Common Polymorphisms Influencing Serum Uric Acid Levels Contribute to Susceptibility to Gout, but Not to Coronary Artery Disease

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Abstract

Background: Recently, a large meta-analysis including over 28,000 participants identified nine different loci with association to serum uric acid (UA) levels. Since elevated serum UA levels potentially cause gout and are a possible risk factor for coronary artery disease (CAD) and myocardial infarction (MI), we performed two large case-control association analyses with participants from the German MI Family Study. In the first study, we assessed the association of the qualitative trait gout and ten single nucleotide polymorphisms (SNP) markers that showed association to UA serum levels. In the second study, the same genetic polymorphisms were analyzed for association with CAD.

Methods and Findings: A total of 683 patients suffering from gout and 1,563 healthy controls from the German MI Family Study were genotyped. Nine SNPs were identified from a recently performed genome-wide meta-analysis on serum UA levels (rs12129861, rs780094, rs734553, rs2231142, rs742132, rs1183201, rs12356193, rs17300741 and rs505802). Additionally, the marker rs6855911 was included which has been associated with gout in our cohort in a previous study. SNPs rs734553 and rs6855911, located in SLC2A9, and SNP rs2231142, known to be a missense polymorphism in ABCG2, were associated with gout (p = 5.6*10^-7, p = 1.1*10^-7, and p = 1.3*10^-7, respectively). Other SNPs in the genes PDZK1, GCKR, LRRC16A, SLC17A1-SLC17A3, SLC16A9, SLC22A11 and SLC22A12 failed the significance level. None of the ten markers were associated with risk to CAD in our study sample of 1,473 CAD cases and 1,241 CAD-free controls.

Conclusion: SNP markers in SLC2A9 and ABCG2 genes were found to be strongly associated with the phenotype gout. However, not all SNP markers influencing serum UA levels were also directly associated with the clinical manifestation of gout in our study sample. In addition, none of these SNPs showed association to the risk to CAD in the German MI Family Study.

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Introduction

Gout is mainly caused by elevated serum uric acid (UA) levels [1]. Several studies showed significant association between single nucleotide polymorphism (SNP) markers in SLC2A9 gene (solute carrier family 2, member 9, also known as GLUT9 gene) and serum UA levels as well as susceptibility to gout [2-6]. Additionally, Dehghgan et al. reported association between markers in genes ABCG2 and SLC17A3 with both, serum UA levels and gout in a large cohort [6]. Very recently, Kolz et al. conducted a meta-analysis of 14 genome-wide association (GWA) studies on serum UA levels including a total of 28,141 participants [7]. Nine loci with significant associations to serum UA levels were found, namely the genes PDZK1, GCKR, LRRC16A, SLC16A9 and SLC22A11 together with the previously reported findings in ABCG2, SLC2A9 and SLC17A1-SLC17A3, as well as the intensively studied SLC22A12 gene encoding for URAT1. Therefore, five novel loci associated with serum UA levels emerged from this meta-analysis [7]. The advantage of this GWA-based meta-analysis is its power to detect novel common variants with relatively small phenotypic effects on serum UA due to the large sample size.

We analyzed these new and known loci for their association with the clinical phenotype gout in a case control study.

Elevated serum UA levels are potentially increasing the risk for coronary artery disease (CAD) and myocardial infarction (MI) [8-10]. We therefore tested additionally for the influence of these SNP markers on the susceptibility to CAD in our German MI Family Study.
Materials and Methods

Ethics Statement

The Ethics committee of the University of Regensburg approved the study protocol and all participants gave their written informed consent at the time of inclusion and again at the time of follow-up investigations. The study was in accordance with the principles of the current version of the Declaration of Helsinki.

Case-Control Samples and Phenotyping

All individuals of this study participated in the German MI Family Study (total n = 7,757). Recruitment process, selection criteria and study details have been reported previously [3]. A total of n = 683 unrelated individuals (n = 480 males, n = 203 females) with the diagnosis of gout were selected from the German MI Family Study. Phenotyping was carried out as reported previously [3]. In brief, the phenotype gout was established using medical history readings and self-reported history of gout.

