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ABCG2 polymorphisms in gout: insights into disease susceptibility and treatment approaches

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Abstract: As a result of the association of a common polymorphism (*rs2231142*, Q141K) in the ATP-binding cassette G2 (ABCG2) transporter with serum urate concentration in a genome-wide association study, it was revealed that ABCG2 is an important uric acid transporter. This review discusses the relevance of ABCG2 polymorphisms in gout, possible etiological mechanisms, and treatment approaches. The 141K ABCG2 urate-increasing variant causes instability in the nucleotide-binding domain, leading to decreased surface expression and function. Trafficking of the protein to the cell membrane is altered, and instead, there is an increased ubiquitin-mediated proteasomal degradation of the variant protein as well as sequestration into aggresomes. In humans, this leads to decreased uric acid excretion through both the kidney and the gut with the potential for a subsequent compensatory increase in renal urinary excretion. Not only does the 141K polymorphism in ABCG2 lead to hyperuricemia through renal overload and renal underexcretion, but emerging evidence indicates that it also increases the risk of acute gout in the presence of hyperuricemia, early onset of gout, tophi formation, and a poor response to allopurinol. In addition, there is some evidence that ABCG2 dysfunction may promote renal dysfunction in chronic kidney disease patients, increase systemic inflammatory responses, and decrease cellular autophagic responses to stress. These results suggest multiple benefits in restoring ABCG2 function. It has been shown that decreased ABCG2 141K surface expression and function can be restored with colchicine and other small molecule correctors. However, caution should be exercised in any application of these approaches given the role of surface ABCG2 in drug resistance.

Keywords: ABCG2, BCRP, gout, urate, uric acid, polymorphism, allopurinol

Introduction

Gout is an inflammatory arthritis caused by an extremely painful but self-limiting innate immune response to monosodium urate (MSU) crystals deposited in synovial fluid.¹ Without effective management, in some individuals, gout can become chronic with the development of tophi (organized lumps of urate and immune cells²) and permanent bony erosion and disability. Gout is also comorbid with other metabolic-based conditions such as heart and kidney disease and type 2 diabetes.³ An elevated concentration of urate (hyperuricemia) is necessary, but not sufficient, for the development of gout. Host-specific and environmental factors are required for the progression from hyperuricemia to gout. Approximately 30 genetic loci, including *ABCG2*, influence serum urate concentrations,⁴ but less is understood about the genetic control of the formation of MSU crystals and the subsequent inflammatory response.⁵ Urate-lowering therapy is a cornerstone of gout management; however, for various reasons, it is often not

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effective.¹ Despite the availability of a range of cost-effective urate-lowering drugs, there is a need for the development of better therapies for acute gout.

The ATP-binding cassette G2 (ABCG2) protein first came to biomedical attention through its contribution to an ATP-dependent multidrug resistance phenotype in a breast cancer cell line.⁶ It is one of a superfamily of 48 human ABC transporters that, in general, transport a wide array of substrates and are dependent on ATP binding to activate transport. The ABCG2 transporter is one of three well-studied multidrug transporters (others are multidrug resistance protein 1 and P-glycoprotein encoded by the *ABCC1* and *ABCB1* genes, respectively). These transporters export a wide range of compounds, including in the case of ABCG2, uric acid from cells. ABCG2 is a “half transporter” ABC protein, containing one membrane-spanning domain of six transmembrane helices, a N-terminal single intracellular portion containing a nucleotide-binding domain (NBD), and a short intracellular C-terminal tail (Figure 1). “Half transporters” like ABCG2

need to homodimerize to function in vitro but more likely exist in higher-order tetrameric or greater assemblages.^{7,8}

ABCG2, also known as breast cancer resistance protein, is overexpressed in human and animal cell lines resistant to chemotherapeutic drugs^{9,10} and transports anticancer drugs such as mitoxantrone and doxorubicin⁶ (reviewed in reference 11). ABCG2 is important in stem cell biology.^{12,13} Also, the wide expression pattern in normal tissues (Figure 2) suggests that ABCG2 may fill an important physiological role as an excretor of environmental and endogenous toxins, for example, heme.¹⁴ This assertion is supported by the susceptibility of ABCG2 knockout mice to diet-induced phototoxicity and protoporphyria.¹⁵ It is expressed in the small intestine (excretion and limiting absorption), blood–brain and blood–placental barriers (mediating distribution), liver and kidney (elimination and excretion), and mammary gland (transporting into milk).¹⁶ The pattern of ABCG2 expression is consistent with its role as a uric acid efflux transporter (reviewed in reference 17).

