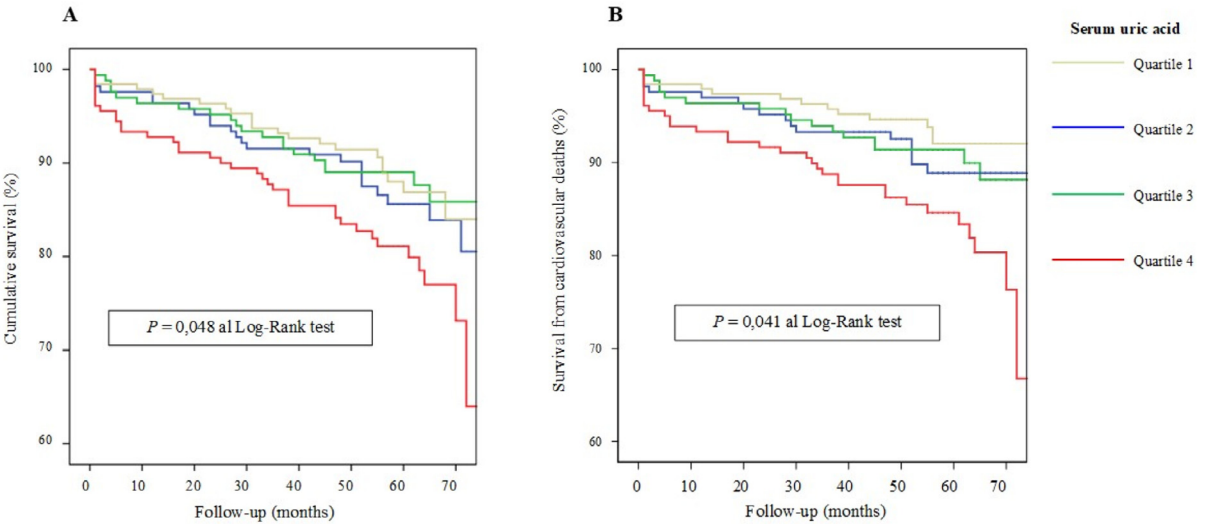

Fig 1. Kaplan-Meier survival curves from total (A) and cardiovascular mortality (B), stratified by serum uric acid levels.

Table 1. Baseline laboratory and clinical characteristic of the study cohort, subdivided on the basis of serum uric acid (SUA) with the 75th percentile as threshold level (0.41 mmol/L)

	Whole study population n = 703	SUA < 0.41 mmol/L n = 523	SUA 0.41 mmol/L n = 180	P*
Age, years	60.6 ± 9.7	61.0 ± 9.6	59.5 ± 10.1	0.085
Male gender, %	81.2	78.6	88.9	0.002
Coronary revascularization	92.5	92.7	91.9	0.705
Previous AMI, %	59.3	57.5	64.4	0.108
LVEF 55	62.1	65.5	52.7	
LVEF: 40-55	29.3	28.4	31.7	<0.001
LVEF <40	8.6	6.1	15.6	
Body mass index, kg/m^2	26.7 ± 3.4	26.5 ± 3.3	27.1 ± 3.7	0.077
Hypertension, %	64.6	61.6	73.3	0.005
Smoking, %	68.9	67.3	73.4	0.134
Diabetes, %	17.1	18.2	13.7	0,172
Serum creatinine, mmol/L	98.8 ± 39.8	93.8 ± 28.3	113.5 ± 59.9	<0.001
Total cholesterol, mmol/L	5.64 ± 1.17	5.53 ± 1.14	5.95 ± 1.20	<0.001
LDL-cholesterol, mmol/L	3.76 ± 1.02	3.66 ± 1.00	4.04 ± 1.01	<0.001
HDL-cholesterol, mmol/L	1.19 ± 0.30	1.20 ± 0.30	1.14 ± 0.30	0.012
Triglycerides, mmol/L	1.96 ± 1.15	1.85 ± 1.04	2.28 ± 1.36	<0.001
hs-CRP, mg/L	3.35 (3.03-3.71)	3.34 (2.97-3.74)	3.39 (2.86-4.06)	0.899
UA, mmol/L	0.36 ± 0.09	0.32 ± 0.06	0.47 ± 0.05	<0.001
Drug therapy at discharge				
Allopurinol, %	7.5	4.4	16.2	<0.001
β-blockers, %	52.5	53.1	50.9	0.616
ACE-inhibitors, %	49.8	49.0	52.4	0.612
Statins, %	51.5	52.4	47.2	0.256
Antiplatelet agents, %	91.9	92.9	89.1	0.117
Oral anticoagulants, %	4.0	4.0	4.2	0.906
All-cause deaths, %	116 (16.5)	74 (14.1)	42 (23.3)	0.004
Cardiovascular deaths, %	83 (12.4)	48 (9.7)	35 (20.2)	<0.001

