Meta-Analysis of 28,141 Individuals Identifies Common Variants within Five New Loci That Influence Uric Acid Concentrations

Citation for published version:

Digital Object Identifier (DOI):
10.1371/journal.pgen.1000504

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher’s PDF, also known as Version of record

Published In:
PLoS Genetics

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Meta-Analysis of 28,141 Individuals Identifies Common Variants within Five New Loci That Influence Uric Acid Concentrations

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Abstract

Elevated serum uric acid levels cause gout and are a risk factor for cardiovascular disease and diabetes. To investigate the polygenetic basis of serum uric acid levels, we conducted a meta-analysis of genome-wide association scans from 14 studies totalling 28,141 participants of European descent, resulting in identification of 954 SNPs distributed across nine loci that exceeded the threshold of genome-wide significance, five of which are novel. Overall, the common variants associated with serum uric acid levels fall in the following nine regions: SLC2A9 (p = 5.2×10⁻₂⁰), ABCG2 (p = 3.1×10⁻²⁰), SLC17A1 (p = 3.0×10⁻¹⁴), SLC22A11 (p = 6.7×10⁻¹⁴), SLC22A12 (p = 2.0×10⁻⁹), SLC16A9 (p = 1.1×10⁻⁹), GCKR (p = 1.4×10⁻⁹), LRRC16A (p = 8.5×10⁻⁹), and near PDZK1 (p = 2.7×10⁻⁹). Identified variants were analyzed for gender differences. We found that the minor allele for rs734553 in SLC2A9 has greater influence in lowering uric acid levels in women and the minor allele of rs2231142 in ABCG2 elevates uric acid levels more strongly in men compared to women. To further characterize the identified variants, we analyzed their association with a panel of metabolites. rs12356193 within SLC16A9 was associated with DL-carnitine (p = 4.0×10⁻²⁸) and propionyl-L-carnitine (p = 5.0×10⁻¹⁳) concentrations, which in turn were associated with serum UA levels (p = 1.4×10⁻³⁷ and p = 8.1×10⁻³⁴, respectively), forming a triangle between SNP, metabolites, and UA levels. Taken together, these associations highlight additional pathways that are important in the regulation of serum uric acid levels and point toward novel potential targets for pharmacological intervention to prevent or treat hyperuricemia. In addition, these findings strongly support the hypothesis that transport proteins are key in regulating serum uric acid levels.
Introduction

Uric acid (UA) is the final catabolic, heterocyclic purine derivative resulting from the oxidation of purines in humans. Due to the loss of hepatic uricase activity during human evolution, UA is excreted as such and is not further metabolized into carbon dioxide and ammonia. A major mechanism underlying hyperuricemia is impaired renal excretion of urate. Most notably, UA is causally involved in the pathogenesis of gouty arthritis that results from deposition of monosodium urate crystals in the joints [1]. Increased UA concentrations have been implicated in cardiovascular disease for more than five decades [2]. In addition, elevated urate is associated with obesity, blood pressure and insulin resistance, and consequently with the metabolic syndrome and type 2 diabetes [2,3]. However, UA also has a positive role as an antioxidant, and is correlated with longevity in mammals [4]. Thus, human physiology is especially sensitive to the precise range of UA levels.

Besides environmental factors, there is evidence for a strong genetic influence upon serum UA concentrations, with heritability estimates of up to 73% [5]. Recently, genome-wide association (GWA) studies have identified single nucleotide polymorphisms (SNPs) in the SLC2A9 gene (solute carrier family 2, member 9 gene), a putative glucose transporter, which are strongly associated with serum UA concentrations and gout [6–9]. This novel gene locus functions as a high-capacity urate transporter in humans [8,10]. This emphasises the power of GWA studies in expanding our understanding at the molecular level of disease mechanisms and in pointing to innovative therapeutic strategies.

The power of GWA studies to detect common variants with modest effects directly depends on the size of the study group. Therefore, the present study sought to detect novel genetic variants related to serum UA levels by conducting a meta-analysis of GWA findings from 14 studies (BRIGHT, CoLaus, CROATIA, Health 2000, KORA F3, KORA S4, ORCADES, PROCARDIS, NSPHS, Sardinia, SHIP, SSAGA, MICROS, and TwinsUK) totaling 28,141 participants. In addition, the meta-analysis was performed independently on sex specific GWA results to address the pronounced gender differences in the regulation of UA concentrations that have previously been reported [1,6]. Identified variants were further analyzed for association with metabolite profiles.

Results

The sample size and participant characteristics for each participating study are shown in Table S1. Meta-analysis of
GWA data of 28,141 individuals of European ancestry yielded 954 SNPs (full list is provided in Table S4) that exceeded the genome-wide significance threshold of \(5 \times 10^{-8}\) (Figure 1A).

