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ORIGINAL ARTICLE

Analysis of ABCG2 and other urate transporters in uric acid homeostasis in chronic kidney disease: potential role of remote sensing and signaling

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Abstract

Background: In the setting of chronic kidney disease (CKD), altered extra-renal urate handling may be necessary to regulate plasma uric acid. The Remote Sensing and Signaling Hypothesis (Nigam S. What do drug transporters really do? *Nat Rev Drug Discov* 2015; 14: 29–44) suggests that multispecific solute carrier (SLC) and ATP-binding cassette (ABC) drug transporters in different tissues are part of an inter-organ communication system that maintains levels of urate and other metabolites after organ injury.

Methods: Data from the Chronic Renal Insufficiency Cohort (CRIC; $n = 3598$) were used to study associations between serum uric acid and single nucleotide polymorphisms (SNPs) on the following uric acid transporters: *ABCG2* (*BRCP*), *SLC22A6* (*OAT1*), *SLC22A8* (*OAT3*), *SLC22A10* (*OAT5*), *SLC22A11* (*OAT4*), *SLC22A12* (*URAT1*), *SLC22A13* (*OAT10*), *SLC17A1-A3* (*NPTs*), *SLC2A9* (*GLUT9*), *ABCC2* (*MRP2*) and *ABCC4* (*MRP4*). Regression models, controlling for principal components age, gender and renal function, were run separately for those of European (EA) and African ancestry (AA), and P-values corrected for multiple comparisons. A twin cohort with participants of EA and normal renal function was used for comparison.

Results: Among those of EA in CRIC, statistically significant signals were observed for SNPs in *ABCG2* (rs4148157; beta-coefficient = 0.68; P = 4.78E-13) and SNPs in *SLC2A9* (rs13125646; beta-coefficient = –0.30; P = 1.06E-5). Among those of AA, the strongest (but not statistically significant) signals were observed for SNPs in *SLC2A9*, followed by SNPs in *ABCG2*. In the twin study (normal renal function), only SNPs in *SLC2A9* were significant (rs4481233; beta-coefficient=–0.45; P = 7.0E-6). In CRIC, weaker associations were also found for *SLC17A3* (*NPT4*) and gender-specific associations found for *SLC22A8* (*OAT3*), *SLC22A11* (*OAT4*), and *ABCC4* (*MRP4*).

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Conclusions: In patients of EA with CKD (CRIC cohort), we found striking associations between uric acid and SNPs on *ABCG2*, a key transporter of uric acid by intestine. Compared with *ABCG2*, *SLC2A9* played a much less significant role in this subset of patients with CKD. SNPs in other *SLC* (e.g. *SLC22A8* or *OAT3*) and *ABC* (e.g. *ABCC4* or *MRP4*) genes appear to make a weak gender-dependent contribution to uric acid homeostasis in CKD. As renal urate transport is affected in the setting of declining kidney function, extra-renal ABCG2 appears to play a compensatory role—a notion consistent with animal studies and the Remote Sensing and Signaling Hypothesis. Overall, the data indicate how different urate transporters become more or less important depending on renal function, ethnicity and gender. Therapies focused on enhancing ABCG2 urate handling may be helpful in the setting of CKD and hyperuricemia.

Key words: ABCG2, CKD, gastrointestinal transport, sex interaction, SLC2A9

Introduction

Alterations of serum uric acid are a common metabolic abnormality associated with diseases such as gout, kidney stones, metabolic syndrome, hypertension, cardiovascular disease and progressive chronic kidney disease (CKD). A number of studies, both clinical and basic, have implicated solute carrier (SLC) and ATP-binding cassette (ABC) transporters in uric acid handling [1–4]. Many of these transporters are also considered 'drug' transporters. Putative uric acid transporters include: ABCG2 (BCRP), SLC2A9 (GLUT9), SLC22A12 (URAT1; originally identified as Rst in mice), SLC17A1-3 (NPTs), SLC22A6 (OAT1), SLC22A8 (OAT3) and SLC22A11 (OAT4) [1–17].

While *in vitro* transport studies and murine knockouts may serve as a guide, the physiology in humans may be substantially different [18]. Furthermore, little is known about coordination between uric acid transporters, including the potential role of non-renal transporters, or the relative importance of various transporters in specific patient populations. Indeed, much of the uric acid is excreted by the kidney [1–4], yet uric acid homeostasis in the setting of the failing kidney has not been extensively studied. While SLC2A9 has been identified as the main uric acid transporter in general cohorts, the role of SLC2A9 and other SLC and ABC transporters in patients with CKD has not been studied. The Remote Sensing and Signaling Hypothesis proposes that multispecific SLC and ABC 'drug' transporters in different tissues are part of an inter-organ and inter-organismal communication network that maintains levels of uric acid, metabolites and signaling molecules in the setting of acute or chronic injury to organs such as the kidney, or other organs [3]. Thus, in the setting of CKD, where uric acid handling by renal urate transporters can be compromised, extra-renal urate transporters, such as ABCG2 in the intestine, may play a more prominent role in uric acid homeostasis. This notion is supported by animal studies [5].

This study explored associations between serum uric acid and single nucleotide polymorphisms (SNPs) in a number of *SLC* and *ABC* genes (proven to regulate uric acid *in vitro* or *in vivo* in human and/or mouse) in a well-characterized cohort of 3591 patients with CKD from the Chronic Renal Insufficiency Cohort (CRIC) study [19]. A twin study ($n = 481$) with participants with hypertension but normal renal function was used as comparison [20, 21]. The African American Study of Hypertension and Kidney Disease (AASK; $n = 508$), a cohort with early renal insufficiency, was used as secondary sample for African Americans [22]. Our results support the view that, as renal function declines, extra-renal ABCG2, most likely in the intestine, compensates to maintain uric acid homeostasis. The results are consistent with animal models of renal decline [5] and support the relevance of the Remote Sensing and Signaling Hypothesis for understanding metabolic disease in humans [3].