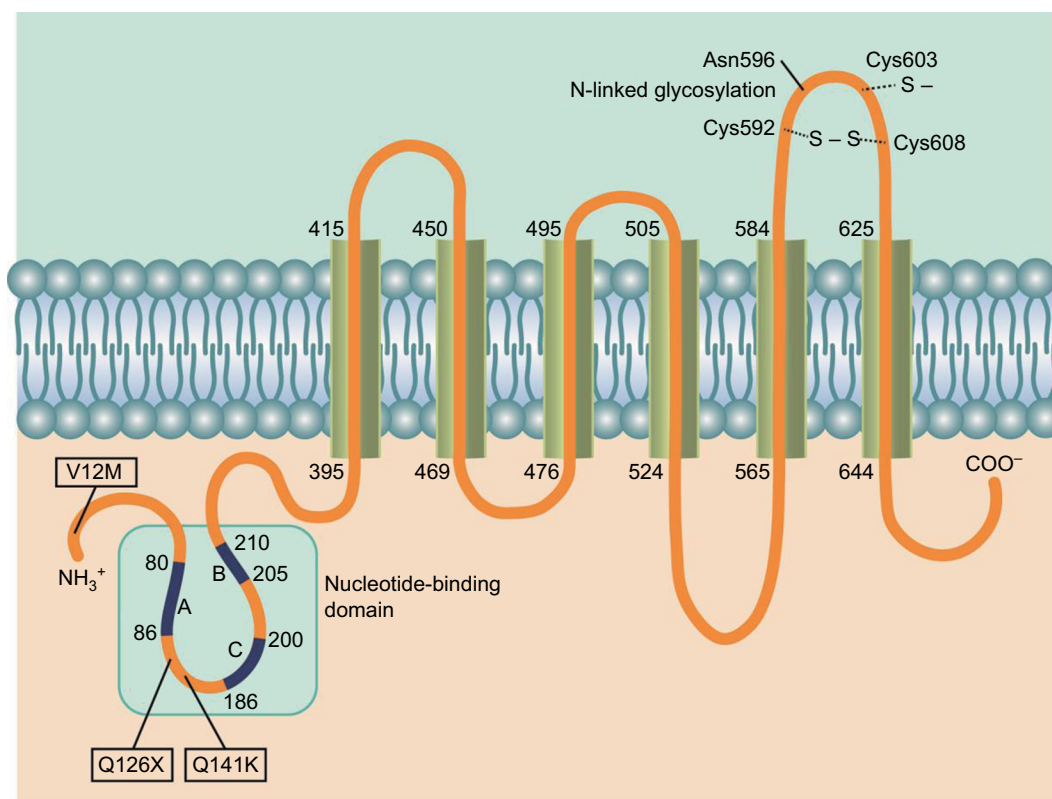


Figure 1 Schematic representation of gout- and hyperuricemia-related polymorphisms in ABCG2. The ABCG2 protein consists of 655 amino acids and can be divided into an intracellular N-terminal domain, a nucleotide binding domain, six transmembrane domains, and a C-terminal end. The nucleotide-binding domain (amino acids 44–288) contains several signature sequences, named Walker A (A), Walker B (B), and the ABC signature sequence (C). The beginning and end amino acid position numbers are given for the signature sequences and the transmembrane domains. The third extracellular loop contains two cysteine residues connected by a disulfide bridge, and a glycosylated asparagine residue, both of which influence stability of the receptor. The cysteine residue at position 603 is important for homodimer formation. The V12M polymorphism is located in the N-terminal domain. The Q126X and Q141K polymorphisms are both located in the nucleotide-binding domain between the Walker A and the ABC signature sequences.

Abbreviation: ABCG2, ATP-binding cassette G2.

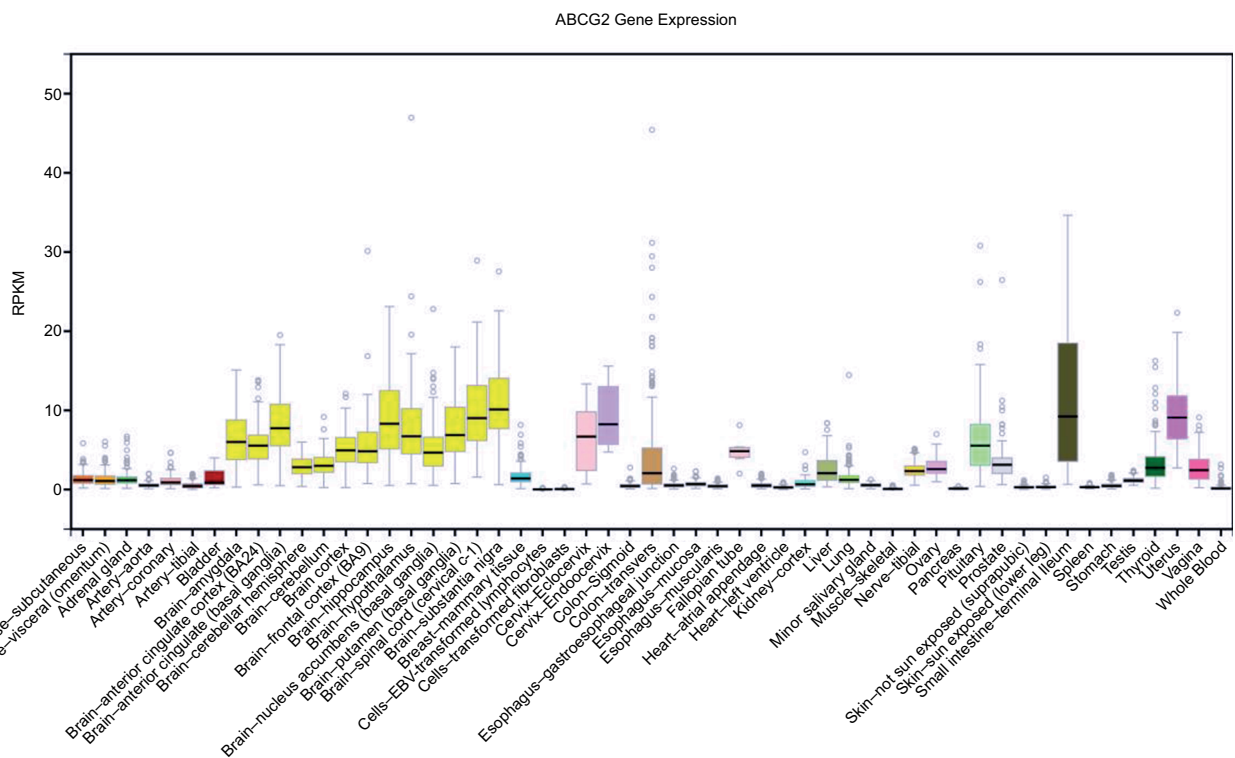


Figure 2 Tissue expression of ABCG2 in humans. Generated from the Genotype Tissue Expression (GTEx) database, derived from deceased people (organ and tissue donors) and surgical donors.

Abbreviations: ABCG2, ATP-binding cassette G2; RPKM, reads per kilobase of exon per million reads mapped.

The ABCG2 transporter was identified as a uric acid efflux transporter only after it was associated with serum urate concentrations in a large genome-wide association study.^{18,19} This review explores the effects of several polymorphisms in the *ABCG2* gene on susceptibility to hyperuricemia, gout, and chronic kidney disease. Furthermore, the role of ABCG2 in inflammatory responses and the possibility of ABCG2 as a treatment target are discussed.

