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Renal Transport of Uric Acid: Evolving Concepts and Uncertainties

Ion Alexandru Bobulescu and **Orson W. Moe**
Departments of Internal Medicine and Physiology and the Charles and Jane Pak Center for Mineral Metabolism and Clinical Research, University of Texas Southwestern Medical Center, Dallas, TX

Abstract

In addition to its role as a metabolic waste product, uric acid has been proposed to be an important molecule with multiple functions in human physiology and pathophysiology and may be linked to human diseases beyond nephrolithiasis and gout. Uric acid homeostasis is determined by the balance between production, intestinal secretion, and renal excretion. The kidney is an important regulator of circulating uric acid levels, by reabsorbing around 90% of filtered urate, while being responsible for 60–70% of total body uric acid excretion. Defective renal handling of urate is a frequent pathophysiologic factor underpinning hyperuricemia and gout. In spite of tremendous advances over the past decade, the molecular mechanisms of renal urate transport are still incompletely understood. Many transport proteins are candidate participants in urate handling, with URAT1 and GLUT9 being the best characterized to date. Understanding these transporters is increasingly important for the practicing clinician as new research unveils their physiology, importance in drug action, and genetic association with uric acid levels in human populations. The future may see the introduction of new drugs that specifically act on individual renal urate transporters for the treatment of hyperuricemia and gout.

Keywords

Urate; hypouricemia; hyperuricemia; URAT1; GLUT9

I. Introduction

Although gout and kidney stones were recognized in antiquity (1), the earliest known description of uric acid dates from the year of the American Declaration of Independence, when German-Swedish chemist Karl Wilhelm Scheele (1742–1786) isolated a substance with acidic properties from a bladder stone, and named it ‘lithic acid’ (from Greek ‘lithos’, stone) (2). George Pearson (1751–1828) and Antoine Fourcroy (1755–1809) later changed

Address correspondence to: I. Alexandru Bobulescu, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX, 75390-8856, Tel: 214-648-0240 | Fax: 214-648-2526, Alexandru.Bobulescu@UTSouthwestern.edu.

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the name from 'lithic' to 'uric', to reflect the presence of this substance in normal urine and its absence from some calculi (3). Fast forward more than 230 years, and our current knowledge of uric acid and its relationship with living organisms, from single genes and molecules to physiology and pathophysiology, could fill a massive treatise. However, several chapters of the uric acid story remain incomplete, with fundamental scientific questions still awaiting response.

We will cover two aspects in this article. First, we provide a summary of the known and potential roles of uric acid in human physiology and pathophysiology, on the backdrop of evolutionary physiology. Second, we discuss the mechanisms of uric acid transport in the kidney, the major regulator of uric acid levels in humans.

II. Uric acid in human biology: The basics

Uric acid (2,6,8-trihydroxypurine, $C_5H_4N_4O_3$) is the end-product of purine metabolism in humans, but it is an intermediary product in most other mammals. It is generated primarily in the liver (Figure 1) by the action of xanthine oxidase, a molybdenum metalloenzyme that can be inhibited pharmacologically by drugs like allopurinol and febuxostat (4, 5). Very little uric acid is normally ingested. Most circulating uric acid is freely filtered in the kidney, with roughly 90% of the filtered load normally reabsorbed along the nephron by mechanisms reviewed later in this article. Renal excretion of uric acid represents approximately 60–70% of total uric acid excretion from the body (6, 7). A smaller proportion of uric acid is secreted in the intestine, and is further metabolized by resident gut bacteria in a process termed intestinal uricolysis (7).

Uric acid is a weak diprotic acid (has two dissociable protons) with $pKa_1 \approx 5.4$ and $pKa_2 \approx 10.3$. At the physiologic pH of 7.4, a proton dissociates from ~99% of uric acid molecules, and thus most uric acid is present in the extracellular fluid as monovalent urate anion (also known as hydrogen urate or acid urate). The divalent urate anion is practically non-existent in the body, because of the very high pKa_2, and thus the term urate is generally used to refer to monovalent urate in the biomedical literature. As the ratio of urate to uric acid in the circulation remains constant with constant pH, the terms urate and uric acid are often used interchangeably to refer to the total pool of uric acid, dissociated and undissociated. In the urine, the ratio of uric acid to urate varies much more with the larger range of pH, and lower urinary pH values result in a greater proportion of uric acid in the undissociated form. Since undissociated uric acid is very poorly soluble in aqueous solutions, unduly acidic urinary pH values increase the propensity for uric acid crystallization and nephrolithiasis (8).

III. Uric acid: friend or foe?

In many organisms, including the majority of mammals, uric acid is metabolized to allantoin by the enzyme urate oxidase (uricase). Over a period of 20–30 million years during the evolution of primates, the uricase gene incurred several independent mutations in its promoter and coding region, resulting in gradual loss of uricase function in the primate lineage. Modern humans and higher primates have non-functional uricase genes (pseudogenes) because of frameshift and missense mutations, and some New World and Old World monkeys harboring promoter mutations have decreased uricase activity compared with other mammals (9).

Although not the main scope of this review, a brief exploration of the biological underpinnings of uricase inactivation is in fact important for understanding the roles of uric acid in *Homo sapiens*, and, by extension, for understanding the logic of urate handling in the kidney.

Physiological roles of uric acid: Clues from evolution

Experimental inactivation of the uricase gene in mice leads to massive deposition of uric acid crystals in the kidney, obstructive nephropathy and death, with most animals succumbing before sexual maturity (10). By contrast, when nature experimented with loss of uricase function in early hominoids, its result were evolutionarily successful organisms. It is possible that uricase loss in our ancestors was perpetuated because higher uric acid levels provided some physiological benefit. However, since higher uric acid can also cause harm in an organism not equipped to handle it (e.g. the uricase deficient mouse), it is also likely that, with uricase loss, other functionally related genes changed to accommodate the increased levels of uric acid. The identity of these 'other' genes is unknown, and future research leading to their identification would be especially edifying.

The fact that multiple independent mutations occurred in the uricase gene during hominoid evolution, with parallel mutations in some New World and Old World monkeys, is compatible with (although not proof of) the hypothesis that uricase inactivation provided some benefit (11). Another argument frequently invoked in support of this hypothesis is the ability of the human kidney to return a large proportion of the filtered urate to the circulation. It is tempting to assume that if uric acid was a metabolic waste product with no physiological value, the kidney would not invest resources in the reabsorption of ~90% of the filtered urate. In fact, one of the great pioneers of renal physiology, the late Robert Berliner (12), specifically commented that it "makes no sense" for the human kidney to reclaim uric acid (13). However, renal reabsorption of a substance does not invariably mean that the substance is needed for the whole organism, as can be exemplified by the case of urea which is only retained to facilitate water conservation before its eventual fate of excretion. It is unlikely that renal handling of urate evolved according to this simple logic, and the intricate pathways for urate transport in the kidney may have evolved for other, more complex reasons (for example, to prevent crystal deposition and protect against kidney disease).

If uric acid does indeed have biological function(s) that made elevated serum urate levels evolutionarily advantageous, what could these functions be? Several hypotheses can be formulated, combining scientific speculation with varying degrees of factual evidence. A comprehensive review of these hypotheses and of all the evidence for and against them is beyond the scope of this article, but a brief summary is provided in the following paragraphs.

Uric acid as metabolic waste

Uric acid has long been viewed as an inert metabolic product, with the sole mission (in humans and higher primates) of shuttling purine waste to the exterior world. This role is undoubtedly important, but could it have been, in itself, a determinant of evolutionary benefit? Organisms that excrete nitrogen primarily as uric acid (uricotelics), such as birds and some terrestrial reptiles, are excellent water conservers (14, 15), because they excrete some or all uric acid as crystals (the white color in bird droppings). In birds and reptiles, this is in part possible because the ureters empty directly into the cloaca, allowing further water reabsorption and uric acid precipitation without retention of calculi. Although urea remains the primary means of nitrogen excretion in hominoids, one could speculate that uricase inactivation, by increasing the proportion of nitrogen excreted as uric acid, may have rendered our ancestors more adept at conserving water (likely an important trait in the hot climate of Africa 30 million years ago). However, this scenario is extremely unlikely, because only uric acid excreted in solid form (not dissolved in the urine) is meaningful for water conservation. Humans and primates do not normally discharge large amounts of uric acid crystals in the urine, and even though there are three nitrogens per urate molecule (as

opposed to two for urea), the amount excreted per day is only a few millimoles, a small fraction of total nitrogen excretion.

Another hypothesis related to the role of uric acid as metabolic waste is that uricase inactivation led to increased disposal of endogenously produced uric acid in the gut, and possibly to changes in the intestinal microbiota, with a higher prevalence of bacteria capable of uricolysis. In turn, these bacteria could have had other advantageous effects on digestion, metabolism, or the immune system. This is highly speculative, not covered to our knowledge by previous publications in the field, but is nevertheless plausible and merits further exploration.

The Jekyll and Hyde of antioxidation

More than 30 years ago, Ames *et al.* (16) hypothesized that higher serum uric acid levels might have been beneficial during hominoid evolution because of the antioxidant properties of uric acid. Loss of L-gulonolactone oxidase, the enzyme responsible for ascorbic acid (vitamin C) synthesis, preceded the loss of uricase during primate evolution, and may have raised the selection pressure for augmentation of an already existing, alternative antioxidant system. Although the antioxidant capacity of uric acid is much smaller than that of vitamin C, (17) uric acid could potentially compensate by its much higher concentration in the extracellular fluid compartment. In addition, uric acid is more effective than vitamin C at neutralizing peroxynitrite, an important oxidant produced from the reaction between nitric oxide and hydrogen peroxide (18).

