The pathophysiological nature of the common ABCG2 gout and hyperuricemia associated variant Q141K (rs2231142) remains undefined. Here, we use a human interventional cohort study (ACTRN12615001302549) to understand the physiological role of ABCG2 and find that participants with the Q141K ABCG2 variant display elevated serum urate, unaltered FEUA, and significant evidence of reduced extra-renal urate excretion. We explore mechanisms by generating a mouse model of the orthologous Q140K Abcg2 variant and find male mice have significant hyperuricemia and metabolic alterations, but only subtle alterations of renal urate excretion and ABCG2 abundance. By contrast, these mice display a severe defect in ABCG2 abundance and function in the intestinal tract. These results suggest a tissue specific pathobiology of the Q141K variant, support an important role for ABCG2 in urate excretion in both the human kidney and intestinal tract, and provide insight into the importance of intestinal urate excretion for serum urate homeostasis.
Uric acid is the terminal metabolite of purine metabolism in humans as a result of the pseudogenization of the uricase (UOX) gene. Increased levels (>7 mg/dL) of serum urate (predominant form at physiological pH) is defined as hyperuricemia and is present in 43 million Americans. Hyperuricemia may lead to saturation and precipitation of the weakly soluble urate as monosodium urate crystals, occasionally in the renal tubules causing kidney stones, but most often in the synovial fluid of joints, causing gout, the most common form of inflammatory arthritis. The heritability of hyperuricemia is high, 70%3,4, predicting important genetic components to hyperuricemia and subsequent risk of gout. In observational studies, hyperuricemia increases risk of hypertension incidence5, and asymptomatic hyperuricemia in the absence of comorbidities increases the risk of hypertension, chronic kidney disease, and obesity 2–3 fold. However, demonstrating causality of hyperuricemia in the pathophysiology of hypertension or metabolic diseases has been complicated by the reciprocal relationship between target organs for these disorders, the kidney and liver respectively, and the function of these organs in urate production and excretion. Further, we lack mechanistic understanding in how urate excretion is mediated and how urate homeostasis is regulated, even as important pieces to these puzzles have been recently identified7,8. Humans excrete urate through the gastrointestinal tract and the kidneys, where urate is freely filtered at the glomerulus, mostly reabsorbed in the early proximal tubule (S1), and secreted and possibly reabsorbed in the S2–S3 segments of the proximal tubule1, although this remains controversial9. Genetic approaches identified a number of kidney and gastrointestinal tract transporters with significant affinity for urate. These include the SLC transporter genes, SLC22A12 (refs.10,11), SLC2A9 (refs.11,12), and the ABC transporter gene, ABCG2 (ref.13). Variants in these three genes alone contribute 5% of measured variability in serum urate (SU), significantly more than all other variants combined14. Functional studies in humans and model systems have demonstrated that ABCG2 (refs.15,16) and SLC2A9/GLUT9 (ref.17) have a role in intestinal excretion, although specific cellular mechanisms remain undescribed. In the kidney, URAT1 (SLC22A12) has proven an important conduit for urate reabsorption18, but the functional roles, expression, localization, and regulation of SLC2A9 and ABCG2 remains poorly understood.

The ABCG2 and SLC2A9 (ref.19) loci harbor common human single nucleotide polymorphisms (SNPs) that associate with increased serum urate levels, including rs2231142, resulting in a missense variation in the ABCG2 protein, p.Gln141Lys (Q141K), found in hundreds of millions of individuals12,16. Interestingly, ABCG2 polymorphisms appear to affect gout risk through pleiotropic pathways, contributing both in the presence of hyperuricemia, and independent of increases in serum urate. One of the most puzzling aspects of understanding the Q141K ABCG2 variant is how and where it affects urate excretion. In vitro, the Q141K protein is a partial loss of function protein13,22 with increased instability and frequency of degradation, resulting in both function and significant abundance defects.23 Mouse models of ABCG2 knockout show missing urate transport in both the intestines and the kidney16, but studies of humans with the Q141K variant have been less consistent. Previous large association studies have reported significant increases24, significant decreases25, or no alterations at all26 in renal fractional excretion of urate for individuals possessing the minor allele (T, corresponding to 141K) of rs2231142. These inconclusive studies have led to doubt for the role of ABCG2 in renal excretion of urate. Interestingly, RNA-seq has found ABCG2 mRNA in the human kidney27,28 and previous studies have documented protein expression and transport function in the apical brush border of renal epithelia29. Fully understanding the pathological role of the Q141K ABCG2 variant allele in urate handling is important for increasing our understanding of the pathogenic nature of urate.

Here, we use a human interventional study and a CRISPR knock-in mouse model of the orthologous Q140K Abcg2 to better understand the role of ABCG2 in urate excretion. Our results support the use of the mouse as a model for ABCG2-mediated urate handling in humans, support the role of ABCG2 in both renal and intestinal excretion of urate, and illuminates the complexity of normal and pathological urate excretion.

**Results**

**Human interventional renal urate handling study.** To understand the impact of ABCG2 and its common variant (ABCG2: rs2231142, Q141K) on renal urate handling, we provoked acute increases in serum urate by oral administration of the purine nucleoside, inosine, to 100 healthy human volunteers. Inosine is quickly metabolized into urate (see "Methods" section) and allows evaluation of the effects of a standardized purine load on both SU concentrations and renal uric acid handling. Participants were young, had no history of gout or being treated with urate-lowering therapies (population characteristics, Supplementary Table 1), and were recruited from two specific populations—people of Polynesian ancestry (New Zealand Māori and Pacific peoples) and people of European ancestry. Oral administration of inosine caused a rapid increase in SU levels in all individuals regardless of ABCG2 genotype (Fig. 1a), and the rates of increases were similar for all participants (ANCOVA, \( P_{\text{rs}} < 0.0001 \)) suggesting that ABCG2 genotypes do not have broad effects on the absorption of inosine nor the metabolism of inosine into urate. The absolute levels of SU differed significantly, however, among the ABCG2 genotypes, both at baseline and at saturation (Fig. 1a), with individuals possessing at least one copy of the ABCG2 risk allele (141K) having significantly elevated SU at baseline and throughout (ANCOVA, \( P_{\text{rs}} < 0.0001, P_{\text{SNP}} = 0.0007 \)) as compared to individuals without the risk allele (Q141). The maintenance of the relative SU differences in individuals with and without 141K across a range of SU levels suggests no energetic homeostatic mechanisms are activated in the time course of the experiment dependent on or affected by ABCG2 genotype. A secondary analysis using a stratification by ancestry showed similar results (Supplementary Fig. 1, see “Methods” section).

The kidney is responsible for the majority of urate excretion in humans, and renal excretion is dependent on filtered urate load (GFR*SU) and the fraction of the filtered load (FEUA) that ultimately ends up in the excreted urine. A comparison of FEUA in participants with (141K) and without (Q141) the ABCG2 risk variant revealed no differences in mean FEUA (Fig. 1b). This is in contrast to the individuals with the previously described urate-lowering associated variant allele of SLC2A9 rs1194223 (ref.30), which exhibited a significant alteration in the renal excretion of urate, consistent with the hypothesized role of SLC2A9 in renal reabsorption (Supplementary Fig. 2). We also measured urinary urate excretion (UUE, calculated by normalizing urinary urate to urinary creatinine) and found UUE increased through time and with urate load, but there were no differences between the Q141 and 141K individuals (Fig. 1c). Together, these data suggest the kidney was passively spilling the increased filtered load, not using active processes to adjust to changing loads.