Materials and methods

CRIC study cohort

The CRIC study was established by the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) to examine risk factors for the progression of CKD and cardiovascular disease in patients with CKD [19]. Data were obtained through authorized access to dbGaP and the NIDDK data repository. Seven clinical centers recruited adults with CKD based on age-based estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) and included women (46%), Caucasians (45%), non-Hispanic Black (46%) and Hispanic (5%); 47% also had diabetes. Participants ($N = 3591$ with genotype data) were between ages 21 and 74 years, with a median age of 58 years. Blood and urine specimens were collected, and information regarding health behaviors, diet, quality of life and functional status was obtained at baseline. Serum uric acid in mg/dL was determined by standard laboratory procedures (uricase/peroxidase enzymatic method; DAX 96; Bayer Diagnostics, Milan, Italy). Baseline measures included renal function, medications and age [23].

Genetics

Genotyping was carried out using the Illumina HumanOmni1-Quad_v1-0_B platform [19]. Gene regions chosen for this study were selected based on previous genome-wide association study (GWAS) of uric acid transporters, including both *in vitro* and animal studies. Variants in Hardy–Weinberg equilibrium (HWE) with a minor allele frequency (MAF) ≥0.05 in European ancestry (EA) and African ancestry (AA) and within 30 kb upstream or downstream of the following genes were investigated: *ABCG2*, *SLC22a12*, *SLC22A6*, *SLC22A8*, *SLC22A13*, *SLC17A1*, *SLC17A2*, *SLC17A3*, *SLC22A9*, *ABCC2* and *ABCC4*. Based on the number of SNPs (980), the significance level used to assess single SNP association after Bonferroni correction for multiple comparison was P = 5.10E-5. Population allele frequencies and linkage disequilibrium (LD) were assessed using Haploview (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview). The strength of association and correlations between SNPs for significant genes were generated using LocusZoom [24]. Correlations were imputed using 1000 Genomes Project, March 2012 (www.1000genomes.org); because of differences in linkage structure, correlations were imputed by EA and AA.

Statistics

Screening/discovery associations

An initial screen for associations between uric acid and SNPs was run with PLINK, an online software program used for genome-wide associations and/or to control genetic associations for

population stratification using a multi-dimensional scaling (MDS) analysis [25]. Data were run separately for those of EA and AA and controlled for principal components age, sex, diuretic, allopurinol, eGFR and a diagnosis of diabetes. Based on previous literature suggesting sex specificity with respect to SLC transporters [26–28], the three most significant SNPs from the discovery analysis were also screened for interactions with sex using PLINK. Models were stratified by sex if the interaction term (SNP*SEX) was significant ($P \leq 0.05$).

Individual SNPs

The most significant SNPs for CRIC were further analyzed with SAS (V 9.1 SAS Institute, Inc., Cary, NC, USA) or STATA (V10, College Station, TX, USA). Multivariate linear regression models were used to determine beta-coefficients and confidence intervals for significant SNPs controlling for population stratification, age, gender, diabetes and eGFR.

Power calculations

Post hoc power calculations, done using G*Power (http://www.gpower.hhu.de/), indicated adequate power to detect relatively small effects in CRIC. Estimates were based on a dominant/recessive genetic model using a two-sided *T*-test and an alpha level of 5.1×10^{-5} (Bonferroni adjustment for multiple comparisons). Among those of EA ($n = 2086$), the CRIC study had ~80% power to detect a 0.35 mg/dL difference in serum uric acid (an estimated small effect size of 0.19, Cohen's D statistic) for SNPs with a MAF of 0.10; among those of AA ($n = 1505$), there was ~80% power to detect a 0.45 mg/dL difference in uric acid (small effect size ~0.25). For rare alleles with a MAF of 0.05, there was ~80% power to detect a 0.38 mg/dL difference (small effect size ~0.21) and 0.45 mg/dL difference (small effect size ~0.25) for EA and AA, respectively.

Twin study

This study population has been described previously [20, 21]. Participants were recruited from the southern California Twin Registry [20]. Additional participants were recruited by newspaper advertisement [21]. The twin cohort included 260 nuclear families (mean size = 2.4, range: 1–9), 60 dizygotic and 161 monozygotic twin pairs. Zygosity was confirmed by microsatellite and SNP markers. For this analysis, individuals of Caucasian (European-American, $n = 535$) or Hispanic (Mexican-American, $n = 81$) ancestry/ethnicity were included ($N = 616$). Ethnicity was based on self-identification and geographic origin of parents and all four grandparents. Age ranged from 14 to 78 years with a median of 39 years. The majority was female (442 females and 174 males).

Genetics

A subset of 481 subjects was genotyped on 592 312 SNPs using the Illumina 610 Quad genotyping array. For each of the 161 monozygotic twin pairs, only one individual was genotyped and the genotype information was used for both twins. As an additional quality control step, unlikely genotypes based on expected inheritance patterns were removed using Merlin's Pedwipe procedure. Imputation was performed using MACH v1.0.16 (http://www.sph.umich.edu/csg/abecasis/MACH/) with the phased haplotypes of 60 HapMapII CEU founders as reference data. A total of 2 028 122 high-confidence SNPs in LD ($r^2 \geq 0.3$) with original genotyped markers were imputed. There were 1256 SNPs on genes listed above in HWE with MAF ≥ 0.01 that were selected for further analysis.

Statistics

Screening/discovery associations

Genetic background heterogeneity was controlled by MDS using PLINK [25]. Association analysis between SNPs and serum uric acid was done using Merlin v1.1.2 (http://www.sph.umich.edu/csg/abecasis/merlin/). Family relatedness and twin status was controlled for using a maximum likelihood estimation test of variance components incorporating a variance-covariance matrix. In addition, age, gender and the first MDS component were included as covariates.

