

# Uric Acid and Cardiovascular Events: A Mendelian Randomization Study

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## ABSTRACT

Obesity and diets rich in uric acid-raising components appear to account for the increased prevalence of hyperuricemia in Westernized populations. Prevalence rates of hypertension, diabetes mellitus, CKD, and cardiovascular disease are also increasing. We used Mendelian randomization to examine whether uric acid is an independent and causal cardiovascular risk factor. Serum uric acid was measured in 3315 patients of the Ludwigshafen Risk and Cardiovascular Health Study. We calculated a weighted genetic risk score (GRS) for uric acid concentration based on eight uric acid-regulating single nucleotide polymorphisms. Causal odds ratios and causal hazard ratios (HRs) were calculated using a two-stage regression estimate with the GRS as the instrumental variable to examine associations with cardiometabolic phenotypes (cross-sectional) and mortality (prospectively) by logistic regression and Cox regression, respectively. Our GRS was not consistently associated with any biochemical marker except for uric acid, arguing against pleiotropy. Uric acid was associated with a range of prevalent diseases, including coronary artery disease. Uric acid and the GRS were both associated with cardiovascular death and sudden cardiac death. In a multivariate model adjusted for factors including medication, causal HRs corresponding to each 1-mg/dl increase in genetically predicted uric acid concentration were significant for cardiovascular death (HR, 1.77; 95% confidence interval, 1.12 to 2.81) and sudden cardiac death (HR, 2.41; 95% confidence interval, 1.16 to 5.00). These results suggest that high uric acid is causally related to adverse cardiovascular outcomes, especially sudden cardiac death.

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Uric acid (UA) is the end product of purine metabolism in humans. In most other mammalian species, UA can be degraded by the enzyme uricase, which mutated in human and ape ancestors about 15 million years ago.<sup>1</sup> The inactivation of uricase and the resulting increased levels of UA are thought to have provided evolutionary advantages by protecting against oxidative damage (antioxidant properties of UA),<sup>2,3</sup> and by maintaining BP under conditions of low salt availability<sup>4</sup> and upright walking.

Increased serum levels of UA have long been recognized as the cause of gout,<sup>5</sup> a disease that has afflicted humans all over the world for thousands of years. Modern lifestyles and the Western diet rich in

UA-raising components such as red meat or sugar, especially fructose,<sup>6</sup> are accompanied by a dramatic rise in UA serum levels. Choi *et al.* compared Third

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National Health and Nutrition Examination Survey participants with >4 servings of soft drinks per day to those with <0.5 servings per day, and reported an increase of 0.42 mg/dl in mean UA concentration.<sup>7</sup> There are also historic examples such as the Maori<sup>8</sup> or Japanese immigrants in the United States.<sup>9</sup> Prevalence rates of cardiometabolic diseases (e.g., hypertension, diabetes mellitus, CKD, coronary heart disease, or stroke) increased together with the increase of UA levels.<sup>10,11</sup> However, whether UA truly represents an independent risk factor for the development of these diseases is still controversial. The increasing evidence that UA plays a key role in cardiovascular disease (CVD) and both existing and emerging medicines to modify its concentration will warrant in-depth investigations to further elucidate answers in the long-lasting debate about UA and CVD.

One strategy to resolve the question of whether a biomarker is causally involved in disease development of a disease is the method of Mendelian randomization (MR). MR is based on the random assortment of genes from parents to offspring that occurs during gamete formation and conception.<sup>12</sup> MR makes use of measured variation in genes of known function. The association between a disease and a polymorphism that mimics the biologic link between a proposed exposure and disease is not generally susceptible to reverse causation or confounding that may distort interpretations of conventional observational studies.

UA concentrations show a substantial degree of heritability. Most studies report heritability estimates of about 40%.<sup>13–15</sup> Recently, a large meta-analysis including >140,000 individuals identified 28 genetic loci that were significantly associated with serum UA concentration genome wide<sup>16</sup> and explained about 7% of its variation. This knowledge of the genetic regulation of UA allows the use of MR to examine a possible causal relationship between UA and cardiovascular risk. One prerequisite for performing MR is that the investigated genetic loci are exclusively associated with the outcome *via* their effect on the concentration of the biomarker in question and not *via* alternative pathways.