Controls (n = 1,563) were unrelated individuals from our German MI Family Study who neither had any indication of gout nor were they medicated with uricostatic or uricosuric agents at any time during follow-up (n = 871 males, n = 692 females). Phenotypic details are shown in Table 1.

Furthermore, a large case-control sample was established from the German MI Family Study including n = 1,473 CAD/MI unrelated cases (n = 856 males, n = 617 females) and n = 1,241 unrelated CAD/MI-free control individuals (n = 336 males, n = 905 females). MI was diagnosed according to MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) diagnostic criteria (http://www.ktl.fi/publications/monica/manual/index.htm). Severe CAD was defined as prior MI, treatment with percutaneous coronary intervention or coronary artery bypass graft.

Cardiovascular risk factors and phenotypic details are summarized in Table 2.

Recent GWA analyses [11,12] using a part of the current study sample (n = 1,021) revealed no population stratification effects within unrelated individuals form the German MI Family Study using the genomic control method [13]. Therefore, no correction for population stratification was carried out.

SNP Selection and Genetic Analyses

Genomic DNA isolation using the PureGene DNA Blood Kit (Qiagen, Hilden, Germany) and genotyping with 5′ exonuclease TaqMan® technology (Applied Biosystems, Foster City, CA, USA) was carried out as previously described [3]. SNPs were selected from a recently published meta-analysis on serum UA levels [7] and our previous study [3].

Statistical Analyses

To determine whether the SNP genotypes of cases and controls deviated from Hardy-Weinberg equilibrium (HWE), actual and predicted genotype counts of both groups were compared by χ²-test. Differences in allele frequencies between dichotomous traits were calculated employing the same method. Prevalence odds ratios (OR) with their 95% confidence intervals (CI) were reported. Continuous parameters were compared by t test for normally distributed values or otherwise by non-parametric tests. Logistic regression was used to adjust for covariates differentially distributed in case-control cohorts. Full adjustment model for gout included gender, medication with diuretics, lipid lowering and antihypertensive therapy, high-density lipoprotein cholesterol (HDL-C), type 2 diabetes, smoking, and BMI. The corresponding model for CAD case-control sample included gender, age at inclusion, hypercholesterolemia, hypertension, type 2 diabetes, smoking, and BMI. Employing a model based on allele dosage,

Table 1. Characteristics of gout case and control study sample.

| Variable                        | Gout cases (n = 683) | Gout-free controls (n = 1,563) | p-value |
|---------------------------------|---------------------|--------------------------------|---------|
| Gender, % male (n)              | 70.3 (480)          | 55.7 (871)                     | <0.0001 |
| Age, years (range) a            | 58.3 ± 9.5 (23–84)  | 58.5 ± 8.6 (28–87)            | n. s.   |
| Medication with diuretics, % (n)| 36.1 (221)          | 22.0 (341)                     | <0.0001 |
| MI or severe CAD, % (n)         | 61.1 (417)          | 58.2 (909)                     | n. s.   |
| Hypercholesterolemia b, % (n)   | 70.5 (481)          | 66.9 (1,046)                   | n. s.   |
| Lipid lowering medication, % (n)| 50.1 (307)          | 44.9 (701)                     | 0.03    |
| LDL-C, mg/dl                    | 150.9 ± 41.0        | 147.8 ± 38.7                   | n. s.   |
| HDL-C, mg/dl                    | 50.7 ± 14.2         | 55.3 ± 15.7                    | <0.0001 |
| Hypertension c, % (n)           | 86.6 (580)          | 83.7 (1,269)                   | 0.05    |
| Antihypertensive therapy, % (n) | 83.5 (512)          | 72.6 (1,134)                   | <0.0001 |
| Systolic blood pressure, mmHg   | 139.0 ± 19.1        | 135.8 ± 18.5                   | 0.0003  |
| Diastolic blood pressure, mmHg  | 84.0 ± 10.3         | 82.0 ± 9.8                     | <0.0001 |
| Type 2 diabetes d, % (n)        | 16.3 (111)          | 10.6 (165)                     | 0.0002  |
| Smoking e, % (n)                | 66.1 (451)          | 60.4 (942)                     | 0.009   |
| BMI, kg/m²                      | 28.1 ± 3.9          | 26.8 ± 3.7                     | <0.0001 |

Values denote means ± standard deviations unless indicated otherwise. n. s., not significant; CAD, coronary artery disease; MI, myocardial infarction; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index.

aAt inclusion to study.

bDefined as LDL-C ≥160 mg/dl or intake of lipid lowering medication.

cDefined as blood pressure ≥140/90 mmHg or ongoing antihypertensive therapy.

dDefined as history of diabetes mellitus or intake of antidiabetic medication.

eFormer or current smoking habit.