Whether this was sufficient to confer an evolutionary advantage for uricase inactivation is not known. What is known, however, is that higher uric acid levels in the modern human are epidemiologically correlated with conditions that are, in turn, associated with increased oxidative stress, such as atherosclerosis, obesity, diabetes, and the metabolic syndrome (19). This could be interpreted as an adaptive response, with more uric acid retained in the circulation in an attempt to offset disease-associated oxidative stress. However, it is equally compatible with the opposite conjecture that uric acid contributes to the pathogenesis of these conditions. Paradoxically, one of the ways in which uric acid has been proposed to contribute to disease is via its conditional pro-oxidant effect. Like most antioxidants, uric acid is in fact a redox agent, capable of both anti-oxidation and pro-oxidation (20, 21). The balance between the two is dictated by a very complex interplay of factors, including concentration of uric acid, the nature and concentration of free radicals, the presence and concentration of other antioxidant mechanisms, and others. It is becoming increasingly clear that uric acid may be anti-oxidant in certain conditions, and pro-oxidant in others.

It is important to note that among all theories related to uric acid, the antioxidant hypothesis is most often presented in the literature as established fact, although the value of uric acid as antioxidant in humans, *in vivo*, is far from proven (22).

Uric acid and blood pressure: good then, bad now?

Johnson and colleagues proposed what is arguably the most intriguing theory for the role of uric acid in human evolution, (11, 23) based on the premise that sodium intake has been low for millions of years in our species' history. According to this theory, the antinatriuretic and vascular effects of elevated uric acid contributed to blood pressure maintenance, during a time when climatic changes forced early hominoids to better conserve sodium. At the time, uricase inactivation was thus advantageous. The advent of increased salt availability and intake over the past 10,000 years, with the most dramatic increases in intake seen during the past century, transformed this system of sodium conservation into an essentially maladaptive trait, postulated to contribute to the modern epidemic of hypertension.

Whether loss of uricase function in the Miocene was indeed related to blood pressure maintenance ("the good") remains speculative, but current evidence, albeit limited, is compatible with a role for uric acid in modern human hypertension ("the bad"). Available data include epidemiological correlations between blood pressure and circulating uric acid, and animal studies in which uric acid levels were manipulated experimentally to explore causality. It should be noted, however, that rodents have functional uricase and are ill equipped to handle hyperuricemia, making them less-than-adequate models for the study of any uric acid related condition.

The most compelling evidence to date for a causal role of uric acid in hypertension comes from one pilot trial of 30 adolescents (11–17 years) with newly diagnosed stage 1 primary hypertension and borderline hyperuricemia (≥360 μmol/L), who were treated with allopurinol and placebo (4 weeks each) in a randomized double-blinded crossover design (24). Adolescents were selected for this study because the potential link between uric acid and hypertension is likely "cleaner" in this age group, while it may be plagued by multiple confounders in older individuals with longer-standing hypertension and associated comorbidities. The fact that blood pressure was decreased by allopurinol in this trial provides an encouraging proof of principle. However, the study had a number of limitations (well detailed by the authors), and does not permit generalization in the absence of further research.

Uric acid and the immune system

It is well known that uric acid crystal deposition in gout causes inflammation, but this has long been considered a non-specific effect. More recent evidence proposed that uric acid is released from injured somatic cells as monosodium urate crystals (MSU), and functions as an innate immunity enhancer or "danger signal", by stimulating the maturation of dendritic cells and augmenting the response of CD8+ T cells to a co-delivered antigen *in vivo* in mice. (25) These findings were specific for MSU, and the effect was not seen when MSU was replaced with other crystals with similar physical properties. Crystalline MSU, but not other crystals, has also been shown to activate the inflammasome, a multiprotein complex that participates in innate immunity and in the initiation of inflammation (26).

Although these findings are extremely provocative, their importance for human biology has not been established, and whether MSU-mediated effects on innate immunity and inflammation differ in species with or without functional uricase is unknown. One could speculate that uric acid, as an enhancer of innate immunity, is beneficial for the organism. It is also possible that some of the detrimental effects of hyperuricemia, beyond gout, are caused by elevated levels of MSU and its effects on inflammatory and immune responses.

Association between hyperuricemia and human disease

Humans have adapted to circulating uric acid levels that are 5 to 20 fold higher than in most other mammals. The definition of hyperuricemia in adults is not universally agreed upon, but commonly used thresholds are in the range of >6–7 mg/dL (>350–400 μM/L), usually higher for men than for women. Hyperuricemia thresholds are based either on the solubility limit of urate in the extracellular fluid compartment at physiologic pH (~420 μM/L), or on distribution curves in normal individuals using classical two standard deviations above the mean, or in some cases are arbitrarily set based on relative risk of uric acid-associated disease. It is important to note however that while cutoffs are convenient for clinical practice, defining hyperuricemia (or sometimes "mild" hyperuricemia) based on precise values has no biological rationale, because one cannot treat a continuous variable such as uric acid as dichotomous.

Urate levels rise above commonly accepted thresholds in conditions of excessive purine intake, endogenous defects in purine metabolism, and/or inadequate uric acid excretion. In turn, persistent clinical hyperuricemia can cause gout and tophi, and is associated with kidney stones. Beyond these classic clinical manifestations, and irrespective of their presence, various degrees of hyperuricemia have also been associated with hypertension (as discussed above), preeclampsia, obesity, the metabolic syndrome, and chronic kidney disease (including but not limited to chronic uric acid nephropathy). However, as always in biology, correlation should not be mistaken for causation. Evidence for causation in all these cases is relatively scarce, and certainly inconclusive.

IV. Renal handling of urate: General Concepts

One unifying element emerges from all the controversies surrounding the roles of uric acid in human biology: whether friend or foe, uric acid is likely much more than a waste product of purine metabolism. Uric acid homeostasis is tightly controlled, with the kidney assuming a pivotal role (Figure 1).

Fractional excretion of urate in humans

Uric acid excretion rate is the product of the filtered load (which can be approximated as plasma ultrafilterable urate x glomerular filtration rate) and fractional excretion of urate (FEUA), which represents the percentage of filtered urate that is excreted in the final urine. FEUA can be estimated from the ratio of urate clearance (C_{UA}) to creatinine clearance (C_{Cr}), calculated using plasma (P) and urinary (U) creatinine and urate values obtained from simultaneous spot urine and blood samples: $FEUA = C_{UA}/C_{Cr} \times 100\% = [(U_{UA} \times \text{Urine sample volume})/P_{UA}]/[(U_{Cr} \times \text{Urine sample volume})/P_{Cr}] \times 100\% = (U_{UA} \times P_{Cr})/(P_{UA} \times U_{Cr}) \times 100\%$. In adult humans under normal conditions FEUA is approximately 10% (range 7–12%); usually higher in women than in men. FEUA is higher in children, averaging 35% in newborns, 13–26% in children less than 1 year old, and then decreasing progressively to adult levels in spite of increasing urate filtered load (27, 28). This fall in FEUA is indicative of maturational changes in renal urate transport, likely involving complex molecular mechanisms (29) that are beyond the scope of this article (see the accompanying article in this journal, “Changes in Serum Urate and Urate Excretion with Age” by B. Stiburkova. Further discussion of urate transport herein refers only to the adult kidney.

Renal handling of urate in other organisms

As with most other biological processes, there are similarities and differences between the physiology of renal urate handling in humans, other mammals, and non-mammalian organisms. Teleologically, the differences can be attributed to two major factors: first, inactivation of uricase in humans and higher primates led to uric acid levels (and thus filtered loads) up to two orders of magnitude higher than in other mammals; second, while humans and other mammals primarily use urea as vehicle for nitrogen excretion (a mode of excretion termed ureotelism), other species such as birds and snakes use primarily uric acid (uricotelism), relying on a combination of high filtered load and FEUA > 100% (net secretion). Consequently, there are wide variations in both filtered load and FEUA among vertebrates, with only higher primates being similar to humans in both respects. This is one of the most serious limitations faced by uric acid research in mice and rats, the most widely used species for laboratory research: even if uric acid levels are manipulated genetically (e.g. uricase knockout) or pharmacologically to more closely resemble the human situation, the rodent kidney is ill equipped to handle such elevated filtered loads (10). Furthermore, although serum uric acid is consistently lower in mice and rats compared with humans (in the absence of genetic or pharmacologic manipulation), there are considerable variations in both serum uric acid and FEUA between different rodent strains (30, 31), indicative of strain

differences in renal urate handling. While studies of urate handling in rodents are certainly key for understanding the functions of individual genes and proteins, the relevance of these studies for human biology has to be interpreted with great caution, and rodent findings should not be directly extrapolated without some form of validation in humans.

The old model

The classic (now obsolete) textbook model of uric acid transport in the human kidney was developed in the 1970s, based primarily on animal studies using microperfusion and micropuncture and on human studies measuring fractional extraction of urate (FEUA), in physiologic conditions and under treatment with drugs that change FEUA without significantly affecting glomerular filtration rate (GFR). This model (Figure 2A) involved four distinct components: glomerular filtration of virtually all circulating urate, reabsorption of 98–100% of the filtered urate, secretion of up to half of this amount (inhibitable by antiuricosuric agents such as pyrazinamide), and postsecretory reabsorption (inhibitable by uricosuric agents such as probenecid). Key for components 3 and 4 of this hypothesis (Figure 2A) was the fact that concomitant treatment with pyrazinamide and probenecid had a similar effect on FEUA as treatment with pyrazinamide alone, which was compatible with (but did not prove) the hypothesis that inhibition of urate secretion by pyrazinamide left too little urate in the lumen at the putative site of postsecretory reabsorption (32, 33). However, this assumption did not consider the possibility that pyrazinamide may not inhibit urate secretion, but rather stimulate reabsorption, as suggested by subsequent studies (34, 35). The classic model also assumed that urate reabsorption and secretion occur in functionally distinct regions of the nephron, while current data strongly suggest that both reabsorption and secretion can occur at the same site in the proximal tubule (36). The actual experiments behind the model depicted in Figure 2A likely remain valid today, but the growing body of data in the field no longer supports their original interpretation, despite the occasional perpetuation of the old model in the recent literature. A revised (albeit oversimplified and incomplete) model that more accurately reflects current knowledge is shown in Figure 2B. We now know that renal urate handling involves a complex interplay of reabsorptive and secretory transport pathways, primarily in the renal proximal tubule, mediated by incompletely understood molecular mechanisms.