Next, we looked closer at the relationship between kidney handling of urate, as assessed by FEUA, and observed variation in SU18. First, we found that FEUA and SU were significantly correlated (Supplementary Fig. 1b) at all time points. Second, we found that SU level was dependent on FEUA for both ABCG2 genotypes, at all time points (Fig. 1c, d, linear regression,
Fig. 1 Human interventional study demonstrates significant alterations in urate handling in individuals with the 141K allele. Effect of ABCG2 genotypes (Q141, n = 79 and 141K, n = 21 participants, Supplementary Table 1 for characteristics of participants) on a serum urate (\(p_{\text{(time)}} < 0.0001; p_{\text{(SNP)}} = 0.0007; p_{\text{(SNP*time)}} = 0.99\)), b fractional excretion of urate (FEUA) (\(p_{\text{(time)}} < 0.0001; p_{\text{(SNP)}} = 0.82; p_{\text{(SNP*time)}} = 0.69\)), and c urinary excretion (UUE = \(U_{\text{UA}}/U_{\text{Cr}}\)) (\(p_{\text{(time)}} < 0.0001; p_{\text{(SNP)}} = 0.12; p_{\text{(SNP*time)}} = 0.86\)) following inosine load to entire group (a–c, statistical analysis: two-tailed ANCOVAs adjusted for age, sex, BMI, and ancestry; ± standard error of the mean [SEM]). Effect of ABCG2 genotypes on dependence of SU on FEUA; each SU and commensurate FEUA measured at each of 5 time points plotted for each individual with the Q141 allele (d) or 141K allele (e) with linear regression fits (\(p < 0.0001\) for all fits) extended until FEUA = 0. Plot of the percentage of SU variability due to renal (\(r^2\)) or extra-renal (1 – \(r^2\)) urate handling across increasing urate loads for Q141 participants (f) or 141K participants (g). h Effect of ABCG2 genotypes on dependence of SU on UUE at baseline (time 0) plotted with linear regression fits (Q141 allele, \(p = 0.86\); 141K allele, \(p = 0.02\)) extended until UUE = 0. Source data are provided as a Source data file.

\(p < 0.0001\) for all fits). Interestingly, a comparison of the dependence of SU on FEUA, as measured by the slope of the linear regression fits, showed no differences between the Q141 and 141K participants at baseline, but did show a significant difference after 180 min (ANCOVA, \(p = 0.0005\), Supplementary Fig. 1b). This suggested that as filtered urate load dynamically increased, a significant difference emerged between the Q141 and 141K participants in the relationship between FEUA and SU.
We then examined the linear regression fits at each time point for each genotype (Fig. 1d, e). In the Q141 individuals, there was a significant change in the slope of the dependence over time (ANCOVA, \( p = 0.0025 \)). Extending the linear regression of each time point in order to model the hypothetical SU with zero FEUA (no renal excretion of urate) revealed the SU would remain \( \leq 8 \) mg/dL in Q141 individuals at the SU loads achieved (Fig. 1d). In the 141K individuals, the dependence of SU on FEUA remained unaltered through time, with only a significant change in the elevation (ANCOVA, \( p < 0.0001 \)). These data hint at a loss of excretion capacity. This hypothesis is supported by modeling where FEUA is zero, revealing an increasing SU over time such that at 180 min the hypothetical SU with zero FEUA was \( \sim 11 \) mg/dL (Fig. 1e). To understand the contribution of renal and extra-renal function to the variability observed in SU, we plotted the coefficient of determination \( (r^2, \text{see "Methods" section}) \) and non-determination \((1-r^2)\) versus SU (Fig. 1f, g). In Q141 participants, the extra-renal contribution was greater than the renal contribution to the observed variability in SU, over time and increasing urate load. Surprisingly, in the 141K individuals, the contribution of renal function was greater than extra-renal contributions (Fig. 1g). A similar comparison of the relationship between SU and FEUA at the beginning of urate loading showed that FEUA was not correlated with SU in the Q141 individuals (Fig. 1b), but was correlated in those with the 141K variant (Pearson, \( p = 0.02 \)). Together, these analyses demonstrate that in individuals with the 141K variant, urate handling and excretion via the kidney was more important in determining SU than in Q141 individuals. This demonstrates a significant role for extra-renal excretion in determining SU in our study population, and critically, a significantly altered role in 141K participants. Although the interventional trial data suggested a significant role for ABCG2 in extra-renal urate excretion, we did not observe an altered FEUA in the 141K individuals. It was unclear whether this was because our study was underpowered to resolve small FEUA changes, ABCG2 plays no significant role in renal urate handling in humans, or that the Q141K variant has no discernable effect on ABCG2 function in human kidneys.

**ABCG2 in human and mouse kidneys.** The role of ABCG2 in human kidney function is controversial. Therefore, we conducted our own investigation into the presence of ABCG2 protein in the human kidney and found ABCG2 present in the cortex (Fig. 2a, b) and medulla (Fig. 2g–j; Supplementary Fig. 3a), colocalized with the proximal tubule marker *Lotus tetragonolobus* lectin (LTL). Surprisingly, the number of tubules with ABCG2 signal appeared greater in the sections taken from the outer stripe of the medulla (Fig. 2g–j), suggesting a prominent role in the straight (S3) portion of the proximal tubule. The immunofluorescence signal corresponded well with western blot analysis of the same tissue (Fig. 2e). ABCG2 localization matched localization of URAT1 (SLC22A12) by western blot studies and localization in LTL-positive proximal tubules of the renal cortex (Fig. 2d, f). The localization of cortical intratubular and intracellular URAT1 does not directly overlap with the LTL marker, but is more apical. In contrast, ABCG2 substantially colocalized with LTL, an observation consistent with a slight difference in the localization of ABCG2 and URAT1 in the cells of the cortical proximal tubule. ABCG2-positive cytosolic puncta were also observed in a number of medullary tubules (Fig. 2g–j). Primary human cortical renal epithelial cells cultured on transwells (Supplementary Fig. 3b) showed significant amounts of ABCG2 protein within the apical compartment/brush border (above the tight junctions, as demarcated by zonula occludens 1 [ZO-1], Fig. 2k). However, there was also a significant portion of the ABCG2 signal localizing to puncta in the cytosol. This contrasted strongly with what was observed with URAT1, which was found exclusively above the nucleus in the apical/brush border compartment (Fig. 2l). This complexity of ABCG2 abundance and localization in human renal epithelia suggests an important role for the regulation of trafficking of renal ABCG2 in urate handling.

For comparison, in the mouse kidney, ABCG2 is highly abundant (Fig. 2m, Supplementary Fig. 3c) and localizes to the S2–S3 portion of proximal tubule (identified as LTL-positive) similar to what was observed in the human kidney tissue. Intratubular and intracellular localization showed that ABCG2 is abundant on the apical brush border surface, apical to the LTL staining (Fig. 2n, o), similar to URAT1 (Fig. 2p–r), but lacked the intracellular localization observed in the human renal tissue. Intriguingly, neither ABCG2 nor URAT1 strongly localize in the early proximal tubule (S1), the proposed site of the majority of urate reabsorption (Fig. 2a).

**Q140K/+ + hyperuricemic mouse model.** To better address the contradictory evidence in humans concerning the pathogenic mechanisms of the Q141K ABCG2 variant, we created an orthologous knock-in of the Q141K human variant in the C57BL6J mouse background using CRISPR-Cas9 genome editing (Fig. 3a, methods). The mouse and human ABCG2 proteins are very similar (81% homology), with the human glutamine at position 141 homologous to mouse ABCG2 Q140. The resulting homozygous animals (Q140K+/++) bred successfully, had no outward phenotypic alterations, and no effect on survival (80-week survival: WT 80%, \( n = 10 \); Q141K+/++ 94%, \( n = 18 \)). The male Q140K+/++ animals displayed a significant increase \((+89.4%; p = 0.0003, \text{Student's } t\text{-test})\) in SU (Fig. 3b). This robust increase in SU was observed in mice on normal chow and with no disruption of uricase function, suggesting that physical transport of urate plays an extremely important role in determining SU. Interestingly, female mice of comparable age showed no alterations in SU (Fig. 3c and Supplementary Fig. 4), mirroring the observed sex differences in SU association with the human Q141K variant (SNP rs2231142)\textsuperscript{14,24}.