ACE, angiotensin converting enzyme; AMI, acute myocardial infarction; HDL, high density lipoprotein cholesterol; hs-CRP, high sensitivity C Reactive Protein; LDL, low density lipoprotiein cholesterol; LVEF, left ventricular ejection fraction; UA, serum uric acid.
Data are expressed in n (%) or mean standard deviation.
*by t-test or χ^2-test, when appropriate.

is, creatinine plasma concentration, hs-CRP levels, diabetes, previous myocardial infarction, ventricular ejection fraction, allopurinol, beta-blocker and statin therapy at discharge (Table 3).

As regards SCL2A9 genotype analysis, the minor allele of rs7442295 polymorphism (G allele) was associated with lower SUA levels and lower prevalence of hyperuricemia (Fig 3A-B). The rs7442295 polymorphism was confirmed as a significant predictor of SUA levels in this study cohort even after adjustment for other main factors influencing SUA variability. With reference to survival analysis, we found no association of

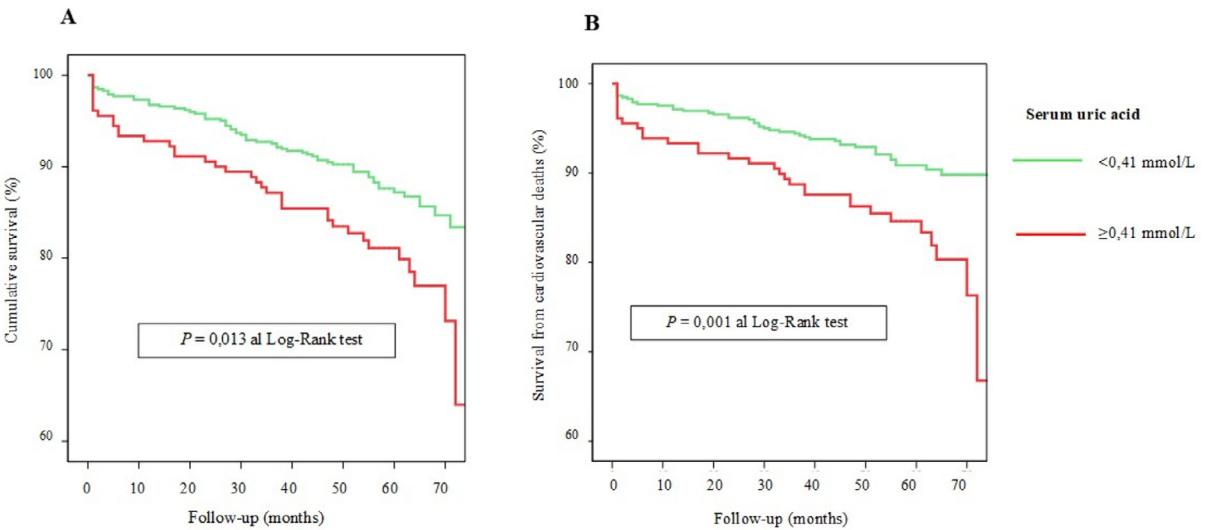

Fig 2. Kaplan-Meier survival curves from total (A) and cardiovascular mortality (B), stratified by quartiles of serum uric acid levels: I quartile <0.30 mmol/L, II quartile 0.30-0.35 mmol/L, III quartile 0.35-0.41 mmol/L, e IV quartile ≥ 0.41 mmol/L.

