Recent insights into the pathogenesis of hyperuricaemia and gout

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Gout is a common rheumatic disease in humans which is characterized by elevation in serum uric acid levels, and deposition of uric acid crystals in the joint. Hyperuricaemia is the primary risk factor for the development of gout and primates have uniquely high levels of serum uric acid due to missense mutations in the uricase gene. Levels of serum uric acid are known to be highly heritable, and mutations in genes which encode enzymes in the purine salvage pathway have long been recognized as rare causes of gout. Until recently, however, little has been known about the genetic determinants of urate metabolism and susceptibility to gout in the general population. Over recent months, a series of large scale genome wide association studies have been performed which have shed new light on the genes which regulate serum uric acid levels and susceptibility to gout. Most of these genes seem to be involved in regulating the renal excretion of uric acid which underscores the importance of reduced urate excretion as opposed to increased endogenous production as a cause of gout. Further work will now be required to investigate the mechanisms by which these genetic variants regulate urate excretion and serum urate levels. However, it seems likely that the genes so far identified will represent new molecular targets for the design of drugs to enhance urate excretion and the genetic variants that predispose to gout might be of value as genetic markers of susceptibility to gout.

INTRODUCTION

Gout is a common rheumatic disease in humans which is characterized by elevation in serum uric acid levels, deposition of uric acid crystals in the joint and an acute inflammatory arthritis (1). The prevalence of gout in both UK and German populations has been reported as 1.4% (2,3) making it the commonest cause of inflammatory arthritis in men. Hyperuricaemia is the most important risk factor for the development of gout with the likelihood of developing gout rising exponentially in line with serum urate levels (4). Despite this strong association the majority of patients with hyperuricaemia do not develop gout; the annual incidence of gout is estimated to be only 5% in patients with levels of serum urate above 9 mg/dl (4,5). Serum levels of urate follow a normal distribution in the general population but levels are higher in men, which probably accounts for the fact that clinical gout is about five times more common in men than in women. Although genetic factors play an important role in regulating serum urate levels, these can also be influenced by increased intake of purines or other factors such as fructose which influence urate metabolism (Fig. 1). Humans have uniquely high basal levels of serum uric acid due to three separate missense mutations resulting in a non-functioning uricase gene (6,7). In an analysis of four hominoid species the human mutations of urate oxidase were identified in chimpanzees and orang utang but an independent deletion event was found in gibbons suggesting that survival advantage accrued from loss of urate oxidase (6).

Genetic factors play an important role in the pathogenesis of gout and regulation of serum uric acid levels. Twin studies have shown that the renal handling of uric acid is strongly heritable (8). This is of relevance to the pathogenesis of gout since, for more than 90% of patients, the primary problem is reduced renal excretion of uric acid (1). Segregation analysis in families has shown that serum uric acid levels also have a significant heritable component (heritability 0.4) with an overall pattern of inheritance that is consistent...
with a complex trait, regulated by an interaction between more than one major gene, several modifying genes and environmental factors (9).

**SINGLE GENE DISORDERS THAT CAUSE HYPERURICAEMIA AND GOUT**

Gout and hyperuricaemia can occasionally be inherited in a Mendelian manner (Table 1). For example, inactivation mutations in hypoxanthine-guanine phosphoribosyl transferase (HGPRT)—a gene involved in the purine salvage pathway—can cause hyperuricaemia and gout in the form of Lesch–Nyhan syndrome (10). This is an X-linked recessive disorder which varies in severity depending on the type of mutation and its effect on catalytic activity of HGPRT.

Another Mendelian form of gout occurs in patients with activating mutations in phosphoribosyl pyrophosphatase synthetase (11) an enzyme that is involved in urate synthesis. This causes X-linked dominant inheritance of primary hyperuricaemia and gout. Gout and hyperuricaemia can also occur in patients with glycogen storage diseases (12) and some other rare inborn errors of metabolism (Table 1).