Power calculations

For the twin study, a power calculation using the genetic power calculator for quantitative trait locus (QTL) association in sibships showed that we were adequately powered (>85% power) to detect the observed variance explained (H2 = 5.55), assuming an additive genetic model, perfect LD between rs4481233 and the QTL, 230 families and 1256 SNPs tested.

AASK study

Because of limited power for African Americans in CRIC, data from the African American Study of Kidney Disease and Hypertension (AASK) were used to increase the sample size. Here, SNPs most significantly associated with uric acid from CRIC were studied. AASK randomized 1094 African American men and women with early hypertensive nephrosclerosis to one of three classes of anti-hypertensive medications. Details of this study have been published [22]. Approximately 41% of this study cohort was female, with an average age of 54 years and mean eGFR of 47 mL/min/1.73 m^2. Baseline serum uric acid was drawn prior to randomization. A subset of this cohort with adequate DNA had a genome-wide scan, using the Affymetrix Genome-Wide Human SNP Array 6.0. After quality control measures, there were 508 successful samples from which data were provided for this study. Associations between SNPs and uric acid were controlled for baseline diuretic use, age and baseline eGFR.

Results

We first briefly describe the overall approach. The uric acid transporter genes that we focused on have previously been shown to function in uric acid handling by *in vitro* assays (e.g. microinjected *Xenopus* oocytes, transfected cells, renal slices), body fluid analysis of knockout mice, on Mendelian conditions affecting uric acid and/or by GWAS. In many cases, the SLC (e.g. SLC2A9) and ABC (e.g. ABCG2) transporters we considered have been validated in several of these kinds of studies and have been recently reviewed [2–4].

The goal was to examine the relative importance of various renal and non-renal urate transporters in the setting of declining renal function. One hypothesis, based on animal physiological data indicating that intestinal ABCG2 transport becomes much more important in the setting of 5/6th nephrectomy [5] was that, in patients with CKD, SNPs in *ABCG2* will be more significant. We sought to examine this in CKD and patients with normal renal function, separately analyzing those of EA and AA.

After an initial screening/discovery analysis in PLINK by EA and AA, multivariate regression models were used to estimate adjusted effects for most significant SNPs with confidence intervals. As power was limited for AA, data from AASK were used to

augment the sample size. A cohort of twins was used as a comparison in individuals with normal renal function. For each of the genes studied here, the first three SNPs most strongly associated with serum uric acid from initial screening in PLINK are shown in Table 1 for CRIC and Table 2 for the twin data set. Results for individual SNPs are shown in Table 3.

CRIC

The strength of associations between serum uric acid and SNPs on *ABCG2* and *SCL2A9* are shown in Figure 1.

European ancestry

Among those of EA, by far the strongest signals were for SNPs in *ABCG2*. This was followed by *SLC2A9*—the latter gene being about eight orders of magnitude less significant than *ABCG2* (~E-13 versus ~E-5). The most significant SNP, *ABCG2* rs4148157 (P = 4.78E-13, beta-coefficient = 0.68) had MAF of 0.10 (Table 1). Serum uric acid was 6.99 [standard deviation (SD): 1.85] mg/dL and 7.64 (SD: 2.05) mg/dL by *ABCG2* rs4148157 GG and GA/AA genotypes (Table 3; unadjusted P < 0.001, not shown). Multivariable regression estimated an adjusted 0.64 mg/dL increase [95% confidence interval (CI): 0.47 to 0.81] in uric acid by *ABCG2* rs4148157 genotypes (GA/AA compared with GG; P < 0.001, Table 3).

Top signals in *SLC2A9* were for SNPs with more common allele frequencies (MAF ≥ 0.20); rs13125646 was the most significant SL2A9 SNP (P = 1.06E-5, beta-coefficient = −0.30; Table 1). Serum uric acid was 7.21 (SD: 1.87) mg/dL, 7.04 (SD: 1.93) mg/dL and 6.86 (SD: 2.08) mg/dL by GG, GA and AA genotypes, respectively (Table 3; unadjusted P = 0.001, not shown). In comparison with GG, multivariate regression estimated a 0.25 mg/dL (95% CI: −0.40 to −0.10; P = 0.001) decrease in uric acid with GA and 0.51 mg/dL (95% CI: −0.81 to −0.22; P = 0.001) decrease in uric acid with AA genotypes (Table 3).

SNPs on *SLC17A3*, *SLC22A10*, *SLC22A8*, *SLC22A11* and *ABCC4* were nominally significant at P ≤ 0.05 (Table 1).

African ancestry

There were no significant SNPs after correction for multiple comparisons among AA (P ≤ 5.10E-5). However, SNPs in *SCL2A9* had the strongest signals, followed by SNPs in *ABCG2*. There was some association at P ≤ 0.05 for SNPs on *SLC17A3*, *SLC22A8* and *ABCC4* (Table 1).

Twin study

After correcting for multiple comparisons, only SNPs in *SLC2A9* were significantly associated with serum uric acid (most significant rs4481233, P = 7.01E-6; beta-coefficient = −0.45; Table 2). Although of marginal significance, associations with *SLC17A1-17A3* (rs11969868, P = 0.014), *ABCG2* (rs2231146, P = 0.029) and *ABCC4* (rs4148499, P = 0.049) were also observed among the twins. Of note, *SLC22A11* (rs3759053, P = 0.016), not observed in CRIC, was also nominally significant (Table 2).

ABCG2 and *SLC2A9* associations among AA (CRIC and AASK combined)

As power was limited for those of AA in the CRIC dataset, CRIC data were combined with AASK, a cohort of African Americans with renal insufficiency. Only the most significant SNPs from CRIC (EA or AA) were studied here. If the same SNP was not available, a SNP in linkage disequilibrium was chosen. Results for multivariate regression models adjusted for sex, age, baseline eGFR, diuretic use and study (CRIC or AASK) for are shown in Table 4.