Silbernagel *et al.* reported a significant association of the serum UA concentration with cardiovascular mortality and sudden cardiac death (SCD) in the Ludwigshafen Risk and Cardiovascular Health (LURIC) study.<sup>17</sup> This prompted us to study whether a genetic risk score (GRS) of variants regulating UA concentrations is associated with prevalent coronary heart disease (cross-sectionally) and with adverse cardiovascular outcomes (prospectively) in the same cohort.

## RESULTS

### Association of Single Nucleotide Polymorphisms with UA

We tested the association of all 28 single nucleotide polymorphisms (SNPs) that were previously found to be significantly associated with the concentration of UA.<sup>16</sup>  $\beta$  coefficients and *P* values

of additive linear regression models are shown in Supplemental Table 1. Four SNPs located at the *SLC2A9*, *TMEM171*, *ABCG2*, and *GCKR* loci were nominally significant ( $P=9.92^{-09}$ ,  $P=0.03$ ,  $P=0.01$ , and  $P=0.01$ , respectively). However, only the *SLC2A9* locus was still significant after Bonferroni correction for 28 tests ( $P<0.002$ ). For 19 loci, the reported risk allele resulted in an increase in UA concentration; for 9 of the candidate loci, the effect was opposite to the reported one.<sup>16</sup>

### Pleiotropic Effects of UA SNPs

One of the prerequisites of MR is that the investigated polymorphisms only affect the outcome *via* the investigated biomarkers. In a classic view, this means that the SNPs should not affect other biomarkers that might influence the outcome; however, because of the complexity and interconnectedness of biologic pathways, this is only an idealized view. It is not straightforward to decipher whether the association of a SNP with other markers is due to “horizontal” pleiotropy (direct association of SNPs with other markers) or a result of an interaction of markers in the same pathways (“vertical” pleiotropy).<sup>18</sup> A method called network Mendelian randomization was recently described to investigate whether some of the effect of an exposure on the outcome may operate through a mediating variable.<sup>19</sup> However, this kind of analysis is beyond the scope of our article.

We checked whether the 28 urate SNPs in the meta-analysis by Köttgen *et al.*<sup>16</sup> showed any association with major cardiovascular risk factors in the LURIC study. We therefore examined potential associations with LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), total cholesterol, triglycerides (TGs), systolic BP (SBP), diastolic BP (DBP), fasting glucose, fasting insulin, body mass index (BMI), high-sensitive C-reactive protein (hsCRP), and  $\gamma$ -glutamyl transpeptidase using an additive model. Fourteen of the 28 SNPs showed an association with at least one trait with a nominal *P* value <0.05 (Supplemental Table 2). Two SNPs (rs11264341 and rs17050272) were associated with LDL-C, two SNPs were associated with total cholesterol (rs17050272 and rs675209), one SNP was associated with TG (rs1260326), three SNPs were associated with BMI (rs2231142, rs10480300, and rs3741414), three SNPs were associated with fasting glucose (rs2231142, rs729761, and rs10480300), two SNPs showed association with fasting insulin (rs1471633, and rs7976059), five SNPs were associated with SBP (rs17632159, rs1165151, rs10480300, rs1171614, and rs2078267), five SNPs showed association with DBP (rs17632159, rs1165151, rs10480300, rs1171614, and rs675209), and one SNP showed association with  $\gamma$ -glutamyl transpeptidase (rs1260326).