Autosomal dominant syndromes of hyperuricaemic nephropathy associated with reduced fractional excretion of uric acid have been described (12) associated with mutations of the uromodulin gene. These are loss of function variants that result in defective transport and apical membrane expression of uromodulin (13). Uromodulin is expressed abundantly in the thick ascending limb of the loop of Henle where it is thought to contribute to water permeability; defects in uromodulin secretion due to protein misfolding (14) correlate with decreased urine osmolarity which in turn correlates directly with increased serum uric acid levels, although the mechanism remains uncertain (15).

**CANDIDATE GENES FOR REGULATION OF HYPERURICAEMIA AND GOUT**

**SLC22A12**

The first specific urate transporter to be identified was SLC22A12 which encodes URAT1. This molecule was identified on the basis of its homology to the organic ion transporter family and is expressed on the apical membrane of renal tubular cells (16) (Fig. 2). Polymorphisms of SLC22A12 have been associated with raised serum urate levels and decreased fractional urate excretion (17), but the functional mechanisms which underlie these associations remain unclear. Although SLC22A12 has not emerged as a significant determinant of uric acid levels in recent genome wide association studies (GWAS) the coverage of URAT1 in most panels of single nucleotide polymorphisms (SNPs) is poor (18). There is little doubt that URAT1 plays an important role in regulating the renal tubular reabsorption of urate, since loss of function mutations in SLC22A12 have been found to cause hypouricaemia in Japanese populations (19) though these findings were not replicated in a Caucasian population (20). Mice lacking
URAT1 have relatively minor changes in urate metabolism (21) suggesting that other transporters may be involved and increasingly a dominant role for URAT1 is being called into question. In a recent meta analysis SLC22A12 was estimated to contribute 0.13% of variance in serum uric acid (22) though this effect remains difficult to interpret due to close linkage disequilibrium (LD) with the solute transporter SLC22A11.

SLC2A9

The SLC2A9 gene was identified as an important regulator of serum urate, uric acid excretion and gout by several GWAS in 2008 (18,23–25). The effect of variation in SLC2A9 is most pronounced in females, accounting for 5–6% of the variance compared with 1–2% in males (18,23–27). Several variants at the SLC2A9 locus have also been shown to be associated with clinical gout (24,25,28). The SLC2A9 gene encodes a membrane protein from the major facilitator superfamily which comprises the characteristic 12 transmembrane helices as well as the signature of sugar transporters. Indeed prior to the studies mentioned above, SLC2A9 was considered to be a fructose transporter (29). Functional studies in Xenopus oocytes have shown that SLC2A9 encodes a highly efficient transporter of uric acid with a capacity far higher than URAT1 (24). The SLC2A9 gene has two major transcript variants; significantly the long isoform of SLC2A9 is expressed in basolateral membranes of proximal renal tubular cells while the short isoform is expressed in the apical membrane of proximal renal tubular cells (30) (Fig. 1). The critical residues within the N-terminus that regulate this cellular localization remain to be established. Both isoforms of SLC2A9 have urate transporter activity (27).

Ten coding SNPs have been reported, five of which give rise to non-synonymous amino acid substitutions. These residues are highly conserved throughout mammalian species, but their functional consequences (if any) have not yet been studied. In only one study has the strongest association been seen with a coding polymorphism (25) and this has not been replicated. This SNP (rs16890979) is in strong LD with previously reported intronic SNPs (D’ 0.91–1). In all other GWAS coding SNPs have shown less significant associations with serum urate than haplotype-tagging intronic or promoter SNPs. In view of this, the many coding polymorphisms are most likely to be in LD with causal variations in promoter or enhancer sequences that predispose to gout by altering levels of gene expression. In this regard, there is evidence that expression levels of SLC2A9 may play a greater role in determining uric acid levels than functional variants (18).

The long isoform of SLC2A9 is strongly expressed in the liver (30). A founder mutation in the orthologue of SLC2A9 is found in Dalmatian dogs, where it is associated with both hyperuricaemia and hyperuricuria, suggesting that both reduced tubular reabsorption and reduced transport into the liver, which contains uricase, are responsible for the defect (31). The importance in humans of urate transport to the liver, the major site of purine synthesis, remains to be established. SLC2A9 is also expressed in human articular chondrocytes (32) a major site of uric acid deposition in gout, raising the possibility that SLC2A9 might play a role in transporting urate within the joint.

