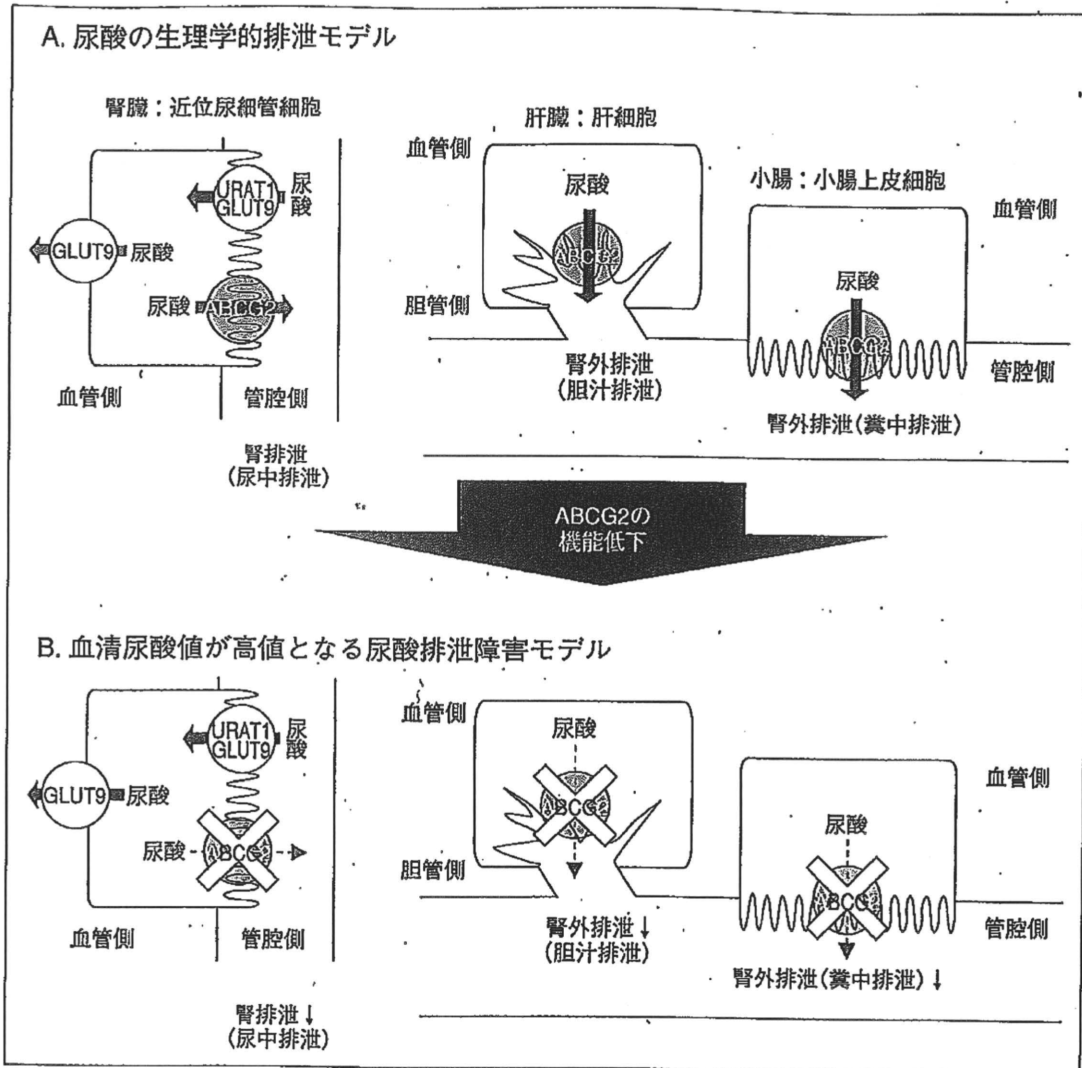

図2　尿酸排泄輸送体ABCG2によるヒト腎臓および腸管からの尿酸排泄機構
輸送体ABCG2は腎近位尿細管細胞，肝細胞および小腸上皮細胞の管腔側に発現していることが報告されている．輸送体ABCG2の機能が低下することで血清尿酸値が上昇することから，ABCG2は尿酸の腎排泄および腎外排泄という生理学的役割を担っていることが示唆された．ABCG2の排泄機能が低下するSNPは高頻度にみられ，このような例では尿酸排泄量が減少し，血清尿酸値が上昇して痛風の発症につながると考えられる．
（文献[3]より引用改変）

ルタミン(Q)に対応する141番目のコドンがリシン(K)に対応するコドンに」それぞれ変異したSNPを指す．日本人において，Q126Xは約5％，Q141Kは約50％に認め，ともに変異としては比較的頻度が高いことがわかっている[3)13)]．

Q126XおよびQ141Kについて，これらの遺伝学的解析および機能解析を行ったところ，以下のことが見出された．すなわち，「Q126Xは輸送体ABCG2の排泄機能を約0％まで消失させ，Q141Kは約50％に半減させる」，「これらのSNPは同じハプロタイプ上には同時に存在しない，すなわち片親からは多くともどちらか1種類のSNPしか遺伝しない」ということである．片親からABCG2の輸送機能が0％，50％または100％となる遺伝子型を受け継ぐことから，「両親の遺伝子型を受け継いだ子の全体としてのABCG2の機能は0％，25％，50％，75％，100％に分類して推定可能である」ということがわかった．たとえば，父親か

らQ126X（機能消失型変異），母親からQ141K（機能半減型変異）を受け継いだ場合，子のABCG2の機能は「(0％＋50％)/2」すなわち25％となることが予想される．同様に，父親からQ141K（機能半減型変異）を受け継ぎ，母親から変異のない遺伝子型を受け継いだ場合，子のABCG2の機能は「(50％＋100％)/2」すなわち75％となることが予想される．

この分類に基づいて，血清尿酸値正常（7 mg/dl以下）の対照群男性865名と痛風患者群男性161症例において，ABCG2の機能低下が及ぼす痛風のリスクについて評価したところ，ABCG2になんらかの機能低下があるヒトでは約3倍以上，25％以下の機能の場合は約26倍の痛風発症リスクを認めることがわかった．さらに，ABCG2になんらかの機能低下を持つヒトは痛風患者の8割を占め，25％以下の機能を持つヒトの割合は，対照群の0.9％に比較して，患者群では約10％であったことからも，*ABCG2*が痛風の主要な病因遺伝子であることが示唆された（図1）．

生理学的尿酸排泄機構の提唱

これまで述べてきたように，輸送体URAT1およびGLUT9については，ヒトの腎近位尿細管に発現し，尿酸の再吸収に関与していることが確認されている．輸送体ABCG2は，近位尿細管のほかにも肝細胞および小腸上皮細胞の管腔側に発現していることが報告されており，前述のわれわれの知見とあわせ，図2-Aに示したような，ABCG2を介した腎排泄および腎外排泄からなる，尿酸の生理学的排泄モデルが提唱された[3]．また，2つのSNPの組み合わせによる輸送体ABCG2の機能異常は，図2-Bに示したような尿酸の排泄障害から高尿酸血症をきたし，これが痛風の発症リスクを高めているものと考えられる．

おわりに

*ABCG2*になんらかの変異を持つヒトは日本人の約半数に見出されており，これらの変異を持つことがただちに高尿酸血症および痛風の発症に結びつくわけではない．むしろ，前述のように，痛風の発症には環境要因と遺伝要因がお互いに重なり合って関与しているものと考えられる．痛風を発症していない対照群の約半数にABCG2の機能低下を認めてはいるが，これはあくまでも遺伝的要因としてのリスクをもつものが対照群の半数を占めているということに過ぎない．逆に，痛風患者の8割がABCG2になんらかの機能低下を持っていたが，機能低下がなくても痛風を発症した患者を約2割認めることから，これらの患者の発症に対しては，他の遺伝要因や環境要因の影響も考えられる．

ここで論じてきたように，GWASなどの遺伝子解析や機能解析などを通したcommon diseaseとしての痛風の遺伝子の探索が現在もさかんに進められている．今後は個人差に基づいた痛風の予防法や治療法の開発が進んでいくものと期待され，更なる研究の発展が期待される．

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* * *

講演会・学会発表

Molecular mechanism of urate reabsorption and excretion in humans

第87回日本生理学大会
2010年5月19-21日, 盛岡

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尿酸トランスポーターによる尿酸再吸収および排泄の分子機構

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Summary

We have previously identified two urate reabsorption transporters, URAT1/SLC22A12 and GLUT9/SLC2A9, through the studies on hypouricemia patients. The loss-of-function mutations in these transporter genes cause renal hypouricemia type 1 and type 2, respectively. These findings, together with their renal expression patterns, showed that URAT1 and GLUT9 physiologically mediated renal urate reabsorption in humans. We also found that ABCG2 is a high-capacity urate secretion transporter and demonstrated that its common variants reduce their urate excretion function and consequently increase serum urate levels. ABCG2 shows apical expression in kidney, liver and intestine. We then propose a molecular model of urate reabsorption and excretion in humans as well as an impaired model as in hyperuricemia patients; urate is reabsorbed by URAT1 and GLUT9 in kidney and excreted by ABCG2 in kidney (renal excretion) and in liver and intestine (gut excretion, or extra-renal excretion), while impaired these functions cause hyperuricemia and gout.