ABCG2 rs4148157

Genotype data were available *for ABCG2* rs4148157, by far the most significant SNP in CRIC among those of EA. Results were similar in the combined CRIC-AASK data for those of AA. Serum uric acid was higher with GA/AA genotypes in comparison with GG genotypes: 8.20 (SD: 2.0) mg/dL versus 7.87 (SD: 1.91) mg/dL (Table 4; P = 0.063, not shown). Multivariable regression estimated a 0.41 mg/dL (95% CI: 0.10 to 0.72) increase in uric acid with the G minor allele (Table 4; P = 0.0095).

SLC2A9 rs13111638, rs6449213 and rs4481233

Three SLC2A9 SNPs available in AASK and CRIC were in LD with rs13125646—the strongest signal observed among those of EA in CRIC (Table 4). There was a 0.32 mg/dL (95% CI: −0.49 to −0.14) decrease with the *SLC2A9* rs13111638 minor allele (P = 0.0004), similar to the results observed in EA for rs13125646 in CRIC. Results for the other two *SLC2A9* SNPs, rs6449213 and rs4481233, in close LD with rs13111638, were similar (Table 4).

Effect modification by sex in CRIC

Interactions between SLC and ABC genotypes and sex are shown in Table 5. Sex was an effect modifier for *SLC2A9* rs3775948 (P = 0.0033, interaction term), *SLC2A9* rs1014290 (P = 0.0079), *SLC22A8* rs3793961 (P = 0.0220), *SLC22A11* rs17300741 (P = 0.0003) and ABCC4 rs1751003 (P = 0.0023). Minor alleles at *SLC2A9 rs3775948* (P < 0.001) and *rs1014290* (P < 0.001) and *SLC22A11* rs17300741 (P = 0.0002) were associated with lower serum uric acid among women only. Conversely, minor alleles on *SLC22A8* rs3793961 (P = 0.004) and *ABCC4* rs1751003 (P = 0.0001) were associated with lower serum uric acid levels among men only.

Discussion

The highly significant association of *ABCG2* with uric acid in patients with CKD may be one of the first human examples suggesting a likely compensatory intestinal extrusion of uric acid in patients with low GFR. Thus, as in animal studies [5], intestinal urate transport by ABCG2 compensates for abnormal urate handling in the setting of declining renal function (Figure 2). As discussed below, this result is compatible with the Remote Sensing and Signaling Hypothesis, which describes how SLC and ABC 'drug' and other transporters in different tissues regulate inter-organ communication in order to maintain normal metabolism and signaling, especially after acute or chronic organ injury [3].

By comparison, the association with SLC2A9, significant in patients in those of EA with CKD, was eight orders of magnitude less (~E-5 for SLC2A9 compared with ~E-13). In striking contrast, among those with normal renal function (the twin study), SLC2A9 but not ABCG2 was associated with altered uric acid levels, consistent with other studies [9–11]. Other SLC and ABC urate transporters such as SLC22A8 (OAT3) and ABCG (MRP4) had only weak gender-dependent effects on uric acid levels. Taken together, our data shed light on the complexity of uric acid homeostasis depending upon renal function, ethnicity and gender.

In this study, *ABCG2* rs4148157 had the strongest association with serum uric acid among those of EA with CKD. The minor allele for this SNP is relatively common among East Asian

Table 1. Discovery analysis[a] CRIC (with CKD)

