Identification of GLUT12/SLC2A12 as a urate transporter that regulates the blood urate level in hyperuricemia model mice

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Recent genome-wide association studies have revealed some genetic loci associated with serum uric acid levels and susceptibility to gout/hyperuricemia which contain potential candidates of physiologically important urate transporters. One of these novel loci is located upstream of *SGK1* and *SLC2A12*, suggesting that variations in these genes increase the risks of hyperuricemia and gout. We herein focused on *SLC2A12* encoding a transporter, GLUT12, the physiological function of which remains unclear. As GLUT12 belongs to the same protein family as a well-recognized urate transporter GLUT9, we hypothesized that GLUT12 mediates membrane transport of urate. Therefore, we conducted functional assays and analyzed *Glut12* knockout hyperuricemia model mice, generated using the CRISPR-Cas9 system. Our results revealed that GLUT12 acts as a physiological urate transporter and its dysfunction elevates the blood urate concentration. This study provides insights into the deeper understanding of the urate regulatory system in the body, which is also important for pathophysiology of gout/hyperuricemia.

GWAS | gout | hyperuricemia | serum uric acid | urate handling

Genes encoding physiologically important urate transporters have been reportedly associated with serum uric acid (SUA) levels and susceptibility to gout/hyperuricemia (1). A previous study has reported a novel locus for SUA on human chromosome 6 near the *SGK1* and *SLC2A12* genes (2), which is consistent with the result derived from our previous genetic study (3). As *SGK1* encodes a kinase protein, we focused on *SLC2A12* encoding a transporter protein known as GLUT12, whose physiological function remains unclear. Since GLUT12 belongs to the same protein family as GLUT9/SLC2A9 [a physiologically important urate transporter (4)], we hypothesized that GLUT12 regulates blood urate concentrations as a urate transporter. To verify this hypothesis, we conducted functional assays and analyzed newly generated *Glut12* knockout (KO) hyperuricemia model mice.

First, using mammalian cells transiently expressing GLUT12, we examined whether GLUT12 can transport urate. After confirming the cellular expression and plasma membrane localization of GLUT12 (Fig. 1 *A* and *B*), we measured the amount of [8-^{14}C]urate incorporated into the cells (Fig. 1*C*). In Krebs buffer mimicking the ionic content of plasma, GLUT12-mediated urate transport was detected. Neither exclusion of sodium from the Krebs buffer nor a high-potassium buffer condition that depolarizes plasma membranes affected GLUT12-mediated urate uptake, indicating that GLUT12 is a sodium-independent and bidirectional urate transporter. Additionally, an enhanced green fluorescent protein (EGFP) tag hardly affected the urate transport activity of GLUT12 (Fig. 1*D*); GLUT12 is more active at lower pH (Fig. 1*E*). As GLUT12-mediated urate uptake increased linearly with time over 10 min (Fig. 1*F*), uptake at 5 min was evaluated in subsequent kinetic analysis. Under the experimentally maximum urate concentration (500 μM), GLUT12-mediated urate uptake was not saturable (Fig. 1*G*). Considering urate concentrations in the blood of healthy (nonhyperuricemia) subjects (<0.41 mmol/L: 6.8 mg/dL) (1), our results suggest that GLUT12 can physiologically maintain urate transport activity in humans. With mouse Glut12, similar results were obtained (Fig. 1 *H–J*).

Next, to address the physiological impact of Glut12 on blood urate concentrations, we analyzed *Glut12* KO mice. Notably, urate is degraded in wild-type mice owing to urate oxidase (Uox), which humans lack. Therefore, we generated two lines of *Glut12* KO mice on *Uox* KO background (5), as *Uox* KO mice spontaneously develop hyperuricemia. The established *Glut12*/*Uox* double-KO (DKO) lines had deletions causing a frameshift in Glut12 (Fig. 1*K*). Each frameshift mutation disrupted the Glut12 urate transport function (Fig. 1*L*), indicating that the DKO lines were deficient in Glut12 function. Then, we examined plasma parameters; urate concentrations in both DKO mouse lines were higher than those in control *Uox* KO mice (Fig. 1*M*), which strongly supported our hypothesis. However, *Glut12* KO did not affect the plasma creatinine concentration (Fig. 1*N*) and fractional renal urate excretion in each DKO line (Fig. 1*O*), suggesting that Glut12 deficiency does not induce severe renal dysfunction or decrease renal urate excretion. Moreover, hepatic urate levels in the DKO mice tended to be lower than those in control mice (Fig. 1*P*), suggesting that the *Glut12* deficiency-dependent elevation of the blood urate concentration is not due to overproduction of uric acid in the liver. However, importantly, the significantly lower values of the liver-to-plasma urate concentration ratio in the DKO mice (Fig. 1*Q*) implied that Glut12 might be involved in urate transport from the blood into the liver. These findings are in line with gene expression

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The authors declare no competing interest.