Those SNPs cluster around nine loci (Table 1), four of which are well known regulators of serum UA levels: SLC2A9 \((p = 5.2 \times 10^{-205})\), ABCG2 \((p = 3.1 \times 10^{-206})\), SLC17A1 \((p = 3.0 \times 10^{-11})\), and SLC22A12 \((p = 2.0 \times 10^{-8})\). The first, SLC2A9, was identified in previous GWA scans (Figure 2C) [6-9]. A total of 788 SNPs reached the genome-wide significance threshold at the SLC2A9 locus. The strongest associated marker was the intronic SNP rs734553 \((p = 5.2 \times 10^{-205})\), Table 1, which is in high linkage disequilibrium \((r^2 = 0.88)\) with the missense mutation rs16897959 previously described [11]. The second locus was on chromosome 4q22, harbouring the ABCG2 gene (Figure 2D). In accordance with previous results, the strongest observed association was at rs2231142 \((p = 3.1 \times 10^{-206})\), Table 1, a coding SNP leading to a glutamine-to-lysine amino acid change at position 141 [11].

The third previously implicated locus influencing UA levels was on chromosome 6p23-p21.3, which contains the SLC17A3 gene (Figure 2F) [11]. The top associated marker was SNP rs1183201 \((p = 3.0 \times 10^{-14})\), Table 1, intronic of SLC17A1, but the association signal encompassed a larger region including the SLC17A1, SLC17A3, SLC17A4 genes and downstream to HIST1H4C, in agreement with the linkage disequilibrium at this locus. SNP rs1183201 is in high linkage disequilibrium \((r^2 = 0.97)\) with rs1165205, a SNP intronic of SLC17A3 gene identified by a previous GWA scan [11].

Among the novel loci identified, the strongest was on chromosome 11q13. One locus was localized upstream and within the SLC22A11 gene, and represented by SNP rs17500741 \((p = 6.7 \times 10^{-11})\), Table 1, Figure 2H. The second signal was SNP rs505802 \((p = 2.0 \times 10^{-8})\), representative of all associated markers falling within and downstream the extensively studied SLC22A12 gene coding for URAT1 (Figure 2F). The p-value plot as well as the LD block structure \((r^2 = 0.09)\) suggested two nearby but independently associated regions, which was verified in multiple regression analysis (Table S5).

The second novel region was on chromosome 2p23.3-p23.2 (Figure 2B). The most significant SNP in this region was SNP rs780094 \((p = 1.4 \times 10^{-5})\), intronic of GCKR, a glucokinase regulator protein recently associated with several quantitative traits including the regulation of triglycerides levels [12]. We also identified genome-wide significant association on chromosome 1q21 (Figure 2A). The top ranking SNP in this region was rs12129861 \((p = 2.7 \times 10^{-8})\), Table 1, located 2 kb upstream of PDZK1I coding for PDZ domain-containing 1 reported to interact with URAT1 [13]. The fourth newly detected region was found on chromosome 6p22.2 (Figure 2E), where the association signal spans two genes, LRRRC16a and SLC7A8, within one highly preserved LD block. The strongest p-value was observed for the SNP rs742132, located within an intron of LRRRC16a \((p = 8.5 \times 10^{-35})\), Table 1. Independence of the LRRRC16a and the SLC17A1 loci \((r^2 = 0.07)\) was verified in multiple regression analysis. P-values and effect estimates only slightly changed between single SNP analysis and multiple regression analysis (Table S5). Finally, we also observed some evidence of association on chromosome 10q21.3 (Figure 2G). One SNP within SLC16A9, rs12356193, reached genome-wide significance \((p = 1.1 \times 10^{-36})\). However, there were several additional SNPs within this gene with borderline significance, supporting the hypothesis that this locus may be a true signal rather than a false positive result.

**Sex-Stratified Meta-Analysis Identifies Male and Female Specific Variants**

We have also performed a meta-analysis of sex specific GWA results using all 14 studies (12,328 males, 15,813 females).

Although the results did not show any additional genome-wide significant locus (Figure 1B and 1C), we were able to query which of the aforementioned SNPs have sex-specific effects on serum UA levels (Table 2). For the SLC24A9 gene, we found that in males the top ranking SNP was still rs734553 \((p = 1.1 \times 10^{-41})\), while for women it was the nearby intronic SNP rs12490712 \((p = 2.4 \times 10^{-16})\). Supporting previously reported results, we found for both markers that the effect size of the minor allele observed in women was twice the effect size observed in men \((p = 3.8 \times 10^{-17})\). Table 2; [6]. The minor allele of rs2231142 in the ABCG2 gene showed a greater effect size in men compared to women \((p = 0.01)\), Table 2. Similar, the effect size of the most significant SNP for males in the ABCG2 gene locus, rs2199936, was greater in men compared to women \((p = 0.008)\), Table 2. The effect sizes of the other SNPs were comparable in men and women (Table 2).

**Association of Identified Variants with Metabolite Profiles**

To further characterize the identified variants, we analyzed their association with a panel of 163 metabolites measured in the KORA F4 survey. After correction for multiple testing, one SNP within SLC16A9, rs12356193, was associated with DL-carnitine concentrations \((\beta = 3.58, p = 4.0 \times 10^{-10})\), while in the turn, we associated with serum UA levels \((\beta = 0.06, p = 1.4 \times 10^{-57})\). In addition, this SNP was associated with propionyl-L-carnitine \((\beta = -0.06, p = 5.0 \times 10^{-5})\). Similar to DL-carnitine, propionyl-L-carnitine concentrations were also strongly associated with serum UA levels \((\beta = 1.78, p = 8.1 \times 10^{-54})\), forming a triangle between SNP, metabolites and UA levels. None of the other SNPs were significantly associated with the measured metabolites.