	European ancestry						African ancestry					
Gene (CHR)	SNP	Location[b]	A1	MAF	BETA	P	SNP	Location	A1	MAF	BETA	P
ABCG2 (4)	rs4148157	Intronic	A	0.10	0.68	4.78E-13[a]	rs10433946	Intronic	G	0.06	−0.42	0.001486
ABCG2 (4)	rs4693924	Intronic	A	0.10	0.68	4.78E-13[a]	rs4148150	Intronic	A	0.06	−0.39	0.001656
ABCG2 (4)	rs2054576	Intronic	G	0.10	0.68	5.08E-13[a]	rs2231137	Missense	A	0.06	−0.37	0.002264
SLC2A9 (4)	rs13125646	Synonymous	A	0.22	−0.30	0.0000106[a]	rs10805346	Intronic	A	0.40	0.22	0.0003544
SLC2A9 (4)	rs4481233	Intronic	A	0.20	−0.30	0.00002923[a]	rs3756231	Intronic	G	0.11	−0.33	0.0006064
SLC2A9 (4)	rs1014290	Intronic	G	0.26	−0.27	0.00003387[a]	rs3775948	Intronic	C	0.33	−0.22	0.0006497
ABCC2 (10)	rs2073337	Intronic	G	0.39	−0.10	0.07911	rs17112266	Intronic	A	0.09	−0.18	0.09621
ABCC2 (10)	rs3740074	Intronic	G	0.38	−0.10	0.0824	rs4148399	Intronic	C	0.07	0.19	0.1024
ABCC2 (10)	rs2756114	Intronic	G	0.39	−0.10	0.08486	rs8187692	Missense	A	0.09	−0.17	0.1067
SLC17A3 (6)	rs9348697	5′ upstream	A	0.37	−0.16	0.005524	rs501220	Intronic	A	0.40	0.15	0.01853
SLC17A3 (6)	rs13198474	5′-UTR	A	0.06	0.25	0.03422	rs17586946	5′ upstream	A	0.07	−0.23	0.05102
SLC17A2 (6)	rs17526722	Intronic	A	0.06	0.23	0.03949	rs670011	5′ upstream	A	0.22	−0.14	0.05353
SLC22A10 (11)	rs1193726	5′ upstream	A	0.45	0.13	0.01853	rs557879	Intronic	G	0.36	0.07	0.2655
SLC22A10 (11)	rs1790218	Nonsense	G	0.45	0.13	0.01892	rs513338	Intronic	A	0.36	0.07	0.2772
SLC22A10 (11)	rs1404608	3′-UTR	A	0.45	0.13	0.01905	rs549144	5′ upstream	C	0.36	0.07	0.2858
SLC22A8 (11)	rs3809069	5′ upstream	G	0.16	−0.18	0.02066	rs4149179	5′-UTR	A	0.05	0.31	0.02004
SLC22A8 (11)	rs4963326	Intronic	A	0.39	0.11	0.05822	rs1004836	Intronic	G	0.24	−0.12	0.09028
SLC22A8 (11)	rs948979	5′ upstream	A	0.25	0.12	0.06354	rs4149181	Intronic	A	0.46	0.10	0.09549
SLC22A11 (11)	rs7936185	5′ upstream	A	0.43	−0.15	0.009676	rs17300741	Intronic	G	0.36	−0.11	0.08903
SLC22A11 (11)	rs12417589	5′ upstream	A	0.43	−0.14	0.01956	rs7124676	5′ upstream	A	0.11	−0.097	0.3353
SLC22A11 (11)	rs4930195	5′ upstream	A	0.45	−0.13	0.02977	rs7104900	5′ upstream	G	0.18	0.058	0.4635
ABCC4 (13)	rs7981095	Intronic	T	0.18	0.19	0.01123	rs4771912	Intronic	G	0.11	0.28	0.004573
ABCC4 (13)	rs4771912	Intronic	G	0.14	0.19	0.01861	rs9516518	3′ downstream	G	0.13	−0.19	0.03153
ABCC4 (13)	rs9524864	Intronic	A	0.49	−0.12	0.03875	rs9561797	Intronic	G	0.32	0.14	0.03369
SLC22A13 (3)	rs9874338	Intronic	C	0.10	−0.085	0.3899	rs7636551	Intronic	G	0.11	−0.16	0.09025
SLC22A13 (3)	rs4679027	Intronic	C	0.05	−0.12	0.3919	rs9827811	Intronic	A	0.05	−0.21	0.135
SLC22A13 (3)	rs1979845	Intronic	A	0.10	−0.072	0.4618	rs9874338	Intronic	C	0.44	0.063	0.314
SLC22A6 (11)	rs4149170	5′-UTR	A	0.09	−0.17	0.07487	rs3017670	Intronic	A	0.06	−0.15	0.2538
SLC22A6 (11)	rs10897312	Intronic	A	0.09	−0.16	0.09564	rs12276943	Intronic	A	0.23	−0.07	0.3517
SLC22A6 (11)	rs3017670	Intronic	A	0.16	0.05	0.5461	rs11828160	3′ downstream	A	0.23	−0.06	0.4009
SLC22A12 (11)	rs3802947	Intron of NRXN2	A	0.05	0.23	0.06009	rs7932437	3′ of NRXN2	G	0.20	−0.12	0.1493
SLC22A12 (11)	rs3741399	3′-UTR of NRXN2	A	0.05	0.23	0.06062	rs11231825	Synonymous	G	0.18	−0.10	0.2178
SLC22A12 (11)	rs475688	Intronic	A	0.25	−0.06	0.3366	rs11606370	Intron of NRXN2	A	0.17	−0.10	0.2415

CKD, chronic kidney disease; SNP, single nucleotide polymorphism; CHR, chromosome; A1, minor allele; MAF, minor allele frequency; BETA, beta-coefficient from PLINK regression; P, P-value for beta-coefficient; UTR, untranslated region.

[a]Adjusted for: population stratification, age, gender, diabetes, estimated glomerular filtration rate, diuretic and allopurinol treatment.

[b]SNP location from dbSNP.

Table 2. Discovery analysis twin data (no CKD)

Gene (CHR)	SNP	Location[a]	A1	MAF	BETA	P
ABCG2 (4)	rs2231146	Intronic	C	0.015	−0.681	0.029
ABCG2 (4)	rs12641369	Intronic	A	0.056	−0.276	0.098
ABCG2 (4)	rs2231137	Missense	T	0.056	−0.276	0.098
SLC2A9 (4)	rs4481233	Intronic	T	0.199	−0.447	7.01E-06
SLC2A9 (4)	rs13111638	Intronic	T	0.2	−0.414	2.16E-05
SLC2A9 (4)	rs6449213	Intronic	C	0.2	−0.414	2.16E-05
ABCC2 (10)	rs10509739	3′ downstream	T	0.067	−0.296	0.060
ABCC2 (10)	rs4148396	Intronic	T	0.361	−0.112	0.154
ABCC2 (10)	rs2756111	Intronic	C	0.058	0.227	0.160
SLC17A1-17A3 (6)	rs11969868	Intronic	G	0.183	−0.241	0.014
SLC17A1-17A3 (6)	rs9461211	5′ upstream	A	0.452	0.163	0.028
SLC17A1-17A3 (6)	rs2275906	Synonymous	G	0.131	−0.247	0.032
SLC22A10 (11)	rs7942667	Intronic	C	0.029	0.345	0.139
SLC22A10 (11)	rs17158018	Intronic	G	0.029	0.345	0.140
SLC22A10 (11)	rs17158022	Intronic	A	0.029	0.342	0.146
SLC22A8 (11)	rs10792369	5′ upstream	C	0.349	0.114	0.171
SLC22A8 (11)	rs4149181	Intronic	G	0.082	0.167	0.213
SLC22A8 (11)	rs4963326	Intronic	A	0.42	0.1	0.259
SLC22A11 (11)	rs3759053	5′ upstream	C	0.474	0.181	0.016
SLC22A11 (11)	rs11231813	5′ upstream	G	0.494	−0.149	0.0496
SLC22A11 (11)	rs17300741	Intronic	T	0.488	−0.025	0.099
ABCC4 (13)	rs4148499	Intronic	T	0.156	−0.216	0.049
ABCC4 (13)	rs12870204	Intronic	T	0.39	−0.135	0.087
ABCC4 (13)	rs9524848	Intronic	G	0.974	−0.397	0.087
SLC22A13 (3)	rs3805040	Intronic	G	0.054	−0.23	0.188
SLC22A13 (3)	rs1979845	Intronic	T	0.114	−0.149	0.224
SLC22A13 (3)	rs2236631	Intronic	T	0.053	−0.147	0.374
SLC22A6 (11)	rs11828074	5′ upstream	T	0.063	0.145	0.339
SLC22A6 (11)	rs11568621	Intronic	A	0.063	0.145	0.339
SLC22A6 (11)	rs11568628	Synonymous	T	0.373	0.031	0.691
SLC22A12 (11)	rs476037	3′-UTR	A	0.136	0.166	0.143
SLC22A12 (11)	rs578829	5′ upstream	C	0.334	0.025	0.759
SLC22A12 (11)	rs11231825	Synonymous	T	0.334	0.025	0.759