V. Renal handling of urate: Molecular Mechanisms

A number of integral plasma membrane transport proteins are candidate participants in renal urate handling. These are depicted schematically in Figure 3, and their more important characteristics and available experimental evidence are summarized in Table 1. For the purpose of this review, the varying levels of evidence that these transporters play a role in urate handling in the human kidney are rated in Table 1 as **weak** (mostly *in vitro* experiments, indirect evidence in animals, no evidence in humans), **moderate** (*in vitro* experiments supported by human genome-wide association studies or knockout mouse data), or **strong** (extensive human data, including genome-wide association studies, loss-of-function mutations and protein localization in the human kidney, in addition to *in vitro* and knockout mouse experiments). It is important to note that weak and moderate evidence levels may be strengthened in the future, with acquisition of new data. Conversely, current evidence levels may be downgraded, and some candidates may even be taken off the list if contradictory data emerges. Also of note, a number of other endogenous organic anions and xenobiotics (e.g. drugs), in addition to urate, serve as substrates for various urate transporters *in vitro*, but the renal handling of these alternative substrates is beyond the scope of this article. Finally, urate reabsorption overall is postulated to be a tertiary active transport process dependent on sodium reabsorption, whereby the basolateral Na^+/K^+ ATPase generates a Na^+ gradient (primary active transport) that drives a number of apical

Na^+-coupled organic anion transporters (secondary active transport), that in turn provide the driving force for urate reabsorption (Figure 3).

The following paragraphs provide more details about the two transporters whose roles in renal urate handling are currently supported by the strongest evidence. The other transporters listed in Table 1 (and shown in Figure 3), are discussed in further detail in the Appendix. For additional perspectives, the interested reader is also referred to several excellent, recent reviews (36–38).

URAT1

Until the early 2000s, very little was known about the molecular mechanisms of renal urate transport. Aided by the publication of the first draft sequence of the human genome in 2001, Enomoto *et al.* (35) searched for genes similar to known members of the organic anion transporter (OAT) family and identified a previously uncharacterized sequence on chromosome 11, now known as solute carrier family 22, member 12 (SLC22A12), or urate transporter 1 (URAT1). When URAT1 was heterologously expressed in *Xenopus* oocytes (a commonly used method to study the properties of transport proteins) it resulted in significantly increased uptake of [^{14}C] urate into oocytes, in exchange for organic monocarboxylate anions such as lactate and nicotinate, with an estimated Michaelis constant (K_m) for urate of around 370 μM (relatively low affinity). URAT1 messenger RNA is highly expressed in the human kidney, but is virtually absent from all other organs examined, including the liver, small intestine and colon. The URAT1 protein specifically localizes to the brush border membrane of the proximal tubule, indicating that it participates in the apical (luminal) uptake of urate from the primary urine to the proximal tubule cell.

The most convincing evidence that URAT1 plays a key role in renal urate handling in humans came from the identification of URAT1 inactivating mutations in homozygous or compound heterozygous state in a number of Japanese patients with idiopathic renal hypouricemia (serum urate 30–60 μM/L or 0.5–1.0 mg/dL) and dramatically increased FEUA (30–90%, from approximately 10% normal) (35, 39–41). In addition to monogenic hypouricemia, sequence variations in the *SLC22A12* gene were associated with serum uric acid levels in genome-wide association studies (studies that examine the statistical association between genetic variants – typically single nucleotide polymorphisms – spread across the entire genome and a specific trait in large populations), including in African Americans, Pacific Islanders and individuals of European descent (42–44). *Taken together, these data strongly suggest that URAT1 is the major luminal pathway for urate reabsorption in humans.* However, given the preservation of some urate reabsorption (indicated by FEUA < 100%) even in patients with complete loss of function of URAT1, other apical entry pathways also exist.

The mouse renal-specific transporter (RST) first identified in 1997 (45) is the murine ortholog of URAT1 (46), with a similar expression profile and properties, albeit with lower affinity for urate ($K_m \approx 1200$ μM). RST knockout mice are grossly normal and only have slightly increased FEUA (from ~3% in wild type to ~5% in knockout mice), nowhere near the increase seen in humans with *SLC22A12* inactivation (47). Once again, this underscores the differences between mouse and human urate handling, and indicates that another yet to be identified transporter handles the bulk of apical urate reabsorption in the mouse nephron.

GLUT9

Cloned in 2000 by Phay *et al.* (52), GLUT9 (*SLC2A9* gene) was classified as a member of the facilitative glucose transporter family, with the ability to transport both glucose and fructose. Human GLUT9 has two splice variants with different expression patterns:

GLUT9a is expressed in multiple tissues, while GLUT9b (also termed GLUT9ΔN) is highly expressed in the kidney, and to a lesser extent in the liver (53). In polarized epithelial cells *in vitro*, GLUT9a was specifically targeted to the basolateral membrane, and GLUT9b to the apical membrane. However, only the basolateral localization was detected by immunohistochemistry in the human proximal tubule, using an antibody that does not distinguish between the two isoforms, so the polarized distribution of the two splice variants is not confirmed *in vivo* (53).

The possible involvement of GLUT9 in urate handling was first suggested in 2007 by a genome-wide association study (GWAS) of serum uric acid levels in two cohorts in Sardinia (54). Subsequent GWAS in other populations confirmed this finding (42, 55–58), with single nucleotide polymorphisms in the GLUT9 gene exhibiting the strongest associations with serum uric acid levels.

A number of allelic variants of the GLUT9 gene, either predicted or demonstrated to lead to loss of transport function, were identified in humans with renal hypouricemia (59–63). Homozygous loss-of-function was described in patients with massive hyperuricosuria, hypouricemia (<12 μM/L or <0.2 mg/dL), FEUA exceeding in some cases 100% (indicative of net urate secretion), and high propensity for nephrolithiasis and exercise-induced acute kidney injury (62, 63). The pathogenesis of exercise-induced acute kidney injury remains unclear, although acute crystal-induced nephropathy has been proposed (but not proven). Interestingly, heterozygous loss-of-function mutations were also identified in patients with less severe hypouricemia (90–160 μM/L or 1.5–2.7 mg/dL), in the absence of mutations in other urate transport candidates (59–61), suggesting that inactivation of only one GLUT9.allele may be sufficient to impair urate reabsorption.

When heterologously expressed in *Xenopus* oocytes, human GLUT9 functions as a urate uniporter, with a K_m for urate around 365 μM (very similar to URAT1). The two isoforms of mouse Glut9, homologous to human GLUT9a and GLUT9b, have transport properties virtually indistinguishable from each other, but have lower affinity for urate, around 600 μM (64). In contrast with humans, mouse Glut9 is expressed in the distal tubule, on both the apical and the basolateral membrane (65). Once again, this highlights the differences in physiology between rodent and human urate handling. At present, it is unknown whether there is urate handling in the distal nephron in rodents, or whether Glut9 functions as a transporter for some other ion in the distal tubule. Whole-body Glut9-null mice have moderate hyperuricemia and hyperuricosuria, much more severe that RST-null animals, resulting in obstructive nephrolithiasis, tubulointerstitial inflammation, fibrosis, and eventually renal failure (31). Hyperuricemia is attributable to the putative role of Glut9 as facilitator of urate entry in in hepatocytes, the major site for uricase-mediated urate breakdown in non-primate mammals. When Glut9 was specifically deleted in the liver, mice developed more severe hyperuricemia and their kidneys remained morphologically normal, most likely because preserved renal Glut9 function in these mice resulted in lower FEUA (31). These findings indicate that in spite of significant differences in expression patterns and transport affinity, GLUT9 is a key player in renal urate handling in both humans and mice.

Taken together, available evidence suggest that GLUT9 may be the principal (and possibly the only) pathway of basolateral urate exit from the proximal tubule cell in the human kidney, with URAT1 and GLUT9 functioning in concert to achieve transcellular urate transport.

VI. URAT1 and GLUT9 as drug targets

As discussed in the previous section, there is strong evidence that apical URAT1 and basolateral GLUT9 are the main renal transporters involved in urate reabsorption. Several existing drugs that affect renal urate handling have been shown to directly regulate the activity of one or both of these transporters, and new drugs targeting them are currently in development for the treatment of hyperuricemia and gout.

Urate transport by URAT1 *in vitro* is significantly decreased (~80–95% inhibition) by uricosuric agents, including probenecid and benzbromarone, and is increased (~25% stimulation) by the antiuricosuric pyrazinamide, suggesting that at least part of the mechanism of action of these drugs is direct regulation of URAT1 (35). Other drugs known clinically to have uricosuric effects also inhibit URAT1 *in vitro*, including losartan (~85% inhibition), and furosemide (~40% inhibition) (35).