For evaluation of kidney function, we used metabolic cages to collect urine over a 24h period with terminal serum, and measured electrolytes, creatinine, and urate (Table 1). Among other differences, we observed an increase in the fractional excretion of sodium (FeNa\(^+\)+) and a decrease in glomerular filtration rate (GFR) in the Q140K+/++ animals, observations potentially related through tubuloglomerular feedback. As with SU, investigation of the female Q140K+/++ mice chemistries demonstrated few of the differences observed in the male mice (Supplementary Table 2), providing further support to sexual dimorphism in the pathogenicity of the Q141K variant. The calculated fractional excretion of urate (FEUA) in the WT male mice was 3.88 ± 0.67% (± standard error of the mean [SEM]; \( n = 9, \text{Fig. 3c} \)) relatively similar to humans (5.68%) at baseline. A comparison of the dependence of SU on kidney urate handling (FEUA) in humans and WT male mice (Fig. 3d) found a remarkable similarity in their dependence of SU on FEUA; only the elevation was altered (ANCOVA, \( p < 0.0001 \)), a difference explained by the substantial difference in urine load. FEUA in the male Q140K+/++ mice was significantly decreased (Fig. 3c; 47%, \( n = 12, \text{ \( p = 0.01 \)} \); no change in female mice, Supplementary Fig. 4, \( n = 7, p = 0.6263, \text{Student's } t\text{-test})\), supporting the role of ABCG2 in the secretion of urate. The reduced FEUA coupled with an increased urate load resulted in our inability to observe a significant increase in UUE (Fig. 3e), though our UUE measurements had a large variability, suggesting that the alteration in renal excretion was not responsible for the
hyperuricemia observed in the Q140K+/+ animals. A comparison of differences in SU dependence on FEUA in WT and Q140K+/+ male mice found, as observed in the human population, that SU is significantly dependent on FEUA (linear regression, WT p = 0.020; Q140K+/+ p = 0.033). Fit comparisons revealed significantly altered slopes (ANCOVA, p = 0.0083) (Fig. 3f), and comparison of the coefficients of determination (r²) showed that in WT animals 51% of SU variation is explained by renal excretion (49% by extra-renal excretion), but the renal contribution is increased to 72% in Q140K+/+ animals (28% by extra-renal) (Fig. 3g).

A relationship between hyperuricemia and metabolic alterations has been previously hypothesized. We, therefore, investigated potential changes in key markers of metabolic health in the Q140K+/+ mice. We found significant increases in serum glucose (p = 0.003, Student’s t-test), insulin (p = 0.018, Student’s t-test), and insulin-like growth factor 1 (IGF1) (P = 0.041, Student’s t-test), but not in body mass (p = 0.15, Student’s t-test) (Fig. 4a). Next, we observed sporadic fatty liver phenotypes in Q140K+/+ males, but not WT animals (Fig. 4b and Supplementary Fig. 5) and found in the same males altered gene expression in markers of fatty liver disease (Pnpla3, p = 0.012; Pplp3, p = 0.050; and G6pd, p = 0.032, Student’s t-tests; Fig. 4c and Supplementary Fig. 5). The female Q140K+/+ mice were not hyperuricemic, and had no alterations in glucose, insulin, or IGF1 (Supplementary Table 2). The observation of significant differences in SU dependence on FEUA led us to consider the unique role of ABCG2 in the proximal tubule of the kidney.

**Fig. 2 Renal ABCG2 localization in the human and mouse proximal tubule.** a, b Representative immunofluorescence micrographs of fixed cortical sections of human kidney samples (Q141) stained for ABCG2 (red), the proximal tubule marker LTL (green), the basolateral marker Na+/K+ ATPase (blue), and colocalization of ABCG2 and LTL (yellow) at 200× (a; scale bar 100 μM) and 400× (b; scale bar 50 μM). c, d Serial sections (from a, b) stained for URAT1 (red), LTL (green), Na+/K+ ATPase (blue), and colocalization of URAT1 and LTL (yellow) at 200× (c) and 400× (d) (a-d represent three independently repeated experiments from n = 3 kidneys). Western blots from cortical samples (n = 3 kidneys) and medullary samples (n = 3 kidneys) for ABCG2 (e) and URAT1 (f). Representative immunofluorescence micrographs of fixed medullary sections of human kidney samples stained for ABCG2 (red), LTL (green), DAPI (blue), and colocalization of ABCG2 and LTL (yellow) at 200× (g; scale bar 100 μM) and 400× (h; scale bar 25 μM), or 400x without LTL (i, j; scale bar 25 μM) (g-i, representative of three independently repeated experiments from n = 3 kidneys). Z projections of cultured human renal epithelial cells stained for ABCG2 (green), Na+/K+ ATPase (red), and ZO-1 (magenta), or URAT1 (green), Na+/K+ ATPase (red), and ZO-1 (blue) (scale bar 5 μM). Representative (similar staining was repeated on tissue from n = 3 mice) micrographs (40×; scale bar 500 μM) from fixed whole mouse kidney slices stained with ABCG2 (m) or URAT1 (p) in green, LTL (red), and colocalization (yellow). Higher magnification (400×; scale bar 20 μM) micrographs on fixed sections from mouse kidney stained for ABCG2 (n, o) or URAT1 (q, r) in red, and in o, r LTL (magenta), Na+/K+ ATPase (blue), and colocalization of ABCG2/URAT1 and LTL (pink). s Diagram of transporter localization. Source data are provided as a Source data file.
Fig. 3 Male Q140K+/+ mice are hyperuricemic and demonstrate a renal urate handling phenotype similar to humans with 141K allele. a Alignment of human and mouse ABCG2 amino acid sequence in region near Q141 residue, and the color-gram of sequencing results of animals heterozygotic for the alterations to the endogenous Abcg2 loci or homozygotic. b Serum urate levels for male and female, WT and Q140K+/+ animals (WT males n = 14; Q140K+/+ males n = 11; WT females n = 9; Q140K+/+ n = 10; p = 0.7113). c Fractional excretion of urate (FEUA) measurements from male WT (n = 9) and male Q140K+/+ (n = 12) (p = 0.01). d Comparison of Q141 human participants (n = 79) to WT mice (n = 10) and their respective relationship between FEUA and SU, fit with linear regression that are not different in slope (p = 0.6390), but are different in elevation (p < 0.0001). e Urinary urate excretion as a function of urinary creatinine was not significantly different between WT (n = 11) and Q140K+/+ (n = 13) mice (p = 0.259). f Dependence of SU on FEUA plotted for each WT (n = 10) or Q140K+/+ (n = 6) is significant for both genotypes (WT p = 0.0195; Q140K+/+ p = 0.0330), but the slope of the linear regression fits are significantly different (p = 0.0083). g Plot of the percentage of SU variability due to renal (r²) or extra-renal (1−r²) urate handling in WT or Q140K+/+ mice. Statistical analysis: b, c, e two-tailed Student’s t-test, ± SEM; d, f Linear regression with slope and elevation comparison using two-tailed ANCOVA. Source data are provided as a Source data file.

ABC22 abundance and localization in Q140K+/+ mouse kidney. The role of reduced ABCG2 protein abundance in explaining altered kidney urate handling in the male Q140K+/+ animals was assessed. We found no difference in ABCG2 mRNA levels between the two genotypes (Fig. 5a). Immunofluorescence investigation of whole kidney slices (Fig. 5b) revealed subtle, but significant differences in ABCG2 immunofluorescent signal of the mutant ABCG2 protein in both the S1 (p = 0.02, Student’s t-test) and the S2 segments (p = 0.0008, Student’s t-test) (Fig. 5c).