**Discussion**

Based on meta-analysis of GWA studies including 28,141 individuals, we have mapped 5 novel loci and confirmed 4 previously implicated loci that influence serum UA levels. Altogether, these associations highlight biological pathways that are important in the regulation of urate concentrations and may point to novel targets for pharmacological interventions to prevent or treat hyperuricemia.

A genome-wide significant p-value was observed for one SNP within SLC16A9 gene locus, coding for monocarboxylic acid transporter 9 (MCT9). This is a member of the monocarboxylate co-transporter family that has been demonstrated to catalyze transport of monocarboxylic acids across cell membranes [14]. MCT9 is expressed in various tissues including the kidney [15]. As other sodium monocarboxylate transporters have been found to influence urate in knockout models this MCT9 isoform might be a sodium-dependent transporter in the kidney. The second newly identified locus was GCKR (glucokinase regulatory protein) a regulator of glucokinase, the first glycolytic enzyme which serves as a glucose sensor, responsible for glucose phosphorylation in the liver. Recently, GWA studies for type 2 diabetes identified the same rs780094 SNP as a potential marker for modulation of triglyceride levels [16]. Meanwhile, GCKR polymorphisms were also shown to be associated with metabolic traits like fasting glucose and, modestly, type 2 diabetes [12,17,18]. Several potential mechanisms have been proposed to link serum UA concentrations with metabolic traits. Exogenous insulin decreases renal sodium and urate excretion [19]. Furthermore, renal clearance of UA is inversely related to the degree of insulin resistance [20]. Finally, insulin resistance is thought to be accompanied by impaired oxidative phosphorylation in hepatic mitochondria, leading to increased concentrations of co-enzyme A esters and thus to increased systemic adenosine concentrations.
Figure 1. Genome-wide association results. Manhattan plots showing significance of association of all SNPs in the meta-analysis for (A) men and women combined, (B) men only, and (C) women only. SNPs are plotted on the x-axis according to their position on each chromosome against association with uric acid concentrations on the y-axis (shown as $-\log_{10}$ p-value).

doi:10.1371/journal.pgen.1000504.g001
Table 1. Nine loci associated with uric acid concentrations.

<table>
<thead>
<tr>
<th>Loci</th>
<th>SNP</th>
<th>Chr*</th>
<th>Position (bp)</th>
<th>Allele</th>
<th>Frequency (Effect allele)</th>
<th>All individuals</th>
<th>Explained variability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N beta [95% CI]</td>
<td>p-value</td>
</tr>
<tr>
<td><strong>PDZK1</strong></td>
<td>rs12129861</td>
<td>1</td>
<td>144437046</td>
<td>A</td>
<td>46.40%</td>
<td>25627 (−0.062) [−0.083; −0.042]</td>
<td>2.68E-09 0.19%</td>
</tr>
<tr>
<td><strong>GCKR</strong></td>
<td>rs780094</td>
<td>2</td>
<td>27594741</td>
<td>T</td>
<td>41.70%</td>
<td>27991 (0.052) [0.035; 0.068]</td>
<td>1.40E-09 0.13%</td>
</tr>
<tr>
<td><strong>SLC2A9</strong></td>
<td>rs734553</td>
<td>4</td>
<td>9532102</td>
<td>T</td>
<td>76.81%</td>
<td>27817 (0.315) [0.294; 0.335]</td>
<td>5.22E-201 3.53%</td>
</tr>
<tr>
<td><strong>ABC2G</strong></td>
<td>rs2231142</td>
<td>4</td>
<td>89271347</td>
<td>T</td>
<td>10.77%</td>
<td>23622 (0.173) [0.141; 0.205]</td>
<td>3.10E-26 0.57%</td>
</tr>
<tr>
<td><strong>LRRC16A</strong></td>
<td>rs742132</td>
<td>6</td>
<td>25715550</td>
<td>A</td>
<td>69.57%</td>
<td>27923 (0.054) [0.036; 0.072]</td>
<td>8.50E-09 0.12%</td>
</tr>
<tr>
<td><strong>SLC17A1</strong></td>
<td>rs1183201</td>
<td>6</td>
<td>25931423</td>
<td>A</td>
<td>48.24%</td>
<td>27908 (−0.062) [−0.078; −0.459]</td>
<td>3.04E-14 0.19%</td>
</tr>
<tr>
<td><strong>SLC16A9</strong></td>
<td>rs12356193</td>
<td>10</td>
<td>61083359</td>
<td>A</td>
<td>82.68%</td>
<td>23559 (0.078) [0.051; 0.105]</td>
<td>1.07E-08 0.17%</td>
</tr>
<tr>
<td><strong>SLC22A11</strong></td>
<td>rs17300741</td>
<td>11</td>
<td>64088038</td>
<td>A</td>
<td>51.06%</td>
<td>27727 (0.062) [0.046; 0.078]</td>
<td>6.68E-14 0.19%</td>
</tr>
<tr>
<td><strong>SLC22A12</strong></td>
<td>rs505802</td>
<td>11</td>
<td>64136648</td>
<td>T</td>
<td>69.83%</td>
<td>27967 (−0.056) [−0.074; −0.038]</td>
<td>2.04E-09 0.13%</td>
</tr>
</tbody>
</table>

*Chromosome.