Major ABCG2 genetic variants

The ABCG2 transporter, expressed on erythrocytes, is the basis of a recently explained blood group system, Junior (Jr).^{20,21} Individuals null for ABCG2 produce an ABCG2 alloantibody upon blood transfusion that can cause transfusion reactions, in extreme cases leading to fatal hemolytic disease of the fetus and newborn. The Jr(a-) phenotype is most prevalent in Japan with the most common Jr(a-) mutations being Q126X (Japanese) and R246K (Europeans).^{19,20}

There are only two common ($\geq 1\%$) missense genetic variants in the *ABCG2* gene (V12M; *rs2231137* and Q141K; *rs2231142*) and one common nonsense variant (Q126X; *rs72552713*) that is specific to South East Asian populations (Figure 1). The Q141K and Q126X variants

influence expression levels of ABCG2 and contribute risk to phenotypes such as serum urate levels, gout, and response to the urate-lowering drug allopurinol (Table 1). Numerous other rare ($<1\%$) variants are identified (<http://www.ensembl.org>), some of which have been phenotyped.^{22–28} The most widely studied variant is Q141K, a variant that is clinically relevant and has significant impact on ABCG2 characteristics. It is prevalent in most major population groups with the 141K allele frequency varying between 1% in African and 29% in South East Asian populations. There is consistent evidence for the 141K variant reducing the amount of ABCG2 protein expressed both in vitro^{19,22–25,28,29} and, in vivo, from human liver samples.³⁰

Residue 141 is located in the E-helix³¹ of the NBD of ABCG2, an area critical for the packing of the soluble portion of the protein. The 141K variant reduces ATPase activity,^{26,27} uric acid transport activity, and total and surface abundance (Table 1 and Figure 3A). These defects are believed to be caused by reduced NBD stability.³² Although proteolysis and thermo-stability as assessed by melting point curves of the isolated 141K NBD showed only small differences from the wild type,³³ work done on the whole protein shows evidence of increased NBD instability in mammalian expression

Table 1 Major genetic variation in ABCG2 and impact on protein expression and phenotype

| Population prevalence (EUR/EAS/SAS/AFR) | Overall level of protein expression | Level of apical membrane protein expression | Level of intracellular protein expression | ATPase activity | Transport activity | Sensitivity to proteasomal degradation | Sensitivity to anticancer drugs | Urate | Gout | Tophus | Allopurinol response |
|---|--|---|---|--------------------------|--|--|--|---------------------------------|--|---------------------------------|---|
| V12M (rs2231137) 0.06/0.33/0.15/0.06 | Unchanged ^{22-26,28} | Same ^{25,27,130} Reduced ²⁶ | Same ²⁷ Reduced ²⁶ None ²⁵ | Same ^{26,27} | Same ²⁵ Equivalent ²⁷ | Same ²² | Same ^{23,27,28} Increased ^{d26} | Decreases ^{4,48} | Decreased risk ^{69,70} | Same ⁶⁹ | - |
| Q126X (rs72552713) 0.00/0.01/0.00/0.00 | Null ⁴ | - | - | - | - | - | - | Increased risk ^{70,73} | Increased risk ^{70,73} | - | - |
| Q141K (rs2231142) 0.09/0.29/0.10/0.01 | Same ²⁶ Reduced ^{22-25,28-30,32,36} | Same ^{25,26} Reduced ^{27,32,33,36,130} | None ²⁵ Same ²⁶ Increased ^{26,27,36} | Reduced ^{26,27} | Reduced ^{26,27} | Increased ^{22,29,32,36} | Increased ^{23,26-28} | Increases ^{4,48} | Increased risk ^{47,88,131,132*} | Increased risk ^{69,87} | Increased Resistance ^{67,117} |
| rs10011796 T: 0.39/0.63/0.48/0.27 | - | - | - | - | - | - | - | Increases ⁴ | Increased risk ⁸⁶ | Increased risk ⁸⁷ | Increased Resistance ⁶⁷ Same ¹¹⁷ |

Notes: Phenotype and prevalence information is given to the minor (alternative) allele. All phenotypes are compared to wild-type. Study systems: naturally occurring humans^{4,30,58,67,69,70,73,86,87,117,130-132}; HEK293 (human embryonic kidney) and derivative cells^{27,25,27-29,26}; LLC-PK1 (porcine epithelial kidney cells)^{25,26}; Sf-9 insect cells^{26,27,33}; PA317 mouse fibroblast cells²³; Xenopus oocytes.²² The Sf-9 cells were used for ATPase activity assays. *Meta-analyses.

systems. Stabilizing the 141K variant protein with either suppressor mutations or with small molecules that may bind the NBD directly³⁴ rescues the trafficking, function, and abundance of 141K ABCG2.³² Furthermore, recent modeling of the 141K NBD suggested that a loop adjacent to the Q141K substitution seems to be shifted outward, caused by a potential clash with a histidine residue (H155) at the top of the loop. This potential packing error in the NBD seems to be corrected by a H155A substitution on the 141K background as evidenced by rescued protein abundance.³⁵ The decreased NBD stability of the 141K variant increases the interaction of the protein with the endoplasmic reticulum-associated degradation pathway and subsequent ubiquitin-mediated proteasomal degradation.²⁹ There is also strong evidence that the 141K variant protein may accumulate in aggregates prior to its degradation.³⁶ In addition, protein expression is also influenced by microRNAs (³⁷ and references therein). In one example, the 3' untranslated region truncation allows increased expression of ABCG2, removing an hsa-miR-519c binding site that blocks translation.³⁸ The 141K variant enhances the hsa-miR-519c-mediated repression of translation.³⁷ Interestingly, the ABCG2 F142 residue is in an analogous position to the Δ F508 cystic fibrosis-causing mutation in the cystic fibrosis transmembrane receptor (encoded by *ABCC7*).³² In vitro deletion of F142 from ABCG2 leads to major processing and folding defects with no surface expression,^{32,33} marking the Q141 and F142 regions as a more general ABC transporter mutational "hot spot."

Regulation of ABCG2 expression

The expression of *ABCG2* is regulated by several transcription factors and hormones. A large number of cis-regulatory regions have been identified in or near the promoter region of ABCG2 (reviewed in ³⁹). Hypoxia-inducible factor-1 (HIF-1 α) stabilizes under hypoxic conditions and has been found to bind to a hypoxia-responsive element in the *ABCG2* promoter and increase expression.^{40,41} Upstream of HIF-1 α , signaling via phosphatase and tensin homolog (PTEN), phosphoinositide 3-kinase (PI3K) and protein B kinase (Akt) regulates ABCG2 expression on the cell membrane.⁴²⁻⁴⁵ Furthermore, nuclear factor E2-related factor 2 (Nrf2), a transcription factor involved in activating oxidative stress responses, can induce *ABCG2* transcription⁴⁶ in addition to inducing the expression of peroxisome proliferator-activated receptor gamma (PPAR γ).^{47,48} Finally, the hormone estrogen has been found to up-regulate ABCG2 gene expression.⁴⁹⁻⁵¹ To our knowledge, however, no polymorphisms in any of these regulatory genes have been associated with serum urate