CKD, chronic kidney disease; SNP, single nucleotide polymorphism; CHR, chromosome; A1, minor allele; MAF, minor allele frequency; BETA, beta-coefficient from PLINK regression; P, P-value for beta-coefficient; UTR, untranslated region.
[a]SNP location from dbSNP.

Table 3. Most significant SNPs in EA (CRIC)

			Multivariable regression[a]		
Gene	*n*	Uric acid mg/dL (SD)	BETA[a]	Adjusted[a] P-value	95% CI[a]
ABCG2 rs4148157					
GG	1627	6.99 (1.85)	Reference		
GA/AA	438	7.64 (2.05)	0.64	<0.001	0.47 to 0.81
SLC2A9 rs13125646					
GG	1211	7.21 (1.87)	Reference		
GA	727	7.04 (1.93)	−0.25	0.001	−0.40 to −0.10
AA	126	6.86 (2.08)	−0.51	0.001	−0.81 to −0.22

SNP, single nucleotide polymorphism; EA, European ancestry; CRIC, Chronic Renal Insufficiency Cohort; BETA, beta-coefficient from PLINK regression; CI, confidence interval.
[a]Adjusted for: population stratification, age, gender, diabetes, estimated glomerular filtration rate, diuretic and allopurinol treatment.

populations (MAF: 0.25–0.30). Other ABCG2 SNPs have been shown to be functional with respect to uric acid transport (rs2231142 [29]) and associated with early onset gout and hyperuricemia in Japanese cohorts (rs2728125 [16, 17, 30]). Among the Japanese population, ABCG2 dysfunction may account for up to 29% of the population-attributable-risk of hyperuricemia [31]. In addition, *SLC2A9* SNPs were also statistically significant among those of EA ($P \leq 5.1E-5$) in the CRIC and the twin dataset, with strong (but not statistically significant) associations among those of AA. The most significant *SLC22A9* SNPs (rs13125646, rs4481233 and rs1014290) are in LD and have been associated with uric acid levels and/or gout in several studies [9, 32, 33].

While SLC2A9 is primarily thought to affect uric acid transport in the proximal tubules of the kidney, the ABCG2