Introduction

<GLUT9>

Renal hypouricemia (MIM 220150) is a common inherited disorder that is characterized by low serum uric acid (urate) levels and impaired renal urate transport; it is typically associated with severe complications such as exercise-induced acute renal failure and nephrolithiasis. We have previously reported that a causative gene for renal hypouricemia is *URAT1*, also called *SLC22A12*. However, the fact of renal hypouricemic patients without *URAT1* mutations implies the existence of another urate transporter. Recent genome-wide association studies have revealed that the most significant single-nucleotide polymorphisms (SNPs) associated with urate concentrations map within *GLUT9*, also known as *SLC2A9* . We then decided to investigate those cases with a large human database.

<ABCG2>

Gout based on hyperuricemia is a common disease with a genetic predisposition. A genome-wide linkage study reported that the *ABCG2* locates in a gout locus on chromosome 4q. Besides its transport of nucleotide analogs that are structurally similar to urate, we have reported that ABCG2 is an exporter that has polymorphic reduced functionality variantsor nonfunctional variants. These findings suggest that ABCG2 could be a urate secretion transporter gene and thus be a promising candidate gene for gout.

Materials and methods

<GLUT9>

Clinicogenetic analysis of hypouricemia

We surveyed the health examination database of about 50,000 personnel of Japan Maritime Self-Defense Force (JMSDF). 50 JMSDF persons and 20 outpatients who had urate levels of ≤ 3.0 mg/dl (178 mM) with written consent was selected. Among them, 23 person who had no mutation in *URAT1* was analysed to find mutation in *GLUT9*.

Mutation analysis

Functional mutant analysis of *GLUT9* mutants were performed using Xenopus oocyte expression system as described elsewhere .

<ABCG2>

Genetic analysis of gout/hyperuricemia

Mutation analysis of all coding regions and intron-exon boundaries of the *ABCG2* gene was performed for 90 Japanese hyperuricemia patients. For QTL analysis of SUA concentrations,genotyping of Q141K in 739 Japanese individuals was performed.

Functional analysis

Vesicles studies were performed for wild-type and mutation ABCG2 with [^{14}C] labeled urate.

Association analysis

228 Japanese male hyperuricemia cases (including 161 gout cases) as well as 871 Japanese male controls (SUA≤7.0mg/dl) were genotyped.

Results < GLUT9 >

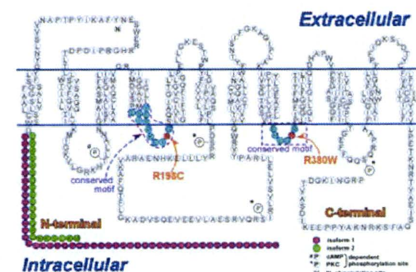

Fig.1. *GLUT9* mutations in patients with renal hypouricemia. Mutation positions in a predicted human GLUT9 membrane topology model.

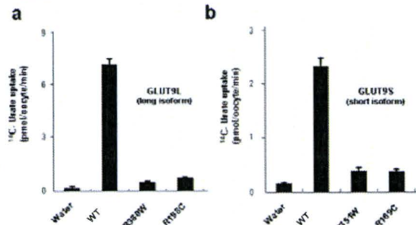

Fig.2. Urate transport activity in oocytes was markedly reduced both in GLUT9L mutants (R380W and R198C) (**a**) and in GLUT9S mutants (R351W and R169C, which correspond to R380W and R198C in GLUT9L) (**b**).

Results < ABCG2 >

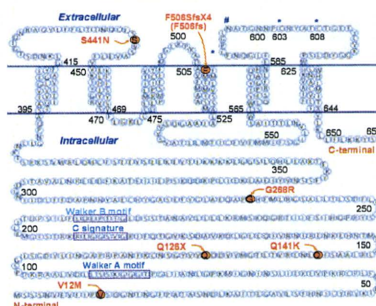

Fig.3. The topological model of ABCG2 and six nonsynonymous mutation sites (magenta) found in hyperuricemic patients.

#, N-linked glycosylation site (N596); *, cysteine residues for disulfide bonds (C592, C603, and C608).

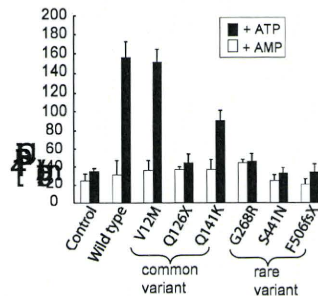

Fig.4. Urate transport of mutated ABCG2.

ATP-dependent transport of urate was reduced by approximately half (46.7%) in Q141K and was nearly eliminated in Q126X, G268R, S441N, and F506SfsX4 mutants.

V12M, Q126X, and Q141K are common variants.

Table 1. Association analysis of *ABCG2* genotype combinationin gout patients.

Estimated transport	Genotype Q126X	Genotype Q141K	Number Gout	Number Control	P-value	OR	95% CI
1/4 function	T/T T/C	C/C A/C	16	8	3.39×10^{-21}	25.8	10.3-64.6
1/2 function	T/C C/C	C/C A/A	37	110	2.23×10^{-9}	4.34	2.61-7.24
3/4 function	C/C	A/C	72	308	2.29×10^{-7}	3.02	1.96-4.65
full function	C/C	C/C	34	439			

Haplotype frequency analysis revealed that there is no simultaneous presence of the minor alleles of Q126X and Q141K in one haplotype.

ABCG2 is then estimated as shown above from these two common variants.OR is obtained by comparing with non-risk genotype combination C/C (Q126X) and C/C (Q141K).

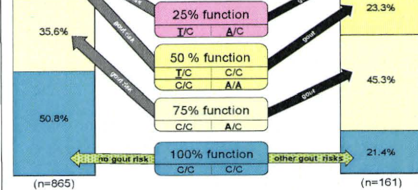

Fig.5. Relation between ABCG2 transport dysfunction and gout

Genotype combinations of Q126X and Q141K are divided into several groups based on estimated ABCG2 transport functions. The Q126X homozygous and heterozygous mutations were identified in up to 13.5% of total gout patients (n = 161). Up to 10.1% of total gout patients have genotype combinations resulting in ≤25% function, whereas the asymptomatic carriers of these genotype combinations, who would have possible risk of gout, were only 0.9% of the normal population (n = 865).

Discussion

<GLUT9>

a **Physiological model**

Proximal tubular cell

Normal serum urate levels

Apical (urine side)

Basolateral (blood side)

Pathogenic mutations GLUT9L (R198C, R380W) GLUT9S (R169C, R351W)

b **Impaired urate reabsorption**

Low serum urate levels (hypouricemia)

Fig.6. Proposed model of renal urate reabsorption in humans.

(a) Based on our findings, we propose a physiological model of renal urate transport via human GLUT9 molecules. Here, GLUT9 mediates renal urate reabsorption on both sides of the proximal tubular cells. URAT1 is expressed only on the apical side and is indirectly coupled with Na⁺-anion cotransporters such as sodium-dependent monocarboxylic acid transporter1/2 (SMCT1/2). (b) An impaired urate reabsorption model. Pathogenic mutations in GLUT9L and GLUT9S markedly reduce urate reabsorption and cause hypouricemia. Pyrazinecarboxylic acid (PZA), a metabolite of pyrazinamide is used for loading test of hypouricemic patients.

<ABCG2>

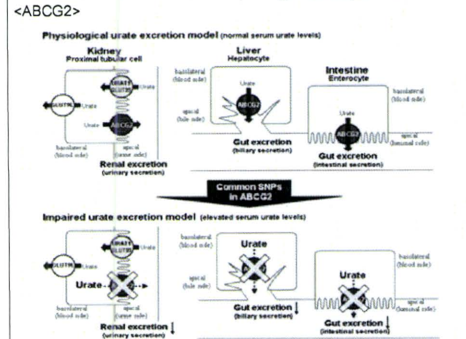

Fig.7. Proposed model of renal and gut urate excetion in human

Proposed model of the renal and gut urate excretion. In the "impaired urate excretion model," ABCG2 variant proteins with common SNPs on the apical side markedly reduce the urate excretion and elevate SUA. In proximal tubular cells, other urate transporters (URAT1 and GLUT9) mediate renal urate reabsorption as shown in the previous figure ."GLUT9L" represents GLUT9 isoform 1 (long isoform) and "GLUT9S" represents GLUT9 isoform 2 (short isoform).