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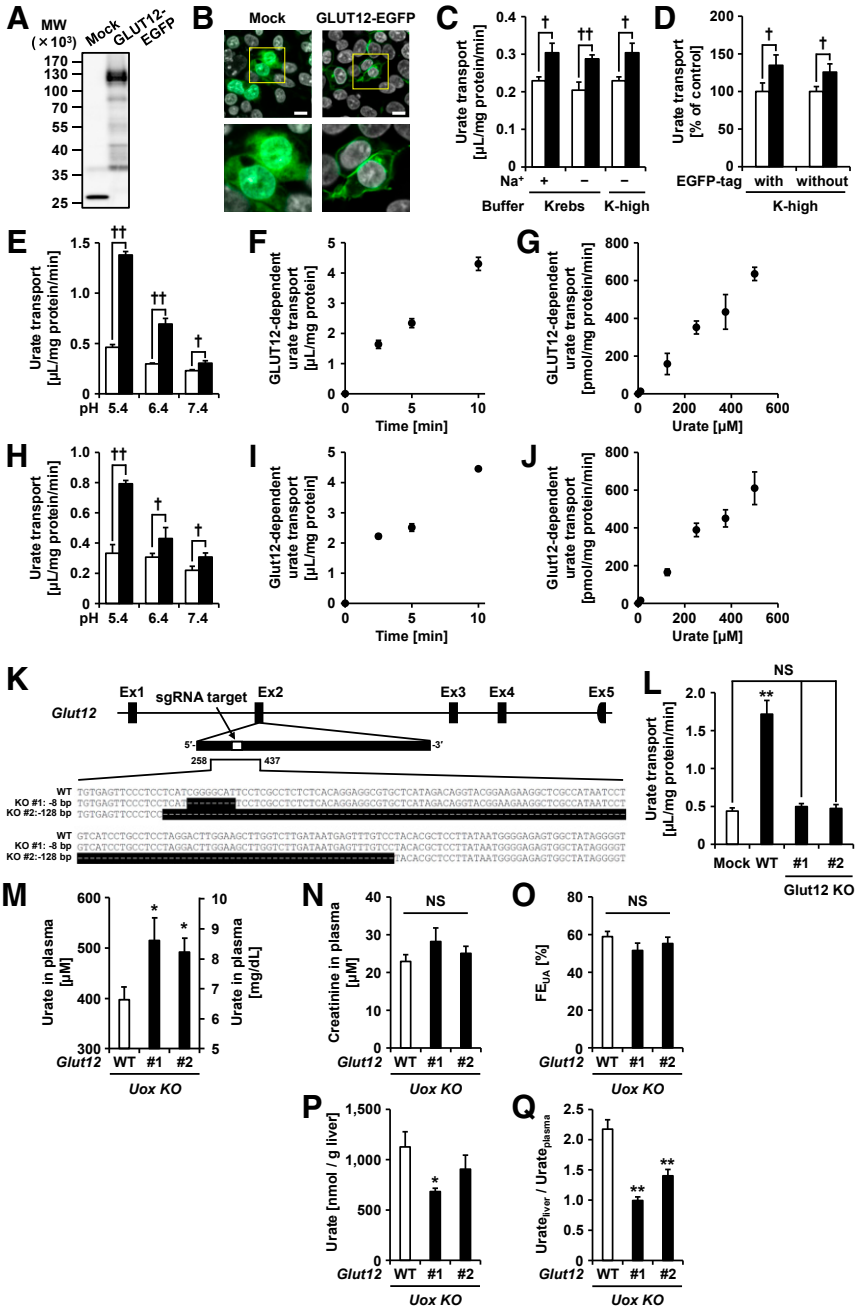