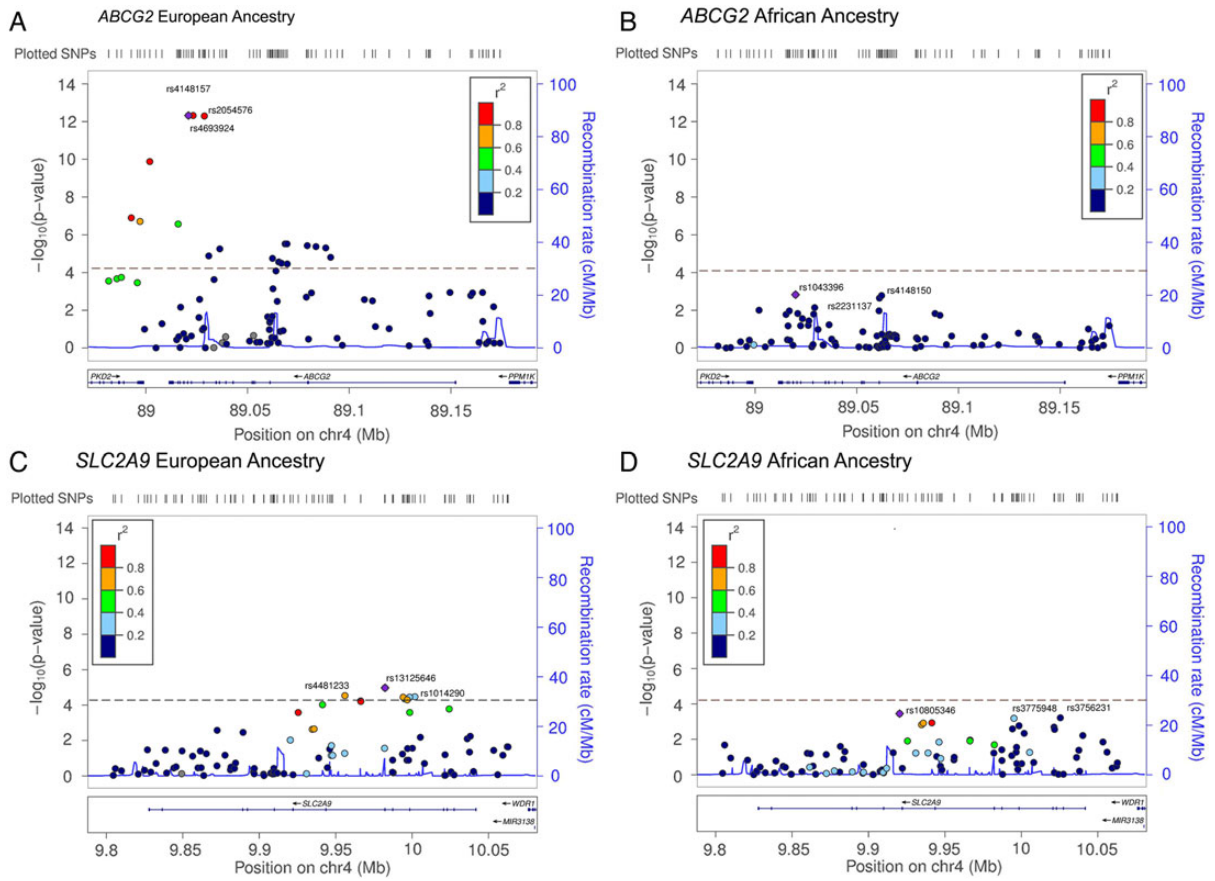

Fig. 1. Strength of association for *ABCG2* and *SLC2A9* gene regions. The strength of the associations (–Log10 P-values) between *ABCG2* and *SLC2A9* SNPs and serum uric acid in the CRIC population stratified by European and African ancestry are shown. The correlation (r^2) between the most highly associated SNPs (indicated by a purple diamond) and all other SNPs tested in the region is represented using the color scheme shown in the legend on the upper left corner of each plot. Correlations were estimated in populations from the 1000 Genomes Project (Mar 2012) with European ancestry or African ancestry. (**A**) ABCG2 SNPs among those of European ancestry. (**B**) ABCG2 SNPs among those of African ancestry. (**C**) SLC2A9 SNPs among those of European ancestry. (**D**) SLC2A9 SNPs among African ancestry. Recombination rates in cM per Mb and Human genome build 19 coordinates (Mb) are also shown. Plots were generated using LocusZoom [24].

Table 4. Most sinificant SNPs in AA (AASK and CRIC)

			Multivariable regression[a]		
	n	Uric acid mg/dL (SD)	BETA[a]	Adjusted P-value[a]	95% CI[a]
ABCG2 rs4148157					
GG	1863	7.87 (1.91)	Reference		
GA/AA	126	8.20 (2.0)	0.41	0.0095	0.10 to 0.72
SLC2A9 rs13111638					
CC	1404	7.97 (1.93)	Reference		
CT/TT	502	7.65 (1.86)	−0.32	0.0004	−0.49 to −0.14
SLC2A9 rs6449213					
AA	1483	7.98 (1.96)	Reference		
AG/GG	509	7.66 (1.78)	−0.33	0.0002	−0.50 to −0.15
SLC2A9 rs4481233					
CC	1649	7.94(1.94)	Reference		
CT/TT	330	7.65 (1.80)	−0.23	0.023	−0.43 to −0.033

SNP, single nucleotide polymorphism; AA, African ancestry; AASK, African American Study of Kidney Disease and Hypertension; CRIC, Chronic Renal Insufficiency Cohort; BETA, beta-coefficient from PLINK regression; CI, confidence interval.
[a]Adjusted for: study population, age, gender, diabetes, estimated glomerular filtration rate, diuretic and allopurinol treatment.

transporter—localized to brush border of renal proximal tubules and intestinal cells—functions to extrude urate [6–9, 14, 16, 17, 28, 30]. While site-directed mutagenesis of *ABCG2* rs2231142 has been shown to significantly decrease renal uric acid transport [27], recent evidence in rodents suggests increased intestinal expression in the setting of renal failure [5]. It is also believed that

Table 5. Sex interaction analysis in CRIC

	Genotype	*n*	BETA	Uric acid mean	Uric acid adjusted mean	P-value	*n*	BETA	Uric acid mean	Uric acid adjusted mean	P-value	P-value interaction
Sex interaction	Men						Women					
SLC2A9 (rs3775948)	CG/CC	995	−0.10	7.72	7.27	0.1805	797	−0.44	6.84	6.40	<0.0001	0.0033
	GG	954		7.67	7.37		776		7.15	6.84		
SLC2A9 (rs1014290)	GA/GG	944	−0.11	7.70	7.26	0.1522	758	−0.41	6.82	6.41	<0.0001	0.0079
	AA	1005		7.69	7.37		814		7.16	6.82		
SLC22A8 (rs3793961)	AA/AG	77	−0.57	7.52	6.77	0.004	92	0.07	7.52	6.71	0.6923	0.0220
	GG	1873		7.70	7.35		1480		6.96	6.65		
SLC22A11 (rs17300741)	GA/GG	1316	0.06	7.67	7.34	0.4792	1020	−0.34	6.77	6.54	0.0002	0.0003
	AA	634		7.75	7.28		553		7.42	6.88		
ABCC4 (rs1751003)	AA/AG	483	−0.34	7.43	7.07	0.0001	363	0.07	7.05	6.70	0.486	0.0023
	GG	1466		7.79	7.41		1210		6.98	6.64		

CRIC, chronic renal insufficiency cohort; BETA, beta-coefficient from PLINK regression.

ABCG2 in the intestine plays a major role in regulation of human uric acid levels [14].

Moreover, *ABCG2* SNPs have been shown to be more specifically associated with gout among patients classified as 'renal overload', whereas *SLC2A9* SNPs are most significant among those classified as 'renal under-excretion' [17]. However, if filtration declines in the setting of CKD, renal secretion may be limited [4]. In this setting, ABCG2—a major luminal intestinal secretory urate transporter—may in part compensate for the loss of renal excretion and thus be more important in net urate excretion in the setting of declining renal function (Figure 2). In this context, it is worth noting that ABCG2 (BCRP) is also a major drug transporter, and many of these drug transporters seem to be substrate inducible [34]. Hence, high levels of uric acid in the setting of CKD may lead to induction of urate transporters in the intestine and other non-renal tissues.