### Calculation of Weighted GRSs

We calculated weighted GRSs for UA concentration based on all 28 SNPs (GRS<sub>28</sub>) and all 14 SNPs with no apparent association to other major risk factors (GRS<sub>14</sub>). Eight of the 14 SNPs that did not reveal pleiotropic effects showed an effect directional in accordance with the published meta-analysis and only these

were used to calculate the final genetic risk score (GRS<sub>8</sub>). The score was defined as the sum of the number of UA-increasing alleles at each locus multiplied by the respective  $\beta$  coefficient reported from the meta-analysis.<sup>16</sup> Histograms of the distribution of the GRS as well as scatter plots showing the correlation between GRS and uric acid are shown in the supplemental Figures 1 and 2. A strong association with the serum concentration of UA was found for all genetic risk scores with  $\beta$  coefficients of 0.751 (SEM 0.107), 0.713 (SEM 0.128), and 0.814 (SEM 0.132) and  $P$  values of  $3.43^{-12}$ ,  $4.02^{-09}$ , and  $8.30^{-10}$  for GRS<sub>28</sub>, GRS<sub>14</sub>, and GRS<sub>8</sub>, respectively (Supplemental Table 1). In all further analyses, we selected GRS<sub>8</sub>, which showed strong association with UA although it contained no SNPs with suspected pleiotropy in the LURIC study.

### Study Characteristics According to GRS<sub>8</sub> Quartiles

The calculated risk score GRS<sub>8</sub> was neither consistently associated with any of the investigated biochemical markers such as blood lipids, blood glucose, BP, or eGFR except for UA, nor with other risk factors such as smoking status or BMI (Table 1). There was no association with the prevalence of coronary heart disease, hypertension, or diabetes mellitus at study entry or with medication (Supplemental Table 3).

### Association of UA and GRS<sub>8</sub> with Disease

Next we compared the association of the serum UA concentration and GRS<sub>8</sub> with a range of cardiovascular and non-cardiovascular conditions. Although association was found between UA concentration and coronary artery disease (CAD), peripheral vascular disease, cardiomyopathies, valve disease, atrial fibrillation, hypertension, as well as diabetes mellitus, there was no association of GRS<sub>8</sub> with any of these disease conditions (Supplemental Table 4). UA concentration was not associated with gout either, but there were only 19 presumed cases of gout in our data set, which were defined by the intake of antigout medication.

We used a two-stage regression estimator to calculate causal odds ratios (CORs) per 1-mg/dl increase in UA (Table 2). In the first stage, a linear regression of UA on GRS<sub>8</sub> was calculated. The predicted UA values from the first stage were then used for logistic regression analysis with the disease as the dependent variable. The 95% confidence intervals (95% CIs) of all calculated CORs gave no evidence for rejecting the null hypothesis of no association. For comparison, odds ratios (ORs) for a 1-mg/dl increase in measured UA concentration were calculated and shown as well.

### Association of UA and GRS<sub>8</sub> with Fatal Events

The association of UA concentration in the LURIC study with all-cause mortality, cardiovascular mortality (CVD), and SCD was previously reported by Silbernagel *et al.*<sup>17</sup> The GRS in our study was not associated with all-cause mortality, but an association was found for CVD and SCD. The lower 95% CI boundary remained  $>1.00$  if we adjusted our model for other risk factors, namely age, sex, LDL-C, HDL-C, smoking, BMI,

diabetes, hypertension, eGFR, TGs, the severity of CAD expressed as Friesinger score,<sup>20</sup> hsCRP, and medication (Supplemental Table 5).

With Cox proportional hazards regression, we calculated causal hazard ratios (CHRs) for the association of UA with all-cause mortality, CVD, and SCD (Table 3). Associations were found for CVD and SCD with CHRs of 1.77 (95% CI, 1.12 to 2.81) and 2.41 (95% CI, 1.16 to 5.00) per 1-mg/dl genetically caused increase of UA concentration, respectively. An equal increase in measured serum UA concentration was also associated with CVD and SCD (hazard ratio [HR], 1.08 [95% CI, 1.03 to 1.13]; and HR, 1.08 [95% CI, 1.00 to 1.17]) in multivariate adjusted models, respectively.