Fig. 1. GLUT12/SLC2A12 is a urate transporter, genetic disruption of which affects the blood urate concentration in genetically induced hyperuricemia model mice. (*A–J*) Identification of human GLUT12 and mouse Glut12 as a novel urate transporter. Immunoblotting (*A*) and confocal microscopic observation (*B*) of GLUT12 transiently expressed in HEK293 cells. Magnified images of representative cells outlined by yellow squares are also shown. (Scale bars, 10 μm.) Urate transport activities of GLUT12 in various transport buffers (*C*). EGFP tag barely affects the urate transport activity of GLUT12 (*D*). White, mock; black, GLUT12. Acidic pH-dependent [^{14}C]urate transport activities of GLUT12 (*E*) and Glut12 (*H*). Time profiles for GLUT12- (*F*) or Glut12- (*I*) mediated [^{14}C]urate uptake into HEK293 cells. Unsaturated GLUT12- (*G*) or Glut12- (*J*) mediated [^{14}C]urate transport activities with increasing urate concentrations. In *E–J*, Krebs–Ringer buffer (pH 5.4) was used. (*K–Q*) Physiological impact of *Glut12* KO on blood urate concentrations in hyperuricemia model mice. Schematic illustration of disrupted *Glut12* gene in each *Glut12*/*urate oxidase* (*Uox*) DKO line: #1, Glut12^{8del}; #2, Glut12^{128del} (*K*). Glut12 KO mutants transiently expressed in HEK293 cells exhibit no urate transport activity in Krebs–Ringer buffer (pH 5.4) (*L*). Plasma urate concentrations are significantly higher in *Glut12*/*Uox* DKO mice than in *Uox* KO mice (*Glut12* wild-type [WT]; control) (*M*). Plasma creatinine concentration (*N*) and fractional renal urate excretion (FE_{UA}, urate clearance/creatinine clearance ratio) (*O*) do not significantly differ; $n = 22$ (WT), 7 (#1), and 13 (#2). The hepatic urate level tends to be lower (*P*) and the liver-to-plasma urate concentration ratios are significantly lower (*Q*) in *Glut12*/*Uox* DKO mice than in *Uox* KO mice; $n = 4$. Data are expressed as the mean ± SD, $n = 3$ (*C–J* and *L*) or the mean ± SEM (*M–Q*). $^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$ (two-sided *t* test). $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control; NS, not significantly different among groups (Dunnett's test).

data published in RefEx (6) showing that GLUT12/Glut12 is expressed relatively ubiquitously, including in the liver.

In summary, we successfully identified GLUT12 as a urate transporter, dysfunction of which elevates the blood urate concentration. Further investigation of the long-term effects of this phenotype on whole-body health including lifespan is warranted. Our findings deepen the understanding of the urate regulatory system in the body, which is also important for pathophysiology of gout/hyperuricemia.

Materials and Methods

All animals received humane care according to criteria outlined in the *Guide for the Care and Use of Laboratory Animals* (7). All animal experiments were performed according to methods approved by the Institutional Animal Care and Use Committee of the University of Tokyo. All animals were maintained as described previously (8). *Uox* KO mice with a C57BL/6J genetic background, originally obtained from The Jackson Laboratory (stock no.: 002223, B6;129S7-Uox^{tm1Bay}/J), were from our previous study (9). Mice with the *Uox*$^{-/-}$ background were administered 180 mg/L allopurinol (Wako Pure Chemical Industries) and 4 g/L Uralyt-U (a standardized mixture of potassium citrate and sodium citrate; Nippon Chemiphar) via drinking water to attenuate hyperuricemia phenotypes that cause serious kidney injury and premature death phenotype (5, 10). *Glut12*/*Uox* DKO mice were generated using the CRISPR-Cas9 system (11), using wild-type eggs and *Uox* KO sperm. After crossing of mice, we designated mice with homozygous Glut^{8del}; Uox^{tm1Bay} and Glut^{128del}; Uox^{tm1Bay} alleles as *Glut12*/*Uox* DKO #1 and *Glut12*/*Uox* DKO #2, respectively. These two alleles had 8 and 128 base deletions in exon 2 of *Glut12*, and both mutations caused a frameshift. Functional analyses and experimental measurements were conducted according to our previous studies (8, 9, 12).

Data Availability. All relevant data are within the paper.

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