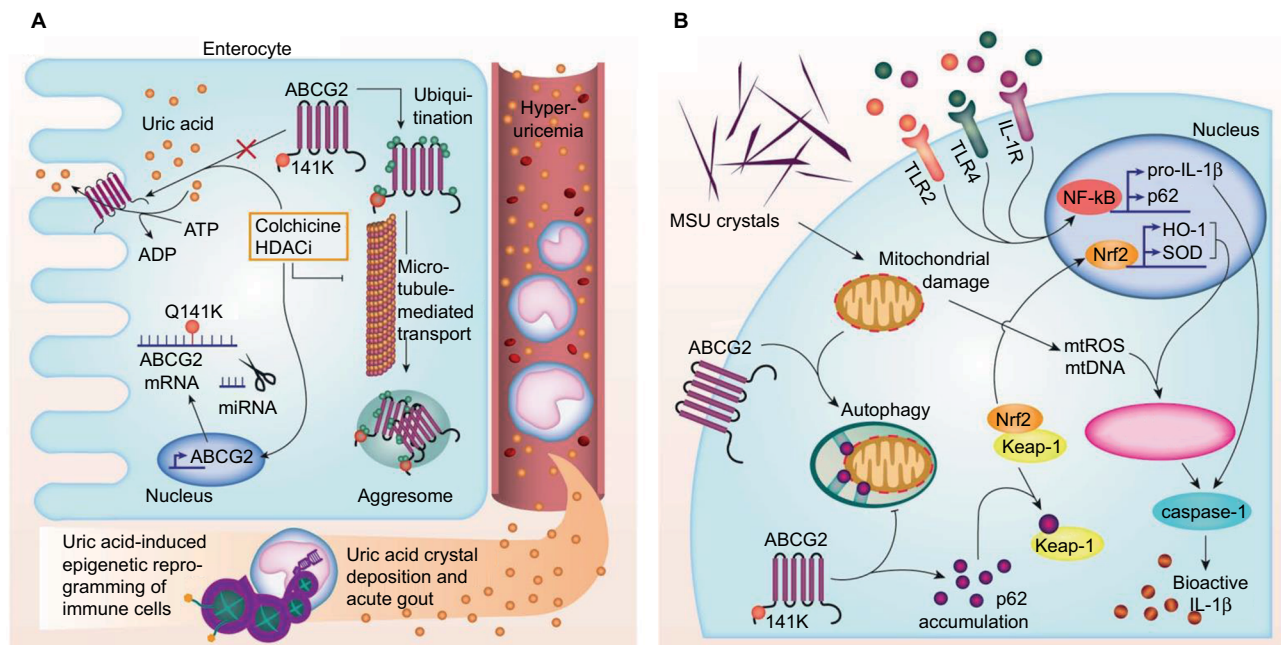


Figure 3 Schematic overview of the roles of ABCG2 in hyperuricemia and gout. **(A)** The ABCG2 transporter is highly expressed in the gut, where it regulates ATP-dependent uric acid excretion. The 141K ABCG2 variant leads to decreased surface expression, targeting of the misfolded protein to aggresomes and proteasomal degradation. In addition, the polymorphism increases miRNA-mediated degradation of mRNA. The resulting increase in circulating urate not only increases the risk of gout but can also induce a more pro-inflammatory state in monocytes due to epigenetic reprogramming.¹²⁹ Colchicine and HDAC inhibitors (HDACi) can restore 141K surface expression and function. **(B)** Monosodium urate crystals can induce mitochondrial damage, leading to the release of mitochondrial reactive oxygen species and DNA (mtROS, mtDNA), which in turn can activate the NLRP3 inflammasome. This can be limited through autophagy-mediated clearance of damaged mitochondria. ABCG2 plays a role in this process. In defective or diminished autophagy, p62 can accumulate, bind to Keap-1, and induce translocation of Nrf2 to the nucleus. Nrf2 induces transcription of heme oxygenase-1 and superoxide dismutase (HO-1, SOD), which can also induce NLRP3 inflammasome activation.

Abbreviations: MSU, monosodium urate; HDACi, histone deacetylase inhibitors; TLR, Toll-like receptor; NLRP3, NBD, leucine-rich family [NLR], pyrin containing 3; ABCG2, ATP-binding cassette G2; IL, interleukin.

levels or gout. However a variant in *PPARGC1B* (*rs45520937*) has been associated with gout in Han Chinese.⁵² *PPARGC1B* encodes the PGC1 β transcriptional repressor, a coactivator for PPAR γ that is involved in estrogen signaling^{53,54} in addition to a key role in maintaining mitochondrial biogenesis.⁵⁵ However the association of *PPARGC1B* with gout has not yet been replicated and it is unknown if *rs45520937* is itself associated with ABCG2 expression. Since reduced mitochondrial DNA copy number is associated with gout⁵⁶ it is possible that systematically linked changes in PGC1 β and associated molecules (including perhaps ABCG2) are factors connecting mitochondrial dysfunction and gout.⁵⁷

ABCG2, urate control, and risk of gout

An individual's serum urate level, like other complex traits, is a function of a suite of inherited genetic variants and environmental exposures.⁵ A genome-wide association study identified the Q141K variant in the *ABCG2* gene as associated with serum urate concentration in people of European ancestry, with the 141K allele associated with increased urate concentration.¹⁸ This genome-wide significant level

of association has been consistently replicated in other genome-wide association studies in people of European and east Asian ancestry^{4,58} but not in people of African-American ancestry.^{59,60} Lack of association in African-Americans in GWAS is likely because of the reduced power because of lower allele frequency in African-Americans ($\leq 3\%$) – there is nominal evidence for the association of the 141K variant with increased serum urate levels in African-Americans¹⁸ and the increased risk of gout.¹⁹ The Q141K variant (and other variants) has been associated with serum urate levels or hyperuricemia in other populations, for example, Tibetan⁶¹ and Korean.⁶² Uric acid transport assays in *Xenopus* oocytes showed that ABCG2 transports uric acid with the 141K variant causing ~50% reduced ability to secrete uric acid.¹⁹ The regulation of ABCG2 protein in epithelial cells is largely unknown. However, similar to other uric acid renal influx transporters, ABCG2 co-immunoprecipitates with the scaffold protein PDZK1,⁶³ suggesting a possible common regulatory pathway.