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epistasis between SNPs was tested using a logistic regression analysis with the second SNP as a covariate. A two-sided \( p \)-value \(< 0.05 \) was considered statistically significant.

Statistical and association analyses were performed using JMP 7.0.2 (SAS Institute Inc, Cary, NC, USA) and PLINK v1.06 [14], respectively. Power analysis was carried out using G*Power 3.0.10 (SAS Institute Inc, Cary, NC, USA) and PLINK v1.06 [14].

RESULTS

Population Characteristics

In our first case-control cohort, gout cases \((n = 683)\) were compared to control individuals \((n = 1,563)\). Prevalence of cardiovascular risk factors and cardiovascular disease was high in both, gout cases and gout-free controls. However, we found no significant difference in number of reported MI or CAD events between gout cases \((61.1\% \text{ CAD/MI})\) and gout-free controls \((58.2\% \text{ CAD/MI})\). The proportion of women was lower in the gout group than in the control group. Gout cases were more often treated with diuretics as compared to controls. In addition and in concordance to the clinical manifestation of gout cases, the prevalence of type 2 diabetes and increased body mass index (BMI) was higher, and gout-free controls showed higher HDL-C levels, even after adjusting for gender \((p < 0.001)\). The prevalence of hypercholesterolemia was equally distributed between the two groups, whereas hypertension and smoking were slightly more prevalent in gout cases (Table 1).

In our second, large case-control sample for CAD/MI the incidence of established cardiovascular risk factors, such as male gender, type 2 diabetes, hypercholesterolemia, hypertension and smoking, as well as increased BMI, was higher in CAD/MI cases \((n = 1,473)\) as compared to controls \((n = 1,241)\) (Table 2). We also found more individuals suffering from gout in our CAD/MI cases compared to CAD/MI-free controls (Table 2).

Genetic Analyses

The cohorts were genotyped for markers listed in Table 3. All SNPs fulfilled our criteria of at least 98% call rate in all sub-samples, except for rs734553 with a total call rate \(= 96.0\%\). Marker rs6855911 is in strong LD with rs734553 \((r^2 = 0.94)\) and, therefore, can to some degree be used as a surrogate. Data for rs734553 were reported for completeness. Strong LD \((r^2 = 0.967)\) exits between rs1183201 \((SLC17A1)\) reported from Kolz et al. [7] and rs1165205 \((SLC17A3)\) described by Dehghan et al. [6]. Therefore, a distinction between these two genes on association level is not possible.

Association analysis of SNPs in the gout case-control sample. Genotype distributions and allele frequencies in gout case-control cohort are shown in Table 4. No deviation from HWE was observed for the ten genotyped markers in gout-free samples. However, as previously reported [3], rs6855911 in \(SLC2A9\) gene showed deviation from HWE in gout cases \((p = 0.01)\). The proximate marker rs734553 also showed nominal deviation from HWE in gout cases \((p = 0.05)\), whereas the other markers exhibited \(p\)-values \(> 0.18\). Significant association with gout was found for rs734553 and rs6855911 located in intron 7 of \(SLC2A9\), even after correction for multiple testing (ten SNPs) with \(p_{\text{corr}} = 5.6 \times 10^{-6}\) and \(p_{\text{corr}} = 1.1 \times 10^{-6}\), respectively. The \(ABCG2\) polymorphism rs2231142 remained significantly associated with gout after correction for multiple testing with \(p_{\text{corr}} = 0.013\). The power to detect nominal association with \(p = 0.05\) and OR \(= 1.2\) for the other SNPs ranged from \(32.8\%\) to \(50.4\%\) (Table 4).