Higher magnification comparison of individual tubules showed no change in the brush border/apical localization of mutant ABCG2 in the proximal tubule (Fig. 5d), but confirmed the subtle yet significant decrease in ABCG2 immunofluorescent signal (p = 0.0007, Student’s t-test, Fig. 5e). Western blot of the male whole kidney lysate confirmed the reduced total abundance of ABCG2 Q140K+/+ protein (p < 0.0001, Student’s t-test, Fig. 5f), but with no statistical difference in the heterozygotic male animals (Supplementary Fig. 2), or females (Supplementary Fig. 4). Critically, we found that FEUA showed a significant correlation (Pearson, p = 0.0033) and dependence (linear regression, p = 0.0066) on renal ABCG2 abundance (both WT and Q141K) confirming a key role for even the mutant protein on renal handling of urate (Fig. 5g). Finally, we investigated the
Table 1 Blood and urine chemistries of male WT and Q140K+/+ mice.

| Blood chemistry | QT140K+/+ (n = 7)* | Blood chemistry | QT140K+/+ (n = 6)* |
|----------------|---------------------|----------------|---------------------|
| Na+ [mmol/l]   | 144.12 ± 1.48       | Na+ [mmol/l]   | 39.60 ± 2.92        |
| K+ [mmol/l]    | 4.60 ± 0.18         | K+ [mmol/l]    | 90.67 ± 5.96        |
| CI− [mmol/l]   | 114.88 ± 1.54       | CI− [mmol/l]   | 90.77 ± 9.23        |
| BUN [mg/dl]    | 26.29 ± 0.78        | BUN [mg/dl]    | 0.47 ± 0.06         |
| Hct [%PCV]     | 35.50 ± 0.88        | Hct [%PCV]     | 33.5 ± 5.7          |
| pH             | 7.10 ± 0.03         | pH             | 1.4 ± 0.25          |
| POCO2 [mmHg]   | 62.08 ± 4.45        | POCO2 [mmHg]   | 4.49 ± 0.69         |
| HCO3 [mmol/l]  | 21.00 ± 1.67        | HCO3 [mmol/l]  | 40.97 ± 9.70        |
| AnGap [mmol/l]| 17.33 ± 0.49        | AnGap [mmol/l]| 808.33 ± 61.03      |
| BECf [mmol/l]  | −11.50 ± 0.58       | BECf [mmol/l]  | 973.67 ± 65.08      |
| Hb [g/dl]      | 12.09 ± 0.30        | Hb [g/dl]      | 39.50 ± 4.89        |
| GFR 207.2 ± 30.0 | GFR 207.2 ± 30.0 | GFR 207.2 ± 30.0 | GFR 207.2 ± 30.0 |
| Osmolarity [mmol/kg] | 808.33 ± 61.03 | Osmolarity [mmol/kg] | 973.67 ± 65.08 |
| UV [ml/24 h]   | 4.49 ± 0.69         | UV [ml/24 h]   | 3.94 ± 0.32         |
| GFR            | 207.2 ± 30.0        | GFR            | 150.9 ± 7.8         |

Blood, serum, and urine chemistries from WT and Q140K+/+ male animals. Blood chemistries acquired using the iSTAT device (see “Methods” section). Bold values are significant; statistical analysis: Student’s t-tests; ±SEM.

BUN blood urea nitrogen, Hct hematocrit, BECf base excess, AnGap anion gap, Hb hemoglobin, FE fractional excretion, UV urinary volume (rate), GFR estimated glomerular filtration rate.

**Group variability; WT: K = n = 6; BUN = n = 7; BECf/AnGap n = 6; Q140K+/+: BECf n = 6; AnGap n = 5.

Methods**: Blood chemistry is measured using an iSTAT device (Abbott). Blood chemistries were acquired from 10 WT male and 11 Q140K+/+ male mice. Group variability was assessed using Student’s t-test.

Fig. 4 Male Q140K+/+ mice are hyperglycemic and develop fatty livers. a Serum levels of glucose (WT n = 7; Q140K+/+ n = 6; p = 0.003), insulin (WT n = 10; Q140K+/+ n = 13; p = 0.018), insulin-like growth factor 1 (IGF1) (WT n = 7; Q140K+/+ n = 7; p = 0.041), and male animal mass (WT n = 28; Q140K+/+ n = 19; p = 0.15) (all ± SEM). b Hematoxylin and Eosin stained representative liver samples from WT and Q140K+/+ male mice; fatty deposits indicated with white arrows (representative of six independent experiments; n = 6 WT and n = 6 Q140K+/+ mice; scale bar 200 µm). c Quantitative real-time PCR analysis of total liver mRNA from WT and fatty Q140K+/+ mice (n = 3 for each) show significant differences in mRNA from genes associated with fatty liver disease, G6pd (p = 0.032), Pnpla3 (p = 0.050), and Pnpla2 (p = 0.012) (±SEM). Statistical analysis: a, c two-tailed Student’s t-test. Source data are provided as a Source data file.
relationship of renal ABCG2 abundance to the overall SU phenotype, and found renal ABCG2 abundance in the WT animals did not significantly correlate with SU (Fig. 5h), suggesting that small changes in ABCG2 abundance left sufficient remaining capacity to handle the low urate loads present with normal gastrointestinal urate excretion. Conversely, in the Q140K+/+ mice, SU showed a significant dependence on renal ABCG2 abundance (linear regression, $p = 0.015$, $r^2 = 0.81$; Fig. 5h), demonstrating that in the absence of significant gastrointestinal excretion and higher urate loads, the remaining ABCG2 capacity in the kidney is critical for setting the SU.

Alterations in intestinal urate secretion in Q140K+/+ mice. Our data from humans with the 141K allele and Q140K+/+ mice, as well as previous work documenting the role for ABCG2 in gastrointestinal excretion of urate\textsuperscript{15,16,31}, led our focus to investigating gastrointestinal-mediated urate excretion defects in the Q140K+/+ mouse model. First, we found the highest ABCG2 protein abundance in the jejunum and ileum of the small intestine; a finding similar to previous mRNA expression studies\textsuperscript{32,33} (Fig. 6a). Immunofluorescence investigation of mouse intestinal sections from the jejunum and ileum localized ABCG2 protein to the apical brush border of the villi cells (Fig. 6b). As in the kidney, ABCG2 apical localization in the villi cells supports the role of ABCG2 secretion of urate into the lumen of the intestines. ABCG2 also localized to the apical surface of crypt cells although with a less obvious physiological consequence (Supplementary Fig. 6a). We found that SLC2A9 strongly colocalizes with the alpha subunit of Na+/K+ ATPase (Fig. 6c) revealing a prominent
basolateral localization consistent with the hypothesized role of SLC2A9 in the intestinal urate secretory pathway. A comparison of localization and abundance of ABCG2 in the WT and Q140K+/+ animals revealed significant differences. First, total ABCG2 protein abundance, but not mRNA levels, in the jejunum was significantly and dramatically reduced in the Q140K+/+ animals (p = 0.0046, Student’s t-test, Fig. 7a, b, Supplementary Fig. 6b). The reduction in protein abundance is far greater than that observed in the kidney of the Q140K+/+ animals (78% vs 44%). The severity of the Q140K ABCG2 protein abundance loss was also observed in a significantly reduced signal in the immunofluorescence of the jejunum of Q140K+/+ mice compared to WT controls (p = 0.018, Student’s t-test, Fig. 7c).

Using an ex vivo intestinal loop assay (Fig. 7d, see “Methods” section), we assessed the effects of Q140K on ABCG2-mediated urate flux. In WT male loops, a net secretory urate flux was observed that was concentration dependent (Fig. 7e), and highly sensitive to a luminal ABCG2 inhibitor topiroxostat (TPX, p = 0.0019, Student’s t-test, Supplementary Fig. 6c). Comparing WT and Q140K+/+ animals revealed that the Q140K+/+ mice had significantly decreased basolateral to luminal urate flux (p < 0.0001, Student’s t-test, Fig. 7f), a large enough decrease to reverse the net secretion of urate to net absorption in the Q140K+/+ intestinal loops (Fig. 7h). Subtraction of the calculated flux sensitive to ABCG2 inhibitor, TPX, revealed about half of WT urate flux is mediated by a TPX-sensitive pathway, but there is little change in TPX-sensitive flux in the Q140K+/+ loops (Fig. 7g). This result is consistent with an almost complete loss of ABCG2-mediated urate secretion in the Q140K+/+ mice. There is no change in urate flux in Q140K+/+ colon derived loops as compared to WT (Fig. 7f), consistent with little ABCG2-mediated secretion in the large intestines. The intestinal experiments in the male Q140K+/+ mouse model support a near complete loss of ABCG2-mediated intestinal secretion of urate in contrast to a largely preserved renal function in the mutant Q140K+/+ animals. Therefore, the loss of intestinal urate secretion appears to drive the hyperuricemia in the Q140K+/+ ABCG2 mice and potentially in the 141K variant carriers in our clinical cohort.