The magnitude of association of Q141K with serum urate levels is the second strongest genome-wide behind SLC2A9, which is consistently the strongest in Europeans and East

Asians. Each copy of the 141K allele associates with a 0.22 mg/dL increase in serum urate in Europeans⁴ and an 0.14 mg/dL increase in east Asians.⁵⁸ The magnitude is stronger in men than women and is modulated by body mass index (BMI), with the magnitude being stronger in lean people than those who are overweight and obese. The sex difference is most marked in lean people.⁶⁴ The sex difference in association with serum urate levels is an interesting observation given the regulation of *ABCG2* expression by estrogen.^{49–51} Compared to other typical genetic variants controlling complex phenotypes, Q141K has a very large magnitude of association, explaining ~1% of variance in serum urate levels. This compares with ~0.3% for the strongest genetic association with BMI at the *FTO/IRX3* locus.⁶⁵

When associations of other SNPs within the *ABCG2* locus with serum urate levels are tested conditionally on Q141K genotype, there is evidence for a second independent association at the *ABCG2* locus, marked by intronic SNP *rs2622629*.⁶⁶ This second association is of clinical relevance as the *rs2622629*-correlated SNPs (ie those SNPs in linkage disequilibrium) include *rs10011796* ($r^2=0.84$ in Europeans). *Rs10011796* is also implicated in gout and in allopurinol response (Table 1).⁶⁷ The molecular mechanism whereby *rs2622629* (or tightly correlated variants) influence serum urate levels is not known. However, it maps within a DNaseI hypersensitivity cluster of ~150 bp (genome.ucsc.edu) identified by the Encode project (www.encodeproject.org) consistent with the effect of the association being mediated through the control of gene expression and/or mRNA editing. Interestingly, Q141K was not associated with gout in a Han Chinese study of 143 patients,⁶⁸ whereas other variants

in *ABCG2* were, further supporting the existence of multiple gout-associated variants in *ABCG2*.

Aside from Q141K, the only other common (>1%) missense variant in *ABCG2* is V12M situated in a short N-terminal section of *ABCG2* prior to the structurally well-defined NBD (Figure 3). This variant is genetically independent of both Q141K ($r^2=0.002$ in Europeans) and *rs2622629* ($r^2=0.009$ in Europeans). However, the impact on *ABCG2* characteristics are, compared to Q141K, very moderate and inconsistent (Table 1). There are some data on association with serum urate and gout, suggesting an independent role for V12M in urate control and risk of gout (given the genetic independence from Q141K and *rs2622629*). Hence, V12M can be considered a third independent effect on gout in *ABCG2*, after Q141K and *rs10011796/rs2622629*. The largest genome-wide association study in serum urate in Europeans reported an increase in serum urate of 0.077 mg/dL⁴ and in East Asians an increase of 0.108 mg/dL⁵⁸ per copy of the 12V allele. In gout, the common 12V allele consistently confers risk in five separate samples drawn from four ancestral groups: Europeans within the UK Biobank (odds ratio [OR] = 1.24 [T Merriman, unpublished data, October 2016]), Taiwanese Aborigines (OR = 1.36),⁶⁹ Han Chinese in Taiwan (OR = 1.33),⁶⁹ Han Chinese in Shanghai (OR = 1.82),⁷⁰ and New Zealand Māori and Pacific (Polynesian) (OR = 1.31 [T Merriman, unpublished data, October 2016]). Any impact of this variant on the uric acid transport activity of *ABCG2* is currently unknown. A combined meta-analysis is shown in Figure 4. Based on the impact of the V12M variant on *ABCG2* characteristics (Table 1), it is not obvious how the 12V allele increases serum urate levels and risk of gout. It is possible that

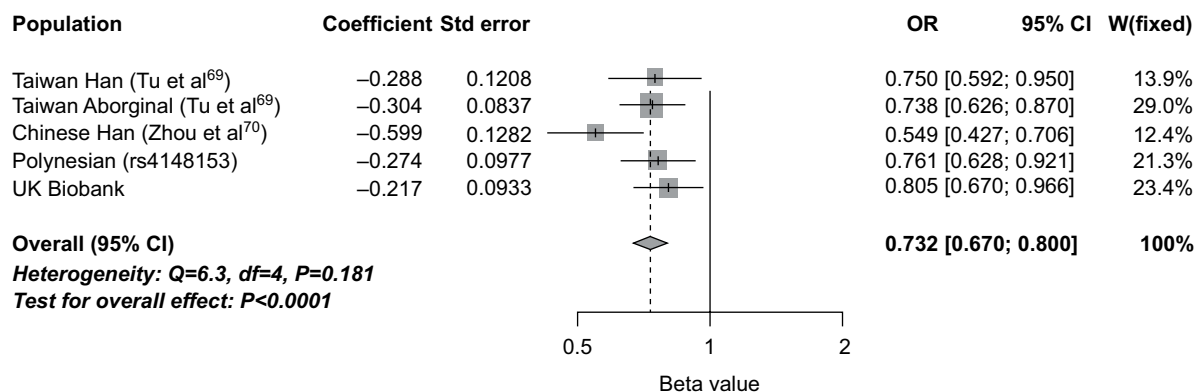


Figure 4 Meta-analysis of association of *ABCG2* V12M (*rs2231137*) with gout: Han Chinese (China),⁷⁰ Han Chinese (Taiwan),⁶⁹ Taiwanese Aboriginal,⁶⁹ New Zealand Polynesian (982 cases and 941 controls), and the UK Biobank (2,422 cases and 152,249 controls). Effect is shown to the minor allele (12M). Variant *rs4148153* is a surrogate marker for *rs2231137* in people of Polynesian ancestry.

Abbreviations: *ABCG2*, ATP-binding cassette G2; OR, odds ratio; CI, confidence interval; Std, standard.

the V12M variant is itself not causal and in genetic correlation (linkage disequilibrium) with a separate causal variant (which is not Q141K or *rs10011796/rs2622629*).