Interaction between SNPs was analyzed using a model based on allele dosage. Nominal significance was observed between SNPs in \(SLC2A9\) (rs734553 and rs6855911) and rs742132 in \(LRRC16A\) with \(p = 0.038\) and \(p = 0.024\), respectively.
Furthermore, we had indication for gender interaction, as separate analyses in females and males revealed association with gout for both SLC2A9 SNPs, whereas ABCG2 SNP rs2231142 only showed significant association in males (Table 5), but not in females (Table 6). Full adjustment for gender, medication with diuretics, lipid lowering and antihypertensive therapy, HDL-C, type 2 diabetes, smoking, and BMI did not change the association results substantially (Table 4). The same model without inclusion of gender was applied in males and females separately and did not lead to a significant change in p-values (Table 5 and Table 6, respectively).

**Association analysis of SNPs in the CAD/MI case-control sample.** Deviation from HWE was not observed for the genotyped markers in CAD/MI cases and CAD/MI-free controls (p > 0.05). No association with CAD was found for any of the analyzed SNPs (Table 7). Again, adjustment for differentially distributed risk factors between CAD cases and controls did not alter the results significantly (Table 7). The power to detect nominal association with CAD was >30.3% for an assumed OR = 1.2 and >79.6% for an OR = 1.4 (Table 7).

**Discussion**

In the present case-control association studies, we evaluated the relationship of common SNPs with gout and their potential influence on CAD. The variants are located in nine different genetic regions, four of which are known and the remaining five loci were only recently identified to be associated with serum UA levels in a large meta-analysis of GWA studies [7]. We were able to confirm significant association between gout and SNPs in two established genes, namely SLC2A9 (rs734553 and rs6855911) and ABCG2 (rs2231142). However, for markers in the other known and novel loci, no association with the clinical phenotype gout was found in our study. Moreover, our results indicate no relevant influence of the investigated polymorphisms on CAD susceptibility in our German MI Family Study.

The strongest association signal with gout was detected for intronic SNPs rs6855911 and rs734553 in the SLC2A9 gene, which is consistent with previous studies on gout and serum UA levels [2–6]. SLC2A9 is coding for GLUT9, a high-capacity urate transporter, which is abundantly expressed in liver and kidney.

### Table 3. SNP marker used in analysis.

| SNP       | Position a | Major allele (1) | Minor allele (2) | Gene name(s) | Function | Call rate b |
|-----------|------------|------------------|------------------|--------------|----------|-------------|
| rs12129861 | Chr1: 144,437,046 | G | A | PDZK1 | 5’ Intergenic | 98.4% |
| rs780094   | Chr2: 27,594,741 | C | T | GCOR | Intron 16 | 99.2% |
| rs734553   | Chr4: 9,532,102 | T | G | SLC2A9 GLUT9 | Intron 7 | 96.0% |
| rs6855911  | Chr4: 9,545,008 | A | G | SLC2A9 GLUT9 | Intron 7 | 99.0% |
| rs2231142  | Chr4: 89,271,347 | G | T | ABCG2 | Exon 5 Q141K | 99.2% |
| rs742132   | Chr6: 25,715,550 | A | G | LRRC1A6 | Intron 34 | 99.4% |
| rs1183201  |Chr6: 25,931,423 | T | A | SLC17A1 | Intron 3 | 98.2% |
| rs12356193 | Chr10: 61,083,359 | A | G | SLC16A9 | Intron 5 | 97.8% |
| rs17300741 | Chr11: 64,088,038 | G | A | SLC22A11 | Intron 4 | 98.2% |
| rs505802   | Chr11: 64,113,648 | T | C | SLC22A12 URAT1 | 5’ Intergenic | 99.3% |

a on human genome build 18.

b in total sample (n = 4,960).