Discussion

Three transporter genes SLC2A9, SLC22A12, and ABCG2 have the largest contribution to risk for hyperuricemia and gout, but where the protein products for these genes, SLC2A9/Glut9, URAT1, and ABCG2, respectively, localize, where they are important for urate excretion, and how they contribute to urate homeostasis remains largely undefined. Here, we have focused on the role of ABCG2, the highest risk gout gene, in urate excretion. Previously, Matsuo et al.31 correlated levels of ABCG2 dysfunction with alterations in both intestinal excretion (renal overload hyperuricemia) and renal under-excretion, demonstrating that even moderate ABCG2 dysfunction may contribute to renal under-excretion. However, in human studies of individuals with at least one copy of the common ABCG2 variant 141K, but not enriched for gout, there has been conflicting conclusions on the effect of renal urate excretion (FEUA)24-26. Even in the studies reporting a significant difference in FEUA associated with the 141K allele24, the difference is very small (0.217 mg/dl), and thus physiological relevance is uncertain. So, what is the quantitative role for ABCG2-mediated urate excretion in the kidney and the gastrointestinal tract?

Here, we approached this problem from two perspectives. First, we used a human interventional trial to assess the contribution of variant genotypes in individuals without gout on renal excretion following a purine challenge. We observed a significantly higher SU in individuals with the 141K allele at baseline and at each time point after the oral inosine load, observations that support the role for ABCG2 in secretion of urate. However, we did not observe any differences in the FEUA or UUE for individuals with
the 141K allele in comparison to the Q141 individuals. These FEUA data are similar to those reported previously, but they also were surprising given the increase in SU in these individuals. More significantly, we observed a critical genotype difference in the role of FEUA in determining the SU, with individuals with the 141K allele losing the extra-renal contribution to excretion of urate. These data are consistent with the previous observations of loss of gastrointestinal-mediated urate secretion (renal overload type) in individuals with ABCG2 dysfunction. Surprisingly, we did not see a genotype difference in UUE, a previously
described component of the renal overload hypothesis\textsuperscript{16,31}. This may be explained by significant differences in the characteristics and genetic background of the study participants. We recruited young and healthy volunteers of both sexes, in contrast to the hyperuricemic and gouty participants of previous association studies\textsuperscript{16,31}. Males and females were not segregated in our analysis, but a secondary analysis showed differences in UUE for the male and female non-carriers (Supplementary Fig. 7). However, analysis with a SNP\textsuperscript{\*} sex interaction term or female only analysis did not reveal any divergence from our segregated analysis (Supplementary Table 3 and Supplementary Fig. 7). Finally, lack of UUE differences in carriers of the Q141K risk allele may also be explained by genetic differences in the respective study populations, East Asian versus European, that have previously demonstrated differences for the association of the rs2231142 variant (Q141K) with poor allopurinol response\textsuperscript{35,36}. An analysis of our data stratified by ancestry did not reveal substantial population differences between Polynesian and European populations.

The interventional trial also revealed more about how increased UA filtered loads are handled by the human kidney (Fig. 8). On the short time scale of the inosine load, we found that increased UA load led to incremental increases in FEUA and UUE, reflecting a passive spilling of UA into the urine with no energetic alterations to UA handling, and reabsorption capacity maximum at baseline. This raises the interesting possibility that under high urate filtered loads, the kidney risks high tubular urate concentrations and UA crystal deposition to protect a specific rate of UA reabsorption (FEUA < 10%), regardless of the SU level. The diminishment of renal contribution to SU in the Q141 participants as the filtered load increased revealed it is the extra-renal contribution that increases commensurate with the SU load to prevent significant alterations in SU (Fig. 8). This observation challenges the concept that the kidney is the primary regulator of baseline SU levels in humans.

Our second approach to understanding the pathology of the Q141K variant was to use a knock-in mouse model of the orthologous Q140K variant. Multiple attributes described here suggest mice present a reasonable model for understanding ABCG2-mediated urate handling in humans. The dependence of SU on FEUA is very similar, as is the prominent localization of ABCG2 protein in the S2–S3 segments of the proximal tubule. There are also significant similarities in the pathological physiology observed between the 141K individuals and the Q140K+/+ mice, but with important exceptions. The Q140K+/+ mice showed altered FEUA, whereas the humans with the 141K allele did not, a difference possibly explained by an allelic dose-response; our mice have two copies and the human participants have only one copy of the 141K allele (with the exception of a single TT individual). Importantly, the urinary excretion of urate is not significantly altered in the Q140K+/+ mice, but the role of the kidney in determining SU was, much like what we observed in the human interventional trial. An additional observation from the human interventional trial was that 141K individuals with high urate loads had an altered relationship between SU and FEUA—they lost the extra-renal contribution to the variation of SU and thus kidney handling of urate became the key determinant in setting absolute circulating urate levels. We observed the same phenomenon in the Q140K+/+ mice, allowing us to carefully dissect the causal mechanisms. We found these animals displayed an almost complete loss of ABCG2 protein and ABCG2-mediated intestinal excretion of urate, but with more subtle changes in renal protein abundance and function. Why does the renal urate excretion become more important in setting SU levels in the Q140K+/+ animals or 141K humans? Increased Q141K ABCG2 (Fig. 5h) abundance reduced the SU through increased secretion, demonstrating that the Q140K protein is still functionally very important, and with the blanket protection of the gastrointestinal excretion lost, small changes in ABCG2 abundance made big differences in SU.

An intriguing finding is the phenotype discrepancy between the male and female Q140K+/+ mice. Female mice do not exhibit hyperuricemia nor changes in FEUA. In addition, they show no alteration in Q140K protein abundance in the kidney, raising the possibility that being female alters the way the variant ABCG2 protein is trafficked and expressed, and thus for females the Q140K variant is essentially fully functional. These data are consistent with findings from human populations that show the Q141K ABCG2 variant has a stronger effect on urate in males than females\textsuperscript{24}. Further understanding of why female mice are protected from the loss of function with the Q140K variant may illuminate how both ABCG2 and urate more generally is regulated in mammals.

Finally, the discrepancies of observed pathology between tissues may help explain the controversy over the role of ABCG2 in humans. Our confirmation of ABCG2 in human tubules and the Q140K+/+ mouse data identifies ABCG2 as important in renal handling of urate and supports previous conclusions\textsuperscript{31} that ABCG2 plays a role in renal urate excretion. Our findings also propose an interesting solution as to why the ABCG2 Q141K variant specifically accumulated in many modern human populations. The Q141K variant results in increases in SU, a likely selective advantage\textsuperscript{37}, but protects the renal handling of urate, without the risk of stones. Is it possible that the intricate scheme of reabsorption and secretion of urate along the proximal tubule is an evolutionary adaptation to urate per se, rather than simply
for the excretion of nitrogenous waste. Work investigated the mechanism of observed increased urinary urate excretion in people with diabetes taking inhibitors of the sodium glucose 2 transporter (SGLT2) found that there is a relationship between tubular glucose and urate reabsorption through URAT1. It is intriguing there may be some role for the recycling of urate in regulating sodium/glucose reabsorption. In human studies, renal clearance of urate was shown to be inversely correlated with insulin resistance and directly correlated with the excretion of sodium. In the Q141K/+ mice, we observed significant hyperglycemia, significant increases in serum insulin, and IGF1, as well as increased fractional excretion of sodium. These data and the literature support a relationship between urate, glucose, and sodium handling along the nephron. In addition, the male Q140K/+ mice presented fatty liver phenotypes and expression changes of genes, including Pnpla2 and Pnpla3, coding for triglycerol lipases implicated in human fatty liver disease, broadening the potential role of urate to disrupt metabolic processes globally. Both intriguing possibilities deserve further exploration.

In conclusion, the minor allele of common human hyperuricemia and gout risk variant Q141K significantly alters the extra-renal contribution of urate excretion and SU determination in both humans and mice. Furthermore, Q140K/+ knock-in mice have hyperuricemia, driven by an almost complete loss of ABCG2-mediated urate excretion in the small intestine. In contrast, the ABCG2 function in kidney is largely preserved indicating an intriguing differential pathalogy of the 141K variant. These conclusions provide support for a role of ABCG2 in human renal urate excretion, provide a better understanding of the respective contributions of the kidney and intestines to serum urate homeostasis, and finally, stimulate new interesting questions about the relationship between glucose, sodium, and urate transport.

Methods

Human interventional study. This study was designed to examine the role of SLC2A9 and ABCG2 on serum urate and FEUA in response to an oral inosine load. Inosine is a purine nucleoside that is metabolized in vivo from AMP as part of the purine salvage metabolic pathway. Inosine is degraded to hypoxanthine, which is, in turn, metabolized to xanthine, and then urate in the purine degradation pathway. Administration of inosine leads to increased serum urate concentrations.