Some of these drugs also regulate the *in vitro* urate transport activity of GLUT9, including probenecid, benzbromarone and losartan (55–70% inhibition), but not pyrazinamide and furosemide (59). Several specific URAT1 inhibitors are in different stages of development, with at least one (Lesinurad) currently in phase 3 clinical trials in patients with gout (ClinicalTrials.gov identifiers NCT01493531, NCT01510158, NCT01510769, NCT01508702).

In addition to its role in the kidney, GLUT9 is the only urate transporter described to date in chondrocytes, where its expression is upregulated by the inflammatory cytokine Interleukin 1 beta (99). Although the exact role of GLUT9 in chondrocytes remains to be established, a potential GLUT9 inhibitor could theoretically increase FEUA and at the same time reduce joint damage in gout.

VII. Conclusion

The past decade has seen enormous advances in our understanding of renal urate handling, including the dethroning of a long-held model of sequential urate transport and the identification of individual transport proteins with direct bearing on uric acid levels in humans. However, much is still unknown, and further research is critical for the elucidation of the exact function of these proteins from whole organism to cellular and molecular level, in both physiology and pathophysiology. Human studies remain critical for the field, because of the major differences in renal urate handling between humans and all commonly used laboratory animals. These efforts are likely to have a direct impact on patient care in the future, by endowing physicians with better understanding of pathophysiology, particularly of renal hyperuricemia, and better tools to treat urate disturbances.

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References

1. Nuki G, Simkin PA. A concise history of gout and hyperuricemia and their treatment. Arthritis Res Ther. 2006; 8(Suppl 1):S1.10.1186/ar1906 [PubMed: 16820040]
2. Richet G. The chemistry of urinary stones around 1800: a first in clinical chemistry. Kidney Int. 1995; 48(3):876–886. [PubMed: 7474678]

3. Thomson, T. Chemistry of Animal Bodies. Edinburgh: 1858. p. 31(digitized version available at http://books.google.com)
4. Brondino CD, Romao MJ, Moura I, Moura JJ. Molybdenum and tungsten enzymes: the xanthine oxidase family. Curr Opin Chem Biol. 2006; 10(2):109–114. [PubMed: 16480912]
5. Schlesinger N. New agents for the treatment of gout and hyperuricemia: febuxostat, puricase, and beyond. Curr Rheumatol Rep. 2010; 12(2):130–134. [PubMed: 20425022]
6. Maesaka JK, Fishbane S. Regulation of renal urate excretion: a critical review. Am J Kidney Dis. 1998; 32(6):917–933. [PubMed: 9856507]
7. Sorensen LB, Levinson DJ. Origin and extrarenal elimination of uric acid in man. Nephron. 1975; 14(1):7–20. [PubMed: 1124137]
8. Sakhaee K, Adams-Huet B, Moe OW, Pak CY. Pathophysiologic basis for normouricosuric uric acid nephrolithiasis. Kidney Int. 2002; 62(3):971–979. [PubMed: 12164880]
9. Oda M, Satta Y, Takenaka O, Takahata N. Loss of urate oxidase activity in hominoids and its evolutionary implications. Mol Biol Evol. 2002; 19(5):640–653. [PubMed: 11961098]
10. Wu X, Wakamiya M, Vaishnav S, et al. Hyperuricemia and urate nephropathy in urate oxidase-deficient mice. Proc Natl Acad Sci U S A. 1994; 91(2):742–746. [PubMed: 8290593]
11. Watanabe S, Kang DH, Feng L, et al. Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. Hypertension. 2002; 40(3):355–360. [PubMed: 12215479]
12. Giebisch, G.; Kennedy, T. National Academy of Sciences Biographical Memoir: Robert W Berliner, 1915–2002. (available at http://www.nasonline.org/publications/biographical-memoirs/memoir-pdfs/berliner-robert-w.pdf)
13. Gutman AB. Renal excretion of uric acid in normal and gouty man. Arthritis Rheum. 1965; 8(5): 665–670. [PubMed: 5859540]
14. Shoemaker VH, Nagy KA. Osmoregulation in amphibians and reptiles. Annu Rev Physiol. 1977; 39:449–471. [PubMed: 322603]
15. Braun EJ. Integration of organ systems in avian osmoregulation. Journal of Experimental Zoology. 1999; 283(7):702–707.
16. Ames BN, Cathcart R, Schwiers E, Hochstein P. Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc Natl Acad Sci U S A. 1981; 78(11):6858–6862. [PubMed: 6947260]
17. Frei B, England L, Ames BN. Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci U S A. 1989; 86(16):6377–6381. [PubMed: 2762330]
18. Kuzkaya N, Weissmann N, Harrison DG, Dikalov S. Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase. Biochem Pharmacol. 2005; 70(3):343–354. [PubMed: 15963955]
19. Gagliardi AC, Miname MH, Santos RD. Uric acid: A marker of increased cardiovascular risk. Atherosclerosis. 2009; 202(1):11–17. [PubMed: 18585721]
20. Hayden MR, Tyagi SC. Uric acid: A new look at an old risk marker for cardiovascular disease, metabolic syndrome, and type 2 diabetes mellitus: The urate redox shuttle. Nutr Metab (Lond). 2004; 1(1):10. [PubMed: 15507132]
21. Sautin YY, Johnson RJ. Uric acid: the oxidant-antioxidant paradox. Nucleosides Nucleotides Nucleic Acids. 2008; 27(6):608–619. [PubMed: 18600514]
22. Hershfield MS, Roberts LJ 2nd, Ganson NJ, et al. Treating gout with pegloticase, a PEGylated urate oxidase, provides insight into the importance of uric acid as an antioxidant in vivo. Proc Natl Acad Sci U S A. 2010; 107(32):14351–14356. [PubMed: 20660758]
23. Johnson RJ, Segal MS, Srinivas T, et al. Essential hypertension, progressive renal disease, and uric acid: a pathogenetic link? J Am Soc Nephrol. 2005; 16(7):1909–1919. [PubMed: 15843466]
24. Feig DI, Soletsky B, Johnson RJ. Effect of allopurinol on blood pressure of adolescents with newly diagnosed essential hypertension: a randomized trial. JAMA. 2008; 300(8):924–932. [PubMed: 18728266]
25. Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. Nature. 2003; 425(6957):516–521. [PubMed: 14520412]

26. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature. 2006; 440(7081):237–241. [PubMed: 16407889]
27. Passwell JH, Modan M, Brish M, Orda S, Boichis H. Fractional excretion of uric acid in infancy and childhood. Index of tubular maturation. Arch Dis Child. 1974; 49(11):878–882. [PubMed: 4441122]
28. Stapleton FB, Linshaw MA, Hassanein K, Gruskin AB. Uric acid excretion in normal children. J Pediatr. 1978; 92(6):911–914. [PubMed: 660356]
29. Baum M, Quigley R, Satlin L. Maturational changes in renal tubular transport. Curr Opin Nephrol Hypertens. 2003; 12(5):521–526. [PubMed: 12920400]
30. Dan T, Tanaka H, Koga H. Mechanism of uricosuric action of AA-193 in DBA/2N mice. J Pharmacol Exp Ther. 1990; 253(2):437–443. [PubMed: 2338641]
31. Preitner F, Bonny O, Laverriere A, et al. Glut9 is a major regulator of urate homeostasis and its genetic inactivation induces hyperuricosuria and urate nephropathy. Proc Natl Acad Sci U S A. 2009; 106(36):15501–15506. [PubMed: 19706426]
32. Diamond HS, Paolino JS. Evidence for a postsecretory reabsorptive site for uric acid in man. J Clin Invest. 1973; 52(6):1491–1499. [PubMed: 4703233]
33. Gutman AB, Yu TF, Berger L. Tubular secretion of urate in man. J Clin Invest. 1959; 38:1778–1781. [PubMed: 13830462]
34. Guggino SE, Aronson PS. Paradoxical effects of pyrazinoate and nicotinate on urate transport in dog renal microvillus membranes. J Clin Invest. 1985; 76(2):543–547. [PubMed: 4031062]
35. Enomoto A, Kimura H, Chairoungdua A, et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature. 2002; 417(6887):447–452. [PubMed: 12024214]
36. So A, Thorens B. Uric acid transport and disease. J Clin Invest. 2010; 120(6):1791–1799. [PubMed: 20516647]
37. Anzai N, Endou H. Urate transporters: an evolving field. Semin Nephrol. 2011; 31(5):400–409. [PubMed: 22000646]
38. Lipkowitz MS. Regulation of uric acid excretion by the kidney. Curr Rheumatol Rep. 2012; 14(2): 179–188. [PubMed: 22359229]
39. Tanaka M, Itoh K, Matsushita K, et al. Two male siblings with hereditary renal hypouricemia and exercise-induced ARF. Am J Kidney Dis. 2003; 42(6):1287–1292. [PubMed: 14655203]
40. Wakida N, Tuyen DG, Adachi M, et al. Mutations in human urate transporter 1 gene in presecretory reabsorption defect type of familial renal hypouricemia. J Clin Endocrinol Metab. 2005; 90(4):2169–2174. [PubMed: 15634722]
41. Ichida K, Hosoyamada M, Kamatani N, et al. Age and origin of the G774A mutation in SLC22A12 causing renal hypouricemia in Japanese. Clin Genet. 2008; 74(3):243–251. [PubMed: 18492088]
42. Kolz M, Johnson T, Sanna S, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009; 5(6):e1000504. [PubMed: 19503597]
43. Tin A, Woodward OM, Kao WH, et al. Genome-wide association study for serum urate concentrations and gout among African Americans identifies genomic risk loci and a novel URAT1 loss-of-function allele. Hum Mol Genet. 2011; 20(20):4056–4068. [PubMed: 21768215]
44. Kenny EE, Kim M, Gusev A, et al. Increased power of mixed models facilitates association mapping of 10 loci for metabolic traits in an isolated population. Hum Mol Genet. 2011; 20(4): 827–839. [PubMed: 21118897]
45. Mori K, Ogawa Y, Ebihara K, et al. Kidney-specific expression of a novel mouse organic cation transporter-like protein. FEBS Lett. 1997; 417(3):371–374. [PubMed: 9409754]
46. Hosoyamada M, Ichida K, Enomoto A, Hosoya T, Endou H. Function and localization of urate transporter 1 in mouse kidney. J Am Soc Nephrol. 2004; 15(2):261–268. [PubMed: 14747372]
47. Eraly SA, Vallon V, Rieg T, et al. Multiple organic anion transporters contribute to net renal excretion of uric acid. Physiol Genomics. 2008; 33(2):180–192. [PubMed: 18270321]