Shown is the most significant SNP for each independent locus associated (p<5×10^{-8}) with uric acid concentrations on meta-analysis in the complete dataset. Position is given for NCBI Build 36. Effect estimates result from additive linear regression on Z-scores of uric acid concentrations. P-values have been combined weighting by the inverse variance. The effect allele is the allele to which the beta (effect) estimate refers.

doi:10.1371/journal.pgen.10000504.t001

Figure 2. Regional association plots of nine urate loci. P-value plots showing the association signals in the region of (A) PDZK1 on chromosome 1, (B) GCKR on chromosome 2, (C) SLC2A9 on chromosome 4, (D) ABC2G on chromosome 4, (E) LRRC16A on chromosome 6, (F) SLC17A1 on chromosome 6, (G) SLC16A9 on chromosome 10, (H) SLC22A11 on chromosome 11, and (I) SLC22A12 on chromosome 11. −log10 p-values are plotted as a function of genomic position (NCBI Build 36). Large diamonds in red indicate the most significant SNP in the region while other SNPs in the region are given as colour-coded smaller diamonds. Red diamonds indicate high correlation with the lead SNP (r^2≥0.8), orange diamonds indicate moderate correlation with the most significant SNP (0.5<r^2<0.8), yellow indicates markers in weak correlation with the most significant SNP (0.2<r^2<0.5), white indicates no correlation with the most significant SNP (r^2<0.2). Estimated recombination rates (HapMap Phase II) are given in light blue, genes as well as the direction of transcription (NCBI) are displayed by green bars.

doi:10.1371/journal.pgen.10000504.g002
Table 2. Gender specific association results at the nine loci.

<table>
<thead>
<tr>
<th>Loci</th>
<th>SNP</th>
<th>Chr*</th>
<th>Position (bp)</th>
<th>Effect Allele</th>
<th>Men: N, beta [95% CI] p-value</th>
<th>Women: N, beta [95% CI] p-value</th>
<th>Difference: Δ beta (men - women) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDZK1</td>
<td>rs12129861</td>
<td>1</td>
<td>144437046</td>
<td>A</td>
<td>11888 -0.080 [-0.10; -0.058] 3.68E-07</td>
<td>13739 -0.047 [-0.075; -0.019] 9.10E-04</td>
<td>-0.033 0.140</td>
</tr>
<tr>
<td>PDZK1</td>
<td>rs1471633</td>
<td>1</td>
<td>144435096</td>
<td>A</td>
<td>12225 0.072 [0.044; 0.099] 2.94E-07</td>
<td>14289 0.040 [0.016; 0.064] 1.10E-03</td>
<td>0.031 0.094</td>
</tr>
<tr>
<td>GCKR</td>
<td>rs780094</td>
<td>2</td>
<td>27594741</td>
<td>T</td>
<td>12225 0.050 [0.023; 0.077] 3.05E-04</td>
<td>15736 0.055 [0.034; 0.077] 3.11E-07</td>
<td>-0.005 0.744</td>
</tr>
<tr>
<td>GCKR</td>
<td>rs780093</td>
<td>2</td>
<td>27596107</td>
<td>T</td>
<td>12243 0.047 [0.020; 0.074] 6.18E-04</td>
<td>15751 0.056 [0.035; 0.076] 2.30E-07</td>
<td>-0.009 0.617</td>
</tr>
<tr>
<td>SLC2A9</td>
<td>rs734553</td>
<td>4</td>
<td>9531102</td>
<td>T</td>
<td>12178 0.220 [0.188; 0.252] 1.13E-41</td>
<td>15639 0.397 [0.371; 0.423] 1.05E-192</td>
<td>-0.177 3.8E-17</td>
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<tr>
<td>SLC2A9</td>
<td>rs12498742</td>
<td>4</td>
<td>9533150</td>
<td>A</td>
<td>12274 0.208 [0.176; 0.239] 1.50E-38</td>
<td>15761 0.395 [0.369; 0.420] 2.36E-196</td>
<td>-0.187 2.1E-19</td>
</tr>
<tr>
<td>ABCG2</td>
<td>rs2231142</td>
<td>4</td>
<td>89271347</td>
<td>T</td>
<td>10324 0.221 [0.171; 0.270] 2.25E-18</td>
<td>13298 0.138 [0.096; 0.181] 1.13E-10</td>
<td>0.083 0.013</td>
</tr>
<tr>
<td>ABCG2</td>
<td>rs2199936</td>
<td>4</td>
<td>89264355</td>
<td>A</td>
<td>10323 0.222 [0.173; 0.272] 1.65E-18</td>
<td>13218 0.133 [0.091; 0.176] 6.85E-10</td>
<td>0.089 0.008</td>
</tr>
<tr>
<td>LRRC16A</td>
<td>rs742132</td>
<td>6</td>
<td>25715550</td>
<td>A</td>
<td>12335 0.062 [0.033; 0.091] 2.68E-05</td>
<td>15688 0.048 [0.024; 0.071] 8.14E-05</td>
<td>0.014 0.449</td>
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<td>SLC17A1</td>
<td>rs1183201</td>
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<td>25931423</td>
<td>A</td>
<td>12206 -0.076 [-0.10; -0.049] 2.52E-08</td>
<td>15702 -0.055 [-0.075; -0.036] 4.48E-08</td>
<td>-0.021 0.224</td>
</tr>
<tr>
<td>SLC17A1</td>
<td>rs9393672</td>
<td>6</td>
<td>25950584</td>
<td>T</td>
<td>12252 -0.074 [-0.10; -0.047] 6.22E-08</td>
<td>15738 -0.056 [-0.076; -0.036] 2.77E-08</td>
<td>-0.018 0.296</td>
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<td>SLC17A1</td>
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<td>6</td>
<td>25957599</td>
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<td>12215 -0.076 [-0.10; -0.049] 2.24E-08</td>
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<td>-0.022 0.198</td>
</tr>
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<td>10</td>
<td>61083359</td>
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<td>64088038</td>
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<td>12120 0.066 [0.039; 0.093] 1.50E-06</td>
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<td>rs505802</td>
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<td>64113648</td>
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<td>15735 0.047 [-0.070; -0.023] 1.02E-04</td>
<td>0.026 0.161</td>
</tr>
</tbody>
</table>