## DISCUSSION

We examined whether a GRS, composed of SNPs significantly associated with serum UA concentration, was also associated with CVD, events, and mortality. We found an association of the GRS with cardiovascular mortality and SCD. This association remained nominally significant after multivariate adjustment. The CHRs were 1.77 (95% CI, 1.12 to 2.81) and 2.41 (95% CI, 1.16 to 5.00) per 1-mg/dl increase of genetically predicted UA, respectively. Bonferroni correction of  $P$  values rendered the association with SCD only borderline significant.

This correlation confirms the findings concerning UA in the analysis of Silbernagel *et al.*<sup>17</sup> By contrast, we did not find an association of the GRS with prevalent CAD. UA might therefore be causally involved in long-term adverse cardiovascular outcomes by mechanisms that are independent of atherosclerosis.

UA has been linked to a wide spectrum of diseases. The strongest evidence is available for hypertension. In almost every prospective study, UA was a strong and independent predictor of hypertension. A recent meta-analysis encompassing data from 97,824 participants showed an increased risk for incident hypertension of 1.15 (95% CI, 1.06 to 1.26) for a 1-mg/dl increase in serum UA.<sup>21</sup> In this study, we observed an increased prevalence rate of hypertension with an OR of 1.16 (95% CI, 1.10 to 1.22) per 1-mg/dl increase. Experiments in rats could convincingly show that inhibition of uricase, which raises the UA serum level, also increases BP. This could be prevented in rat experiments by xanthine oxidase inhibitors (*i.e.*, blockade of UA synthesis).<sup>22</sup> Treatment with allopurinol resulted in a reduction of BP in adolescents.<sup>23</sup> However, it is noteworthy that a recent MR analysis reported no causal relationship between UA and SBP or DBP.<sup>24</sup>

The association of hyperuricemia with adverse cardiovascular outcomes has been found in a number of studies. An association with high serum UA levels has been reported for stroke and heart failure,<sup>25–27</sup> which are major consequences of hypertension. A recent meta-analysis encompassing the data of  $>172,000$  individuals confirmed that UA is an independent predictor of cardiovascular mortality.<sup>28</sup> Thus, hyperuricemia

**Table 1.** Patient characteristics according to quartiles of urate GRS

Characteristic	All Patients	GRS <sub>8</sub>				P Value*
		First Quartile (≤0.63), n=764	Second Quartile (0.64–0.94), n=828	Third Quartile (0.95–1.03), n=813	Fourth Quartile (≥1.04), n=655	
UA (mg/dl)	5.11±1.69	4.90±1.61	5.02±1.69	5.20±1.74	5.33±1.71	<0.001
Men	70.1	71.6	67.5	71.3	69.8	0.25
Age (yr)	62.71±10.63	62.6±10.6	62.7±11.0	63.0±10.6	62.7±10.4	0.91
BMI (kg/m <sup>2</sup> )	27.5±4.0	27.6±4.13	27.5±4.1	27.2±3.9	27.6±4.0	0.23
eGFR (ml/min per 1.73 m <sup>2</sup> )	81.7±20.2	82.0±20.2	81.9±20.9	81.9±20.0	82.0±19.7	0.99
DBP (mmHg)	80.9±11.4	81.1±11.3	80.9±11.6	80.9±11.5	80.6±11.1	0.90
SBP (mmHg)	141.1±23.6	141.1±23.0	140.1±24.0	142.2±24.0	140.1±23.6	0.36
Fasting glucose (mg/dl)	102.4 (93.7–118.5)	102.1 (93.2–116.8)	103.0 (94.2–120.6)	102.6 (94.7–119.4)	101.0 (93.4–116.0)	0.24
LDL-C (mg/dl)	116.5±34.5	115.5±34.2	117.9±35.5	117.0±35.1	115.4±32.7	0.41
HDL-C (mg/dl)	38.7±10.8	38.7±11.0	38.7±10.0	38.7±11.0	38.6±10.7	0.99
TGs (mg/dl)	147.0 (109.0–201.0)	141.0 (106.0–195.0)	147.0 (110.0–202.0)	143.0 (106.0–189.7)	145.5 (110.0–208.3)	0.09
hsCRP (mg/L)	3.46 (1.32–8.76)	3.93 (1.36–9.59)	3.38 (1.34–8.52)	2.96 (1.17–8.40)	3.75 (1.46–8.61)	0.03
Smoking (no/ex/current)	35.8/41.0/23.2	34.8/41.8/23.4	35.7/41.2/23.2	37.1/39.1/23.8	35.9/41.0/22.2	0.52
CAD	79.0	79.7	78.6	77.5	80.6	0.89
Hypertension	72.7	74.0	71.0	75.0	70.4	0.52
Diabetes	40.4	40.1	43.4	38.6	39.2	0.31
All-cause mortality	31.9	27.7	28.9	31.0	29.8	0.64
Cardiovascular mortality	18.4	17.7	17.3	17.3	21.9	0.35
SCD	8.9	9.1	7.3	8.5	11.2	0.17