48. Bahn A, Hagos Y, Reuter S, et al. Identification of a new urate and high affinity nicotinate transporter, hOAT10 (SLC22A13). J Biol Chem. 2008; 283(24):16332–16341. [PubMed: 18411268]
49. Gopal E, Umapathy NS, Martin PM, et al. Cloning and functional characterization of human SMCT2 (SLC5A12) and expression pattern of the transporter in kidney. Biochim Biophys Acta. 2007; 1768(11):2690–2697. [PubMed: 17692818]
50. Thangaraju M, Ananth S, Martin PM, et al. c/ebpdelta Null mouse as a model for the double knock-out of slc5a8 and slc5a12 in kidney. J Biol Chem. 2006; 281(37):26769–26773. [PubMed: 16873376]
51. Frank H, Groger N, Diener M, et al. Lactaturia and loss of sodium-dependent lactate uptake in the colon of SLC5A8-deficient mice. J Biol Chem. 2008; 283(36):24729–24737. [PubMed: 18562324]
52. Phay JE, Hussain HB, Moley JF. Cloning and expression analysis of a novel member of the facilitative glucose transporter family, SLC2A9 (GLUT9). Genomics. 2000; 66(2):217–220. [PubMed: 10860667]
53. Augustin R, Carayannopoulos MO, Dowd LO, et al. Identification and characterization of human glucose transporter-like protein-9 (GLUT9): alternative splicing alters trafficking. J Biol Chem. 2004; 279(16):16229–16236. [PubMed: 14739288]
54. Li S, Sanna S, Maschio A, et al. The GLUT9 gene is associated with serum uric acid levels in Sardinia and Chianti cohorts. PLoS Genet. 2007; 3(11):e194. [PubMed: 17997608]
55. Dehghan A, Kottgen A, Yang Q, et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet. 2008; 372(9654):1953–1961. [PubMed: 18834626]
56. Doring A, Gieger C, Mehta D, et al. SLC2A9 influences uric acid concentrations with pronounced sex-specific effects. Nat Genet. 2008; 40(4):430–436. [PubMed: 18327256]
57. Vitart V, Rudan I, Hayward C, et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat Genet. 2008; 40(4):437–442. [PubMed: 18327257]
58. Wallace C, Newhouse SJ, Braund P, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. Am J Hum Genet. 2008; 82(1):139–149. [PubMed: 18179892]
59. Anzai N, Ichida K, Jutabha P, et al. Plasma urate level is directly regulated by a voltage-driven urate efflux transporter URATv1 (SLC2A9) in humans. J Biol Chem. 2008; 283(40):26834–26838. [PubMed: 18701466]
60. Matsuo H, Chiba T, Nagamori S, et al. Mutations in glucose transporter 9 gene SLC2A9 cause renal hypouricemia. Am J Hum Genet. 2008; 83(6):744–751. [PubMed: 19026395]
61. Kawamura Y, Matsuo H, Chiba T, et al. Pathogenic GLUT9 mutations causing renal hypouricemia type 2 (RHUC2). Nucleosides Nucleotides Nucleic Acids. 2011; 30(12):1105–1111. [PubMed: 22132964]
62. Dinour D, Gray NK, Campbell S, et al. Homozygous SLC2A9 mutations cause severe renal hypouricemia. J Am Soc Nephrol. 2010; 21(1):64–72. [PubMed: 19926891]
63. Dinour D, Gray NK, Ganon L, et al. Two novel homozygous SLC2A9 mutations cause renal hypouricemia type 2. Nephrol Dial Transplant. 2012; 27(3):1035–1041. [PubMed: 21810765]
64. Bibert S, Hess SK, Firsov D, et al. Mouse GLUT9: evidences for a urate uniporter. Am J Physiol Renal Physiol. 2009; 297(3):F612–619. [PubMed: 19587147]
65. Keembiyehetty C, Augustin R, Carayannopoulos MO, et al. Mouse glucose transporter 9 splice variants are expressed in adult liver and kidney and are up-regulated in diabetes. Mol Endocrinol. 2006; 20(3):686–697. [PubMed: 16293642]
66. Eraly SA, Hamilton BA, Nigam SK. Organic anion and cation transporters occur in pairs of similar and similarly expressed genes. Biochem Biophys Res Commun. 2003; 300(2):333–342. [PubMed: 12504088]
67. Cha SH, Sekine T, Kusuhara H, et al. Molecular cloning and characterization of multispecific organic anion transporter 4 expressed in the placenta. J Biol Chem. 2000; 275(6):4507–4512. [PubMed: 10660625]

68. Ekaratanawong S, Anzai N, Jutabha P, et al. Human organic anion transporter 4 is a renal apical organic anion/dicarboxylate exchanger in the proximal tubules. J Pharmacol Sci. 2004; 94(3):297–304. [PubMed: 15037815]
69. Hagos Y, Stein D, Ugele B, Burckhardt G, Bahn A. Human renal organic anion transporter 4 operates as an asymmetric urate transporter. J Am Soc Nephrol. 2007; 18(2):430–439. [PubMed: 17229912]
70. Yang Q, Kottgen A, Dehghan A, et al. Multiple genetic loci influence serum urate levels and their relationship with gout and cardiovascular disease risk factors. Circ Cardiovasc Genet. 2010; 3(6): 523–530. [PubMed: 20884846]
71. van der Harst P, Bakker SJ, de Boer RA, et al. Replication of the five novel loci for uric acid concentrations and potential mediating mechanisms. Hum Mol Genet. 2010; 19(2):387–395. [PubMed: 19861489]
72. Ho HT, Ko BC, Cheung AK, et al. Generation and characterization of sodium-dicarboxylate cotransporter-deficient mice. Kidney Int. 2007; 72(1):63–71. [PubMed: 17410095]
73. Leal-Pinto E, Tao W, Rappaport J, et al. Molecular cloning and functional reconstitution of a urate transporter/channel. J Biol Chem. 1997; 272(1):617–625. [PubMed: 8995305]
74. Lipkowitz MS, Leal-Pinto E, Rappoport JZ, Najfeld V, Abramson RG. Functional reconstitution, membrane targeting, genomic structure, and chromosomal localization of a human urate transporter. J Clin Invest. 2001; 107(9):1103–1115. [PubMed: 11342574]
75. Hyink DP, Rappoport JZ, Wilson PD, Abramson RG. Expression of the urate transporter/channel is developmentally regulated in human kidneys. Am J Physiol Renal Physiol. 2001; 281(5):F875–886. [PubMed: 11592946]
76. Werner A, Moore ML, Mantei N, et al. Cloning and expression of cDNA for a Na/Pi cotransport system of kidney cortex. Proc Natl Acad Sci U S A. 1991; 88(21):9608–9612. [PubMed: 1946375]
77. Chong SS, Kristjansson K, Zoghbi HY, Hughes MR. Molecular cloning of the cDNA encoding a human renal sodium phosphate transport protein and its assignment to chromosome 6p21.3–p23. Genomics. 1993; 18(2):355–359. [PubMed: 8288239]
78. Ruddy DA, Kronmal GS, Lee VK, et al. A 1.1-Mb transcript map of the hereditary hemochromatosis locus. Genome Res. 1997; 7(5):441–456. [PubMed: 9149941]
79. Biber J, Custer M, Werner A, Kaissling B, Murer H. Localization of NaPi-1, a Na/Pi cotransporter, in rabbit kidney proximal tubules. II. Localization by immunohistochemistry. Pflugers Arch. 1993; 424(3–4):210–215. [PubMed: 8414908]
80. Ishibashi K, Matsuzaki T, Takata K, Imai M. Identification of a new member of type I Na/phosphate co-transporter in the rat kidney. Nephron Physiol. 2003; 94(1):10–18.
81. Jutabha P, Anzai N, Kitamura K, et al. Human sodium phosphate transporter 4 (hNPT4/SLC17A3) as a common renal secretory pathway for drugs and urate. J Biol Chem. 2010; 285(45):35123–35132. [PubMed: 20810651]
82. Biber J, Hernando N, Forster I, Murer H. Regulation of phosphate transport in proximal tubules. Pflugers Arch. 2009; 458(1):39–52. [PubMed: 18758808]
83. Urano W, Taniguchi A, Anzai N, et al. Sodium-dependent phosphate cotransporter type 1 sequence polymorphisms in male patients with gout. Ann Rheum Dis. 2010; 69(6):1232–1234. [PubMed: 19556210]
84. Hollis-Moffatt JE, Phipps-Green AJ, Chapman B, et al. The renal urate transporter SLC17A1 locus: confirmation of association with gout. Arthritis Res Ther. 2012; 14(2):R92. [PubMed: 22541845]
85. Iharada M, Miyaji T, Fujimoto T, et al. Type 1 sodium-dependent phosphate transporter (SLC17A1 Protein) is a Cl(−)-dependent urate exporter. J Biol Chem. 2010; 285(34):26107–26113. [PubMed: 20566650]
86. Doyle LA, Yang W, Abruzzo LV, et al. A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc Natl Acad Sci U S A. 1998; 95(26):15665–15670. [PubMed: 9861027]
87. Woodward OM, Kottgen A, Coresh J, et al. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc Natl Acad Sci U S A. 2009; 106(25):10338–10342. [PubMed: 19506252]