Participants. One hundred healthy participants were recruited by public advertising. Indigenous New Zealanders (Māori) and Pacific people living in New Zealand have a high prevalence of hyperuricemia and gout, and for this reason the sampling framework in the study protocol specified recruitment of 50 participants of Māori or Pacific ethnicity (Polynesian), and 50 participants of New Zealand European ethnicity. Inclusion criteria were: the ability to provide written informed consent, and estimated glomerular filtration rate (eGFR) > 60 ml/min/1.73 m². Exclusion criteria were: ethnicity other than Māori, Pacific, or New Zealand European, first degree relative of another study participant (consistent with our prior short term intervention studies in which the primary aim was an analysis of specific genetic variants), history of gout, history of kidney stones, history of diabetes mellitus, diuretic use, or urine pH ≤ 5.0. All visits took place at a clinical research center. The study was approved by the New Zealand Health and Disability Ethics Committee, and each participant gave written informed consent. The study was prospectively registered by the Australian Clinical Trials Registry (ACTRN12615001302549).

Primary and secondary endpoints. The primary endpoint was the change from baseline in serum urate concentration over 3 h following an inosine challenge. The secondary endpoint was change in fractional excretion of urate up to 3 h following an inosine challenge (the fractional excretion of urate is the ratio between the renal clearance of urate and the renal clearance of creatinine, expressed as a percentage) or the calculation of urinary urate excretion (UUE).

Protocol. Participants attended the study visit within two weeks of the screening visit. At the study visit, a venous catheter was inserted for blood collection. Following an overnight fast, participants took three 500 mg inosine tablets (Source Naturals) orally over a 5 min period between 0800 and 0930. Blood was obtained for urate and creatinine prior to ingestion, and then 15, 30, 60, 120, and 180 min after ingestion. Urine was obtained for urate and creatinine prior to ingestion, and then 0, 30, 60, 120, and 180 min after ingestion. Urine volume was measured at each time point. Drinking water was also provided at each time point (30, 60, 120, and 180 min) to a volume equivalent to the collected urine volume. Complete study protocol is provided in the Source data file.

Laboratory testing. Serum and urine chemistry was tested using the Roche Modular P (Hitachi) analyzer. The FEUA was calculated as the ratio between the renal clearance of uric acid to the renal clearance of creatinine, expressed as a percentage. Urinary excretion (UUE) was also calculated: Urinary excretion (UUE) = (urate x creatinine)/(urate x creatinine) × 100

Statistical analysis. Sample size calculations were based on the variability of change in serum urate at each time point in our previous study of fructose-induced hyperuricemia and of inosine use for Parkinson’s disease. We assumed that their interaction 30% of participants would have the lower urate-raising allele, and that approximately 30% of participants would have the ABCG2 rs2231142 urate-raising allele based on our previous studies. The proposed study was adequately powered (90% at the 5% significance level for a two-tailed test) to detect a difference in the change in serum urate between the groups of at least 0.20 μmol/L. Sample size calculations were performed using PASS 2002 (Hintze, J (2006) Kaysville, Utah).

Data are presented as mean (standard deviation (SD)) or n (%) for descriptive purposes; and plotted as least squares adjusted (for age, sex, BMI, and ancestry) mean ± standard error of the mean. The primary endpoint was SU and secondary endpoint (SU was FEUA or UUE) was tested using linear regression using SAS (v9.4, SAS Institute Inc, Cary, NC, USA) on an intention to treat basis. All tests were two-tailed. To understand the relationship between SU and FEUA or SU and UUE, we compared Pearson’s coefficient to test if there was a correlation (two-tailed test). We secondarily tested whether SU was dependent on FEUA or UUE using linear regression models with goodness of fit calculations and also compared different equations and slopes of different data sets. To calculate the percentage of the total SU variability that was dependent on FEUA, we calculated the coefficient of determination from the linear regression fit (r2, a percentage of SU variation accounted for by FEUA or renal urate handling) and for the percentage of total SU variability dependent on renal excretion we calculated the coefficient of non-determination (1− r2, percentage of SU variability accounted for by extra-renal function).

Cell culture and immunostaining in human cells. Normal human kidney epithelial cells from three different adult human kidneys were received from the Baltimore PKD Research and Clinical Core Center (reviewed by the UMB IRB and determined to not be human research, requiring no further IRB review). Primary cell cultures were established by plating cells directly onto Corning Transwells (12 mm tissue culture treated, Costar 3460). Cells were cultured with the Baltimore PKD renal epithelial cell media (REC): 1:1 mixture of RENaLife Complete Medium (Lifeline Cell Technology LL-0025) and Advanced MEM medium (Fisher Scientific #12492) with 5% FBS (ThermoFisher #26140-079), 2% Pen/Strep (Fisher Scientific #30-002-C2), 0.6% L-α-linolenate (Gemini Bio-products #400-106), and 0.03% Gentamicin (Quality Biological #120-098-661). Human primary cells were cultured for 5 days past confluence to establish a polarized monolayer. On day ten of culture, cells were fixed with 3% parafomaldehyde (Electron Microscope Solutions 15714-S), permeabilized with 0.1% triton (Sigma T-9284), and blocked in 0.1% BSA in 1× PBS. The culture was incubated with primary antibodies: anti-ABCG2 (Millipore 05-369, 1:200), anti-α-actinin (Millipore #30-002-Cl), 0.6% L-alanyl-Glutamine (Gemini Bio-products #400-106), and 0.03% Gentamicin (Quality Biological #120-098-661). Human primary cells were cultured for 5 days past confluence to establish a polarized monolayer. On day ten of culture, cells were fixed with 3% parafomaldehyde (Electron Microscope Solutions 15714-S), permeabilized with 0.1% triton (Sigma T-9284), and blocked in 0.1% BSA (Sigma A6003) as per standard protocols. Following blocking, polarized cells were incubated overnight at 4°C with primary antibodies: anti-ABCG2 (Cell Signaling Technology 42078, 1:200), rabbit anti-URAT1 (BMP B0564, 1:300), rat anti-zerumbone oculudens 1 (Santa Cruz sc-33725, 1:50), mouse anti-NaKATPase (Millipore 05-369, 1:200) in 1× PBS. After washing the primary antibody with 1× PBS, the culture was incubated for an extra 90 min with primary antibody and 1× PBS. Finally, the culture was incubated for 1× PBS. The culture was incubated for an extra 90 min with primary antibody and 1× PBS. The culture was incubated for an extra 90 min with primary antibody and 1× PBS. Following this incubation, transwells were washed with 1× PBS and then excised from the plastic mold. The membrane was mounted onto a glass slide with Vectashield Mounting Media with DAPI (Vector Labs H1200). Samples were imaged on a Nikon W-1 Spinning Disk.

Animal studies. Animal studies were performed in accordance to the NIH Guide for the Care and Use of Laboratory Animals and approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. Mice were housed in groups of two to five per cage on a 12:12 h light/dark cycle; with...
lights on at 6 a.m. Tissues, blood, and urine samples were collected between 8 and 10 am. Food and water were available ad libitum.

**Metabolic cage studies.** Renal function was evaluated using metabolic cages. After acclimation (2 days) in metabolic cages (Nalgene; Thermo Scientific # NALGENE650-0322), food and water consumption was recorded and urine and fecal samples were collected. Urine samples were collected several times a day to prevent contamination from food, water, and fecal matter. Kidney electrolyte handling was assessed in 24-hour measurements of fractional excretion (FE), which are calculated by creatinine clearance (see below), and Px is the plasma concentration.