*Chromosome.

Shown are the gender-specific loci for the most significant SNP at the nine associated loci. Positions are given according to NCBI Build 36. Effect estimates result from additive linear regression on Z-scores of uric acid concentrations when only males (or females) were considered for the analysis. P-values have been calculated using weighting by the inverse variance. The effect allele is the allele to which the beta (effect) estimate refers. When different from the main meta-analysis, the most associated marker in males (females) is also listed.

doi:10.1371/journal.pgen.1000504.t002
[21]. Increased adenosine, in turn, may result in renal retention of sodium, urate, and water [21,22]. This provides a putative mechanism for hyperuricemia via both the break down of adenosine to urate and increased renal urate retention [21,22].

We also found evidence for association in a region containing two genes, LRRC16A and SCGN. The strongest association was located within LRRC16A coding for CARMIL. This large protein is most abundant in kidney and epithelial tissues and serves as an inhibitor of the heterodimeric actin capping protein (CP), an essential element of the actin cytoskeleton which binds to the barbed ends of actin filaments and regulates their polymerization [23]. The multiple biochemical functions associated with CARMIL raise many possibilities for its mechanism of action in cells, but the relation of CARMIL to UA concentration is thus far unclear. The nearby SCGN is coding for Secretagogin, a calcium-binding protein selectively expressed in neuroendocrine tissue and pancreatic beta-cells. The function of Secretagogin is unknown, but it has been suggested to influence calcium influx and insulin secretion [24].

We also demonstrated association of SNPs in SLC22A11 and SLC22A12 with UA concentrations. SLC22A12 encodes the extensively studied URAT1, a member of the organic anion transporter (OAT) family [25]. URAT1, a well known candidate gene for UA accumulation/transport, mediates the non-voltage-dependent exchange of urate for several organic anions [1]. SLC22A11 codes for OAT4, an OAT isoform which, like URAT1, is localized at the apical membrane of the proximal tubules. OAT4 serves as an organic anion–dicarboxylate exchanger, which mediates urate transport across the apical membrane of kidney [26,27]. In combination with these findings, we also identified genome-wide significant association of SNPs in and upstream of PDZK1, coding for PDZ domain containing 1, a scaffolding protein reported to interact with OAT4, URAT1 and NPT1 (SLC17A1) via their C-terminal PDZ motifs [13,28]. It has been proposed that the PDZ scaffold may form a bidirectional transport system by linking URAT1 (reabsorption) and NPT1 (secretion) leading to a functional complex responsible for the balanced urate transport regulation at the apical membrane of renal proximal tubules [1,28].

In accordance with previous genome-wide studies, the strongest effect on serum UA concentrations was detected for SLC2A9, the gene for GLUT9, which has been shown to be strongly associated with hyperuricemia and gout and to serve as a high-capacity urate transporter in humans [8,10]. Additional confirmed loci include ABCG2 and SLC17A1 [11]. ABCG2 is a member of the ATP-binding cassette (ABC) superfamily of membrane transporters, while the SLC17A1 locus, located directly downstream of the recently identified SLC17A3 locus (NPT4), encodes NPT1 (renal sodium phosphate transport protein 1). The human NPT1 is localized at the apical membrane of renal proximal tubules and serves as a voltage-driven UA transporter in model systems [28].

Although several of the SNPs associated with uric acid concentrations in this meta-analysis are located within genes that are plausible candidates for influencing uric acid concentrations, our association approach is not able to identify underlying genes or mechanisms in the regions of association signals. Therefore, other genes might be responsible for the observed associations and functional studies are warranted to identify the causal variants and provide insights in the underlying biological mechanisms.

Pronounced gender differences in the regulation of serum UA concentrations have been reported for both humans and animals [1,6]. In line with our previous findings, [6] the strongest gender-specific effect was observed for the minor allele of rs734553 (SLC2A9), resulting in a 2-fold larger effect size on serum UA concentrations in women compared to men. For ABCG2, the effect of the minor allele of rs2231142 demonstrated a larger effect on UA concentrations in men compared to women. For the other loci, effect sizes did not significantly differ by gender.