Heterozygous mutations in SLC2A9 associated with partial reductions in urate transport activity (about 50–75% of normal activity) have been reported in individuals with hypouricaemia, most of whom were asymptomatic but some of whom developed nephrolithiasis or exercise-induced acute renal failure (EIARF) (24,33,34). Recently, homozygous loss-of-function mutations have also been reported in individuals with hypouricaemia, nephrolithiasis or EIARF but even

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Phenotype</th>
</tr>
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<tbody>
<tr>
<td>Syndromes of altered purine metabolism</td>
<td></td>
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<tr>
<td>HPRT related</td>
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<td>XD</td>
<td>Hypoxanthine guanine phosphoribosyl transferase (HPRT 1)</td>
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<td>AD</td>
<td>Unknown</td>
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<td>AD/AR</td>
<td>Uromodulin</td>
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<tr>
<td>Familial juvenile hyperuricemic nephropathy</td>
<td>16p12.3</td>
<td>AD</td>
<td>Uromodulin</td>
<td>Progressive renal dysfunction, variable hyperuricaemia, early onset gout</td>
</tr>
</tbody>
</table>

Adapted from (12). XD, X-linked dominant; AD, autosomal dominant; AR, autosomal recessive.
lower residual urate transport activity (19–38% of normal), high fractional excretion of uric acid (>150%) and very low serum urate concentrations (mean 0.17 mg/dl) (D. Dinour et al., submitted). The occurrence of EIARF was attributed to reduced blood antioxidant capacity and oxygen free radical induced renal vasoconstriction (35, D. Dinour et al.). This is consistent with the observation that urate accounts for ~60% of the antioxidant capacity in blood (36).

**ABCG2**

Polymorphisms in the **ABCG2** gene were identified as a significant determinant of serum uric acid levels and gout by a GWAS in participants of the Rotterdam and Framingham studies with replication in participants of the Atherosclerosis Risk in Communities study (25). The **ABCG2** gene encodes a transporter of the ATP-binding cassette (ABC) family which is expressed in the apical membrane of human kidney proximal tubule cells and is known to transport purine nucleoside analogues. The strongest association was with a coding SNP in exon 5 of the gene (rs2231142), which leads to a glutamine-to-lysine amino acid substitution (Q141K). This SNP also emerged as the strongest predictor of urate levels after **SLC2A9** in a meta-analysis of genome wide searches (22). The glutamine residue in **ABCG2** is highly conserved across species, suggesting that this may be the functional variant, although functional analysis on the effects of this allele on urate transport activity have not yet been performed.

**SLC17A3 locus**

Polymorphic variants at a locus on 6p21 containing three members of the **SLC17** gene family (**SLC17A3, SLC17A1** and **SLC17A4**) were identified as a significant predictor of uric acid levels and gout in the GWAS mentioned above (25). The strongest association was with rs1165205 within intron 1 of **SLC17A3**, but several other SNP within the locus reached genome wide significance. The **SLC17A3** gene encodes a sodium phosphate transporter (NPT4) which is expressed at the apical membrane of renal proximal tubule cells. The potential role of **SLC17A3** as a urate transporter has not yet been studied. The **SLC17A1** gene lies immediately downstream of **SLC17A3** and encodes NPT1, which is expressed in the human kidney and can transport uric acid *in vitro* (37) though has not yet been shown to be physiologically important. A missense SNP was identified in **SLC17A1** (T269I) that reached genome wide significance in the Framingham study, but was in strong LD with rs116205 within **SLC17A3**. Furthermore a different SNP (rs1183201) within **SLC17A1** was identified as the strongest predictor of serum urate in a meta-analysis of GWAS (22). Because of the strong LD in this locus, further studies will be required to
identify the causal SNP that regulate uric acid levels and susceptibility to gout.