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Pathogenic GLUT9 mutations in renal hypouricemia type 2

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Summary

Renal hypouricemia (MIM 220150) is a hereditary disease characterized by low serum uric acid (SUA) levels, and has severe complications such as exercise-induced acute renal failure and nephrolithiasis. We have previously reported that *URAT1/SLC22A12* encodes a renal urate-anion exchanger and its mutations cause renal hypouricemia type 1. With a large health examination database of Japan Maritime Self Defense Force, we searched hypouricemia patients and identified two heterozygous mutations in *GLUT9/SLC2A9*. We found that *GLUT9* encodes another renal urate-anion exchanger and that its mutations cause renal hypouricemia type 2. R380W and R198C (mutation sites in GLUT9L) are highly conserved amino acid motifs in "sugar transport proteins signatures" which are observed in GLUT family transporters. The corresponding mutations in *GLUT1* (R333W and R153C) are known to cause GLUT1 deficiency syndrome. Arginine residues in this motif are reported to be an important determinant of membrane topology of human GLUT1, and the same may be true in GLUT9 on the basis of membrane topology. Their mutants showed markedly reduced urate transport in oocyte expression study, which would be the result of loss of positive charges of those amino acid motifs. We additionally performed mutational analysis of *GLUT9* in another 50 hypouricemia patients, and identified a new hypouricemia patient who have R380W mutation in *GLUT9* gene. Our findings, together with previous reports on GLUT9 localization, suggest that these *GLUT9* mutations cause renal hypouricemia by their decreased urate reabsorption on both sides of the renal proximal tubules. These findings also enable us to propose a physiological model of the renal urate reabsorption and can be a promising therapeutic target for gout and related cardiovascular diseases.

Introduction

Renal hypouricemia is a common inherited disorder that is characterized by low serum uric acid (urate) levels and impaired renal urate transport; it is typically associated with severe complications such as exercise-induced acute renal failure and nephrolithiasis (1,2). We have previously reported that a causative gene for renal hypouricemia is *URAT1*, also called *SLC22A12* (3). However, the fact of renal hypouricemic patients without *URAT1* mutations (4,5) implies the existence of another urate transporter. Recent genome-wide association studies have revealed that the most significant single-nucleotide polymorphisms (SNPs) associated with urate concentrations map within *GLUT9*, also known as *SLC2A9* (6-8). Because neither the physiological role of GLUT9 *in vivo* nor human cases with functional GLUT9 deficiency has been reported previously, we decided to investigate those cases with a large human database.

Materials and methods

Mutation analysis and construction of mutant cDNA: For the GLUT9 sequence determination, we used primers described by S. Li with slight modification (Table 1). Some primer sequences were newly selected according to the genomic structure of the human GLUT9 (see fig. S3). High molecular weight genomic DNA was extracted from peripheral whole blood cells (3, 4), and was amplified by PCR. The PCR products were sequenced in both directions using a 3130xl Genetic Analyzer (Applied Biosystems). Functional mutant analysis of GLUT9 mutants were performed using Xenopus oocyte expression system as described elsewhere (3).

Clinicogenetic analysis of hypouricemia with GLUT9 mutations: The following flowchart was used for clinicogenetic analysis (Fig.1).

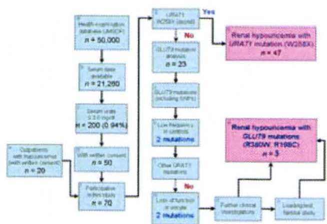

Fig.1 The flowchart for clinicogenetic analysis of hypouricemia with GLUT9 mutations.

Urate (mg/dl)	Frequency	Cumulative Frequency	Relative Frequency (%)	Cumulative Relative Frequency (%)
0.0–0.5	3	3	0.01	0.01
0.6–1.0	27	30	0.13	0.14
1.1–1.5	2	32	0.01	0.15
1.6–2.0	7	39	0.03	0.18
2.1–2.5	29	68	0.14	0.32
2.6–3.0	132	200	0.62	0.94
3.1–	21060	21260	99.06	100.00

Table.1 Frequency of hypouricemia of the Japan Maritime Self-Defense Force

Results

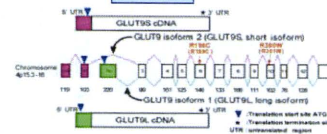

Fig.2 Genomic structure of the human *GLUT9* gene.
The structure of the *GLUT9* gene and cDNAs. The alternative splicing results in two transcripts: GLUT9 isoform 1 (GLUT9L) and isoform 2 (GLUT9S).

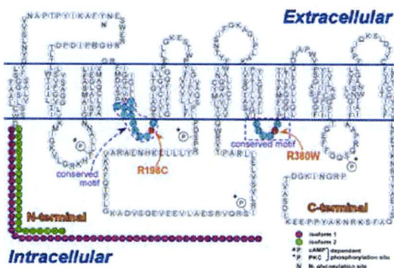

Fig.3 Topology model of GLUT9 and its mutation sites
Mutation positions in a predicted human GLUT9 membrane topology model.

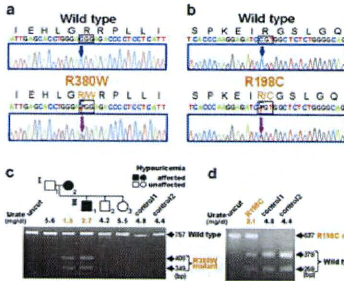

Fig.4 *GLUT9* mutations in patients with renal hypouricemia.
(a, b) Heterozygous mutations (1138C>T [R380W] and 592C>T [R198C]; magenta arrows) in the renal hypouricemia patients. (c, d) Genotyping by restriction enzyme analysis (*BtsC*I and *Alw*I). The response of PZA loading test targeting URAT1 was normal in these patients.

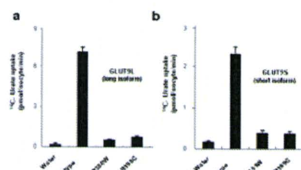

Fig.5 Markedly Reduced Urate Transport Activities in Oocytes that Express Mutant GLUT9 Isoforms
Urate transport activity in oocytes was markedly reduced both in GLUT9L mutants (R380W and R198C) (a) and in GLUT9S mutants (R351W and R169C, which correspond to R380W and R198C in GLUT9L) (b).

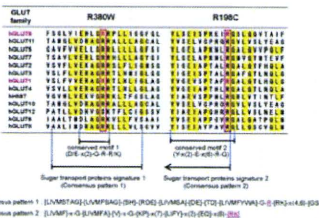

Fig.6 Amino acid conservation in the GLUT family transporters.

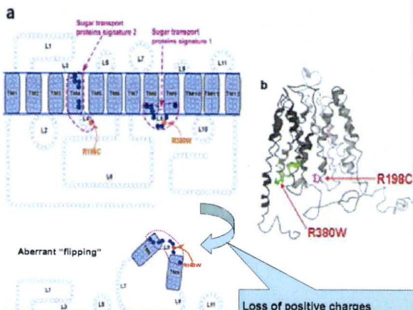

Fig.7 Pathogenic mutations and possible mechanisms
(a) Topology model of GLUT9L. Both mutations are at equivalent positions within the cytoplasmic loops, which cause a loss of positive charge. (b) Three-dementional model of GLUT9L. The pathogenic mutation sites are shown in green (R380) and in magenta (R198). Sugar transport proteins signatures 1 and 2 are shown in light green and pink, respectively.

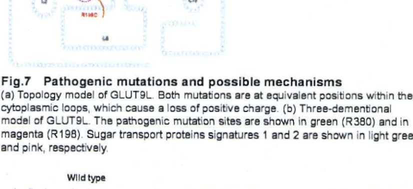

Fig.8 Heterozygous mutation (R380W) in another renal hypouricemia patient

We additionally performed mutational analysis of *GLUT9* in another 50hypouricemiapatients, and identified a new hypouricemia patient whohave R380W mutation in *GLUT9* gene.

Discussion

GLUT9 mutations in renal hypouricemia patients may change its topology

We identified loss-of-function mutations of *GLUT9* in renal hypouricemic patients having no *URAT1* mutations. Mutation sites in GLUT9 (R380W and R198C) are highly conserved amino acid motifs in "sugar transport proteins signatures", which is observed in GLUT family transporters. The corresponding mutations in GLUT1 (R333W and R153C) is known to cause GLUT1 deficiency syndrome (9). Arginine residues in this motif are reported to be an important determinant of membrane topology of human GLUT1 (10), and the same may be true in GLUT9 on the basis of membrane topology.

Physiological importance of GLUT9 in human urate transport

The urate metabolism in humans is quite different from that in mice due to the lack of uricase (11). Therefore, it is a great significance to identify the inactivating human *GLUT9* mutations using the large human population. In MDCK cells, GLUT9L and GLUT9S show basolateral and apical localization, respectively. Since inactivating mutations of either GLUT9L or GLUT9S dramatically reduced the urate transport activity, renal hypouricemia caused by these mutations may be ascribed to the decreased urate reabsorption on both sides of the renal proximal tubules, where GLUT9 expresses. Based on our findings, we propose a physiological model of renal urate transport, in which GLUT9 isoforms play a key

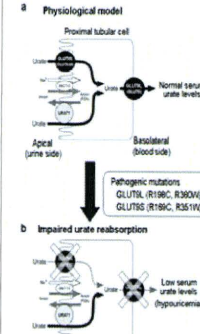

Fig.9 Proposed model of renal urate transport in humans.