The kidney is the major regulator of serum urate levels, which are determined by the balance of secretion and reabsorption. This is controlled by reuptake transporters such as GLUT9 (*SLC2A9*) and URAT1 (*SLC22A12*) and secretory transporters ABCG2⁷¹ and NPT1/NPT4 (*SLC17A1*, *SLC17A3*),⁷² all of which are genetically associated with serum urate concentration.⁴ Expression of most of these transporters is relatively high in the kidney or, for URAT1, restricted to the kidney. However, expression of ABCG2 is also relatively high in the gut (Figure 2).⁷¹ Recent work in a Japanese gout cohort has demonstrated the role of ABCG2 in uric acid excretion in both the gut and kidney. In this well-defined Japanese population sample, it was possible to create grades of ABCG2 dysfunction based on Q141K and Q126X genotype combinations. Individuals positive for the dysfunctional variants 126X and 141K had the highest serum urate levels and highest risk for gout, and those homozygous for 141Q and 126Q had the lowest serum urate levels and lowest risk for gout.⁷³ ABCG2 dysfunction has been shown to influence extra-renal excretion by correlating with an increase in total urinary uric acid excretion. This results in a renal overload (ROL) type of hyperuricemia.⁷⁴ The presence of 141K (and 126X) alleles reduces excretion through the gut and adds to the circulating urate, overloading the kidney excretion system, resulting in increased urinary uric acid levels. Further study also revealed that ABCG2 dysfunction resulted in a renal under-excretion (RUE) type of hyperuricemia consistent with a critical role in ABCG2-mediated uric acid secretion in the kidney.⁷⁵ Other studies support a primary role for ABCG2 in excretion of uric acid by the gut. A Spanish study demonstrated considerably stronger association of the 141K allele with gout in patients classified as normo-excretors than in those classified as under-excretors,⁷⁶ and two New Zealand studies demonstrated 141K-positive individuals not to be renal under-excretors of uric acid.^{77,78}

Predictably, therefore, ABCG2 Q141K associates with gout in most, but not all, ancestral groups analyzed – European, Han Chinese, Japanese, Korean, African American, Taiwanese Aborigine, and Pacific Islanders but not New Zealand Māori.^{69,79–84} The 126X variant, specific to East Asian populations, confers a higher risk to gout than 141K in Han Chinese and Japanese sample sets.⁷³ In the context that the magnitude of association of *ABCG2* on serum urate concentration is less than that of *SLC2A9*,⁴ it is interesting that the magnitude of association of ABCG2 Q141K on the

risk of gout is consistently larger than that of *SLC2A9*.^{4,5,85} This does not equate with the hypothesis that ABCG2 influences gout solely through contributing to hyperuricemia. Emerging evidence (described in the ‘ABCG2: a regulator of gouty inflammation?’ section) indicates that ABCG2 has pleiotropic effects in gout, contributing to the etiology of gout in the presence of hyperuricemia.

ABCG2: a regulator of acute gouty inflammation?

The key initial checkpoint in gout is hyperuricemia.¹ Further checkpoints are formation of MSU crystals, the acute inflammatory response to these crystals (gout flare), and tophus formation. A gout flare typically presents as an acutely inflamed and extremely painful lower limb joint. The symptoms peak over the first 24 h and then gradually resolve over the next 10–14 days. Flares are usually recurrent, and the patient is well in between flares. In some patients with prolonged hyperuricemia, tophi can develop both in the joint and non-articular tissues; these are collections of MSU crystals surrounded by a chronic inflammatory granulomatous response.²

As described earlier, it is well established from genome-wide association studies that *ABCG2* variants are associated with elevated serum urate concentrations. However, as pointed out above, these studies show that the influence of *ABCG2* variants on serum urate is lower than *SLC2A9* variants. In contrast, *ABCG2* variants are more strongly associated with risk of gout than *SLC2A9*; Köttgen et al⁴ reported ORs for gout of 1.73, $P=1.7\times 10^{-39}$ for *ABCG2* and 1.56, $P=1.9\times 10^{-31}$, for *SLC2A9*. In a more recent analysis,⁸⁶ *ABCG2* was associated with an increased risk of gout; compared with asymptomatic hyperuricemia controls, the OR for gout was 1.83, $P=2.6\times 10^{-14}$, in European people, and was 2.35, $P=3.9\times 10^{-5}$, in Polynesian people. In contrast, *SLC2A9* variants were not associated with increased risk of gout when compared with asymptomatic hyperuricemia controls.⁸⁶ These are intriguing findings that suggest a role for ABCG2 in developing gout once hyperuricemia is established. They do, however, require replication in other sample sets.

ABCG2 variants have also been associated with tophaceous disease in people with gout. In a Taiwanese study of people with gout, 141K was associated with tophi in both Han and Aboriginal Taiwanese populations (OR 1.51 and 1.50, respectively, for the 141K allele, pooled OR 1.55, $P=7.8\times 10^{-5}$).⁶⁹ Recently, a New Zealand study of people with gout has reported that risk alleles for two *ABCG2* SNPs, Q141K and *rs10011796*, were associated with tophi (compared with gout patients without tophi), with effects

observed in participants of Western Polynesian (Samoan, Tongan, Niuean, and Tokelaun) ancestry (OR for *rs2231142* 1.71, $P=0.017$ and for *rs10011796*, 3.76, $P=0.002$) but not in people of other ancestries (European and Eastern Polynesian [NZ Māori, Cook Island Māori]).⁸⁷ The *ABCG2* associations persisted in Western Polynesian people even after accounting for serum urate and disease duration in the regression models. Given that only two studies have thus far associated *ABCG2* with tophus in gout, further studies are required to substantiate this relationship.

These genetic data suggest a role of *ABCG2* in the presentation of gout that is, at least in part, independent of hyperuricemia. Supporting this, cyclosporine inhibits *ABCG2* in vitro,⁸⁸ hyperuricemia and gout can be induced by cyclosporine,⁸⁹ and there is an interaction between cyclosporine and colchicine⁹⁰ (colchicine is commonly used to treat gout attacks and is able to correct the dysfunctional internalization of *ABCG2* 141K³⁶). Collectively, these observations suggest that *ABCG2* may be influenced by off-target effects of cyclosporine. It is possible that *ABCG2* influences other checkpoints, such as MSU crystal formation or the inflammatory response to these crystals. Although the mechanisms of MSU crystal formation are incompletely understood, it is conceivable that *ABCG2* variants lead to preferential transport of urate into the joint (or reduced efflux out of the joint), resulting in higher saturating urate concentrations and MSU crystal formation.