Additional genes

Several other genetic variants have been identified as being associated with serum uric acid levels by meta-analysis of 14 GWAS with a total of over 28,141 participants (Table 2). They include the SLC22A11 gene, the glucokinase regulatory protein (GCKR) gene, Carmil (LRRC16A), and near PDZ domain containing 1 (PDZK1) gene (22). The SLC22A11 gene encodes a solute transporter in the same family as URAT1. The scaffolding proteins PDZK1 and Na/H exchange regulatory factor-1 have been reported to interact directly with several key transporters and appear to involve in the assembly of a transporter complex at the apical membrane of renal tubular cells (Fig. 1) (38). The GCKR gene is predominantly expressed in liver where it stabilizes and regulates glucokinase and mice with targeted inactivation of GCKR have impaired glucose tolerance (39). It has been suggested that GCKR variants may cause hyperuricaemia by causing insulin resistance (22). The LRRC16A gene product Carmil has been shown to be expressed in kidney and epithelial tissues and is a key inhibitor of actin capping protein (40). The mechanisms by which variants at this gene locus regulate uric acid remain unclear.

**HYPERURICAEMIA AND THE METABOLIC SYNDROME**

Hyperuricaemia is strongly associated with metabolic syndrome (41) and cardiovascular disease (42), although a cause and effect relationship has not been established (43). Metabolic syndrome is a state of insulin resistance associated with elevated blood pressure, plasma glucose and triglyceride, decreased high-density lipoprotein cholesterol and abdominal obesity (44). The reported association of serum uric acid and metabolic syndrome was clearly evident in our Tayside cohort, where 78% of gout cases had metabolic syndrome (P = 8.9 × 10⁻³³; OR = 3.8).

There is increasing evidence of a cardioprotective effect of the xanthine oxidase inhibitor allopurinol in hyperuricaemic patients. Allopurinol has been shown to improve endothelial function (45), to be associated with decreased cardiovascular mortality (46) and to reduce blood pressure in hyperuricaemic teenagers (47). Importantly, it does not follow from this that hyperuricaemia is itself a cause of vascular morbidity. The endothelial effects of allopurinol have been shown to be independent of its uric acid lowering effect and mediated by a reduction in oxidative stress (48). This suggests that the generation of oxygen radicals by xanthine oxidase in the process of generating uric acid (Fig. 3) causes endothelial damage rather than hyperuricaemia per se. This is compatible with the long acknowledged role of uric acid as a powerful antioxidant (49) and the demonstration that exogenous uric acid has been shown to have beneficial effects on endothelial function (36). In turn this may explain a protective effect on endothelial function of elevating levels of uric acid by increased renal reabsorption or reduced uricase degradation.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>P-value</th>
<th>Explained variability (%)</th>
</tr>
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<tbody>
<tr>
<td>PDZK1</td>
<td>rs12129861</td>
<td>2.68E⁻⁰⁹</td>
<td>0.19</td>
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<td>GCKR</td>
<td>rs780094</td>
<td>1.40E⁻⁰⁹</td>
<td>0.13</td>
</tr>
<tr>
<td>SLC2A9</td>
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<td>5.22E⁻²⁰¹</td>
<td>3.53</td>
</tr>
<tr>
<td>ABCG2</td>
<td>rs2231142</td>
<td>3.10E⁻²⁶</td>
<td>0.57</td>
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<tr>
<td>LRRC16A</td>
<td>rs742132</td>
<td>8.50E⁻⁰⁹</td>
<td>0.12</td>
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<td>3.04E⁻¹⁴</td>
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<td>SLC22A12</td>
<td>rs505802</td>
<td>2.04E⁻⁰⁹</td>
<td>0.13</td>
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</tbody>
</table>

The data are derived from recent meta-analysis of GWAS in relation to the phenotype of serum uric acid. The estimated percentage of variance ascribed to the strongest SNP from each locus is shown. Data from Ref. (22).

However, the pathological consequences of elevated uric acid levels may vary between tissues, depending on the balance of import and export into cells, whether intracellular or extracellular concentrations are elevated and on the ascorbate and glutathione antioxidant status.