(a) Based on our findings, we propose a physiological model of renal urate transport via human GLUT9 molecules. Here, GLUT9 mediates renal urate reabsorption on both sides of the proximal tubular cells. URAT1 is expressed only on the apical side and is indirectly coupled with Na+-anion cotransporters such as sodium-dependent monocarboxylic acid transporter1/2 (SMCT1/2). (b) An impaired urate reabsorption model. Pathogenic mutations in GLUT9L and GLUT9S markedly reduce urate reabsorption and cause hypouricemia. Pyrazinecarboxylic acid (PZA), a metabolite of pyrazinamide is used for loading test of hypouricemic patients.

GLUT9 as a novel therapeutic target

Taken together, we have identified *GLUT9* as a causative gene for renal hypouricemia and demonstrated that human GLUT9 physiologically regulates serum urate levels *in vivo*. Our results indicate that GLUT9 can be a promising therapeutic target for hyperuricemia, gout and related cardiovascular diseases.

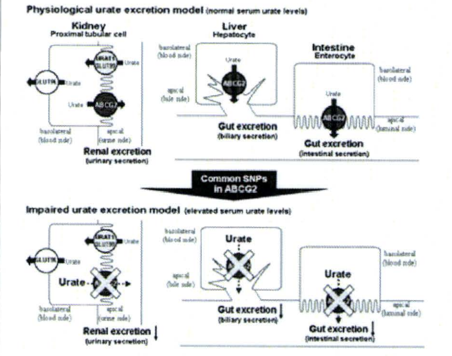

Fig.10 Proposed model of renal and gut urate excetion in human

Recently, we found that ABCG2 is a high-capacity urate secretion transporter and identified that ABCG2 is a major causative gene for gout (ref.13). We also proposed model of renal and gut urate excretion in human (ref.13). In the "impaired urate excretion model," ABCG2 variant proteins with common SNPs on the apical side markedly reduce the urate excretion and elevate SUA.

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ABCG2/BCRP as a major causative gene for gout

60th Annual ASHG Meeting
Nov 2-6, 2010, Washington DC

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Summary

Gout based on hyperuricemia is a common disease with a genetic predisposition. Recent genome-wide association study also showed that serum uric acid (SUA) levels and gout relates to *ABCG2* gene, which is reported to locate in a gout-susceptibility locus (MIM 138900) on chromosome 4q revealed by a genome-wide linkage study. We previously reported that ABCG2 is an exporter that has polymorphic reduced functionality variants. As ABCG2 exports nucleotide analogs structurally similar to urate, these findings suggest that ABCG2 could be a urate secretion transporter and a cause of gout. Mutation analysis of 90 Japanese hyperuricemia patients in *ABCG2* revealed six nonsynonymous mutations: V12M, Q126X, Q141K, G268R, S441N and F506SfsX4. ATP-dependent transport of urate was reduced by approximately half (46.7%) in Q141K, and was nearly eliminated in Q126X, G268R, S441N and F506SfsX4. Among these variants, relatively frequent two dysfunctional SNPs, Q141K (31.9%) and Q126X (2.8%), were then analyzed. Haplotype frequency analysis revealed that there is no simultaneous presence of Q126X and Q141K in one haplotype. As Q126X and Q141K are assigned to nonfunctional and half-functional haplotype, respectively, their six genotype combinations are divided into five functional groups. Gout risk of 75% function was increased with an OR of 3.02 (95% CI, 1.96-4.65; $P=2.29\times10^{-7}$) and that of 50% function was with an OR of 4.34 (95% CI, 2.61-7.24; $P=2.23\times10^{-9}$). Gout risk of ≤25% function was remarkably increased with an OR of 25.8 (95% CI, 10.3-64.6; $P=3.39\times10^{-21}$). 10.1% of gout patients had these genotypes of ≤25% function, while only 0.9% of control males have the same genotype combinations. In addition, genotype combinations of full function are detected in 50.8% of the control subjects but only in 21.4% of gout patients. Our function-based genetic analysis showed that combinations of dysfunctional variants are major causes for gout, thereby providing evidence for "a common disease common variant " hypothesis. We will show the latest progress on our study in this meeting.

Introduction

Gout based on hyperuricemia is a common disease with a genetic predisposition. *ABCG2* is reported to locate in a gout-susceptibility locus on chromosome 4q, and is recently identified to relate to serum uric acid (SUA) and gout by genome-wide association studies. Besides its transport of nucleotide analogs that are structurally similar to urate, we have reported that ABCG2 is an exporter that has polymorphic reduced functionality variants. We also found that ABCG2 is a urate exporter and that its common variants reduce the transport function. We then hypothesized that common variants of ABCG2 might cause gout.

Materials and Methods

Functional analysis

- Urate transport analysis via wild-type and mutated ABCG2 (Fig1a, b, e-g)

Genetic analysis

- Sequencing analysis of all coding regions of *ABCG2* gene in 90 Japanese patients with hyperuricemia (Fig1c, d)
- Additional genotyping of *ABCG2* SNPs: 228 hyperuricemia (161 gout) patients (Fig1k-n, q)
- Haplotype frequency analysis (Fig1o)
- Genotype combination analysis (Fig1p, q)

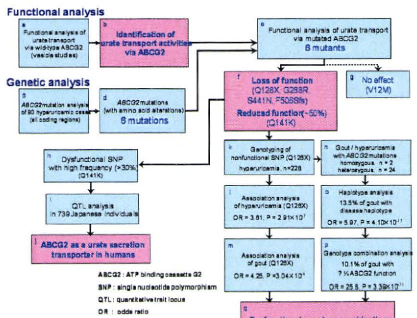

Fig.1. Flowchart for molecular-function-based clinicogenetic analysis of gout with *ABCG2* polymorphic variants.

Results

Table 1. Nonsynonymous mutations in *ABCG2* in 90 hyperuricemia patients

Amino acid change	SNP ID dbSNP (NCBI)	Exon	Type of mutation	Number of hyperuricemia patients: Wild type	Hetero-zygote	Homo-zygote	Allele Frequency (%) (in hyperuricemia)	Allele Frequency* (%) (in Japanese population)
Q141K	rs2231142	5	missense	29	47	14	41.67	31.9
V12M	rs2231137	2	missense	64	23	3	16.11	19.2
Q126X		4	nonsense	80	10	0	5.56	2.8
G268R		7	missense	89	1	0	0.56	N.D.
S441N		11	missense	89	1	0	0.56	0.3
F506SfsX4		13	frameshift	89	1	0	0.56	0.3

* Data from Maekawa et al, Drug Metab. Pharmacokinet. 2006. N.D., not detected.

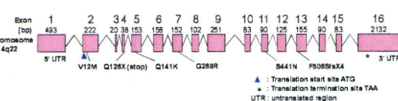

Fig.2. Genomic structure and mutation sites of the human *ABCG2* gene.

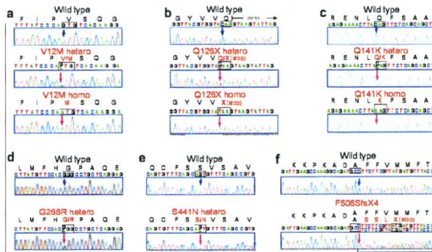

Fig.3. Results of sequence analysis of *ABCG2* gene.

Both heterozygous and homozygous mutations of V12M [(a) c.34G>A], Q126X [(b) c.376C>T] and Q141K [(c) c.421C>A] were identified in hyperuricemia patients. Heterozygous mutations of G268R [(d) c.802G>A], S441N [(e) c.1322G>A] and F506SfsX4 [(f) c.1515delC] were also identified. Mutations are indicated by magenta arrows.

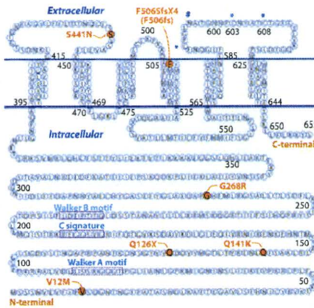

Fig.4. Topological model of ABCG2

The topological model of ABCG2 and six nonsynonymous mutation sites (magenta) found in hyperuricemic patients. #, N-linked glycosylation site (N596); *, cysteine residues for disulfide bonds (C592, C603, and C608).

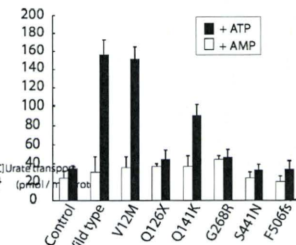

Fig.5. Urate transport of mutated ABCG2

Vesicles prepared from HEK293 cells expressing the wildtype or variants of ABCG2 were incubated with ^{14}C-labeled urate with or without ATP. The amount of ^{14}C-labeled urate was measured after 5 min. Results are expressed as means ± SD.