Sample collection, preparation, and analysis. Animals were anesthetized with isoflurane (>4.5%). Once an animal was unconscious, blood samples were collected via cardiac puncture. Blood chemistry and gases (Na+, K+, Cl−, HCO3−, hematocrit, and BUN) were measured from a 100 µl aliquot of whole blood using an i-STAT EC8+ cartridge and an i-STAT1 Handheld Analyzer (Abaxis). The organs were next harvested (as described below). The remaining fraction of blood was immediately centrifuged (1000 g) to separate formed elements and plasma, the latter was subsequently isolated and frozen for later analysis of urine, and IGF1 levels. Urine sodium, potassium, and chloride analysis was performed using an Easylyte Analyzer (Medica Corporation). Plasma and urine creatine levels were measured using the QuantiChrom Creatinine Assay Kit (BioAssay Systems) following the manufacturer’s protocol. Serum and urine urea levels were measured using the colorimetric method (Sigma #D1032-30) with a RIPA lysis buffer (1% deoxycholic acid, 1% triton X-100, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 10 mM Tris HCl pH 7.5 and 1% protease inhibitor (complete protease inhibitor, Sigma, USA)). The samples were incubated at 37 °C for 30 min with 5x laemmli buffer and 5% 2-mercaptoethanol. The samples were run on precast 10% Stain-Free Gels (Biorad, USA), transferred to nitrocellulose membrane using the Bio-Rad Transblot transfer system (Biorad, USA), and blocked in 5% nonfat milk. The appropriate primary antibody diluted 1:100 in 5% normal goat serum in PBS for 1 h at room temperature. Subsequently, sections were incubated with primary antibody diluted (1:100) in 5% normal goat serum in PBS overnight at 4 °C followed by FITC- or Alexa-conjugated goat secondary antibody (1:200), and images were obtained using a confocal microscope. Liver samples were dissected and either flash frozen or fixed in 3% PFA before embedding and staining with hematoxylin and eosin.

**Immunostaining fixed intestinal and liver tissues.** For staining of mouse intestinal tissue sections, the intestine was dissected, and the ileum was rinsed with ice-cold saline and fixed in 10% formalin before paraffin embedding.45 Individual sections of each fixed and deparaffinized tissue followed by microwave treatment in 0.1 M sodium citrate solution (pH 6.0) for 5 min, followed by rinsing in PBS, and then washed in 1× PBS and blocked with 5% normal goat serum in PBS for 1 h at room temperature. Subsequently, sections were incubated with primary antibody diluted (1:100) in 5% normal goat serum in PBS overnight at 4 °C followed by FITC- or Alexa-conjugated goat secondary antibody (1:200), and images were obtained using a confocal microscope. Liver samples were dissected and either flash frozen or fixed in 3% PFA before embedding and staining with hematoxylin and eosin.

**Western blot protocol.** Freshly dissected kidney tissue was homogenized using the BeadBug™ (Benchtop Scientific) using 3.0 mm beads (Benchmark Scientific, #D1032-30) with a RIPA lysis buffer (1% deoxycholic acid, 1% triton X-100, 0.1% SDS, 150 mM NaCl, 1 mM EDTA, 10 mM Tris HCl pH 7.5 and 1% protease inhibitor (complete protease inhibitor, Sigma, USA)). The samples were incubated at 37 °C for 30 min with 5x laemmli buffer and 5% 2-mercaptoethanol. The samples were run on precast 10% Stain-Free Gels (Biorad, USA), transferred to nitrocellulose membrane using the Bio-Rad Transblot transfer system (Biorad, USA), and blocked in 5% nonfat milk. The appropriate primary antibody diluted 1:100 in 5% normal goat serum in PBS overnight at 4 °C followed by FITC- or Alexa-conjugated goat secondary antibody (1:200), and images were obtained using a confocal microscope. Liver samples were dissected and either flash frozen or fixed in 3% PFA before embedding and staining with hematoxylin and eosin.

**Quantitative real-Time PCR (qRT-PCR).** Whole kidney (or intestine or liver) preserved in RNALater™ (Qagen, #P1017980) was homogenized by bead beating using a BeadBug™ (Benchtop Scientific) using 3.0 mm beads (Benchmark Scientific, #D1032-30). RNA was isolated using TRIzol™ (Ambion, #15596062) using Phase Lock Gel Heavy tubes (Quantabio, #2302830) for phase separation followed by RNA clean up using the RNeasy® Mini Plus Kit (Qagen, #74134). 5 µg of RNA was then transcription into cDNA using the SuperScript™ III First-Strand Synthesis System (Invitrogen, #18080051). 10 ng of the resulting cDNA was used as template for qPCR using PowerUp™ SYBR® Green Master Mix (Applied Biosystems, #A25742), run using the QuantStudio 3 Real Time PCR System (Applied Biosystems). Manufacturer’s specifications were followed for all reagents listed above. Primers were validated using the following sequences: ABCG2 F- CAAATGGCTCGGAAAACCC, R-CTCTAGCCTATTTGTACCC; GAPDH F- CTTTGACCTGATTGGAAGGCC, R- TCGAGGAGATGTCTTG.
Uric acid transport study in ex vivo intestinal loop. Following anesthesia by isoflurane, a laparotomy was performed, and experimental loops of 3–5 cm length were constructed at the small intestine by tying with nonabsorbable silk. For a typical experiment, 4–6 loops were isolated from the small intestine and placed into 100 mm × 20 mm dishes containing prewarmed (37 °C) Ringer’s (in mM: 140 NaCl, 5 KCl, 1 MgCl2, 2 CaCl2, 10 HEPES, 10 glucose, pH 7.4) of specific urine concentration, then Ringer’s (100–200 μl) was injected in each loop via a tuberculin syringe through the ligated end of the loop to allow for transport of urate from serosal (s) to mucosal (m). After 10 mins, these loops were placed in ice cold Ringer’s (no urate). The fluid from each loop was collected, and the amount of urate in the loop with respect to the surface area of the loop (μM/cm²/time) was calculated. For mucosal to serosal urate transport loops, were filled with 1 mM urate and submersed into an Eppendorf tube containing 150–200 μl Ringer’s solution at 37 °C. Unidirectional fluxes were directly measured and used to calculate the overall net direction of urate movement (JUAnet) across the epithelium.