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### Table 4. Association analysis results in gout case-control sample.

| SNP       | Gout case genotypes | Gout-free control genotypes | Allelic OR | Adjusted \( \text{OR}^{a} \) | Power b |
|-----------|---------------------|----------------------------|------------|--------------------------|---------|
| rs12129861 | 187 (134, 194) | 572 (507, 703) | 0.472 | 0.500 | 0.083 | 0.504 | 0.953 |
| rs780094   | 240 (255, 225) | 456 (432, 512) | 0.406 | 0.417 | 0.350 | 0.490 | 0.950 |
| rs734553   | 429 (391, 465) | 553 (516, 590) | 0.253 | 0.224 | 0.109 | 0.418 | 0.909 |
| rs6855911  | 429 (391, 465) | 553 (516, 590) | 0.253 | 0.224 | 0.109 | 0.418 | 0.909 |
| rs2231142  | 500 (475, 525) | 299 (270, 328) | 0.104 | 0.137 | 0.973 | 0.430 | 0.918 |
| rs742132   | 330 (303, 357) | 144 (125, 163) | 0.251 | 0.285 | 1.10–1.80 | 0.239 | 0.663 |
| rs1183201  | 187 (150, 224) | 314 (270, 356) | 0.478 | 0.507 | 0.157 | 0.452 | 0.932 |
| rs12356193 | 475 (450, 500) | 436 (415, 458) | 0.137 | 0.385 | 0.933 | 0.504 | 0.953 |
| rs17300741 | 176 (150, 192) | 158 (135, 181) | 0.487 | 0.502 | 0.105 | 0.452 | 0.932 |
| rs505802   | 317 (290, 343) | 682 (650, 714) | 0.315 | 0.385 | 1.02–1.16 | 0.328 | 0.819 |

Numbers of genotypes (11, 12, 22) according to alleles from Table 3.

\( ^{a} \) Model including gender, medication with diuretics, lipid lowering and antihypertensive therapy, HDL-C, type 2 diabetes, smoking, and BMI.

\( ^{b} \) Power was calculated for the given OR using the respective MAF in controls and a two-tailed \( p = 0.05 \).

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It is noteworthy that both SNPs located in SLC2A9 gene showed deviation from HWE in gout cases, which can in some degree support a true association [19,20]. In addition, we found a significant association between the exonic SNP rs2231142 in ABCG2 and gout, again supporting the results of a prior GWA on serum UA levels and gout [6]. This is the only marker examined that leads to a missense mutation with an amino acid exchange from glutamine to lysine at position 141 in ABCG2 transporter and therefore could have a direct and causal influence on development of the disease [6]. It is notable, however, that the effect of this variant on susceptibility to gout is only present in our male subcohort.

A recent meta-analysis documented an additional locus on chromosome 6p23-p21.3 encompassing three members of the solute carrier family 17 (SLC17A1, SLC17A3 and SLC17A4) to be associated with serum UA levels [7]. Interestingly, their top marker rs1183201 in the SLC17A1 gene did not show significant association with the qualitative trait of gout in our study. Another SNP marker, rs1165205 in SLC17A3, which is in strong LD with rs1183201 in SLC17A1 was previously found to be related to serum UA levels and also representing a risk factor for gout [6]. A possible explanation for these discrepancies may lie in the different recruitment strategies of the study populations and the distinct definition of the phenotype “gout”. While Kolz et al. [7] in their meta-analysis examined participants of European ancestry from 14 different study cohorts with widely varying initial inclusion criteria – potentially concealing a substructure which could lead to false positive results – our ascertainment approach was to recruit individuals with a strong familial history of CAD from all over Germany with a concomitant accumulation of cardiometabolic risk factors, such as gout. On the other hand, Deghan et al. [6] included participants from three large population-based studies (Framingham cohort, Rotterdam cohort and the Atherosclerosis Risk in Communities (ARIC) study) with different definitions of gout in each of the study cohorts. It is important to notice, that the allele frequencies between Deghan et al. (rs1165205) [6], Kolz et al. [7] and our present study (both rs1183201) did not differ substantially (47%, 48% and 48%, respectively).