Table 2. Predictors of total and cardiovascular mortality by univariate and sex- and age-adjusted Cox regression models

	Total mortality				Cardiovascular mortality			
	Univariate		Sex- and age - adjusted		Univariate		Sex- and age - adjusted	
	HR	*P*	HR	*P*	HR	*P*	HR	*P*
SUA (0.1 mmol/L)	1.23 (1.01-1.51)	0.043	1.30 (1.06-1.60)	0.012	1.38 (1.08-1.75)	0.008	1.46 (1.16-1.85)	0.001
SUA ≥0.41 mmol/L (y/n)	1.62 (1.10-2.37)	0.014	1.76 (1.20-2.58)	0.004	2.11 (1.36-3.27)	0.001	2.34 (1.50-3.65)	<0.001
Age (10 year)	1.5 (1.29-1.95)	<0.001	1.67 (1.34-2.08)	<0.001	1.79 (1.39-2.30)	<0.001	1.86 (1.43-2.42)	<0.001
Diabetes (y/n)	1.88 (1.23-2.86)	0.003	1.87 (1.22-2.86)	0.004	2.16 (1.34-3.05)	0.002	2.13 (1.31-3.46)	0.002
Previous AMI (y/n)	1.73 (1.16-2.58)	0.008	1.90 (1.26-2.85)	0.002	1.95 (1.19-3.19)	0.008	2.17 (1.32-3.57)	0.002
LVEF <40% (y/n)	3.14 (1.89-5.21)	<0.001	2.97 (1.78-4.94)	<0.001	3.93 (2.23-6.94)	<0.001	3.63 (2.05-6.42)	<0.001
hs-CRP (mg/L)	1.30 (1.13-1.61)	0.001	1.20 (1.08-1.55)	0.004	1.38 (1.12-1.70)	0.003	1.31 (1.07-1.62)	0.011
Serum creatinine (mcmol/L)	1.06 (1.04-1.08)	<0.001	1.06 (1.04-1.08)	<0.001	1.06 (1.04-1.08)	<0.001	1.07 (1.05-1.09)	<0.001
Statins (y/n)	0.51 (0.32-0.81)	0.005	0.54 (0.34-0.87)	0.011	0.46 (0.26-0.81)	0.007	0.50 (0.28-0.88)	0.017
β-blockers (y/n)	0.44 (0.29-0.66)	<0.001	0.48 (0.32-0.73)	0.001	0.36 (0.22-0.59)	<0.001	0.41 (0.24-0.68)	0.001
Allopurinol (y/n)	3.64 (2.20-6.03)	<0.001	3.93 (2.37-6.52)	<0.001	4.48 (2.55-7.87)	<0.001	4.74 (2.70-8.33)	<0.001

AMI, acute myocardial infarction; HR, hazard ratio; hs-CRP, high sensitivity C reactive protein; LVEF, left ventricular ejection fraction; SUA, serum uric acid; y/n, yes/no.

Table 3. Predictors of total (3A) and cardiovascular mortality (3B) in multi-adjusted Cox regression model

3A		
Total mortality		
	Multi-adjusted Cox regression model*	
	HR	***P***
SUA ≥ 0.41 mmol/L (y/n)	1.87(1.05-3.34)	0.033
Previous AMI (y/n)	3.07 (1.52-6.21)	0.002
Serum creatinine (10 mcmol/L)	1.11 (1.05-1.18)	<0.001
Statins (y/n)	0.30 (0.14-0.63)	0.001
β-blockers (y/n)	0.46 (0.25-0.83)	0.011
3B		
Cardiovascular mortality		
	Multi-adjusted Cox regression model *	
	HR	***P***
SUA ≥ 0.41 mmol/L (y/n)	2.09 (1.03-4.25)	0.041
Age (10 years)	1.54 (1.02-2.33)	0.040
Previous AMI (y/n)	3.00 (1.19-7.57)	0.020
LVEF < 40% (y/n)	2.45 (1.01-5.99)	0.049
Serum creatinine (10 mcmol/L)	1.14 (1.07-1.21)	<0.001
Statines (y/n)	0.27 (0.10-0.74)	0.010
β-blockers (y/n)	0.42 (0.18-0.98)	0.046

AMI, acute myocardial infarction; LVEF, left ventricular ejection fraction; UA, serum uric acid; y/n, yes/no.
*by Cox regression analysis including in the model sex, age, coronary revascularization procedure, and all the other predictors of mortality at univariate analyses (ie, plasma creatinine concentration, hs-CRP levels, diabetes, previous myocardial infarction, ventricular ejection fraction, allopurinol, beta-blocker and statin therapy at discharge). Final models were obtained with backward stepwise logistic regression models, with $P > 0.10$ as the critical value for excluding variables in the model.

rs7442295 polymorphism with either total or cardiovascular mortality (Fig 4A-B).

Discussion

The main result of this study is that increased levels of SUA are associated with total and cardiovascular mortality in an Italian cohort with angiographically demonstrated and clinically stable CAD.