Table 2. Association analysis of *ABCG2* variants in gout patients

Pheno-type	SNP*	Case 1/1	Case 1/2	Case 2/2	Case MAF*	Control 1/1	Control 1/2	Control 2/2	Control MAF*	Genotype** P-value	Allele Frequency mode P-value	OR*	95% CI*
Gout	Q126X	1	21	139	0.071	0	31	840	0.018	1.74×10^{-7}	3.04×10^{-6}	4.25	2.44-7.38
	Q141K	31	87	41	0.469	87	316	462	0.281	5.80×10^{-15}	5.54×10^{-11}	2.23	1.75-2.87
	V12M	3	43	112	0.155	30	306	526	0.212	0.055	0.020	0.68	0.49-0.94
Hyper-uricemia	Q126X	2	24	202	0.061	0	31	840	0.018	1.91×10^{-6}	2.91×10^{-7}	3.61	2.14-6.08
	Q141K	45	113	68	0.449	87	316	462	0.281	5.32×10^{-15}	1.53×10^{-11}	2.06	1.67-2.55
	V12M	7	55	163	0.153	30	306	526	0.212	0.006	0.005	0.67	0.51-0.89

* SNP = single nucleotide polymorphism; MAF = minor allele frequency; OR = odds ratio; 95% CI = 95% confidence interval.
** Minor allele was referred to as allele 1 and major allele as 2.
Allele 1 is T and allele 2 is C in Q126X. Allele 1 is A and allele 2 is C in Q141K. Allele 1 is A and allele 2 is G in V12M.

Table 3. Haplotype frequency analysis of variants

Allele V12M	Q126X	Q141K	Frequency Gout	Control	P-value	OR*	95% CI*
G	C	A	0.465	0.284	2.26×10^{-13}	2.50	1.94-3.20
G	T	C	0.071	0.018	4.10×10^{-12}	5.97	3.39-10.51
G	C	C	0.306	0.486	-	-	-
A	C	C	0.155	0.212	-	-	-

* OR = odds ratio; 95% CI = 95% confidence interval.
OR is obtained by comparing with the non-risk haplotypes GCC and ACC.
Risk alleles for Q126X and Q141K are underlined.

Table 4. Association analysis of *ABCG2* genotype combinationin gout patients

Estimated transport	Genotype Q126X	Q141K	Number Gout	Control	P-value	OR*	95% CI*
≤ 1/4 function	T/T T/C	C/C A/C	16	8	3.39×10^{-21}	25.8	10.3-64.6
1/2 function	T/C C/C	C/C A/A	37	110	2.23×10^{-9}	4.34	2.61-7.24
3/4 function	C/C	A/C	72	308	2.29×10^{-7}	3.02	1.96-4.65
full function	C/C	C/C	34	439			

*OR = odds ratio; 95% CI = 95% confidence interval.
OR is obtained by comparing with non-risk genotype combination C/C (Q126X) and C/C (Q141K).
Risk alleles for Q126X and Q141K are underlined.

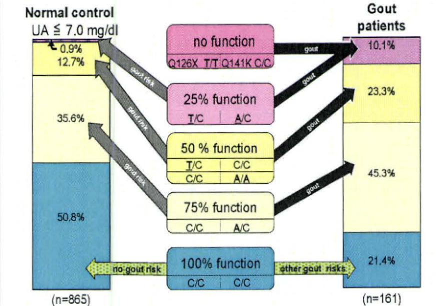

Fig.6. Relation between ABCG2 transport dysfunction and gout

Genotype combinations of Q126X and Q141K are divided into several groups based on estimated ABCG2 transport functions. The Q126X homozygous and heterozygous mutations were identified in up to 13.5% of total gout patients (n = 161). Up to 10.1% of total gout patients have genotype combinations resulting in ≤25% function, whereas the asymptomatic carriers of these genotype combinations, who would have possible risk of gout, were only 0.9% of the normal population (n = 865).

Table.5 Frequency of ABCG2 dysfunction in 2150 Japanese individuals

Estimated function	Genotype Q126X	Q141K	Male Number (%)	Female Number (%)	Male + Female Number (%)
≤1/4 function	T/T T/C	C/C A/C	12 (1.2)	15 (1.4)	27 (1.3)
1/2 function	T/C C/C	C/C A/A	144 (13.8)	134 (12.1)	278 (12.9)
3/4 function	C/C	A/C	405 (38.9)	450 (40.6)	855 (39.8)
Full function	C/C	C/C	481 (46.2)	509 (45.9)	990 (46.0)
Total			1042 (100.0)	1108 (100.0)	2150 (100.0)

Discussion and Conclusion

1. ABCG2 is a high capacity urate secretion transporter.

2. High-frequent SNPs (Q126X: 2.8%, Q141K: 31.9%) result in loss of and reduced function.

↓

Increased serum urate levels due to urate secretion disorder.

3. The combinations of these dysfunctional variants increase gout risk.

4. *ABCG2/BCRP* is a major causative gene for gout and hyperuricemia.

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Feb 18-21, 2011, Tokyo, Japan 国際学会賞受賞

ABCG2 is a high-capacity urate transporter and its genetic impairment increases serum uric acid levels in humans

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Summary

The ATP-binding cassette, subfamily G, member 2 (*ABCG2/BCRP*) gene encodes a well-known transporter which exports various substrates including nucleotide analogs such as 3'-azido-3'-deoxythymidine (AZT). *ABCG2* is also reported to be located in a gout-susceptibility locus (MIM 138900) on chromosome 4q, and is recently identified to relate to serum uric acid (SUA) and gout by genome-wide association studies. Since urate is structurally similar to nucleotide analogs, we hypothesized that ABCG2 might be a urate exporter. To demonstrate our hypothesis, transport assays were performed with membrane vesicles prepared from ABCG2-overexpressing cells. Transport of estrone-3-sulfate (ES), a typical substrate of ABCG2, is inhibited by urate as well as AZT and ES. ATP-dependent transport of urate was then detected in ABCG2-expressing vesicles but not in control vesicles. Kinetic analysis revealed that ABCG2 is a high-capacity urate transporter and maintained its function even under high-urate conditions. The calculated parameters of ABCG2-mediated transport of urate were a Km of 8.24 ± 1.44 mM and a Vmax of 6.96 ± 0.89 nmol/min per milligram of protein. Moreover, quantitative trait locus (QTL) analysis was performed in 739 Japanese individuals and revealed that a dysfunctional variant of ABCG2 increased SUA as the number of minor alleles of the variant increased ($P = 6.60 \times 10^{-5}$). Since ABCG2 is expressed on the apical membrane in several tissues including kidney, intestine and liver, these findings indicate that ABCG2, a high-capacity urate exporter, has a physiological role of urate homeostasis in the human body through both renal and extra-renal urate excretion.

Introduction

Gout based on hyperuricemia is a common disease with a genetic predisposition. *ABCG2* is reported to locate in a gout-susceptibility locus on chromosome 4q, and is recently identified to relate to serum uric acid (SUA) and gout by genome-wide association studies. Besides its transport of nucleotide analogs that are structurally similar to urate, we have reported that ABCG2 is an exporter that has polymorphic reduced functionality variants. We then hypothesized that ABCG2 might be a urate exporter and affect SUA levels.

Materials and Methods

Functional analysis
- Urate transport analysis via wild-type and mutated *ABCG2* (Fig.1. a, b, e-g)

Genetic analysis
- Sequencing analysis of all coding regions of *ABCG2* gene in 90 Japanese patients with hyperuricemia (Fig.1. c, d)
- Quantitative trait locus (QTL) analysis in 739 Japanese individuals (Fig.1. h-j)

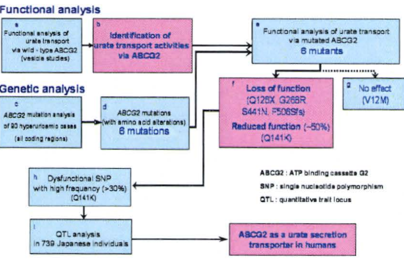


Fig.1. Flowchart for this study.

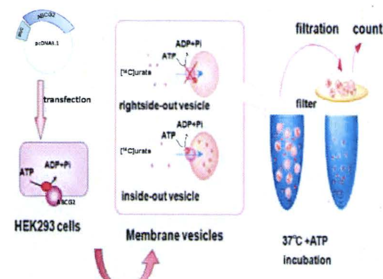


Fig.2. Experimental protocol for vesicle transport assays.

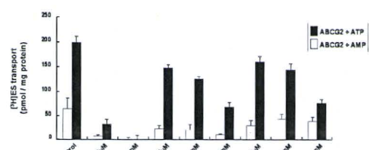


Fig.3. Urate inhibits ABCG2-mediated transport.

Vesicles prepared from HEK293 cells expressing ABCG2 were incubated with 500 nM [^{3}H]estron-3-sulfate (ES) plus the indicated inhibitors or unlabeled ES with or without ATP. The amount of [^{3}H]ES was measured after 1 minute. Results are expressed as means ± S.D.