The rapidly evolving field of metabolomics aims at a comprehensive measurement of endogenous metabolites in a cell or body fluid [29]. Based on screening of 163 metabolites, we have observed an association of one of the identified variants, rs12356193, with DL-carnitine and propionyl-L-carnitine. Moreover, DL-carnitine and propionyl-L-carnitine were strongly correlated with serum UA levels, forming a triangle between SNP, metabolites and UA levels. Carnitine is acquired from diet and endogenous biosynthesis. Its primary function is in the transport of long chain fatty acids. After strenuous physical exercise, both acylcarnitine and UA levels increase in the serum of healthy humans [30]. In spontaneously hypertensive rats, L-carnitine decreases serum UA levels and the age-dependent rise in serum UA [31,32]. Kidneys absorb 95% of carnitine from the glomerular filtrate via an active Na+-dependent transport mechanism [33]. Impairment of this reabsorptive function can lead to carnitine deficiency, in which hyperuricemia may be present because carnitine competes for renal tubular excretion [34]. Although experimental data are few, currently available data suggest that urinary acylcarnitine, which reflects the balance between dietary intake of carnitine and renal excretion, may be linked to serum UA via oxidative stress pathways [35]. Given that palmitoyl carnitine inhibits binding of Ca²⁺ channel ligands to rat brain cortical membranes and to inhibit voltage-activated Ca²⁺ channel currents, acylcarnitines may also have direct influences on MCT9 [36].

Overall, serum UA concentrations are mainly determined by the balance between urate production and renal excretion. We have identified nine loci that are associated with serum UA levels and six of them harbor genes that code for renal transport proteins. Most notably, five of these transport proteins belong to the family—and moreover, to one phylogenetic cluster within this family [37]. These findings strongly support the hypothesis that genetic variation in urate transport proteins are the key influences upon regulation of serum UA levels in humans.

Materials and Methods

Study Participants

The present meta-analysis combined data from 14 GWA scans: British Genetics of Hypertension (BRIGHT), Cohorte Lausannoise (CoLaus), Vis Island Isolate Study (CROATIA), Health 2000 cohort (Health 2000), two surveys of the Cooperative Health Research in the Region of Augsburg (KORA F3, KORA S4), Orkney Complex Disease Study (ORCADES), Precocious Coronary Artery Disease (PROCARDIS), Northern Swedish Population Health Study (NSPHS), Sardinia Study of Aging (Sardinia), Study of Health in Pomerania (SHIP), Semi-Structured Assessment for Genetics of Alcoholism (SSAGA), Microisolates in South Tyrol (MICROS), and UK Adult Twin Register (TwinsUK). Altogether, the meta-analysis comprises 28,141 individuals (12,328 males, 15,813 females) of European ancestry with measured serum UA concentrations (Table S1). Approval was obtained by local ethic committees for all studies and informed consent was given from the study participants. A detailed individual description of study designs is provided in Text S1.

Genome-Wide Genotyping and Imputation

Six different platforms/arrays were used for genotyping: the Affymetrix 500 K GeneChip array (4 cohorts, n = 13,103), the
Meta-Analysis on Uric Acid Levels

Association testing Z-scores were calculated in each stratum separately. Study-specific results of the most significant SNP at each locus are presented in Table S3. The results from all 14 GWA scans were combined into a fixed-effects meta-analysis with inverse variance weighting, using the METAL package (www.sph.umich.edu/csg/abecasis/metal). The individual studies were corrected for residual inflation of the test statistic using genomic control methods for genotyped and imputed SNPs combined [40]. For the overall meta-analysis, the inflation factor was 1.029, no further correction was applied. Quantile-quantile plots of the association results are shown in Figure S1, study-specific quantile-quantile plots are illustrated in Figure S2 and S3. Associations were considered genome-wide significant below p = 5×10⁻⁸, which corresponds to a Bonferroni correction for the estimated one million independent common variant tests in the human genome of European individuals [41]. We also tested whether the effect estimate resulting from the gender-specific fixed effect meta-analysis differed significantly between men and women by applying a t-test comparing effect and standard error estimates in men with the effect and standard error estimates in women. Genome-wide significant SNPs were tested for independent associations, by including all SNPs in a multiple regression model, and then performing inverse variance weighted meta-analysis, across all cohorts (except for Health 2000), of the coefficient for each SNP. The analysis of metabolites was performed using the same linear regression adjusted by sex and gender as in the genome-wide scan. To specify the dependency of uric acid on metabolite concentration, a univariate regression model without further transformation or adjustment was used. The multiple regression and metabolite analysis were performed using either posterior expected allele dosages, or on best-guess imputed genotypes, with the statistical analysis software R.

Accession Numbers

The OMIM (http://www.ncbi.nlm.nih.gov/OMIM) accession numbers for genes mentioned in this article are PDZK1 (603031), GCKR (606042), SLC2A9 (600142), ABCG2 (603756), LRRN16A (609593), SLC17A1 (182308), SLC22A11 (607097), and SLC2A12 (607096). The HGNC (http://www.gene.ucl.ac.uk) accession number for SLC16A9 is 23520.

Supporting Information

Figure S1 Quantile-quantile plots of association results. Meta-analysis was performed using sample-size weighted Z-scores after cohort-specific genomic control. Shown are expected p-values plotted against observed p-values resulting from meta-analysis based on all subjects (1st row), only males (2nd row) and only females (3rd row) for all analysed SNPs (left column) and for all analysed SNPs excluding the SLC2A9 region (GLUT9, right column).

