ABCG2: the molecular mechanisms of urate secretion and gout

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ABCG2: the molecular mechanisms of urate secretion and gout. Am J Physiol Renal Physiol 309: F485–F488, 2015. First published July 1, 2015; doi:10.1152/ajprenal.00242.2015.—The human propensity for high levels of serum uric acid (SUA) is a trait that has defied explanation. Is it beneficial? Is it pathogenic? Its role in the human diseases like gout and kidney stones was discovered over a century ago; but today emerging new genetic and epidemiological techniques independently increase risk for diseases like hypertension and chronic kidney disease. More recent work suggests hyperuricemia increases the risk for other human related disorders like stroke, metabolic disorders, and CKD, where SUA has been implicated as an increasing risk for incident CKD (relative risk of CKD was 1.22 per mg/dl of SUA).

Hyperuricemia results primarily from underexcretion, which occurs via two primary pathways, the gut (30%) and the kidney (70%) (5, 22, 24). Work to describe the physiology of kidney excretion of uric acid was begun a half-century ago by the pioneering team of Gutman and Yu, when they proposed the three-component hypothesis of kidney uric acid filtration and excretion (5, 9, 10, 24): 1) uric acid is freely filtered at the glomerulus; 2) most uric acid is reabsorbed in the proximal tubule; and 3) some uric acid is actively secreted within the proximal tubule, distal to reabsorption. Subsequent decades only saw slight revisions of this theory, including a fourth postsecretory reabsorption component (24), but the central idea of a balance between reabsorption and secretion determining overall excretion remained. However, the advent of new molecular and genetic tools led to the molecular identification of most if not all of the key uric acid transporters in humans and allowed for the first time a high-resolution understanding of uric acid transport in the kidney [see Mandal and Mount for an excellent overview of the molecular players of uric acid homeostasis (13)]. Here, we focus exclusively on one gene and transporter gene product, ABCG2, its critical role in uric acid secretion, and a common ABCG2 causal variant for gout and hyperuricemia. Additional significant uric acid dose effect (29). Increased hypertension risk also translates to increased risk for other related disorders like stroke, metabolic disorders (22, 23), and chronic kidney disease (CKD), where SUA has been implicated as an increasing risk for incident CKD (relative risk of CKD was 1.22 per mg/dl of SUA).
The story of ABCG2 and uric acid began with an unbiased genetic screening tool, the genome-wide association study (GWAS). Dehghan et al. (3) conducted a GWAS on SUA and identified the first single nucleotide polymorphisms (SNPs) in a small region of chromosome 4 that associated with increased levels of SUA and gout (3). We showed that the gene ABCG2, located in the same region of chromosome 4, and which encodes an ABC (ATP-binding cassette) transporter (2), was a heretofore unknown urate efflux transporter (30). Using a Xenopus oocyte expression system, we demonstrated ABCG2 to be a high-capacity urate transporter with ABCG2-mediated c-14 uric acid efflux highly dependent on the intracellular concentration, and could be blocked by a specific ABCG2 inhibitor, FTC, or with a single amino acid substitution, S187A. Furthermore, endogenous ABCG2 in proximal tubule cells is localized to the apical brush border and critical for apical secretion of urate (30). Study of the most significant SNP that associated with SUA, rs2231142, revealed it codes for an amino acid Q-to-K substitution at position 141. We found the Q141K variant had similar total and surface expression levels in the Xenopus oocytes but showed a 54% reduction in urate transport, marking Q141K as a loss-of-function mutation (17, 18, 30). A population-based study of 14,783 individuals supported rs2231142 (Q141K) as a causal variant for gout and increased SUA levels, marking the rs2231142 as a rare example in support of the common disease-common variant hypothesis (30).

Subsequent, but independent work from Matsuo et al. (15) confirmed many of our findings. In a series of elegant papers, these same authors make a compelling argument for the critical importance of ABCG2 in gout and hyperuricemia risk. Matsuo et al. compared gouty and normal cohorts of Japanese males and found two ABCG2 mutations, Q141K (50% function) and Q126X (no function), and used these to correlate ABCG2 function with age of gout onset. They found that 76.2% of their gout cohort had some level of ABCG2 dysfunction and that severe ABCG2 dysfunction substantially increased the risk of early-onset gout (OR 22.2) (14). Nakayoma et al. (20) further calculated the population-attributable risk percentage (PAR%) for the major known hyperuricemia risk factors, including obesity, heavy drinking, age, and ABCG2 dysfunction. They calculated a PAR% for ABCG2 dysfunction at 29.2%, almost twice the next greatest contributor to risk, obesity (18.7%) (20). Taken with the high mutant allele frequency [the minor allele frequency of those of European decent is 0.11 (31), for Japanese is 0.31 (31), and for Han Chinese is 0.31 (33)], ABCG2 dysfunction potentially puts hundreds of millions of individuals at increased risk for hyperuricemia and gout as well as hypertension, stroke, and metabolic diseases (7). Further large-scale genetic studies have also added significant support to the critical importance of ABCG2 in uric acid secretion. Kottgen et al. (12) used a GWAS to identify a total of 28 genome-wide significant loci that associated with SUA from a population of 140,000 individuals, predominantly of European origin (12). Of the 28 loci, the ABCG2 loci (rs2231142/ Q141K mutation) resulted in the highest OR for gout risk (1.73) and contributed the largest increases in SUA (0.217 mg/dl) (12).