Although urate required a higher concentration than did unlabeled ES to inhibit [^{3}H]ES transport via ABCG2, the potency of urate was similar to that of the previously reported substrate, 3'-azido-3'-deoxythymidine (AZT).

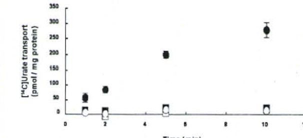


Fig.4. ABCG2-mediated urate transport.

ATP-dependent transport of [^{14}C]urate was detected in ABCG2-expressing vesicles but not in control vesicles after indicated periods. Results are expressed as means ± S.D.

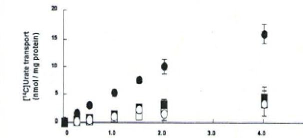


Fig.5. ABCG2 transports urate with high capacity.

Concentration dependence of ABCG2-mediated transport of [^{14}C]urate was detected with 5-minute incubation. Results are expressed as means ± S.D.

Kinetic analysis revealed that ABCG2 mediated the high-capacity transport of urate, remaining their function even under high-urate conditions. Calculated parameters of ABCG2-mediated transport of urate were a Km of 8.24 ± 1.44 mM and a Vmax of 6.96 ± 0.89 nmol/min/mg protein. The calculated Km value exceeded the highest concentration in the experimental condition. This is due to the low-solubility limitation of urate, a property related to the monosodium urate crystal formations in gout patients.

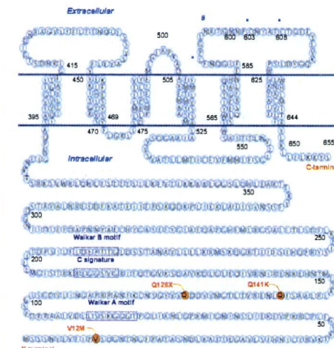


Fig.6. Topological model of ABCG2.

#: N-linked glycosylation site (N596)
*: cysteine residues for disulfide bonds (C592, C603 and C608)

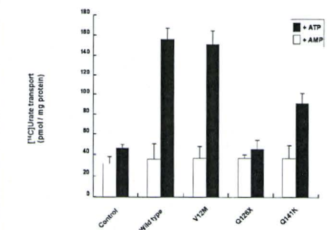


Fig.7. Urate transport analysis of mutated ABCG2.

Vesicles prepared from HEK293 cells expressing the wild type or variants of *ABCG2* were incubated with [^{14}C]urate with or without ATP. The amount of [^{14}C]urate was measured after 5 minutes. Results are expressed as means ± S.D.

ATP-dependent transport of urate was reduced by approximately half (46.7%) in Q141K and was nearly eliminated in Q126X. The V12M variant did not show any changes in urate transport relative to wild-type ABCG2.

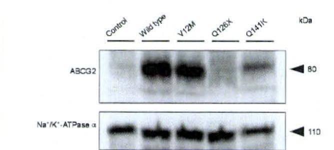


Fig.8. Western blot analysis of wild-type and mutated ABCG2.

Western blot analysis showed a band of approximately 80 kDa in wild-type ABCG2. V12M showed a similar ~80 kDa band of almost the same density. Half-reduced expression in Q141K and no expression in Q126X were observed. As a loading control, the expression of Na^+/K^+-ATPase α was detected.

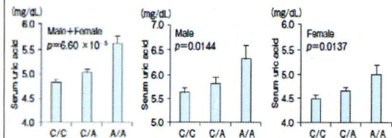
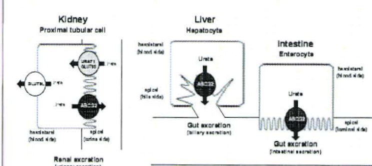


Fig.9. QTL analysis of *ABCG2* Q141K and serum uric acid levels.

Quantitative trait locus (QTL) analysis of the high-frequency dysfunctional variant Q141K in *ABCG2* and serum uric acid levels (SUA) was performed in 739 Japanese individuals from a random sample of Japanese population, including 245 male and 494 female subjects. "C/C," "C/A," and "A/A" indicate wild-type subjects, heterozygous mutation carriers, and homozygous mutation carriers of Q141K, respectively. Results are expressed as means ± S.E.

The analysis revealed that SUA significantly increased as the number of minor alleles of Q141K increased ($P = 6.60 \times 10^{-5}$). These findings indicate that ABCG2 controls SUA *in vivo*, and that there could be great inter-individual differences in this function because of its polymorphic nature.

a Physiological urate excretion model (normal serum urate levels)



Common SNPs in *ABCG2*

b Impaired urate excretion model (elevated serum urate levels)

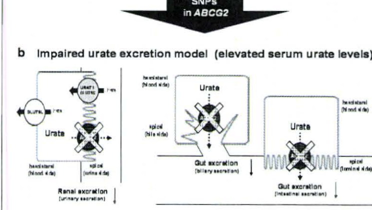


Fig.10. Proposed model of renal and gut urate excetion in human.

In the "impaired urate excretion model," ABCG2 variant proteins with common SNPs on the apical side markedly reduce the urate excretion and elevate SUA. In proximal tubular cells, other urate transporters (URAT1/SLC22A12 and GLUT9/SLC2A9) mediate renal urate reabsorption as shown in previous reports. "GLUT9L" represents GLUT9 isoform 1 (long isoform) and "GLUT9S" represents GLUT9 isoform 2 (short isoform).

Reference

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5. H. Matsuo, et al. Mutations in glucose transporter 9 gene *SLC2A9* cause renal hypouricemia. Am. J. Hum. Genet. 83, 744–751 (2008).
6. C. Kondo, et al. Functional analysis of SNPs variants of BCRP/ABCG2. Pharm. Res. 21, 1895–1903 (2004).
7. A. Dehghan, et al. Association of three genetic loci with uric acid concentration and risk of gout: A genome-wide association study. Lancet 372, 1953–1961 (2008).
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10. M. Huls, 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).
11. D. A. Sica, A. Schoolwerth. Elements of normal renal structure and function: Renal handling of organic anions and cations, in Brenner and Rector's The Kidney, B. M. Brenner, Ed. (Saunders, Philadelphia, ed. 7, 2004), pp. 645–649.
12. O. M. Woodward, A. Köttgen, et al. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc Natl Acad Sci U S A 106, 10338-10342 (2009).
13. H. Matsuo, T. Takada, 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. 1, 5ra11 (2009).

Acknowledgements

Acknowledgments: We thank all of the patients and healthy volunteers involved in this study. We also thanks T. Chiba, Y. Kusanagi, S. Tadokoro, Y. Kawamura, Y. Utsumi, Y. Kato and H. Sasaki for technical support.

尿酸トランスポーター異常症

東京薬科大学病態生理学
市田公美

尿酸トランスポーター異常症とは:

尿酸トランスポーターの異常により、高尿酸血症または低尿酸血症をきたすこと。

1　尿酸動態について

2　URAT1　　腎性低尿酸血症

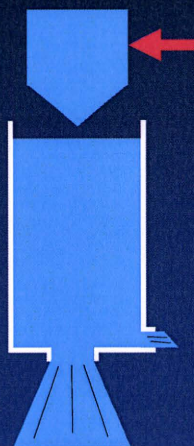
3　GLUT9　　腎性低尿酸血症

4　ABCG2　　高尿酸血症

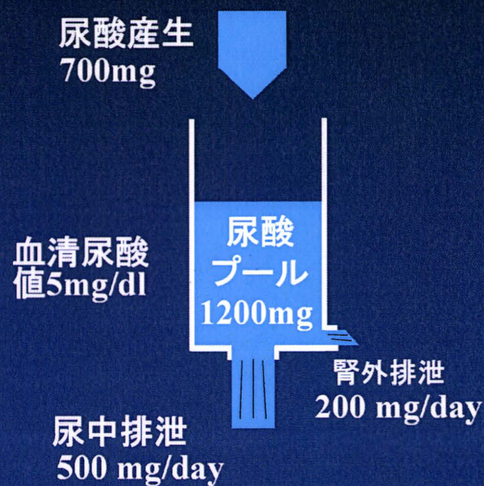
5　その他

高尿酸血症の病型

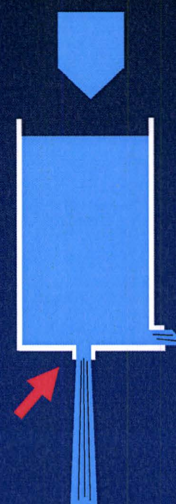
産生過剰型

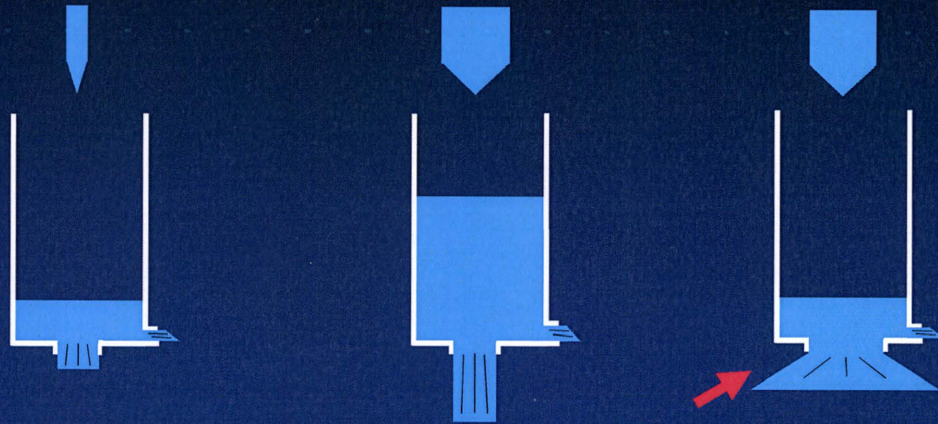


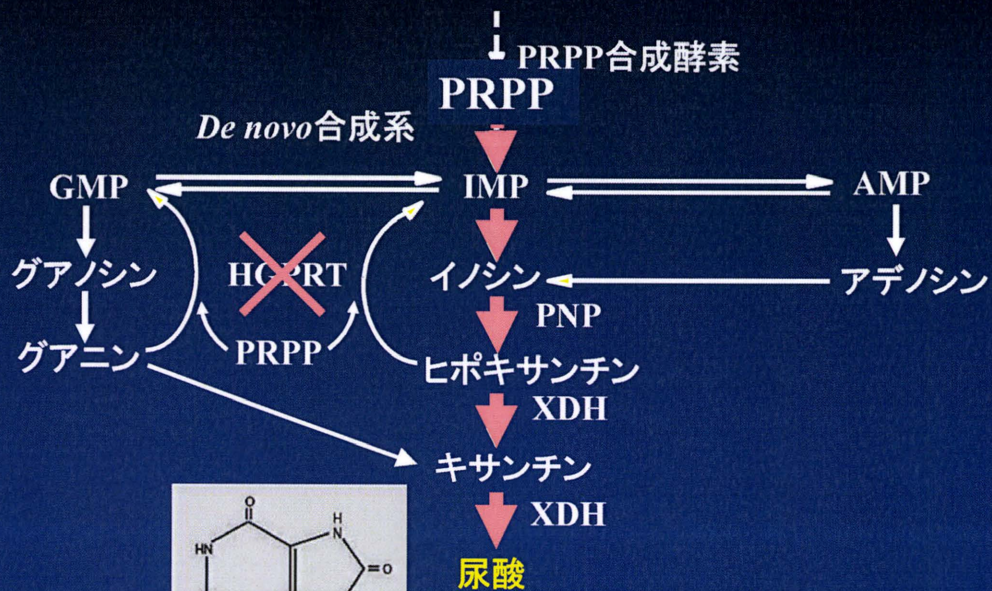
正常

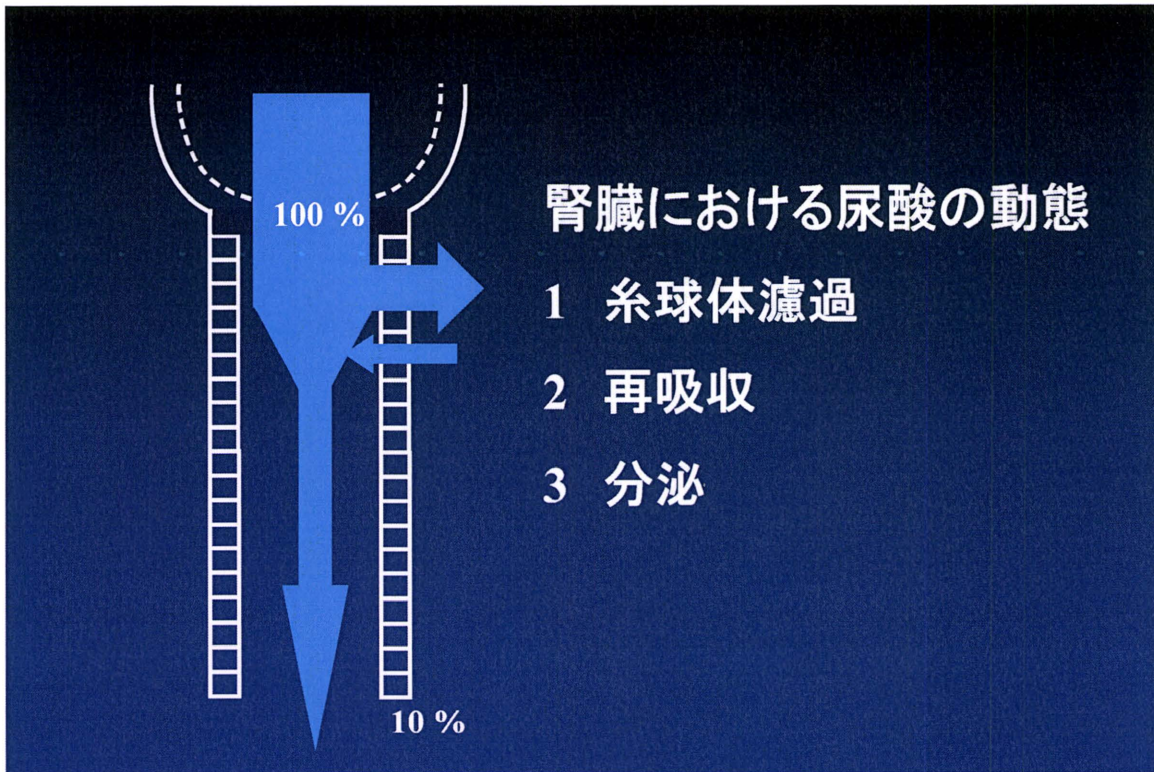


排泄低下型




低尿酸血症の病型
産生低下型
正常
排泄亢進型


ヒトにおけるプリン代謝
PRPP合成酵素
PRPP
De novo合成系
GMP
IMP
AMP
グアノシン
HGPRT
イノシン
アデノシン
PNP
グアニン
PRPP
ヒポキサンチン
XDH
キサンチン
XDH
尿酸