Data are given as percentages, mean±SD, or median (interquartile range), unless otherwise indicated. Variables with skewed distribution were log transformed before entering analysis.

\*Chi-squared test for categorical variables. ANOVA for continuous variables.

apparently predicts CVD, but it has thus far not been established as a causal cardiovascular risk factor.<sup>29–31</sup> In the LURIC study, the UA concentration was associated with a number of pathologic conditions. By contrast, CORs were not significant for any of these conditions when calculated using the GRS derived from SNPs associated with UA concentration. This is in line with a meta-analysis from the Cohorts for Heart and Aging Research in Genomic Epidemiology consortium,

which did not find an association of a genetic urate score with incident CAD,<sup>32</sup> and Palmer *et al.*, who found no association of UA with prospectively assessed ischemic heart disease in their recent MR study.<sup>24</sup> Because this study only used one SNP in the *SLC2A9* gene as an instrumental variable, we also calculated CORs and CHRs using a single SNP in *SLC2A9* (rs12498742), which is in high linkage disequilibrium with the one used by Palmer *et al.* The COR for prevalent CAD

**Table 2.** CORs from MR analysis for association of UA with prevalent disease

Disease	No. of Cases	OR per 1-SD Increase in GRS <sub>8</sub>		COR per 1-mg/dl Increase in UA		UA per 1-mg/dl Increase	
		OR (95% CI)	P Value	COR (95% CI)	P Value <sup>a</sup>	OR (95% CI)	P Value
CAD	2418	0.99 (0.91 to 1.09)	0.90	0.99 (0.62 to 1.58)	0.97	1.18 (1.11 to 1.24)	<0.001
Peripheral vascular disease	295	0.92 (0.82 to 1.04)	0.18	0.76 (0.40 to 1.43)	0.39	1.16 (1.09 to 1.23)	<0.001
Cardiomyopathy	316	1.00 (0.89 to 1.12)	0.93	0.87 (0.47 to 1.62)	0.66	1.31 (1.24 to 1.39)	<0.001
Valve disease	538	1.08 (0.99 to 1.19)	0.10	1.29 (0.78 to 2.13)	0.33	1.09 (1.04 to 1.14)	0.001
Arrhythmia	444	0.98 (0.88 to 1.08)	0.64	1.12 (0.65 to 1.92)	0.69	1.03 (0.98 to 1.09)	0.29
Atrial fibrillation	368	1.03 (0.93 to 1.15)	0.57	1.19 (0.66 to 2.14)	0.57	1.14 (1.08 to 1.20)	<0.001
Hypertension	2225	0.98 (0.90 to 1.06)	0.56	0.86 (0.56 to 1.32)	0.49	1.18 (1.12 to 1.24)	<0.001
Diabetes mellitus	1236	0.94 (0.88 to 1.01)	0.10	0.83 (0.57 to 1.23)	0.36	1.20 (1.15 to 1.25)	<0.001
Cancer	226	0.95 (0.83 to 1.08)	0.41	0.77 (0.38 to 1.58)	0.48	1.05 (0.98 to 1.13)	0.19
Gout	19	1.15 (0.72 to 1.82)	0.56	1.91 (0.15 to 24.08)	0.62	0.79 (0.57 to 1.09)	0.16

<sup>a</sup>After Bonferroni correction for 10 tests, P<0.01 would be considered significant.