![Fig. 1. Q141K gout-causing mutation changes NBD structure in a model of ABCG2. A: model comparing Wt and Q141K NBDs as described in Woodward et al. (32) reveals a shift in an adjacent loop (black arrow, Q141K in blue, Wt in green). B: the H155 residue at the top of the loop clashes with the mutant 141K, causing a loop shift and is corrected in the model with a 155A substitution. C: biochemical confirmation of the model shows the Q141K mutant expression levels can be rescued with the secondary 155A substitution. HEK293 cells were transiently transfected with ABCG2 constructs. Total protein was measured and normalized to GAPDH loading control and Wt ABCG2 expression from same Western blot. For details, see Ref. 32. D: summary data. Values are means ± SE, Student’s t-test; n = 4, **P < 0.0001.]

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Recent work has focused on the molecular defect caused by the Q141K mutation of ABCG2 as both a potential therapeutic target and also as a model for understanding the basic structure/function biology of ABC transporters. The Q141K mutation occurs in a residue of the nucleotide-binding domain, a position believed critical for interactions with the intracellular loops of the transmembrane portion of the protein. Interestingly, the Q141 residue is adjacent to F142, a phenylalanine homologous to the F508 of CFTR, another human ABC transporter (ABCC7). This F508 is deleted in >90% of all cystic fibrosis patients, and considerable effort has been made to characterize its molecular defect (16, 21). A comparative analysis between the Q141K ABCG2 mutant and the ΔF508 CFTR mutant reveals striking similarities. Both mutants reduce innate function and result in significant reduction in total and surface expression (32). Both mutants can be corrected with low-temperature incubation techniques (4, 32), and both can be corrected with small molecules like 4-PBA (32). However, there are striking differences as well. The ΔF508 CFTR mutant is characterized by an unstable NBD domain and disruptions in interdomain interactions that lead to errors in protein folding (16, 21). The Q141K ABCG2 mutant appears to only cause instability in the NBD domain. We recently demonstrated that artificially stabilizing the NBD domain of the Q141K mutant corrects the molecular defect. Using either small molecules like the drug VRT-325 (11), or by using the suppressor mutation G188E to enhance NBD sandwich formation (25), we were able to rescue expression, trafficking, and function (32). Interestingly, we found that deleting the F142 residue in ABCG2 did phenocopy the more severe defect found in the homologous ΔF508 CFTR, including disrupted interdomain interactions with a dimerization defect. However, this conclusion remains controversial (32). Saranko et al. (26), in a follow-up study, found that the ΔF142 ABCG2 protein can dimerize when the mutant ABCG2 is expressed in the SF9 insect cell expression system grown at temperatures low enough to rescue misfolded mammalian proteins (4, 32). These conflicting findings need resolution as this may identify critical portions of the protein involved in protein folding and dimerization.

The specific source of the Q141K instability has remained unresolved. Recently, we have found that modeling the Q141K and Wt ABCG2 NBD domains (32) suggested a loop adjacent to the site of the Q141K substitution (see Fig. 1A) appears to be shifted outward, resulting from a clash between the mutant 141K residue and a histidine residue at the top of the loop. Replacing the histidine with an alanine appeared to resolve the shift and also significantly increased total Q141K (H155A) and mature, glycosylated protein abundance (Fig. 1, B–D) when expressed in HEK293 cells.

The study of uric acid in human health and physiology has entered a renaissance. Increased epidemiological data and small clinical trials have marked uric acid as a causal risk factor for hypertension and other important human diseases. These recent implications have been paralleled by the discovery of the molecular identities for many of transporters regulating the SUA levels, including the dominant secretory transporter ABCG2. Moving forward, learning more of how ABCG2 and the other urate transporters are physiologically regulated and the pathophysiology of their disease-causing