88. Yamagishi K, Tanigawa T, Kitamura A, et al. The rs2231142 variant of the ABCG2 gene is associated with uric acid levels and gout among Japanese people. Rheumatology (Oxford). 2010; 49(8):1461–1465. [PubMed: 20421215]

89. Phipps-Green AJ, Hollis-Moffatt JE, Dalbeth N, et al. A strong role for the ABCG2 gene in susceptibility to gout in New Zealand Pacific Island and Caucasian, but not Maori, case and control sample sets. Hum Mol Genet. 2010; 19(24):4813–4819. [PubMed: 20858603]

90. Huls M, Brown CD, Windass AS, et al. The breast cancer resistance protein transporter ABCG2 is expressed in the human kidney proximal tubule apical membrane. Kidney Int. 2008; 73(2):220–225. [PubMed: 17978814]

91. Matsuo H, Takada T, Ichida K, et al. Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. Sci Transl Med. 2009; 1(5):5ra11.

92. Hosomi A, Nakanishi T, Fujita T, Tamai I. Extra-renal elimination of uric acid via intestinal efflux transporter BCRP/ABCG2. PloS one. 2012; 7(2):e30456. [PubMed: 22348008]

93. Ichida K, Matsuo H, Takada T, et al. Decreased extra-renal urate excretion is a common cause of hyperuricemia. Nat Commun. 2012; 3:764. [PubMed: 22473008]

94. Maliepaard M, Scheffer GL, Faneyte IF, et al. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res. 2001; 61(8): 3458–3464. [PubMed: 11309308]

95. van Aubel RA, Smeets PH, Peters JG, Bindels RJ, Russel FG. The MRP4/ABCC4 gene encodes a novel apical organic anion transporter in human kidney proximal tubules: putative efflux pump for urinary cAMP and cGMP. J Am Soc Nephrol. 2002; 13(3):595–603. [PubMed: 11856762]

96. Lee K, Belinsky MG, Bell DW, Testa JR, Kruh GD. Isolation of MOAT-B, a widely expressed multidrug resistance-associated protein/canalicular multispecific organic anion transporter-related transporter. Cancer Res. 1998; 58(13):2741–2747. [PubMed: 9661885]

97. Bakhiya A, Bahn A, Burckhardt G, Wolff N. Human organic anion transporter 3 (hOAT3) can operate as an exchanger and mediate secretory urate flux. Cell Physiol Biochem. 2003; 13(5):249–256. [PubMed: 14586168]

98. Ichida K, Hosoyamada M, Kimura H, et al. Urate transport via human PAH transporter hOAT1 and its gene structure. Kidney Int. 2003; 63(1):143–155. [PubMed: 12472777]

99. Shikhman AR, Brinson DC, Valbracht J, Lotz MK. Cytokine regulation of facilitated glucose transport in human articular chondrocytes. J Immunol. 2001; 167(12):7001–7008. [PubMed: 11739520]

100. Hosoyamada M, Takiue Y, Morisaki H, et al. Establishment and analysis of SLC22A12 (URAT1) knockout mouse. Nucleosides Nucleotides Nucleic Acids. 2010; 29(4–6):314–320. [PubMed: 20544513]

101. Sharma S, Sundararajan A, Suryawanshi A, et al. T cell immunoglobulin and mucin protein-3 (Tim-3)/Galectin-9 interaction regulates influenza A virus-specific humoral and CD8 T-cell responses. Proc Natl Acad Sci U S A. 2011; 108(47):19001–19006. [PubMed: 22052881]

102. Assem M, Schuetz EG, Leggas M, et al. Interactions between hepatic Mrp4 and Sult2a as revealed by the constitutive androstane receptor and Mrp4 knockout mice. J Biol Chem. 2004; 279(21):22250–22257. [PubMed: 15004017]

103. Van Aubel RA, Smeets PH, van den Heuvel JJ, Russel FG. Human organic anion transporter MRP4 (ABCC4) is an efflux pump for the purine end metabolite urate with multiple allosteric substrate binding sites. Am J Physiol Renal Physiol. 2005; 288(2):F327–333. [PubMed: 15454390]

104. Shimada H, Moewes B, Burckhardt G. Indirect coupling to Na+ of p-aminohippuric acid uptake into rat renal basolateral membrane vesicles. Am J Physiol. 1987; 253(5 Pt 2):F795–801. [PubMed: 3120599]

105. Kaufhold M, Schulz K, Breljak D, et al. Differential interaction of dicarboxylates with human sodium-dicarboxylate cotransporter 3 and organic anion transporters 1 and 3. Am J Physiol Renal Physiol. 2011; 301(5):F1026–1034. [PubMed: 21865262]

Appendix

Description of candidate transport proteins involved in urate handling in the human proximal tubule, other than URAT1 and GLUT9 (which are discussed in detail in the main text of the article). Transporters are listed in the same order as in Table 1 and Figure 3.

OAT10

First identified by Bahn *et al* in 2008, OAT10 is expressed at the messenger RNA level in the human kidney, and possibly at lower levels in the colon and other tissues (48). The rat OAT10 homolog is present in the proximal tubule brush border and cortical collecting duct, but the localization of OAT10 protein in the human kidney has not been described. Human OAT10 and URAT1 are 33% identical at the amino acid level. OAT10 can function as an urate-monocarboxylate exchanger when expressed in *Xenopus* oocytes, but may have a lower affinity for urate than URAT1, and in contrast with URAT1 can also exchange urate for para-aminohippurate. However, no data are presently available to confirm a role for OAT10 in urate handling *in vivo*.

SMCT1/2

SMCT1 and SMCT2 are Na^+-coupled monocarboxylate cotransporters expressed in multiple tissues, and localized at the apical membrane of the proximal tubule in mice (49). SMCT1 and SMCT2 may participate in urate transport by utilizing the Na^+ gradient provided by the Na^+/K^+-ATPase to drive cellular uptake of monocarboxylates (secondary active transport), thus creating the driving force required for urate uptake via URAT1 and OAT10 (tertiary active transport) (Figure 3). Indirect evidence for this model was provided by a knockout mouse deficient for the c/ebpδ transcription factor, in which decreased reabsorption of urate was attributed to reduced expression of both SMCT1 and SMCT2, in part because mRNA levels of URAT1, OAT1, and NaDC3 were unaffected (50). Although intriguing, this animal model does not definitively prove that renal SMCT1/2 are important for urate transport in the mouse kidney, because lack of c/ebpδ may have affected the transcription of other proteins involved in the regulation of urate handling. SMCT1-null mice have impaired lactate reabsorption and lactaturia (51), but no reported defects in urate handling (possibly because of SMCT1/2 redundancy for urate transport, but not for lactate transport). The localization and function of SMCT1/2 in the human kidney remain uncertain.

OAT4

The organic anion transporter OAT4 is 52% identical at the amino acid level with URAT1, and the OAT4 and URAT1 genes (*SLC22A11* and *SLC22A12* respectively) are immediately adjacent on chromosome 11. Such occurrence of related genes in tandem, also encountered for other mammalian ion transporters (including some discussed later in this article), may indicate that OAT4 and URAT1 originated from a single gene by duplication, and could thus be linked to similarities in function, regulation and expression patterns (66). No homolog of OAT4 has been found in the mouse genome (though homologs are present in other non-primate mammals such as the dog), and thus no mouse studies are available. In humans, OAT4 is expressed in the placenta and in the apical membrane of the renal proximal tubule (67, 68). Experiments in the *Xenopus* oocyte expression system suggest that human OAT4 mediates urate-dicarboxylate exchange, with a lower estimated affinity for urate compared with URAT1 (69).

Though not identified in individual GWAS, an association between variants of the *SLC22A11* gene and uric acid levels was reported by two large meta-analyses of multiple

GWAS (42, 70), and later confirmed in an independent cohort (71). Statistical analyses suggested that the association of *SLC22A11* variants with serum urate is independent (i.e. not attributable to concomitant variation) of *SLC22A12,* in spite of the close physical proximity of the two genes on chromosome 11 (42). However, no loss-of-function mutations of OAT4 with effects on urate handling have been described to date, and the exact role and relative importance of OAT4 in the renal transport of urate *in vivo* remains to be established.