**Table 3.** CHR from MR analysis for association of UA with mortality, cardiovascular mortality, and SCD

Model	HR per 1-SD increase in GRS <sub>8</sub>		CHR per 1-mg/dl Increase in UA		HR per 1-mg/dl Increase in UA	
	HR (95% CI)	P Value	CHR (95% CI)	P Value <sup>a</sup>	HR (95% CI)	P Value
Model 1						
All-cause mortality	1.01 (0.95 to 1.08)	0.78	1.05 (0.74 to 1.49)	0.78	1.22 (1.18 to 1.26)	<0.001
Cardiovascular mortality	1.09 (1.00 to 1.19)	0.04	1.62 (1.02 to 2.56)	0.04	1.27 (1.28 to 1.32)	<0.001
SCD	1.14 (1.00 to 1.31)	0.05	2.06 (1.00 to 4.24)	0.05	1.31 (1.23 to 1.39)	<0.001
Model 2						
All-cause mortality	1.01 (0.94 to 1.08)	0.81	1.04 (0.73 to 1.49)	0.81	1.17 (1.13 to 1.21)	<0.001
Cardiovascular mortality	1.09 (1.00 to 1.19)	0.04	1.61 (1.02 to 2.57)	0.04	1.22 (1.18 to 1.27)	<0.001
SCD	1.13 (0.99 to 1.30)	0.08	1.94 (0.93 to 4.03)	0.08	1.26 (1.18 to 1.34)	<0.001
Model 3						
All-cause mortality	1.02 (0.96 to 1.09)	0.55	1.11 (0.78 to 1.58)	0.55	1.08 (1.03 to 1.12)	<0.001
Cardiovascular mortality	1.12 (1.02 to 1.22)	0.01	1.79 (1.13 to 2.83)	0.01	1.12 (1.07 to 1.18)	<0.001
SCD	1.16 (1.01 to 1.33)	0.04	2.18 (1.05 to 4.51)	0.04	1.14 (1.05 to 1.23)	0.001
Model 4						
All-cause mortality	1.02 (0.95 to 1.09)	0.59	1.10 (0.78 to 1.57)	0.59	1.04 (0.96 to 1.08)	0.09
Cardiovascular mortality	1.11 (1.02 to 1.21)	0.02	1.77 (1.12 to 2.81)	0.02	1.08 (1.03 to 1.13)	0.003
SCD	1.18 (1.03 to 1.35)	0.02	2.41 (1.16 to 5.00)	0.02	1.08 (1.00 to 1.17)	0.05

Median follow-up time was 10 years. Model 1 was unadjusted. Model 2 was adjusted for age and sex. Model 3 was additionally adjusted for LDL-C, HDL-C, smoking, BMI, diabetes, hypertension, eGFR, TG, Friesinger score,<sup>20</sup> and hsCRP. Model 4 was additionally adjusted for medication use (diuretics, ACE inhibitors,  $\beta$ -blockers, calcium-antagonists, statins, acetyl salicylic acid, oral antidiabetics, and insulin).

<sup>a</sup>After Bonferroni correction for three tests,  $P < 0.02$  would be considered significant.

was not significant (data not shown) and estimates for fatal events derived from this analysis were very similar compared with the results obtained for GRS<sub>8</sub> (Supplemental Table 6), which is expected because the *SLC2A9* SNP shows by far the strongest association with UA.