Attempts to clarify the relationship between serum uric acid and metabolic syndrome or hypertension through genetic association studies have provided no support for a causal effect (24,27). Variants in SLC2A9, which account for two-thirds of the explained variance in serum uric acid concentration (22) showed no association with metabolic syndrome in 3751 cases and 1678 controls (24), no association with hypertension in a meta-analysis involving 11,897 participants, or with blood pressure in a meta-analysis with 11,629 participants (24,27). Similarly a case–control study looking at 1473 patients with severe coronary artery disease or myocardial infarction and 1241 healthy controls found no association of SLC2A9 variants and disease (28). A large case control study analyzing the GCKR rs780094 polymorphism found it to be associated with dyslipidaemia but a reduced risk of type 2 diabetes (50). Conversely, GWAS looking at obesity related quantitative traits (51) or type 2 diabetes (52) showed no overlap with the genes so far associated with hyperuricaemia.

Recently, both fructose intake (53) and alcohol intake (54) as well as dietary purine intake (55) have been demonstrated in careful prospective studies to be associated with an increased risk of clinical gout. Alcohol intake is thought to increase uric acid levels through increases in lactic acid which can exchange with urate either in the kidney or liver (a similar phenomenon is observed in diabetic ketoacidosis). However, given that beer consumption is a greater risk factor than consumption of spirits or wine (54) it seems that there is at least as important a role for carbohydrate and purine content of beer in the aetiology of hyperuricaemia. The renal handling of monocarboxylates such as lactate and urate depends on coupled Na/anion and urate/anion co-transporters (56). Sodium dependent monocarboxylate transporters SLC5A8 and SLC5A12 have both been shown to be expressed on the apical membrane of renal proximal tubular cells (57,58).
Fructose is known to increase uric acid levels (59) and this increase occurs within minutes of the administration of intravenous fructose and has been attributed to an increase in the degradation of purine metabolites (60). Interestingly the effect is enhanced in patients with a history of gout (59). The identification of SLC2A9 as a novel uric acid transporter when it had formerly been thought to be a fructose transporter fuelled speculation that a direct interaction of the two substrates may occur. SLC2A9 certainly transports both glucose and fructose although it is considerably more active as a urate transporter (24,27). It has also been shown that urate efflux from Xenopus oocytes can be trans-stimulated by glucose or fructose (27), but there is no cis-inhibitory effect of these hexoses on urate transport. The physiological significance of these observations is unclear but it is possible that dietary fructose enhances renal reuptake of uric acid and sustains the acute elevation of uric acid caused by fructose ingestion. Diverse mechanisms for fructose induced metabolic syndrome include contributions to insulin resistance, lipogenesis as well as hyperuricaemia (61) Animal studies showing that urate lowering therapy directly attenuates the effect of fructose in causing metabolic syndrome have been interpreted as showing fructose induced hyperuricaemia to be a critical contributor of risk (62) but they do not address the diverse actions of allopurinol (see above).

**CLINICAL IMPLICATIONS**

Gout is the commonest cause of inflammatory arthritis in men (2,3) and its incidence is increasing (63). Recognizing that the care of patients with gout is highly variable and often disappointing (64), both the European League against Rheumatism and British Society for Rheumatology have recently introduced guidelines for the management of gout (65,66). The mainstay of treatment remains xanthine oxidase inhibition (predominantly with allopurinol) rather than uricosuric therapy which is used second line because of lesser efficacy or increased risk of side effects (66). This is rather counterintuitive given that reduced fractional excretion of uric acid is the dominant cause of hyperuricaemia and gout. The identification of the key transporters in the renal tubule that mediate this effect allows the direct assessment of current therapeutics on uric acid transport (24,27) and should in turn allow identification of new drugs to treat gout.

At the present time asymptomatic hyperuricaemia is not considered to be an indication for urate lowering therapy (66) and in clinical practice, prophylactic treatment for gout is only introduced in patients who have had two or more attacks in a 12 month period. The identification of alleles that increase the risk of gout could in the future be used in targeting high-risk patients for treatment, without the need for further attacks to occur. At present, however, the relative contribution of the different genes to uric acid variability has been estimated at between 0.12 and 3.53% (Table 2) and so it remains to be seen whether genetic screening would prove clinically valuable.

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**REFERENCES**

cases and a review of 196 previously reported cases. Mutat. Res., 463, 309–326.