NaDC1/3

Similar to the Na^+-monocarboxylate cotransporters SMCT1/2 that may be functionally coupled with URAT1 and/or OAT10, the apical Na^+-dicarboxylate cotransporter NaDC1 may create the dicarboxylate gradient that allows for urate reabsorption via OAT4 (Figure 3). Analogously, the basolateral Na^+-dicarboxylate cotransporter NaDC3 may be functionally coupled with OAT1 and/or OAT3 (potentially important transporters discussed later in this article) to facilitate basolateral urate uptake, thus contributing to proximal tubule secretion of urate. These functional interactions remains purely speculative, and whether NaDC1 and NaDC3 have any contribution to renal urate handling *in vivo* is unknown. Genetic inactivation of NaDC1 in mice results in increased urinary excretion of citrate, succinate and other Krebs cycle intermediates, but no reported uric acid disturbances (72).

hUAT

Better known to cell biologists as Galectin 9, the rat homolog of hUAT was first linked to urate transport when it reacted with an anti-uricase antibody during a whole kidney complementary DNA library screen for urate-binding proteins, and was then shown to function as a urate channel/uniporter *in vitro*, in artificial lipid bilayers (73, 74). hUAT is a member of the ubiquitously expressed, multifunctional family of galectins, or galactoside-binding proteins (*LGALS1-14* genes). hUAT was localized to the cytosol and toward the apical pole of human proximal tubule cells (75); however, it should be noted that immunohistochemistry was performed with the same antibody against pig liver uricase originally used for screening, thus leaving ample possibility for non-specific binding (including to other urate transporters that were not known at the time). In theory, hUAT could function at the luminal membrane as a urate channel/uniporter moving urate down its electrochemical gradient, which depending on local conditions and on the activity of other transporters could mean either reabsorption or secretion. Although hUAT remains historically important as the first protein proposed to transport urate in the kidney, there is currently no evidence that it plays such a role *in vivo*.

NPT1/4

NPT1 (also known as NaPi-1) was first cloned from a rabbit kidney complementary DNA library screened for Na^+-dependent phosphate uptake in *Xenopus* oocytes, and was thus classified as a Na^+-phosphate cotransporter (76). The human gene *SLC17A1* (NPT1) and the highly related *SLC17A3* (NPT4) were then identified by homology and found to be located in tandem on chromosome 6 (77, 78). Although both proteins are expressed in the luminal membrane of the proximal tubule (79–81), current evidence strongly suggest that renal phosphate reabsorption relies on other transporters with much higher affinity for phosphate: NaPi-2a, NaPi-2c and Pit-2 (*SLC34A1*, *SLC34A3* and *SLC20A2* genes respectively) (82).

As with other urate transporters, key evidence for the potential roles of NPT1 and NPT4 in urate handling *in vivo* came from GWAS (55) or GWAS meta-analysis followed by independent confirmation (42, 83, 84). NPT1 and NPT4 were both found to transport urate *in vitro* when expressed in oocytes or reconstituted in proteoliposomes, and several variants

associated with hyperuricemia and gout in GWAS resulted in decreased urate transport when studied *in vitro* (81, 85). Together with the proximal tubule luminal localization, these findings suggest that NPT1 and NPT4 may be key players in renal urate secretion, and, importantly, that a defect in renal secretion may be sufficient to cause hyperuricemia. However, no cases of familial hyperuricemia to date have been attributed to loss-of-function mutations of *SLC17A1* or *SLC17A3*.

ABCG2

First identified as a multidrug resistance protein (termed BCRP, for breast cancer resistance protein) (86), *ABCG2* (ATP-binding cassette subfamily G member 2) is yet another transporter linked to uric acid metabolism by GWAS (42, 55). At least one common *ABCG2* variant was associated with gout in multiple populations, including African Americans, Asians, Caucasians, and Pacific Islanders (87–89). ABCG2 protein is expressed in the human proximal tubule brush border (90), transports urate *in vitro*, and several variants of the *ABCG2* gene leading to reduced or abolished *in vitro* transport were identified in gout patients, in either heterozygous or homozygous state (87, 91). These findings suggest that ABCG2 may be another important participant in renal urate secretion, besides NPT1/4.

However, genetic inactivation of AbcG2 in mice that were already hyperuricemic led to increased renal urate excretion, in apparent contradiction with the proposed role of ABCG2 in the kidney (92, 93). This was attributed to a demonstratable decrease in intestinal urate secretion in knockout versus wild-type mice, leading to “renal urate overload” and compensatory increase in renal excretion, via AbcG2-independent mechanisms. Compatible with these findings, ABCG2 is highly expressed in the human intestinal epithelium (94), and variants of ABCG2 with decreased transport activity *in vitro* were associated with higher renal urate excretion rates among 644 hyperuricemic men, including 575 with gout (93). Taken together, these data suggest that decreased intestinal secretion of urate may contribute to the pathogenesis of hyperuricemia in humans, and ABCG2 may be more important for urate export in the intestine than in the kidney.

ABCC4

Similar to ABCG2, ABCC4 is an ATP-binding cassette transporter and multi-drug resistance protein (also termed MRP4), expressed in the luminal membrane of the human proximal tubule in addition to other tissues (95, 96). ABCC4 transports urate *in vitro,* but there is no evidence to date for such a role *in vivo* in the kidney.

OAT1/3

The *SLC22A6* and *SLC22A8* genes are located in tandem on chromosome 11, and the resulting OAT1 and OAT3 proteins are 48% identical at the amino acid level. OAT1 and OAT3 are dicarboxylate-anion exchangers expressed on the basolateral membrane of the proximal tubule, transport urate *in vitro*, and their individual deletion in mice leads to decreased urate excretion (47, 97, 98). These data are compatible with a role for OAT1 and OAT3 in basolateral urate uptake, with overall function in renal urate secretion. No studies to date have confirmed this hypothesis in humans.

Clinical Summary

- Uric acid or urate may have multiple physiologic roles, including blood pressure regulation, immune modulation and antioxidation/prooxidation balance.
- Hyperuricemia has been associated with prevalent diseases including hypertension, metabolic syndrome and chronic kidney disease, but whether uric acid has a causal role in the pathogenesis of these conditions is not yet known.
- Renal handling of urate has a pivotal role in uric acid homeostasis and is achieved through a complex interplay of reabsorption and secretion primarily in the proximal tubule; urate transport is unlikely to follow the classic four-component model of filtration, presecretory reabsorption, secretion and postsecretory reabsorption, although this model is still perpetuated at times in the literature.
- Of the multiple transport proteins that may play a role in renal transport of urate, the best characterized to date are apical URAT1 and basolateral GLUT9, both with demonstrated effects on uric acid levels and direct regulation by known uricosuric drugs; these transporters present attractive targets for current and future drug development.

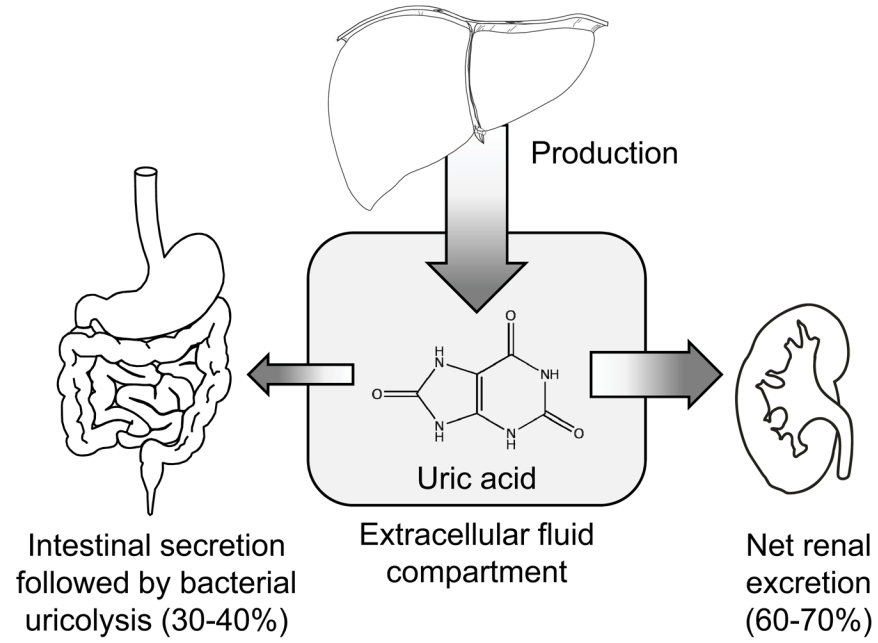

Figure 1.
Schematic representation of the key determinants of uric acid homeostasis in humans.

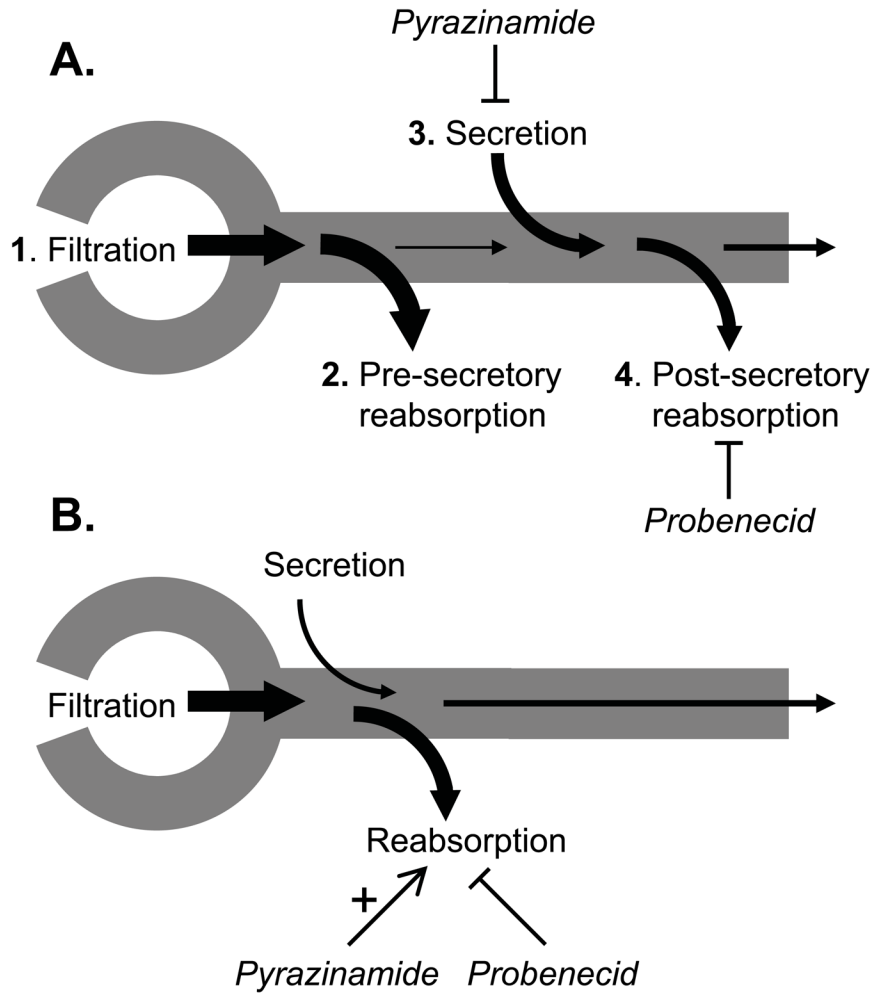

Figure 2.
A. Traditional model and **B.** Revised model of urate transport in the kidney (see text for details).

