

ABCG2: The molecular mechanisms of urate secretion and gout

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Abstract

High levels of serum uric acid or urate (SUA) is a human 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 (22, 23) but today emerging new genetic and epidemiological techniques have revived an age old debate over whether high uric acid levels (hyperuricemia) independently increases risk for diseases like hypertension and chronic kidney disease (6-8, 29, 34). Part of the mystery of the role uric acid plays in human health stems from our lack of understanding of how humans regulate uric acid homeostasis, an understanding that could shed light on uric acid’s historic role in human adaptation and its present role in human pathogenesis. This review will highlight the recent work to identify the first important human uric acid secretory transporter, ABCG2, and the identification of a common causal ABCG2 variant, Q141K, for hyperuricemia and gout.

Uric acid is a terminal metabolite of the purine metabolic pathway in humans, and is only weakly soluble in water or blood. In the mammalian lineage there appears a concerted selection for decreasing the function of the enzyme that metabolizes uric acid, uricase, an adaptation culminating in the complete loss of uricase function in humans and other great apes (1). The selective advantage of uricase loss and higher SUA levels remains unknown, but even humans can have too much uric acid. The clinical disorder Hyperuricemia, afflicting 43 million Americans, causes gout and urate kidney stones as a direct result of precipitation of urate in the form of monosodium urate crystals in either synovial fluid (gouty arthritis) or in the kidney tubule (uric acid kidney stones)(19). Gout is the best characterized result of hyperuricemia and afflicts 2-3% of Americans (13)[see Richette and Bardin (22) and Mount (19) for recent reviews on gout]. More recent work suggests hyperuricemia increases risk for other human diseases as well. Feig et al. (7) recently argued that hyperuricemia has a causal role in hypertension in part based on a small scale clinical trial of obese adolescents with pre-hypertension and hyperuricemia that when given urate lowering therapy demonstrated a significant reduction of blood pressure (27). On a larger scale, a recent meta-analysis of incident hypertension from 25 studies and 97,824 individuals found that overall SUA levels contributed a relative risk (RR) of 1.35, with an 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 increasing risk for incident CKD (relative risk of CKD was 1.22 per mg/dl of SUA)(34).

Hyperuricemia results primarily from under excretion, 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 a 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 to this theory, including a fourth post-
secretory reabsorption component (24), but the central idea of a balance between reabsorption and secretion determining overall excretion remained. But 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 excellent overview on 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 (28, 30-32).

The story of ABCG2 and uric acid began with an unbiased genetic screening tool, the genome wide association study (GWAS). Dehghan et al. 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 hereto 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. Further, 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 the 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. confirmed much of our findings (15). 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 2 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. further calculated the population attributable risk percent (PAR%) for the major known hyperuricemia risk factors including obesity, heavy drinking, age, and ABCG2 dysfunction (20). 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 100's of millions of individuals at increase 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. used a GWAS to identify a total of 28 genome wide significant loci that associated with serum UA 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).

Recent work has focused on the molecule 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 over 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 ∆F508 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). But there are striking differences as well. The ∆F508 CFTR mutant is characterized by an unstable NBD domain and disruptions in inter-domain 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 ∆F508 CFTR, including disrupted inter-domain interactions with a dimerization defect. However, this conclusion remains controversial (32). Saranko et al., in a follow up study, found that the ∆F142 ABCG2 protein can dimerize (26) 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 1B-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 clinic 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 variants will shed light on the long disputed role of uric acid in human evolution and human health.

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References


Figure 1:
Q141K gout causing mutation changes NBD structure in 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 H155A
substitution, (D) summary data (P<0.0001, Student’s T-Test, n=4 ± SEM). (Methods:
HEK293 cells transiently transfected with ABCG2 constructs. Total protein measured
and normalized to GAPDH loading control and Wt ABCG2 expression from same
western blot, for details see (32)).
**Q141K** and **H155A**

**A.** ICL

**B.** Q141K / Wt ABCG2

**C.** Q141K ABCG2

**D.** Q141K ABCG2 abundance relative to Wt

**GAPDH**