References

1. Woodward, O. M. ABCG2: the molecular mechanisms of urate secretion and gen. Am. J. Physiol. Renal. Physiol. 309, F485–F488 (2015).
2. Major, T. J., Dalbeth, N., Stahl, E. A. & Merriman, T. R. An update on the genetics of hyperuricaemia and gout. Nat. Rev. Rheumatol. 14, 341–353 (2018).
3. Krishnan, E., Lessov-Schlaggar, C. N., Krasnow, R. E. & Swan, G. E. Nature Genetics of hyperuricaemia and gout. Nat. Rev. Rheumatol. 5, 464 (2014).
4. Huysmans, D., Liu, S. & Miner, J. N. Urate Handling in the Human Body. Curr. Rheumatol. Rep. 18, 34 (2016).
5. Wang, J. et al. Hyperuricemia and risk of incident hypertension: a systematic review and meta-analysis. JAMA Intern. Med. 181, 1519–1526 (2019).
6. Kuwabara, M. et al. Asymptomatic hyperuricemia without comorbidities and uncertainties. Adv. Chronic Kidney Dis. 19, 358–371 (2012).
7. Ketharaththan, S. et al. A non-coding genetic variant maximally associated with serum urate levels is functionally linked to HNF4A-dependent PDX1 expression. Hum. Mol. Genet. 27, 3964–3973 (2018).
8. Leask, M. et al. Functional urate-associated genetic variants influence expression of lincRNAs LINC01229 and MAFTRR. Front. Genet. 9, 733 (2018).
9. Bobulescu, I. A. & Moe, O. W. Renal transport of uric acid: evolving concepts and uncertainties. Adv. Chronic Kidney Dis. 19, 358–371 (2012).
10. Enomoto, A. et al. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. Nature 417, 447–452 (2002).
11. Tan, A. et al. Large-scale whole-exome sequencing association studies identify rare functional variants influencing serum urate levels. Nat. Commun. 9, 4228 (2018).
12. Vitart, V. et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat. Genet. 40, 437–442 (2008).
13. Woodward, O. M. et al. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc. Natl Acad. Sci. USA 106, 10338–10342 (2009).
14. Tin, A. et al. Target genes, variants, tissues and transcriptional pathways influencing human serum urate levels. Nat. Genet. 51, 1459–1474 (2019).
15. Hosomi, A., Nakanshi, T., Fujita, T. & Tamai, I. Extra-renal elimination of uric acid via intestinal efflux transporter BCRP/ABCG2. PLoS ONE 7, e30456 (2012).
16. Ichida, K. et al. Decreased extra-renal urate excretion is a common cause of hyperuricemia. Nat. Commun. 3, 76 (2012).
17. DeBosch, B. J., Kluth, O., Fujiwara, H., Schurmann, A. & Moley, K. Early-onset metabolic syndrome in mice lacking the intestinal uric acid transporter SLCA29. Nat. Commun. 5, 4642 (2014).
18. Hyndman, D., Liu, S. & Miner, J. N. Urate Handling in the Human Body. Curr. Rheumatol. Rep. 18, 34 (2016).
19. Tai, V., Merriman, T. R. & Dalbeth, N. Genetic advances in gout: potential applications in clinical practice. Curr. Opin. Rheumatol. 31, 144–151 (2019).
20. Woodward, O. M., Kottgen, A. & Kottgen, M. ABCG transporters and disease. PERS. 278, 312–324 (2018).
21. Geophas, M. C. et al. ABCG2 polymorphisms in gout: insights into disease susceptibility and treatment approaches. Pharmacogenomics Pers. Med. 10, 129–147 (2017).
22. Matsuo, H. et al. Common defects of ABCG2, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. Sci. Transl. Med. 5, 2069111 (2013).
23. Woodward, O. M. et al. Gout-causing Q141K mutation in ABCG2 leads to instability of the nucleotide-binding domain and can be corrected with small molecules. Proc. Natl Acad. Sci. USA 110, 5233–5238 (2013).
24. Kottgen, A. et al. Genome-wide association studies identify 18 new loci influencing plasma uric acid levels. Nat. Genet. 45, 145–154 (2013).
25. Matsuo, H. et al. Genome-wide association study of clinically defined gout identifies multiple risk loci and its association with clinical subtypes. Ann. Rheum. Dis. 75, 652–659 (2015).
26. Kannagara, D. R. et al. Hyperuricaemia: contributions of urate transporter ABCG2 and the fractional renal clearance of urate. Ann. Rheum. Dis. 75, 1363–1366 (2016).
27. Wu, H. et al. Single-cell transcriptomics of a human kidney allograft biopsy specimen defines a diverse inflammatory response. J. Am. Soc. Nephrol. 29, 2069–2080 (2018).
28. Wu, H. et al. Comparative analysis and refinement of human PSC-derived kidney organoid differentiation with single-cell transcriptomics. Cell Stem Cell 20, 869–881 (2018).
29. Huls, M. et al. The breast cancer resistance protein transporter ABCG2 is expressed in the human kidney proximal tubule apical membrane. Kidney Int. 73, 220–225 (2008).
30. Hollis-Moffatt, J. E. et al. Role of the urate transporter SLCA29 gene in susceptibility to gout in New Zealand Maori, Pacific Island, and Caucasian Test-control sample sets. Arthritis Rheum. 60, 3485–3492 (2009).
31. Matsuo, H. et al. ABCG2 dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload. Sci. Rep. 4, 3755 (2014).
32. Haber, A. L. et al. A single-cell survey of the small intestinal epithelium. Nature 551, 333–339 (2017).
33. Gutmann, H., Hruz, F., Zimmermann, C., Beglinger, C. & Drewe, J. Distribution of breast cancer resistance protein (BCRP/ABCG2) mRNA expression along the human GI tract. Distribution of breast cancer resistance protein (BCRP/ABCG2) mRNA expression along the human GI tract. Biochem. Pharm. 70, 695–699 (2005).
34. Miyata, H. et al. Identification of Febuxostat as a New Strong ABCG2 Inhibitor: Potential Applications and Risks in Clinical Situations. Front. Pharm. 7, 518 (2016).
35. Stamp, L. K. et al. ABCG2 rs2231142 (Q141K) and oxypurinol concentrations in people with gout receiving allopurinol. Drug Metab. Pharmacoceon. 33, 241–242 (2018).
36. Zhang, K. & Li, C. ABCG2 gene polymorphism rs2231142 is associated with gout comorbidities but not allopurinol response in primary gout patients of a Chinese Han male population. Hereditas 156, 26 (2019).
37. Johnson, R. J., Lanasa, M. A. & Gaucher, E. A. Uric acid: a danger signal from the RNA world that may have a role in the epidemic of obesity, metabolic syndrome, and cardio renal disease: evolutionary considerations. Semin. Nephrol. 31, 394–399 (2011).
38. Novikov, A. et al. SLGT2 inhibition and renal urate excretion: role of luminal glucose, GLUT9, and URAT1. Am. J. Physiol. Renal. Physiol. 316, F173–F185 (2019).
39. Perez-Ruiz, F. et al. Renal clearance of uric acid is linked to insulin resistance and lower excretion of sodium in gout patients. Rheumatol. Int. 35, 1519–1524 (2015).
40. Cohen, J. C., Horton, J. D. & Hobbs, H. H. Human fatty liver disease: old questions and new insights. Science 332, 1519–1523 (2011).
41. BasuRay, S., Wang, Y., Smagris, E., Cohen, J. C. & Hobbs, H. H. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. *Proc. Natl Acad. Sci. USA* **116**, 9521–9526 (2019).

42. Zimmermann, R. et al. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* **306**, 1383–1386 (2004).

43. Lanasa, M. A. et al. Uric acid stimulates fructokinase and accelerates fructose metabolism in the development of fatty liver. *PLoS ONE* **7**, e47948 (2012).

44. Parkes Disease Study Group. et al. Inosine to increase serum and cerebrospinal fluid urate in Parkinson disease: a randomized clinical trial. *JAMA Neurol.* **71**, 141–150 (2014).

45. Winnard, D. et al. National prevalence of gout derived from administrative health data in Aotearoa New Zealand. *Rheumatology* **51**, 901–909 (2012).

46. Dalbeth, N. et al. Population-specific influence of SLC2A9 genotype on the acute hyperuricaemic response to a fructose load. *Ann. Rheum. Dis.* **72**, 1868–1873 (2013).

47. Dalbeth, N. et al. Influence of the ABCG2 gout risk 141 K allele on urate metabolism during a fructose challenge. *Arthritis Res Ther.* **16**, R34 (2014).

48. Fabre, S. et al. Accuracy of the HumaSens(plus) point-of-care uric acid meter using capillary blood obtained by fingertip puncture. *Arthritis Res Ther.* **20**, 78 (2018).

49. Sarkar, P. et al. Zinc ameliorates intestinal barrier dysfunctions in shigellosis. *Science* **360**, 1383–1386 (2004).

50. Murtazina, R. et al. Tissue-specific regulation of sodium/proton exchanger isoform 3 activity in Na(+)/H(+) exchanger regulatory factor 1 (NHERF1) null mice. cAMP inhibition is differentially dependent on NHERF1 and exchange protein directly activated by cAMP in ileum versus proximal tubule. *J. Biol. Chem.* **282**, 25141–25151 (2007).

51. Martovetsky, G., Bush, K. T. & Nigam, S. K. Kidney versus liver specificity of PNPLA3 on lipid droplets and development of hepatic steatosis. *Hepatology* **61**, 108–118 (2015).

52. Jin, L. et al. Role of glucokinase in the subcellular localization of glucokinase regulatory protein. *Int. J. Mol. Sci.* **16**, 7377–7393 (2015).

53. Kakuma, T. et al. Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. *Proc. Natl Acad. Sci. USA* **97**, 8536–8541 (2000).

54. Smagris, E. et al. Poplin3148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. *Hepatology* **61**, 108–118 (2015).

55. Wu, J. W. et al. Deficiency of liver adipose triglyceride lipase in mice causes progressive hepatic steatosis. *Hepatology* **54**, 122–132 (2011).

56. Nobrega-Pereira, S. et al. G6PD protects from oxidative damage and improves healthspan in mice. *Nat. Commun.* **7**, 10894 (2016).

57. Le Lay, S., Lefrere, I., Trautwein, C., Dugail, I. & Krief, S. Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein beta as an SREBP-1C target. *J. Biol. Chem.* **277**, 35625–35634 (2002).

58. Clarke, L. L. A guide to Ussing chamber studies of mouse intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* **296**, G1151–G1166 (2009).

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**Additional information**

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