### Table 5. Association analysis results in male gout case-control sample.

| SNP          | Gout case genotypes | Gout-free control genotypes | Allelic | Allelic OR | Adjusted a |
|--------------|---------------------|----------------------------|---------|------------|------------|
|              | 11      12   22  | 11     12   22  | p-value | (95% CI)   | p-value    |
| rs12129861   | 135    232  104 | 218   415  228 | 0.056   | 0.86(0.73–1.00) | 0.145 |
| rs780094     | 164    225  87  | 315   411  138 | 0.277   | 1.09(0.93–1.28) | 0.293 |
| rs734553     | 289    159  12  | 453   318  64  | 1.1*10⁻⁴ | 0.68(0.56–0.83) | 6.0*10⁻⁴ |
| rs6855911    | 291    174  12  | 445   349  69  | 2.2*10⁻⁵ | 0.67(0.55–0.80) | 3.0*10⁻⁴ |
| rs2231142    | 345    124  7   | 686   172  7   | 4.4*10⁻³ | 1.41(1.11–1.78) | 3.3*10⁻³ |
| rs742132     | 224    203  50  | 438   354  73  | 0.122   | 1.15(0.96–1.36) | 0.511 |
| rs1183201    | 126    232  109 | 230   440  188 | 0.757   | 1.03(0.87–1.20) | 0.991 |
| rs12356193   | 334    127  9   | 586   252  22  | 0.237   | 0.88(0.71–0.99) | 0.334 |
| rs17300741   | 116    236  120 | 228   426  199 | 0.295   | 1.09(0.93–1.28) | 0.421 |
| rs505802     | 215    214  48  | 386   384  92  | 0.812   | 0.98(0.83–1.16) | 0.781 |

Numbers of genotypes (11, 12, 22) according to alleles from Table 3.

*aModel including medication with diuretics, lipid lowering and antihypertensive therapy, HDL-C, type 2 diabetes, smoking, and BMI.

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### Table 6. Association analysis results in female gout case-control sample.

| SNP          | Gout case genotypes | Gout-free control genotypes | Allelic | Allelic OR | Adjusted a |
|--------------|---------------------|----------------------------|---------|------------|------------|
|              | 11      12   22  | 11     12   22  | p-value | (95% CI)   | p-value    |
| rs12129861   | 52     102  45  | 176    337  166 | 0.720   | 0.96(0.77–1.20) | 0.315 |
| rs780094     | 76     100  25  | 243    336  109 | 0.493   | 0.88(0.70–1.11) | 0.734 |
| rs734553     | 140    52   2   | 393    235  39  | 0.235   | 1.4*10⁻⁴ | 0.55(0.40–0.75) |
| rs6855911    | 138    59   3   | 384    254  45  | 0.252   | 2.0*10⁻⁴ | 0.58(0.43–0.77) |
| rs2231142    | 155    44   2   | 555    127  5   | 0.100   | 4.1*10⁻⁴ | 1.22(0.86–1.74) |
| rs742132     | 106    73   23  | 326    290  72  | 0.315   | 0.426   | 0.91(0.71–1.16) |
| rs1183201    | 61     88   52  | 163    351  166 | 0.502   | 0.816   | 0.91(0.73–1.13) |
| rs12356193   | 141    55   5   | 483    184  18  | 0.161   | 0.958   | 1.01(0.75–1.36) |
| rs17300741   | 60     101  38  | 181    344  156 | 0.482   | 0.194   | 0.86(0.69–1.08) |
| rs505802     | 102    84   16  | 335    298  56  | 0.298   | 0.687   | 0.95(0.74–1.21) |

Numbers of genotypes (11, 12, 22) according to alleles from Table 3.

*aModel including medication with diuretics, lipid lowering and antihypertensive therapy, HDL-C, type 2 diabetes, smoking, and BMI.