Mechanistic aspects of *ABCG2* in inflammation

A schematic diagram of the role of *ABCG2* in inflammation is shown in Figure 3B. NLRP3 (NBD, leucine-rich family [NLR], pyrin containing 3) inflammasome activation leading to mature interleukin (IL)-1 β release through caspase-1 is a key step in the initiation of the acute gout flare.⁹¹ An initial signal (signal 1) is required to stimulate the expression of pro-IL-1 β and inflammasome components.⁹² Signal 1 includes Toll-like receptor-4 (TLR4) and TLR2 ligands, as well as unsaturated long-chain free fatty acids,^{93,94} and can be inhibited by omega-3 fatty acids and HDL-cholesterol.^{95,96} MSU crystals act as signal 2, inducing NLRP3 inflammasome complex oligomerization and activation of caspase-1.⁹¹ Genes in this system have been associated with gout. A variant in *TLR4*, that likely influences *TLR4* expression, has been associated with gout in two studies.^{97,98} Two SNPs in NLRP3-related genes have been associated with gout.^{99–101} The first one is a nonsense variation in the caspase recruitment domain-containing protein 8 (*CARD8*, *rs2043211*), a negative regulator

of the NLRP3 inflammasome. One of the studies also found a multiplicative interaction between this SNP and an IL-1 β polymorphism (*rs1143623*)¹⁰¹ consistent with a pathogenic model whereby greater inflammasome activity from reduced *CARD8* expression, combined with higher levels of pre-IL-1 β expression, leads to increased production of mature IL-1 β and an amplified immune response. Furthermore, the *PPARGC1B* SNP associated with gout (*rs45529037*) upregulates the expression of NLRP3 and IL-1 β .⁵²

The role of *ABCG2* in regulating inflammation has been described in nonarticular cells. In HEK293 cells cultured with H₂O₂, *ABCG2* inhibited reactive oxygen species generation, enhanced anti-oxidant capacity of the cells, and protected cells from reactive oxygen species-induced toxicity.¹⁰² This may be mediated by the role of *ABCG2* in exporting urate, an established pro-oxidant in the intracellular setting¹⁰³ by mechanisms that include promotion of mitochondrial dysfunction.¹⁰⁴ In animals, *ABCG2* also inhibited NF- κ B and the expression of the inflammatory gene IL-8 induced by reactive oxygen species.¹⁰² In rheumatoid arthritis, a chronic autoimmune inflammatory arthritis, *ABCG2* is highly expressed in the intimal lining layer and on macrophages and endothelial cells in the synovial sub-lining layer.¹⁰⁵ The synovium is also a very important tissue in gouty arthritis. In several cell culture systems, the expression and function of *ABCG2* is reduced when stimulated with pro-inflammatory cytokines.^{106–108} Collectively, these results suggest both a suppressive effect of *ABCG2* on pro-inflammatory signaling pathways and that *ABCG2* is itself a target of pro-inflammatory cytokines, where its expression and activity is suppressed. To date, the influence of *ABCG2* variants on the inflammatory response to MSU crystals has not been reported, although genetic and other data suggest a role for the 141K variant in promoting gout once hyperuricemia is established (see the ‘*ABCG2*: a regulator of gouty inflammation?’ section).

ABCG2 and autophagy

In addition to its effects through increased serum urate concentration and the inflammatory responses described earlier, *ABCG2* has been reported to play a role in autophagy. In a study by Ding et al,¹⁰⁹ *ABCG2* overexpressing cell lines were more resistant to stressors such as nutrient deprivation due to increased autophagy. The authors observed more degradation of autophagy receptor p62 and increased accumulation of LC3-II, a protein essential for autophagosome formation and function. Knockdown of *ABCG2* in these cells subsequently abolished these effects.¹⁰⁹ It was shown previously that effective autophagy is essential in acute gouty arthritis

to clear mitochondria that have been damaged by MSU crystals. This prevents the release of mitochondrial DNA and the production of reactive oxygen species, which can activate the NLRP3 inflammasome.^{110,111} When autophagy is not effective, p62 accumulates, causing nuclear factor E2-related factor 2 (Nrf2), a transcription factor involved in oxidative stress responses, to be released from its repressor, kelch-like ECH-associated protein 1 (keap-1), and translocate to the nucleus. Previously, Nrf2 has been shown to induce transcription of heme oxygenase-1 and superoxide dismutase, which can in turn also activate the NLRP3 inflammasome.¹¹² Finally, ABCG2 expression has been shown to be upregulated by Nrf2, which promotes cancer stem cell survival.⁴⁶ Taken together, ABCG2 plays a role in autophagy and its expression is induced through Nrf2. Decreased or a lack of ABCG2 function could therefore induce or prolong NLRP3 inflammasome activation in acute gout through defective autophagy of damaged mitochondria in response to MSU crystals.

ABCG2 as a drug target

Restoring the expression and function of ABCG2 molecules that harbor detrimental genetic polymorphisms may be an important next step to limit urate levels and inflammatory responses. Recently, several papers have shown that histone deacetylase (HDAC) inhibitors can restore the function of the 141K ABCG2 variant. Woodward et al demonstrated that the HDAC inhibitor 4-phenylbutyrate restores trafficking and dimer expression of 141K ABCG2.³² Basseville et al showed that several different HDAC inhibitors, as well as colchicine, inhibit targeting of 141K ABCG2 to the aggresome and promote relocalization on the cell surface.³⁶ Colchicine is an anti-inflammatory agent that acts by inhibiting microtubule polymerization through binding to tubulin. This suggests that HDAC inhibitors, as does colchicine, inhibit trafficking of ABCG2 to the aggresome along microtubules. Finally, in addition to restoring surface localization of 141K ABCG2, the HDAC inhibitor romidepsin was shown to increase gene transcription of ABCG2 by activating the aryl hydrocarbon pathway.¹¹³ Recently, small molecules that are effective at rescuing the trafficking, abundance, and function of the 141K variant protein have been found. The compounds VRT-325 and 4a, both from the corrector class of molecules found in efforts to rescue mutant CFTR protein, are effective rescue agents for mutant ABCG2 as well.³² These results should encourage further research into the use of less toxic small molecules than HDAC inhibitors to restore defective ABCG2 function in patients with the 141K polymorphism.