Found at: doi:10.1371/journal.pgen.1000504.s001 (1.25 MB TIF)

Figure S2 Study-specific quantile-quantile plots. Shown are expected p-values plotted against observed p-values resulting from each single study before (black dots) and after (blue dots) genomic control correction. The study-specific λ-values were λ = 1.007 (BRIGHT), λ = 1.025 (CoLaus), λ = 1.013 (CROATIA), λ = 1.024 (Health 2000), λ = 1.006 (KORA F3), λ = 1.016 (KORA S4), λ = 1.246 (MICROS), λ = 1.253 (NSPHS), λ = 1.182 (ORCADES), λ = 1.022 (PROCARDIS), λ = 1.090 (SardiNIA), λ = 1.031 (SHIP), λ = 1.022 (SSAGA) and λ = 1.122 (TwinsUK). For the overall meta-analysis, the inflation factor was 1.028.

Found at: doi:10.1371/journal.pgen.1000504.s002 (1.95 MB TIF)

Metabolite Measurements

Metabolomic analyses were conducted in 2020 randomly selected participants (ages 32–81 years) of the KORA F4 survey, a follow-up survey of KORA S4. Genotype information was available for 1814 of these participants. Fasting blood samples were collected in 2006–2008. Blood was drawn into serum gel tube in the morning between 8 and 10 am. The tube was gently inverted two times, followed by 30 minutes resting at room temperature to obtain complete coagulation, and finally centrifugation of blood was performed at 2750 g, 15°C for 10 minutes for serum collection. Serum was aliquoted and kept at 4°C for a maximum of 6 hours, after which it was frozen at −80°C until analyses. Liquid handling of serum samples (10 μl) was performed with Hamilton Star (Hamilton Bonaduz AG, Bonaduz, Switzerland) robot and prepared for quantification with AbsoluteIDQ kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). Sample analyses were done on API4000 Q TRAP LC/MS/MS System (Applied Biosystems, Darmstadt, Germany) equipped with Schimadzu Prominence LC20AD pump and SIL-20AC auto sampler. The complete analytical process (e.g. the targeted metabolite concentration) was performed using the MetIQ software package, which is an integral part of the AbsoluteIDQ Kit. A total of 163 metabolites were measured. The metabolomics dataset contains 14 amino acids, one sugar, 41 acylcarnitines, 15 sphingolipids, and 92 glycerophospholipids.

Statistical Analysis

GWA scans were made using an additive genetic model on Z-scores, calculated by adjusting serum UA levels for age and sex using linear regression and standardizing residuals. In sex-specific

Uric Acid Measurements

Non-fasting blood samples were obtained from study participants of BRIGHT, KORA, NSPHS, SardiNIA, SHIP and TwinsUK and fasting samples from those of CoLaus, PROCARDIS, CROATIA, Health 2000, MICROS, ORCADES and TwinsUK. UA analyses were carried out on fresh samples in all studies except from BRIGHT, NSPHS, CROATIA, MICROS and SSAGA, where frozen serum was used that was stored at −20°C (BRIGHT) or −70°C (NSPHS, SSAGA, CROATIA, MICROs). UA concentrations were measured using an uricase/peroxidase method (CROATIA, MICROs, NSPHS and ORCADES: DVI1650-Autoanalyzer, Siemens Healthcare Diagnostics) or an uricase method (BRIGHT: Hitachi, Roche Diagnostics; CoLaus: uricase PGP, Roche Diagnostics; Health 2000: Thermo Fisher Scientific, Vantaa; KORA F3: URCA Flex, Dade Behring; KORA S4: UA Plus, Roche; PROCARDIS: Hitachi 917, Roche Diagnostics; SardiNIA: Bayer; SHIP: UA PAP, Boehringer; SSAGA: Hitachi 747, Boehringer; TwinsUK: Ektachem/Vitros system, Johnson & Johnson Clinical Diagnostics).

Table S1 Study sample characteristics. Characteristics are shown by study for British Genetics of Hypertension (BRIGHT), Cohorte Lausannoise (CoLaus), Vis island isolate study (CROATIA), Health 2000 cohort (Health 2000), two surveys of the Cooperative Health Research in the Region of Augsburg (KORA F3, KORA S4), Orkney Complex Disease Study (ORCADES), Precocious Coronary Artery Disease (PROCARDIS), Northern Swedish Population Health Study (NSPHS), SardiniNIA Study of Aging (SardiNIA), Study of Health in Pomerania (SHIP), Semi-Structured Assessment for Genetics of Alcoholism (SSAGA), Microisolates in South Tyrol (MICROS) and UK Adult Twin Register (TwinUK). Age is given as mean and range in brackets. Urinary acid concentrations (UA) are given as mean and appropriate standard deviation (SD). NA indicates not applicable.

Table S2 Genotyping, imputation and analysis procedures by study. Shown are the genotyping platforms, quality control (QC) filters applied before imputation, imputation software, number of SNPs and genotype-phenotype association software.

Table S3 Study-specific results. Shown are study-specific results of the most significant SNP at each locus.