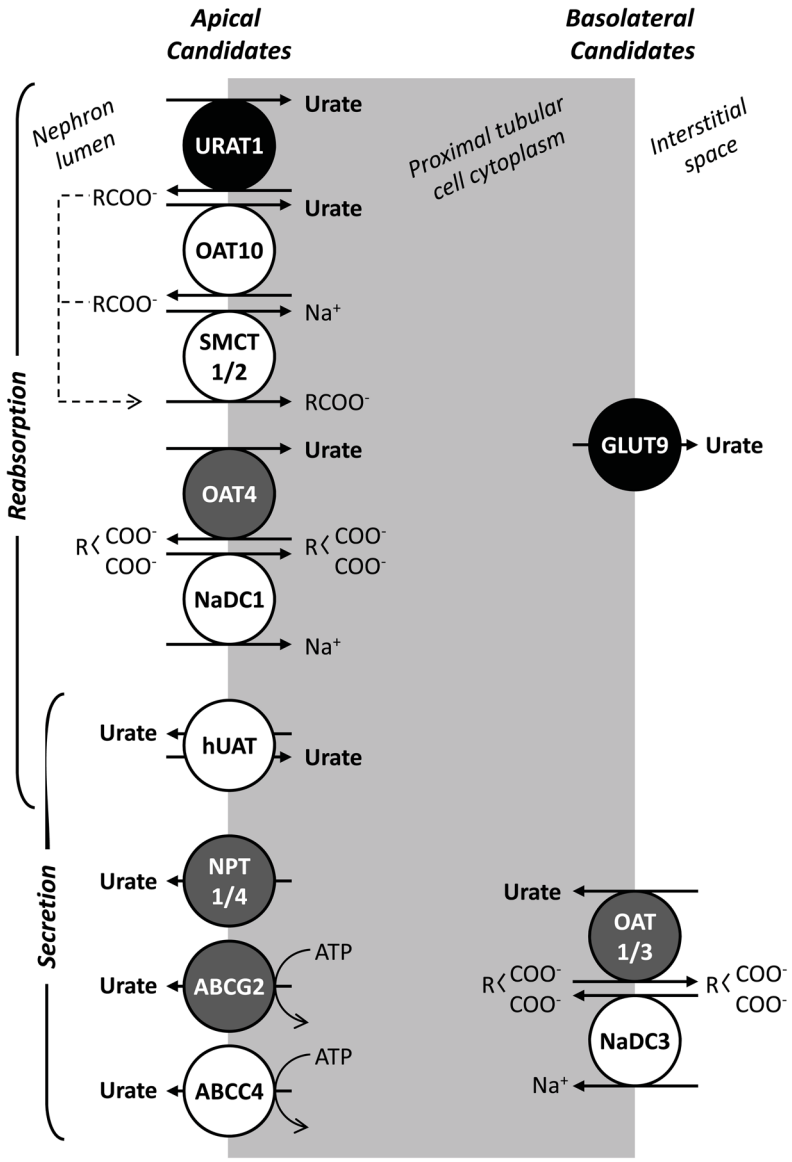

Figure 3.

Candidate transport proteins involved in urate handling in the human proximal tubule. Circles representing individual transporters are colored according to the level of evidence (see text and Table 1 for details): black, grey and white circles represent strong, moderate and weak evidence respectively.

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Table 1

Plasma membrane ion transporters involved or potentially involved in renal urate handling (ordered as in Figure 3, from top to bottom and from left to right, for ease of reference).

Human gene name(s) and location(s)	Protein name(s)	Protein function(s)	Level and type of evidence for role in renal urate transport	Knockout mouse phenotype	Human mutations/allelic variants	Refs
SLC22A12 (11q13.1)	**URAT1** (RST, OAT4L)	Urate-monocarboxylate exchanger; reabsorbs urate at the apical membrane.	**Strong** (human mutations, GWAS *, oocytes, KO * mouse); apical localization confirmed in humans.	Slightly higher FEUA *, but reabsorption of UA * largely preserved.	Homozygous or compound heterozygous loss-of-function → Increased FEUA (30– 90%), hyperuricosuria and hypouricemia (30–60 μM/L or 0.5–1.0 mg/dL).	(35, 39– 42, 47, 100)
SLC22A13 (3p21.3)	**OAT10**	Similar to URAT1, but lower affinity	**Weak** (oocytes)	N/A	N/A	(48)
SLC5A8 (12q23.1) and **SLC5A12** (11p14.2)	**SMCT1** and **SMCT2**	Na^+-coupled monocarboxylate cotransporters; may function in concert with URAT1 (and possibly OAT10) to achieve Na^+-coupled urate reabsorption.	**Weak** (based primarily on reduced SMCT1/2 expression and decreased urate reabsorption in mice null for the c/ebpδ transcription factor).	Lactaturia in SMCT1 KO mouse, no described abnormalities of urate handling	N/A	(49–51)
SLC2A9 (4p16.1)	**GLUT9** (GLUTX; URATv1)	Urate uniporter; reabsorbs urate at the basolateral membrane.	**Strong** (human mutations, strongest association with serum urate in GWAS, oocytes, KO mice); basolateral localization confirmed in humans.	Moderate hyperuricemia and massive hyperuricosuria; more severe hyperuricemia in liver-specific knockout.	Heterozygous loss-of-function →hyperuricosuria and hypouricemia (90–160 μM/L or 1.5–2.7 mg/dL); Homozygous loss-of-function → marked hyperuricosuria, hypouricemia (<12 μM/L or <0.2 mg/dL), FEUA can exceed 100% (net secretion).	(31, 54–64)
SLC22A11 (11q13.1)	**OAT4**	Urate-dicarboxylate exchanger; reabsorbs urate at the lumen; lower affinity than URAT1	**Moderate** (meta-analysis of GWAS, oocytes)	N/A (no identified mouse ortholog)	N/A	(42, 68, 69)
SLC13A2 (17p13.2)	**NaDC1**	Apical Na^+-coupled dicarboxylate transporter; may function in concert with OAT4 to achieve Na^+-coupled urate reabsorption.	**Weak** (theoretical)	No described abnormalities of urate handling	N/A	(72)
LGALS9 (17q11.2)	**hUAT** (Galectin 9)	Functions *in vitro* as urate channel; may mediate apical bidirectional transport of urate.	**Weak** (artificial lipid bilayers)	No described abnormalities of urate handling	N/A	(73–75, 101)

Human gene name(s) and location(s)	Protein name(s)	Protein function(s)	Level and type of evidence for role in renal urate transport	Knockout mouse phenotype	Human mutations/allelic variants	Refs
SLC17A1 (6p22.2) and **SLC17A3** (6p22.2)	**NPT1** (NaPi-1, OATv1) and **NPT4**	Apical urate exporters; originally classified as Na^+-phosphate transporters.	**Moderate** (human allelic variants, GWAS, oocytes, proteoliposomes)	N/A	NPT1 and NPT4 allelic variants leading to lower urate transport *in vitro* are linked with hyperuricemia and gout.	(42, 55, 81, 85)
ABCG2 (4q22)	**ABCG2** (BCRP, MRX)	Apical ATP-binding cassette transporter G2 functions as a urate extrusion pump.	**Moderate**(human allelic variants, GWAS, oocytes, but KO mouse data indicate a more important role for ABCG2 in the intestine)	No urate abnormalities at baseline; raising UA levels with oxonate leads to decreased UA secretion in the intestine and increased renal UA excretion in KO vs. control mice.	ABCG2 allelic variants leading to reduced or abolished urate transport *in vitro* are linked with hyperuricemia and gout.	(87, 90, 91, 93)
ABCC4 (13q32.1)	**ABCC4** (MRP4, MOAT-B)	May function similarly to ABCG2	**Weak** (membrane vesicles from Sf9 cells)	No described abnormalities of urate handling	N/A	(95, 102, 103)
SLC22A6 (11q12.3) and **SLC22A8** (11q12.3)	**OAT1** and **OAT3**	Organic anion-dicarboxylate exchangers; may mediate basolateral uptake of urate.	**Moderate** (transfected cells, KO mice)	Decreased UA excretion in both KO models, no hyperuricemia	N/A	(47, 97, 98)
SLC13A3 (20q13.12)	**NaDC3**	Na^+-coupled dicarboxylate transporter; may function in concert with OAT1/3 to achieve Na^+-coupled basolateral uptake of urate.	**Weak** (theoretical)	N/A	N/A	(104, 105)

*Abbreviations: GWAS, human genome-wide association studies with serum uric acid levels; UA, urate; FEUA, fractional excretion of urate; KO, knockout