ABCG2 and allopurinol response

Allopurinol is the most commonly used urate-lowering therapy for people with gout. It is rapidly metabolized to oxypurinol which is responsible for the majority of the urate-lowering effect through the inhibition of xanthine oxidase. Despite widespread use of allopurinol, many people fail to achieve the recommended target serum urate of <0.36 mmol/L. For example, in the Febuxostat versus Allopurinol Controlled Trial, only 21% of those receiving allopurinol 300 mg daily achieved the primary endpoint of the last three serum urates being ≤ 0.36 mmol/L.¹¹⁴ Although poor adherence and restricted dosing based on kidney function are frequently associated with failure to reach target serum urate, genes encoding the enzymes involved in the metabolism, mechanism of action, and excretion of allopurinol may also have a role.¹¹⁵

Most studies related to the pharmacogenetics of allopurinol have focused on adverse events rather than predicting efficacy.¹¹⁶ However, two recent studies have highlighted a potential role for ABCG2 in allopurinol response. A significant association between ABCG2 141K and reduced allopurinol response was initially identified in a genome-wide association study.⁶⁷ However, the definition of response used in this study was allopurinol-related change in serum urate, and there was only assessment of adherence with allopurinol using administrative data. A subsequent study, in which poor response was defined as serum urate ≥ 0.36 mmol/L despite allopurinol >300 mg daily, and with adherence confirmed by plasma oxypurinol concentrations, replicated the finding with 141K conferring a significantly increased risk of poor response to allopurinol (OR 2.71 (1.70–4.48), $P=6.0 \times 10^{-5}$).¹¹⁷ This effect remained significant after adjustment for various variables that influence serum urate and allopurinol response including age, sex, BMI, ethnicity, eGFR, diuretic use, and serum urate off urate-lowering therapy. The association between ABCG2 Q141K and allopurinol response is a very interesting observation that should be further replicated in further studies.

The mechanism by which ABCG2 modulates allopurinol response is not clear. Wen et al⁶⁷ reported ABCG2 to be an efficient allopurinol and oxypurinol efflux pump. HEK 293 cells transfected with 141K had significantly impaired ABCG2 function leading to intracellular accumulation of allopurinol and oxypurinol.⁶⁷ These authors suggested that the ABCG2 141K variant might impair allopurinol response by increasing allopurinol and oxypurinol concentrations in renal tubular cells and decreasing concentration in the tubule fluid, thereby reducing the inhibition of uric acid influx pumps within the kidney. However, this theory is unproven, and modeling the complexity of the relative contribution

of reabsorbed allopurinol¹¹⁸ versus nonsecreted allopurinol is extremely difficult; therefore, *in vivo* experiments are required to further explore this possible mechanism.

ABCG2 and chronic kidney disease

It is clinically established that chronic kidney disease is a cause of hyperuricemia and gout. However, the physiological response to disrupted renal uric acid handling in kidney disease is a question of clinical interest. Bhatnagar et al¹¹⁹ addressed this question by evaluating the strength of genetic association with urate of variants in a suite of genes encoding uric acid transporters (including *SCL2A9*, *ABCG2*, *SLC17A1-A3*, and *SLC22A11-A12*) in 3,598 individuals with chronic kidney disease. In contrast to the situation in healthy individuals where *SLC2A9* has the strongest magnitude of association on serum urate levels (0.37 mg/dL increase in urate per risk allele compared with 0.22 mg/dL for *ABCG2* in sex-adjusted analysis),⁴ each *ABCG2* risk allele increased serum urate by 0.68 mg/dL in people of European ancestry, compared with 0.30 mg/dL for *SLC2A9* in sex-adjusted analysis.¹¹⁹ Although this finding requires replication, it does suggest that the extra-renal (gut) urate handling of *ABCG2* necessarily compensates in renal injury.¹¹⁹ This is consistent with the observation, in nephrectomized rats, of increased gut expression of *ABCG2* and maintenance of normal serum urate concentration.¹²⁰ Bhatnagar et al¹¹⁹ evoke the “remote sensing and signaling” hypothesis whereby the action of multi-specific SLC and ABC transporters is critical in disrupted homeostasis resulting from organ injury.¹²¹ The hypothesis posits that the transporters cooperate in sensing and controlling fluctuations in important substances, with the ABC (and SLC) transporters facilitating interorgan communication through small molecules,¹²¹ including those important in oxidant status such as urate.¹²² One specific example of this is the ability of *ABCG2* to transport and transfer heme, through an extracellular loop, to albumin.¹²³

In a study of fructose-induced hyperuricemia in humans, the urate-increasing 141K allele was associated with a lower increase in serum urate but greater increase in fractional excretion of uric acid when compared with the 141Q allele,⁷⁸ consistent with the previously reported ROL type of hyperuricemia. Supporting *ABCG2* as a “remote sensor and signaller,” the 141K allele was associated with a lower glucose response to the fructose load.⁷⁸ Whether or not the urate and glucose effects are related is unclear; however, the remote sensing hypothesis could relate to differential ATP utilization by the 141Q and 141K alleles given that the 141K variant has significantly reduced ATPase activity.³²

Hyperuricemia predicts reduced renal function and chronic kidney disease, independent of measured confounders, in observational studies.¹²⁴ However, whether or not urate is causal of reduced renal function is unresolved. Mendelian randomization studies, in which urate-associated genetic variants inherited at conception are used as an un-confounded proxy for exposure to urate,¹²⁵ suggest that urate is not causal of reduced renal function in healthy people¹²⁶ but could promote progression of declining renal function in chronic kidney disease.¹²⁷ Based on the observation in mice that uric acid may compete with toxins as an efflux substrate of *ABCG2*, Dankers et al postulate that, in the kidney, hyperuricemia promotes reduced renal *ABCG2*-mediated secretion of toxic molecules.¹²⁸

Conclusion

The *ABCG2* transporter is an important molecule in urate excretion. Decreased *ABCG2* expression and function due to genetic polymorphisms leads to both ROL hyperuricemia and RUE hyperuricemia. The most extensively studied genetic variant is Q141K. Besides significantly increasing serum urate concentration, the 141K *ABCG2* variant has also been associated with acute gout, tophaceous gout, and poor allopurinol response. In addition, 141K-induced hyperuricemia may lead to excessive inflammatory responses and decreased *ABCG2* function may cause defective autophagy. All of these effects warrant further research to the restoration of 141K *ABCG2* function and surface expression, for example, by small molecules.

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Disclosure

The authors report no conflicts of interest in this work.

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