Regarding all-cause mortality and cardiovascular mortality, most studies find that serum UA is an independent predictor of cardiovascular end points, especially in patients with CAD,<sup>17,30,33–35</sup> but some studies reported the opposite.<sup>29,36,37</sup> Possible explanations for diverging results could be sex-specific effects,<sup>38</sup> an interaction with kidney function,<sup>39</sup> or the possibility that UA might turn pro-oxidant under certain conditions (*e.g.*, CAD).<sup>40</sup> In the LURIC study, the UA concentration is associated with CVD and SCD after multivariate adjustment. Calculated CHRs using a GRS as instrumental variable were equally significant for CVD and SCD and were higher than the HR calculated for a 1-mg/dl increase in measured UA. Yang *et al.*<sup>32</sup> similarly calculated a GRS in their meta-analysis and found no association with the incident CAD end point, which was defined as a mixture of fatal and nonfatal events; however, they could not exclude a modest association due to insufficient power. From our point of view, their failure to find a significant association might be due to the broadly defined end point.

Although UA may not be causally associated with atherosclerotic processes, it might potentially be involved in the pathogenesis of SCD. It has consistently been reported that UA concentration is associated with left ventricular hypertrophy,<sup>41,42</sup> a major risk factor for SCD and incident ventricular arrhythmia in patients with left ventricular hypertrophy,<sup>43</sup> which in turn is associated with a high incidence of ventricular tachycardia and SCD. In the LURIC study, we also found UA to

be associated with left ventricular hypertrophy, whereas genetically predicted UA was not (Supplemental Table 7).

In our study, we did not detect any association of UA with prevalent atrial fibrillation but a large prospective population-based study recently reported a significant association with incident atrial fibrillation.<sup>44</sup> Another study reported that hyperuricemia was a significant predictor of stroke in a population of patients suffering from atrial fibrillation.<sup>45</sup>

UA might potentially trigger arrhythmia as an endogenous danger signal.<sup>46</sup> Monosodium urate crystals are phagocytized by immune cells and activate the NOD-like receptor protein 3 inflammasome,<sup>47</sup> which in turn secretes the proinflammatory cytokines IL-1 $\alpha$  and IL-1 $\beta$ . Recently, it was shown in isolated rat ventricular myocytes that TNF- $\alpha$  and IL-1 $\beta$  increased sarcoplasmic reticulum Ca<sup>2+</sup> leak leading to depressed contractility and also increased the susceptibility to spontaneous sarcoplasmic reticulum Ca<sup>2+</sup> release, which may contribute to arrhythmia.<sup>48</sup> Of course, activation of the NOD-like receptor protein 3 inflammasome and secretion of proinflammatory cytokines would also be suspected to increase or promote atherosclerosis; however, in contrast with SCD, we obtained no evidence of UA being causally involved in atherosclerosis in our MR study. Another possible link between UA and SCD could be *via* calmodulin (CAM). It has been shown that UA can interfere with the binding of CAM to endothelial nitric oxide synthase,<sup>49</sup> possibly by binding directly to CAM. CAM represents an important molecule mediating many disease-associated signaling pathways, and missense mutations in its gene have been linked to certain inherited forms of catecholaminergic polymorphic ventricular tachycardia that greatly increase the risk of SCD.<sup>50</sup>

This study has some limitations. All participants were of European origin and were recruited at a tertiary referral center. Therefore, our findings may not be representative of a random population sample or applicable to other ethnicities. Furthermore, UA was only measured once at baseline. Hence, we were not able to control for intraindividual variability. Some potential confounding variables such as data about socioeconomic status were not available and thus we could not adjust for these. The major strengths of the LURIC cohort are the precise clinical and metabolic characterization of the participants, including the availability of coronary angiograms, and its cross-sectional and prospective design.

Although the concentration of UA was associated with a range of pathologic conditions in the LURIC study, the CORs calculated (by using a GRS composed of UA-raising alleles as an instrumental variable) were not significant for any of the investigated prevalent cardiometabolic disease conditions. Regarding incident fatal events, both the serum concentration of UA and the GRS were predictors of CVD and SCD in Cox regression models adjusted for conventional risk factors and medication. CHRs were higher than the HRs for the equal increase in measured UA. This might be explained by the fact that those patients with high GRS have been exposed to elevated UA throughout their lives.

