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A 'complexity' of urate transporters

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Genetic variation in the *SLC2A9* gene is a new genetic risk factor for low fractional excretion of uric acid, hyperuricemia, and gout. Its gene product, GLUT9, was previously known as a type II glucose/fructose transporter but is now known to function as a high-capacity uric acid transporter that is expressed in kidney, liver, and several other tissues. Follow-up meta-analyses, including one with data from 28,141 individuals, implicated a total of nine additional loci influencing serum urate concentrations, including six other membrane transporters (*SLC17A1*, *SLC17A3*, *SLC22A11*, *SLC22A12*, *SLC16A9*, and *ABCG2*). Variants in these genes together account for about 5% of the variance in serum urate, two-thirds of which is due to *SLC2A9*. Using these variants in 'Mendelian randomization' analyses provides a powerful means of dissecting the role of urate in cardiovascular and metabolic diseases, where cause-and-effect influences are difficult to discern due to potential confounding. The results highlight the complex interplay of membrane transporters involved in urate metabolism. They also show how variants of weak effect identified by genome-wide association studies can still be important in identifying novel pathways, including a 'complexity' of new and potentially druggable targets for modifying urate transport.

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Uric acid is the end product of purine metabolism in humans, higher primates, and only a small number of other species.[1] In other (ureotelic) mammals, urate is metabolized by urate oxidase (uricase) to the more soluble product allantoin, which is readily excreted in the urine. This is not the case in lineages leading to the lesser apes and great apes/humans, respectively, because of two independent mutations in the uricase gene occurring 10–20 million years ago, suggesting adaptive evolution, although the proposed adaptive advantage remains unclear.[2,3] Species lacking uricase have up to 10-fold higher serum urate levels compared with other mammals.

Uric acid behaves as a weak acid (pK_{a1} 5.75) and occurs predominantly (98%) as urate anion at physiological pH, although more is in the uric acid form in urine at pH ~5–6, which affects its solubility and transport.[4] Urate anion cannot readily pass through cell membranes in the absence of transporters. The water solubility of monosodium urate is limited (405 μmol/l) so that urate often exists in plasma at concentrations close to or above this limit, in a state of supersaturation, when there is an increasing risk of crystal deposition. Uric acid is substantially less soluble than the anion in urine at pH 5.

GENOME-WIDE ASSOCIATION STUDIES REVEAL NEW URATE TRANSPORTERS

Body urate levels reflect the balance of formation and loss. Urate is formed both from dietary purines (for example, seafood, meat) and by the metabolism of endogenously synthesized purines. The major site of urate formation is the liver, where it is formed from xanthine by the enzyme xanthine oxidoreductase, which, in its oxidase form, can form superoxide anion and hydrogen peroxide as by-products. In the presence of transition metals, these can form damaging hydroxyl ions, or in the presence of nitric oxide, peroxynitrite. Xanthine oxidase is abundant in liver and small intestine but is present in lower amounts in endothelial cells, brain, kidney, and several other cell types. Urate is excreted by proximal renal tubules (two-thirds of daily turnover) and by liver hepatocytes into the bile canaliculi and gastrointestinal tract (one-third of daily turnover). Almost all serum urate is initially filtered by the renal glomeruli but about 90% is reabsorbed into the blood. In humans, this is achieved by proximal renal tubule

transporters, such as URAT1 (encoded by the *SLC22A12* gene), a member of the major facilitator superfamily.[5] The major facilitator superfamily are secondary carriers (facilitated transport) capable of carrying small molecule solutes down chemiosmotic ion gradients. URAT1 exchanges intracellular anions for filtered urate and is expressed on the apical surface of the proximal renal tubules—facing the lumen—whereas the basolateral transporter carrying urate through to the interstitial fluid and blood was until recently unknown.