Table S4 Full list of genome-wide significant SNPs. Shown is a full list of SNPs that exceeded the threshold of genome-wide significance (p<3x10^-8). Position is given for NCBI Build 36. Effect estimates result from additive linear regression on Z-scores of uric acid concentrations. P-values have been calculated using weighting by the inverse variance. The effect allele is the allele to which the beta (effect) estimate refers.

Table S5 Multiple regression analysis. Genome-wide significant SNPs were tested for independent associations, by including all nine SNPs in a multiple regression model, and then performing inverse variance weighted meta-analysis, across participating cohorts (except for Health2000), of the coefficient for each SNP.

Text S1 Study design. This section describes additional study specific characteristics.

Acknowledgments

The authors would like to acknowledge those who agreed to participate in the contributing studies. The computations for CoLaus imputation were performed in part at the Vital-IT center for high performance computing of the Swiss Institute of Bioinformatics. The CoLaus authors thank Volandre Barreau, Mathieu Furrmann, Vladimir Mayor, Anne-Lise Bastian, Binasam Ramic, Martine Morvanville, Martine Baumer, Marcy Sagette, Jeanne Ecoffey, and Sylvie Mermoud for data collection. The CROATIA authors are grateful to Professor Pavao Rudan and the staff of the Institute for Anthropological Research in Zagreb, Croatia (organization of the field work, anthropometric and physiological measurements, and DNA extraction); Professor Ariana Vorko-Jovic and the staff and medical personnel of the Andrijas Stupar School of Public Health of the Faculty of Medicine, University of Zagreb, Croatia (questionnaires, genealogical reconstruction and data entry). The CROATIA authors acknowledge the Wellcome Trust Clinical Research Facility (Edinburgh) for performing the genotyping. The KORA authors acknowledge the contribution of P. Lichtner, G. Eckstein, G. Fischer, L. Geistlinger, and N. Klopp and all other members of the Helmholtz Zentrum München genotyping staff for generating the SNP data. The KORA authors also want to thank C. Prehn and W. Romisch-Margl for support and advice, and A. Sabunchi, H. Chavez, B. Hochstrat and T. Halex for technical assistance during the metabolite analyses as well as all members of field staffs who were involved in the planning and conduct of the KORA Augsburg studies. The MICROS authors are grateful to the primary care practitioners Raffaela Stocker, Stefan Waldner, Toni Pizzeco, Josef Planger, Ugo Marcadent, and the personnel of the Hospital of Silandro (Department of Laboratory Medicine) for their participation and collaboration in the research project. The NSPHS authors are grateful to the Upplands Genome Center for performing the genotyping, the contribution of samples from the Medical Biobank in Örebro and the contribution of the district nurse Svea Hennix in the Kareusandno study. DNA extractions for ORCADES were performed at the Wellcome Trust Clinical Research Facility in Edinburgh. The ORCADES authors would like to acknowledge the invaluable contributions of Lorraine Anderson and the research nurses in Orkney, the administrative team in Edinburgh and the people of Orkney. The PROCARDIS authors acknowledge the technical contributions of Anuj Goel, Simon Heath, Ivo Gut, and Anne-Christine Syvainen. The SardiNIA authors thank the team of the physician, the nurses and the genotyping team of biologists: Gianlucca Usala, Fabio Busonero, Andrea Maschio, Sandra Lai, Mariano Dei, and Antonella Mulas. The SHIP authors are grateful to Stefan Funke for the opportunity to use his Server Cluster for SNP imputation, and to the contribution of Florian Ernst, Anja Hoffmann, and Astrid Petersmann in generating the SNP data. The SSAGA authors thank Professors Nicholas Martin and Grant Montgomery and all members of the Genetic Epidemiology and Molecular Epidemiology Laboratories who were involved in the planning and conduct of the SSAGA studies. The TwinUK authors thank the staff from the TwinsUK, the DNA Collections and Genotyping Facilities at the Wellcome Trust Sanger Institute for sample preparation; Le Centre National de Génotypage, France, led by Mark Lathrop, for genotyping; Duke University, North Carolina, United States of America, led by David Goldstein, for genotyping; and the Finnish Institute of Molecular Medicine, Finnish Genome Center, University of Helsinki, led by Aarno Palotie. The UA measurements (ORCADES, MICROS, CROATIA) were performed in Regensburg in the laboratory of Professor Schmitz. The details and full memberships of the consortia are listed at http://www.procardis.org for the PROCARDIS consortium, http://www.helmholtz-muenchen.de/ kora/ for the KORA study, http://www.euengage.org for the ENGAGE consortium and http://homepages.ed.ac.uk/s0565445/ for the EURO-SPAN consortium. See Text S1 for membership and affiliations of the Wellcome Trust Case Control Consortium.

Author Contributions

Conceived and designed the experiments: AFW JA JBW UG JFW IR PP HW AD HEW TDS LP HV RN PV MC TI CG. Performed the experiments: HP RWS. Analyzed the data: MK TJ SS AT VV MP MM EA CW MF AJ DRN YA CL PS RWS CG. Contributed reagents/materials/analysis tools: JSB SB MB MB HC JC AD GH MM TM VM PM MN JPS VS DS DL UV GW DW TI. Wrote the paper: MK TJ CG. Conducted meta-analysis: MK TJ EA CG.
References