The association between SUA and CVD has been investigated for several decades, but with not conclusive results so far.[1,5-7] More precisely, if the correlation between SUA levels and CVD appears unquestionable,

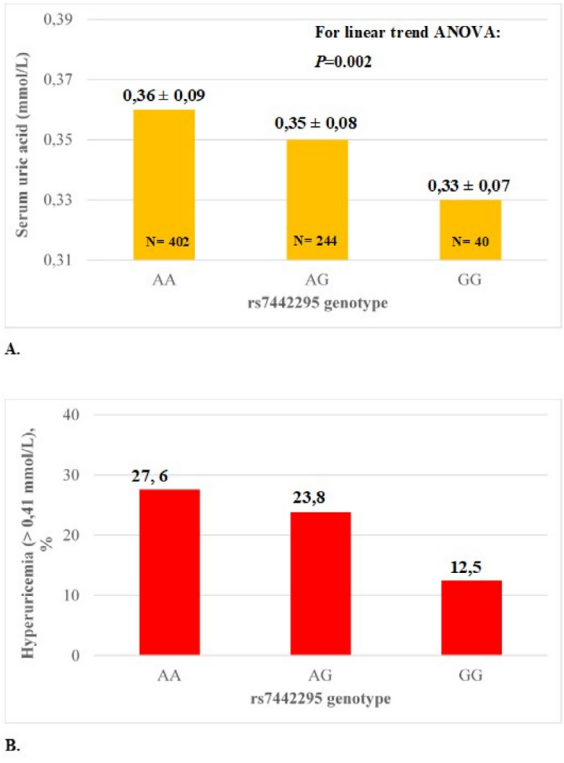

Fig 3. Serum uric acid levels (A) and prevalence of hyperuricemia (B) according to rs7442295 polymorphism of the SCL2A9 gene. AA: omozigote wild type. AG: heterozygous. GG: omozigote.

the issue of a causal role remains still debated. High levels of SUA are well known to associate with several cardiovascular risk factors, such as obesity, diabetes, dyslipidaemia, hypertension, renal insufficiency, as well as with iatrogenic settings, like the use of diuretics. Therefore, it has been proposed that SUA may be merely an epiphenomenon of other conditions linked with CVD.[7]

In our analysis SUA levels remained a predictor of mortality even after a complete series of adjustments for potential confounding factors, including diabetes and renal function, as well as some drug therapies at discharge, thereby suggesting SUA as an independent prognostic marker in CAD.

Our results are consistent with other studies linking SUA and CVD. In the Japanese Coronary Artery Disease (JCAD) Study, elevated SUA was an independent predictor of cardiovascular events and all-cause mortality in patients with severe CAD.[20] Noteworthy, JCAD Study was

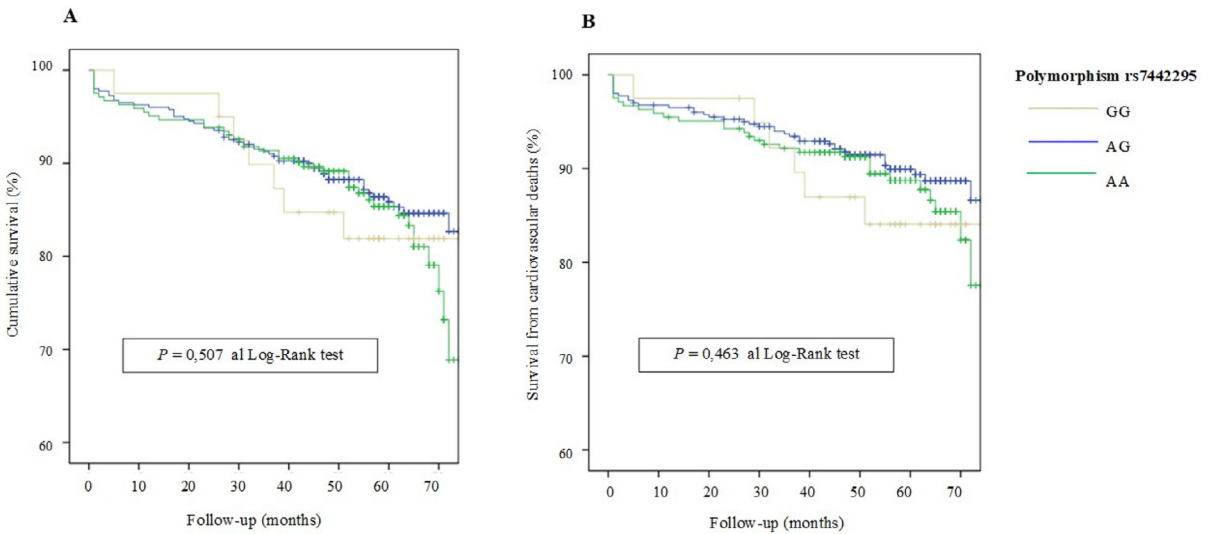

Fig 4. Kaplan-Meier survival curves from total (A) and cardiovascular mortality (B), stratified by rs7442295 polymorphism of the SCL2A9 gene.