Last year, four groups independently identified one or more variants in the *SLC2A9* gene that exerts a major influence on serum urate concentrations following genome-wide association studies (GWAS).[6–9] *SLC2A9* was known to encode GLUT9, a class II glucose/fructose transporter,[10] and common variants were found to be associated with 5–6% of the variance in serum urate in females and 1–2% in males. The identified variants were also associated with gout and low-fractional excretion of uric acid.[8,9,11] Surprisingly, GLUT9 was then shown to be a high-capacity/low-affinity ($K_m \sim 1$ mmol/l) urate transporter using *Xenopus* oocytes.[8] This has since been confirmed by four other groups.[11–14] The urate transport activity of GLUT9 is 45- to 60-fold faster than glucose/fructose transport.[11] In one study, in which the membrane potential was carefully controlled, GLUT9-mediated urate transport was shown to be electrogenic—resulting in charge imbalance across the cell membrane—and strongly voltage dependent,[14] suggesting that urate is transported with a net negative charge into the cell. Large changes in the transmembrane gradients of either Na^+ or Cl^- did not influence GLUT9-mediated urate transport rate and reports of trans-stimulation by glucose or fructose were contradictory,[11,12,14,15] so it is unclear whether trans-stimulation is secondary to membrane potential changes. The simplest model that fits the current data is that GLUT9 functions as a urate anion uniporter, but it may also show a parallel, weakly coupled exchange transporter function.[14] A GLUT9 urate uniporter is now widely believed to represent the missing basolateral membrane exit pathway in proximal renal tubules, which would be facilitated by, rather than working against, the cell's negative membrane potential. In contrast, the apical uptake of urate from the tubular lumen occurs through a nonelectrogenic mechanism using the urate-anion exchanger URAT1.[5] However, analysis of GLUT9 isoforms suggests a more complex picture.

GLUT9 has two isoforms, both of which transport urate in oocytes and are expressed in proximal renal tubular cells.[8,11,13,16] Isoform 1 (GLUT9) is most strongly expressed in basolateral membranes of proximal renal tubular cells, as well as in liver and pancreas, whereas isoform 2 (GLUT9ΔN) is most strongly expressed in apical membranes of proximal renal tubules and placenta.[16] This begs the question as to the function of a potentially electrogenic apical urate transporter. Current models of renal urate transport in humans involve free filtration by glomeruli, most of which is reabsorbed by proximal convoluted tubules, a proportion of which is secreted back into the urine, so that about 10% of filtered urate is finally excreted.[17] The GLUT9ΔN isoform may therefore have a role in apical secretion (down the electrical gradient), perhaps in exchange for glucose[15] or in facilitating urate uptake from the lumen (down the concentration gradient but against the electrical gradient), perhaps indirectly coupled to the secretion of an anion.[8] GLUT9 is also expressed in chondrocytes from articular cartilage, raising interesting questions about its role in gout. GLUT9-mediated urate transport in oocytes shows limited sensitivity to conventional uricosuric drugs, such as benzbromarone, probenecid, or losartan,[8,11,12] but is sensitive to the glucose-transport inhibitor phloretin.[14]

Until recently, the transport of urate in and out of the liver was poorly understood. Dogs express uricase but Dalmatians were recently shown to have a mutation in *SLC2A9*, associated with raised plasma urate and increased urinary excretion of urate.[18] The raised serum urate appears to result from failure of GLUT9 to transport urate from the sinusoidal blood vessels into the basolateral surface of liver hepatocytes for destruction by uricase, whereas renal reuptake of filtered urate is also blocked (perhaps at the basolateral transporter step in proximal tubules), resulting in excessive urinary excretion and recurrent urate stone formation. It is likely that urate transport into human liver, where GLUT9 is strongly expressed, is also mediated by this transporter. A complete *Slc2a9* knockout mouse was also recently reported to show increased blood and urinary urate.[19] This was again attributed to impaired urate transport into hepatocytes and destruction by uricase, as well as severely impaired renal urate reabsorption in the distal convoluted tubules, where mouse Slc2a9 was mainly located, in contrast to the human kidney. An early-onset urate nephropathy was also present, with tubulointerstitial inflammation and fibrosis secondary to uric acid crystal deposition and severe hyperuricosuria.

A follow-up meta-analysis of serum urate GWAS of 11,847 normal individuals from the Framingham and Rotterdam cohorts, revealed two loci in addition to *SLC2A9* that are associated with both serum urate and gout.[20] These loci explained 1.3% (*ABCG2*) and 0.7% (*SLC17A3*) of the variance in serum urate levels compared with 5.3% for *SLC2A9* in the Framingham cohort. *ABCG2* encodes an ATP binding cassette transporter, expressed in the apical membranes of several tissues, including liver and human proximal renal tubular cells, where it transports a wide variety of substrates, including urate.[21] Recently, *ABCG2* has been shown to encode a high-capacity urate secretion transporter.[21,22] Several loss-of-function *ABCG2* variants have also been identified that confer an increased risk of gout, including a common missense mutation (Q141K) within the nucleotide binding domain (next to the equivalent phenylalanine residue commonly mutated in cystic fibrosis), which was shown to reduce urate efflux in *Xenopus* oocytes by 53%.[21] This variant appears to be the causal *ABCG2* variant associated with raised serum urate and gout in GWAS meta-analyses.[20,23] It confers an adjusted odds ratio of