There is a growing body of data on the potential role of SLC and ABC transporters in inter-organ communication via small molecules that might include those with antioxidant properties such as uric acid [35–39]. Taken together, our data support the notion that uric acid transporters in remote organs (intestine versus kidney) may regulate serum urate levels, as suggested in the Remote Sensing and Signaling Hypothesis [3, 35–38]. That this appears most important in the setting of chronic renal injury is also consistent with this hypothesis, which also suggests that Remote Sensing and Signaling via multispecific ABC and SLC 'drug' transporters is particularly critical in the setting of perturbed homeostasis due to organ injury. Moreover, because of the clustering of SLC22 transporters in the genome (human chromosome 11) and, in particular, the pairing of the *SCL22A6* (*OAT1*) with *SLC22A8* (*OAT3*) and *SCL22A11* with *SLC22A12* [40], as well as tissue-selective expression patterns (e.g. kidney versus gastrointestinal) [5, 14], it has been suggested that there might be coordinate regulation of these genes [3, 37, 40, 41].

The effects of several SLC and ABC transporters on serum uric acid also varied by gender (Table 4). Similar to other studies, *SLC2A9* rs3775948 appeared only to be significant in women [11, 26–28]. The effect of *SLC2A9* variation on uric acid is also influenced by environmental factors, including diet and body mass index [42]. Sex was also an effect modifier in the association between serum uric acid and *SLC22A8*, the association being significant among women only; in contrast, *SLC22A11* and *ABCC4* were significant in men only.

Probably because of differences in linkage structure and allele frequencies, the most significant SNPs were different between those of EA and AA. While associations in AA generally trended in a similar fashion to those of EA, the associations were of marginal statistical significance, even when CRIC and AASK data were combined. This is most likely the result of inadequate coverage for the AA genome on commercial chips. As CRIC is a unique study cohort with CKD, there are few comparable cohorts for replication. Thus, the role of ABCG2 in patients with CKD remains to be verified in larger clinical cohorts; the clinical significance of *ABCG2* rs4148157 in populations of East Asian descent who have a relatively high MAF (~0.30) warrants further study. Interestingly, URAT1 was not significantly associated with uric acid levels in either CRIC or the twin data set despite adequate power to detect a modest effect size in the study populations considered here.

By these analyses, we uncovered a notable highly significant association between *ABCG2* serum uric acid among patients of EA with CKD, with a considerably less significant association with SLC2A9. Serum uric acid homeostasis in CKD settings may in large part be maintained by intestinal secretion by transporters such as ABCG2, suggesting that remote effects on

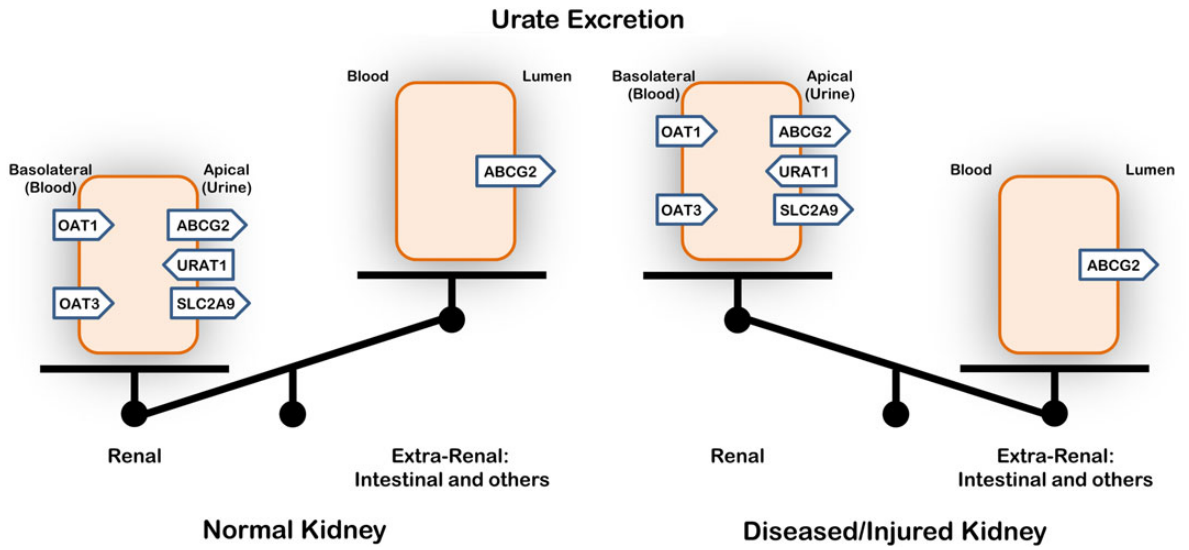

Fig. 2. Uric acid excretion with normal or decreased renal function. The results of this analysis of human data—as well as physiological data from rodent models with renal dysfunction [5]—support the view that urate transport by ABCG2, most likely in the intestine, compensates for altered renal urate handling in the setting of poor kidney function.

transporters in distant tissues (i.e. intestine versus kidney) may be critical in the setting of injury to one tissue or the other [3, 38]. Results presented here indicate that transporters may be more or less dominant in serum uric acid homeostasis depending on patient population characteristics (disease state, sex, ethnicity). Alteration in uric acid levels is relatively common and genetic effects may very well depend on the patient population and be a function of medical illness (e.g. renal disease) and genetic substructure. To more thoroughly evaluate these issues, in addition to large meta-analyses that combine patient populations, it will be important for future studies to address effects in a well-characterized cohort with a focus on confounding variables and effect modification. Additional transporters, such as those of the SLC22 family, which has over 30 mammalian members [43]—several of which are known to transport urate—may eventually be implicated in human urate handling. To the best of our knowledge, the highly significant association of *ABCG2* with uric acid in patients with CKD may be among the clearest human evidence to date suggesting a remote compensatory role of intestinal-expressed ABCG2 patients with low GFR, consistent with the Remote Sensing and Signaling Hypothesis [3, 35–38]. Therapeutic strategies aimed at enhancing extra-renal ABCG2 urate extrusion may have clinical value in CKD patients with hyperuricemia.

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Conflict of interest statement

None declared.

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