angiographically-controlled like VHS and its results were very similar to our findings with a clear increase of the risk only in the highest SUA quartile, that is, SUA ≥6.8 mg/dL, a threshold substantially equivalent to that in our study cohort. As regards the SUA threshold level, it is worthy to note that in both JCAD Study and VHS cohorts the increase of mortality risk was evident at SUA levels corresponding approximately to those conventionally accepted for defining hyperuricemia (>6.5 mg/dL in women and >7.0 mg/dL in men), which in turn are near to SUA solubility limit (6.8 mg/dL at pH = 7). In a Taiwanese cohort of CAD patients undergoing coronary revascularization both higher SUA variability and average levels were significantly associated with an increased occurrence of cardiovascular events and mortality.[21] Elevated SUA levels have been associated with increased cardiovascular mortality in diabetic subjects[22] and in patients with acute coronary syndrome[23] or acute myocardial infarction,[24] as well as in general non-institutionalized population within the First National Health and Nutrition Examination Survey (NHANES I) and NHANES I Epidemiologic Follow-up Study (NHEFS).[25] Within the Taiwan I-Lan Longitudinal Aging Study, high SUA levels were correlated with cardiovascular risk estimated by Framingham risk score in non-hypertensive and non-diabetic individuals.[26] In asymptomatic individuals, high SUA level was an independent predictor of non-calcified plaques as detected by coronary computed tomography angiography, thus suggesting a link with subclinical coronary atherosclerosis.[26] SUA levels have been associated with early-onset CAD in patients younger than 50 years, in whom high SUA concentration was also a marker of a more advanced involvement of coronary vessels.[27] A large meta-analysis of cohort studies of over a million subjects found a positive dose-response association between SUA and CVD mortality risk.[28]

Recent studies have suggested that the relationship between SUA and mortality would be not linear, but could show a U-shaped curve, with both low and high SUA levels associating with increased with total and cardiovascular mortality.[29,30] Taking into account that circulating UA may protect vascular endothelial cells from oxidative stress and UA contributes by itself to more than 50% of human plasma antioxidant capacity,[31] it has been claimed that hypouricemia may represent a decrease of total antioxidant protection, thereby favouring the development of vascular atherosclerotic diseases.[30] On the other hand, it should be noted that low SUA may be an indirect marker of malnutrition and has been related with other nutritional defects, like vitamin C and D deficiency, which in turn play a role in anti-inflammatory and anti-oxidant protection.[32,33] According with the latter view, in a large cohort of Taiwanese elderly

subjects (age ≥65 years) low SUA levels predicted cardiovascular mortality only in malnourished people and consistent with the severity of malnutrition.[29]

While data linking hypouricemia and CVD are limited so far, there is a substantial bulk of epidemiological data supporting the association of hyperuricemia and cardiovascular risk in the setting of both primary and secondary prevention.[29-33] However, the precise mechanism driving this association and the potential causal role of UA remain still unclear. Several biological processes have been proposed as molecular mechanisms linking hyperuricemia and CVD. UA has been found to impair nitric oxide synthesis resulting in vascular endothelial dysfunction,[34,35] to stimulate directly vascular smooth muscle cell proliferation,[36] and to promote the calcification of blood vessel.[37] High levels of UA can activate the nucleotide-binding leucine-rich repeat-containing pyrin receptor 3 (NLRP3) inflammasome and induce the production of interleukin-1β and interleukin 18, eventually stimulating the inflammatory cascade.[38] Moreover, UA may promote chemokine production and adhesion molecule expression in vascular endothelium via Nuclear Factor-kappa B (NF-kB) signalling.[39] Finally, UA can induce epithelial-mesenchymal transition of renal tubular epithelial cells via the TLR4/NF-kB pathway, contributing to kidney fibrosis.[40] On the other hand, reflecting xanthine oxidase activity which is well known to promote peroxidation and reactive oxygen species production, UA may be also merely an indirect marker of oxidative stress[8] and a surrogate of the real pathogenic mechanism.[41] An increased xanthine oxidase activity has been associated with coronary lipid-rich plaque in patients with stable CAD undergoing elective percutaneous coronary intervention under near-infrared spectroscopy intravascular ultrasound.[42] Moreover, there are some therapeutic evidences, which address the role of xanthine oxidase and the related generation of oxidative free radicals. Allopurinol, the historical inhibitor of xanthine oxidase, has been proposed to reduce vascular oxidative stress, decrease endothelial dysfunction, and improve cardiovascular outcomes,[43-46] whereas the beneficial effects for CVD − even though with some controversial results − have been observed less frequently in patients treated with uricosuric agents,[47-50] thereby suggesting that upregulated xanthine oxidase activity rather than UA by itself is actively involved in cardiovascular harmful effects.[51] In our study cohort at univariate analysis therapy at discharge with allopurinol was associated with an increased risk of mortality. But such result, at first glance contradictory, can be explained bearing in mind that our present observational study is not a clinical trial and that patients taking allopurinol at discharge were usually allocated