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The same might hold true for the other SNPs that were genotyped in our present study but showed no significant association with the phenotype gout. However, one has to emphasize that in our study, we explicitly investigated the role of SNP markers with clinically manifest gout or gouty arthritis, for which elevated serum UA levels are an important but not a mandatory risk factor [21,22]. Therefore, differences in the pathophysiological pathways of the development of elevated serum UA levels and the ignition of the inflammatory process of gout or gouty arthritis may account for the distinct findings of our study. This is also reflected by the clinical observations that many patients with high serum UA levels never experience an attack of gout, whereas other people in the absence of hyperuricemia suffer from severe and recurrent flares of gouty arthritis [22]. One can speculate that other pathophysiological mechanisms might be involved, or a complex interplay of genes and their variants lead to the manifestation of the disease. For example, the well-known URAT1 transporter, encoded by SLC22A12 gene, is involved in renal urate exchange [23], and a SLC22A12 polymorphism is also linked to serum UA levels [7]. Therefore, this gene is a strong candidate for gout, but does not show significant association with the clinical phenotype in this and a previous study [6]. Another possible explanation is the small effect size of some polymorphisms on serum UA levels that could directly impact susceptibility to gout. Our present study showed a high degree of association between gout and SNPs in ABCG2 and SLC249, those polymorphisms that were reported to have highest effects on serum UA levels found in the previous meta-analysis (explaining 0.57% and 3.53% of variability, respectively) [7] and that showed ORs for gout between 1.37 and 1.52 in our study. All other SNPs were significant on a genome-wide level, but explained less the variability of serum UA levels (below 0.2%) [7]. Therefore, either power was not sufficient for detection of association between these SNPs and gout in the present study, or their relevance on the clinical phenotype gout is not evident.

Additionally, we found only weak epistatic interaction between SNPs in SLC249 and LRRC16A on gout, making a relevant additive effect of SNPs influencing serum UA levels on the qualitative trait unlikely. Potential confounders, such as different medications and prevalence of type 2 diabetes, smoking or BMI, did not influence the association results significantly. Taken together, it is obvious that SNPs with highest influence on serum UA levels could be directly linked to susceptibility to gout, whereas the relevance of less contributing polymorphisms is still arguable.

More complex functional studies are warranted in the future to elucidate the pathways with which the newly identified genes impact serum UA levels and development of gout.

Furthermore, the presence of hyperuricemia and gout has often been discussed to be a cardiovascular risk factor [24–28]. We thus examined the SNPs being associated with elevated UA serum levels in our second case-control study consisting of CAD cases and controls from the general population. Here, we did not detect a direct genetic relationship between the tested SNPs and CAD. One possible explanation may be limited power: polymorphisms with a small effect on disease susceptibility require very large study samples to be detected. Therefore, we cannot rule out a causal link between the SNPs influencing serum UA levels and CAD. On the other hand, CAD is possibly a more heterogeneous disorder than gout, even on genetic level. For example, no genes known to influence serum UA levels were identified by recent GWA studies on CAD, but genetic loci involved in several different pathways were found [11,12,29–32].

There are limitations in our study design that have to be considered. First, we do not have measurement of serum UA levels in our cohort. Hence, we can not directly replicate the findings of Kolz et al. on serum UA levels [7]. However, we did not aim in replication of serum UA level association but in expansion of these results to clinical manifestation of the phenotypes gout and CAD. Second, all phenotypes were assessed retrospectively from patient documentations and medical history readings. When gout was diagnosed by a physician according to ICD-9 code 274, the phenotype gout was considered as confirmed. In case of self-reported gout, additional intake of uricosuric medication was required to affirm the diagnosis of gout. We have follow-up data from more than 80% of our study participants and, therefore, validation of clinical phenotypes is available. Third, the power to analyze gender effects in our study is limited. As previously described, association of serum UA levels depends to some degree on gender [4]. Our findings on gender-specific association between male but not female gout patients and rs2231142 in ABCG2 gene are likely to be true positive results but some other gender effects may have been overlooked. Forth, assuming that gout is a risk factor for CAD, we expected to observe significantly more CAD patients in the gout sample than...
in gout-free controls. However, based on our initial ascertainment strategy where we retrospectively identified gout patients and gout-free controls from a MI/CAD study cohort, we did not find a significant coincidence of CAD and gout. On the other hand, in our CAD case-control sample we found that the clinical phenotype of gout seems to be associated with CAD.

In conclusion, we performed a comprehensive analysis on association with susceptibility to gout and CAD of recently published polymorphisms known to be linked with serum UA levels. Markers in SLC2A9 and ABCG2 genes are strongly associated with clinical manifestation of gout in the German MI Family Study. With the knowledge of a comprehensive number of genetic polymorphisms contributing to gout, genetic testing as a supportive diagnostic tool would be conceivable.

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