## CONCISE METHODS

### LURIC Study

The LURIC study includes 3316 Caucasian patients hospitalized for coronary angiography between 1997 and 2000 at a tertiary care center in Southwestern Germany.<sup>51</sup> Clinical indications for angiography were chest pain or a positive noninvasive stress test suggestive of myocardial ischemia. To limit clinical heterogeneity, individuals suffering from acute illnesses other than acute coronary syndrome, chronic noncardiac diseases, and a history of malignancy within the past 5 years were excluded. The study was approved by the ethics committee at the Ärztekammer Rheinland-Pfalz and was conducted in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants. Both genotypes and UA concentrations were available in 3060 LURIC participants.

### Follow-Up

During a median follow-up of 10.0 years (range, 0.1–11.9 years), information on vital status was obtained from local person registries and death certificates, and medical records of local hospitals and autopsy data were reviewed to classify the causes of death. Deceased patients were classified into those who died from cardiovascular and noncardiovascular causes. Cardiovascular deaths included SCD, fatal myocardial infarction, death due to congestive heart failure, death immediately after intervention to treat CAD, fatal stroke, and deaths due to other cardiac causes. Noncardiovascular deaths included fatal infection, fatal cancer, and other noncardiovascular causes of death. Two experienced clinicians who were blinded to any data on the baseline characteristics of the study participants independently

classified the causes of death. In cases of disagreement or uncertainty concerning the coding of a specific cause of death, the decision was made by a principal investigator (W.M.).

### Laboratory Procedures

Fasting blood samples were obtained by venipuncture in the early morning. Blood glucose, cholesterol, and TGs were measured by standard laboratory procedures.<sup>51</sup> HDL-C was measured after separating lipoproteins with a combined ultracentrifugation-precipitation method.<sup>51</sup> Genomic DNA was prepared from EDTA anticoagulated peripheral blood by using a common salting-out procedure. GFR was estimated by using the 2012 Chronic Kidney Disease Epidemiology Collaboration  $eGFR_{\text{creat-cys}}$  equation as previously described.<sup>52</sup> The extent of CAD was determined semiquantitatively according to Friesinger.<sup>20</sup> Serum UA was measured using a photometric color test on a Hitachi 717 analyzer (Roche, Mannheim, Germany) with reagents from Rolf Greiner Biochemica (Flacht, Germany).

### Genotyping

Genotyping was done using the Affymetrix Human SNP Array 6.0. Samples with an individual call rate <95% or ambiguous sex were excluded before genotype imputation as well as SNPs with a call rate <98%, minor allele frequency <1%, or deviation from Hardy-Weinberg equilibrium with  $P < 10^{-4}$ .

Imputation was performed using MACH 1.0<sup>53,54</sup> and HapMap II CEU (release 22, NCBI build 36, dbSNP 126) samples as a reference. After imputation, 2,543,887 SNPs were available. SNPs with a squared correlation of  $\geq 0.3$  between imputed and true genotypes were considered well imputed. SNPs associated with serum UA concentration<sup>16</sup> were imported in IBM SPSS (version 20) and R (version 3.0.2) software for further statistical analysis.

### Statistical Analyses

Continuous data are presented as means and SDs when normally distributed or as medians and interquartile ranges for non-normally distributed variables. All continuous variables were checked for normality and those showing a skewed distribution were logarithmically transformed before entering analysis; between-group comparisons were made by univariate ANOVA. Categorical data are shown as percentages, and chi-squared tests were used for comparisons between groups.

Cross-sectional associations of GRS or UA with disease conditions at the baseline examination were analyzed by logistic regression. Cox proportional hazard regression analysis was applied to assess the effect of variables on survival prospectively. CORs and CHRs were calculated analogous to the method described for CORs by Palmer *et al.*<sup>55</sup> SPSS 20.0 (IBM SPSS) and R (version 3.0.2; <http://www.r-project.org>) were used for all analyses.

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## DISCLOSURES

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