within the subgroup of subjects with hyperuricemia. Consistently with these considerations, including in the adjusted Cox regression model both hyperuricemia and allopurinol, only the former remained associated with mortality.

The link between UA and CVD remains anyway still debated. The controversies about such association come to light distinctly also by considering the studies on genetic determinants of SUA levels. SCL2A9 gene locus, although identified by genome-wide association studies as main genetic determinant of SUA and clearly associated with gout, has not been found to associate with CVD.[14-16,52,53] Therefore, an evident proof of mendelian randomization is still lacking for the association between UA and CVD. In our study cohort, consistent with these results, SCL2A9 rs7442295 polymorphism although significantly associated with SUA did not predict total and cardiovascular mortality. However, it should be noted that this polymorphism accounts for only a little variability – about 5% – of SUA levels[16] and such small variation may not have any clinically relevant effect on a complex phenotype like CVD. On the other hand, we emphasize that in the present study we assessed only 1 gene polymorphism modulating SUA levels and specifically involved in renal excretion of UA, but we did not provide any data on other genotypes potentially influencing SUA levels. From this point of view, a recent phenome-wide mendelian randomization study in UK Biobank cohort demonstrated a robust association between SUA and many diseases including gout, CVD, hypertension, and metabolic disorders of lipids, but the causal role of UA was only supported in gout. Genetic polymorphism involved in renal handling of UA were confirmed to be associated with gout but not with CVD, while the association between SUA and CVD could be due to the pleiotropic effects of other genetic variants on urate and metabolic traits, like hypertension, plasma lipids, and oxidative damage.[53] Therefore, the authors of this study hypothesized that the negative results of earlier mendelian randomization studies may be imputed to the selection of genes selectively involved in renal clearance of UA, while a substantial portion of cardiovascular risk is probably due to pleiotropic genes controlling xanthine oxidase activity and UA production.[53]

Our analysis has some significant limitations which should be acknowledged, from the small sample size to the unbalance between males and females in the study cohort, from the lack of clinical data (eg, food and alcohol intake, prescription of diuretics) to the incomplete assessment of genetic determinants of SUA.

Conclusions

Our results support in a well-characterized and angiographically-demonstrated CAD population a solid role of SUA levels as prognostic marker in the setting of secondary prevention of clinically stable CAD. On the other hand, the rs7442295 polymorphism of SCL2A9 gene, which plays a crucial function in modulating renal clearance of UA and then in influencing SUA levels, does not predict total or cardiovascular mortality in the same study cohort, thereby being consistent with the hypothesis that renal handling of UA is not linked with a substantial cardiovascular risk. Further studies investigating UA-related molecular pathways, as well as larger prospective studies and clinical trials on different UA-lowering therapies are needed to elucidate the complex quiddity of UA in CVD.

Authors' Contributions

CM performed the interpretation of data and wrote the manuscript. DG conceived the study, performed the statistical analysis and revised the manuscript. AS, JC, and FS collected the data and contributed to data interpretation. FP, SF, and OO contributed significantly to interpretation of data and revising the intellectual content. NM conceived the study, performed the statistical analysis and wrote the manuscript.

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Ethical approval

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (include name of committee + reference number) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards." The Ethical Committee of the Azienda Ospedaliera Universitaria Integrata Verona approved the study.

Informed consent

Informed consent was obtained from all individuals participants included in the study.

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