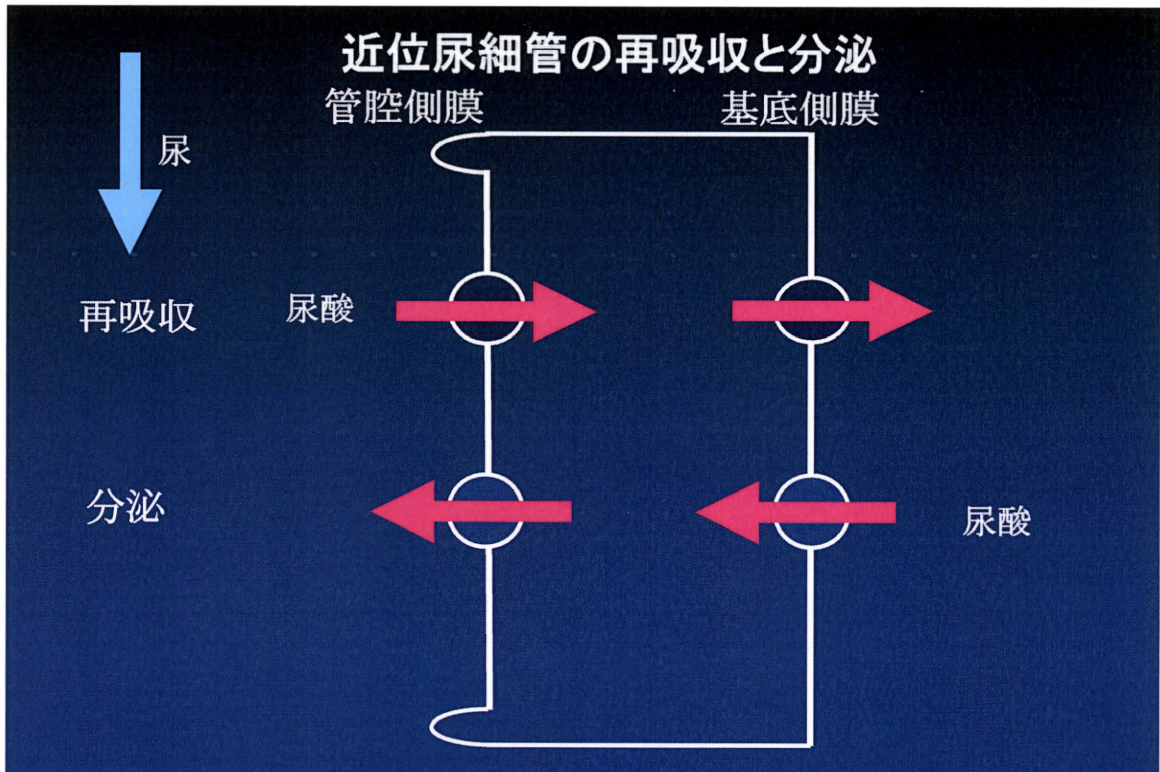
1　尿酸動態について

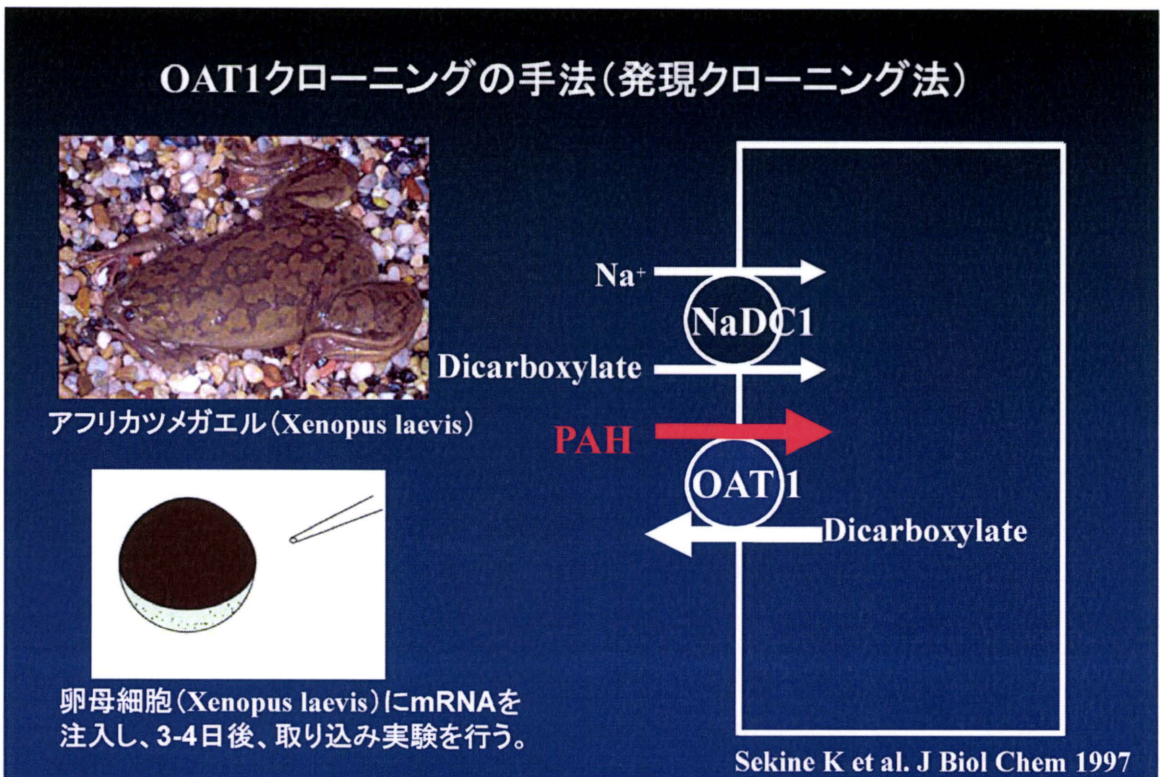
2　URAT1　腎性低尿酸血症

3　GLUT9　腎性低尿酸血症

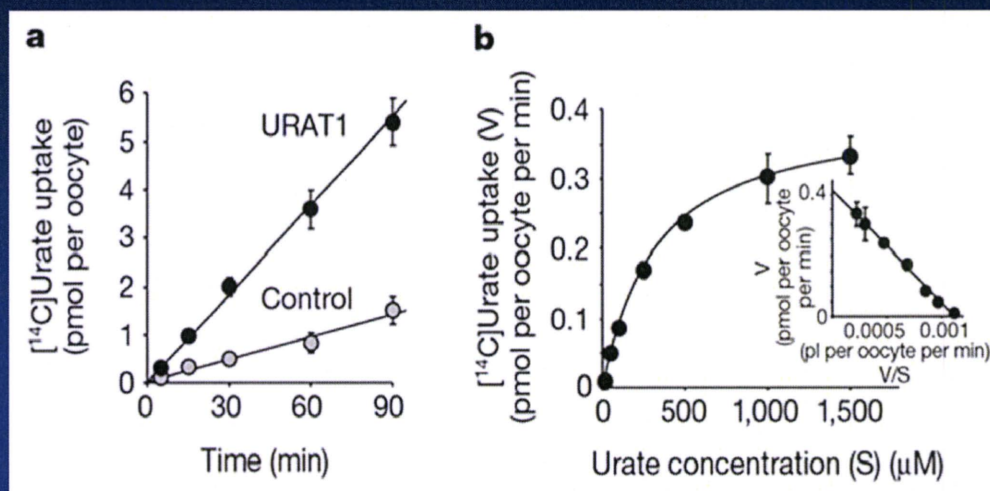
4　ABCG2　高尿酸血症

5　その他


近位尿細管の再吸収と分泌
管腔側膜
基底側膜
尿
再吸収
尿酸
分泌
尿酸


OAT1クローニングの手法（発現クローニング法）
アフリカツメガエル（Xenopus laevis）
Na+
NaDC1
Dicarboxylate
PAH
OAT1
Dicarboxylate
卵母細胞（Xenopus laevis）にmRNAを
注入し、3-4日後、取り込み実験を行う。
Sekine K et al. J Biol Chem 1997

URAT1による尿酸輸送



Enomoto A et al. Nature, 2002.

URAT1の基質特異性(Cis)

Control	100	Phenylbutazone (1mM)	29.1
Urate (1mM)	18.3	Sulfinpyrazone (1mM)	39.6
L-Lactate (1mM)	64.6	Furosemide (1mM)	38.4
Nicotinate (1mM)	26.1	Bumetanide (1mM)	26.2
Probenecid (1mM)	19.1	PAH (1mM)	91.5
Benzbromarone (50μM)	6.9	Salicylic acid (1mM)	21.9
PZA (1mM)	27.7	Indomethacin (1mM)	8.9
Allopurinol (1mM)	89.9	Losartan (1mM)	15.9
Ketoglutarate (1mM)	97.2	EXP-3174 (1mM)	23.1
Orotic acid (1mM)	26.5		

(% of urate uptake)
Enomoto A et al. Nature, 2002.

腎性低尿酸血症

腎性低尿酸血症は、尿酸排泄亢進により起こる先天性の低尿酸血症であり、尿細管における尿酸のトランスポーターの異常が原因である。日本人に多いと考えられている。

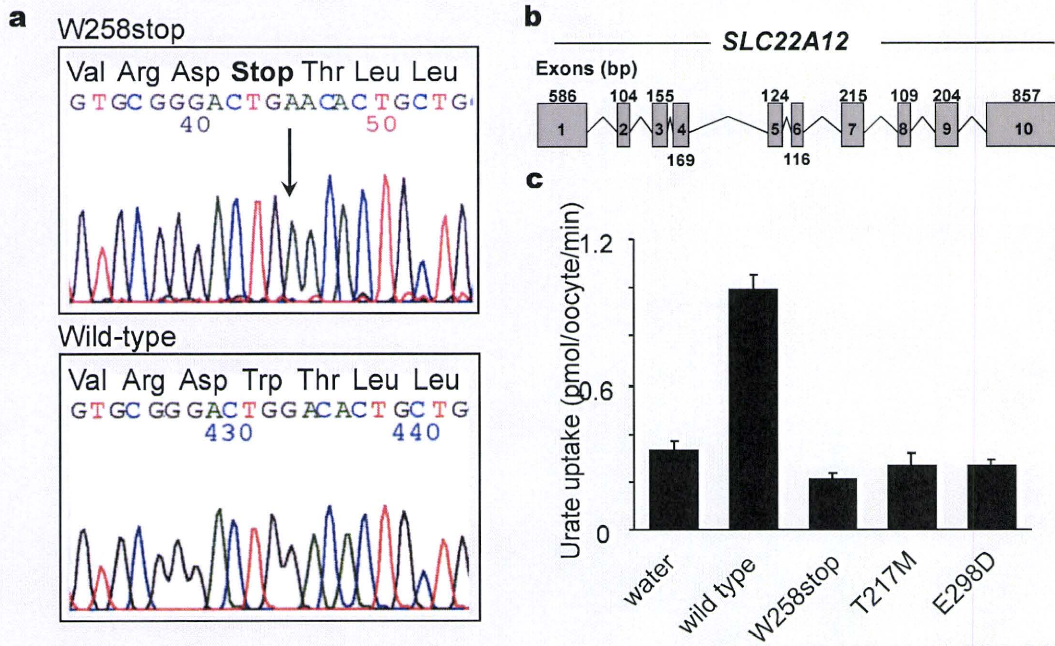
臨床上の特徴として、運動後急性腎不全や尿路結石の合併が多い。さらに腎性低尿酸血症は、pyrazinamide、probenecid等の尿酸排泄に影響を及ぼす薬物により、いくつかのsubtypeに分類されてきた。

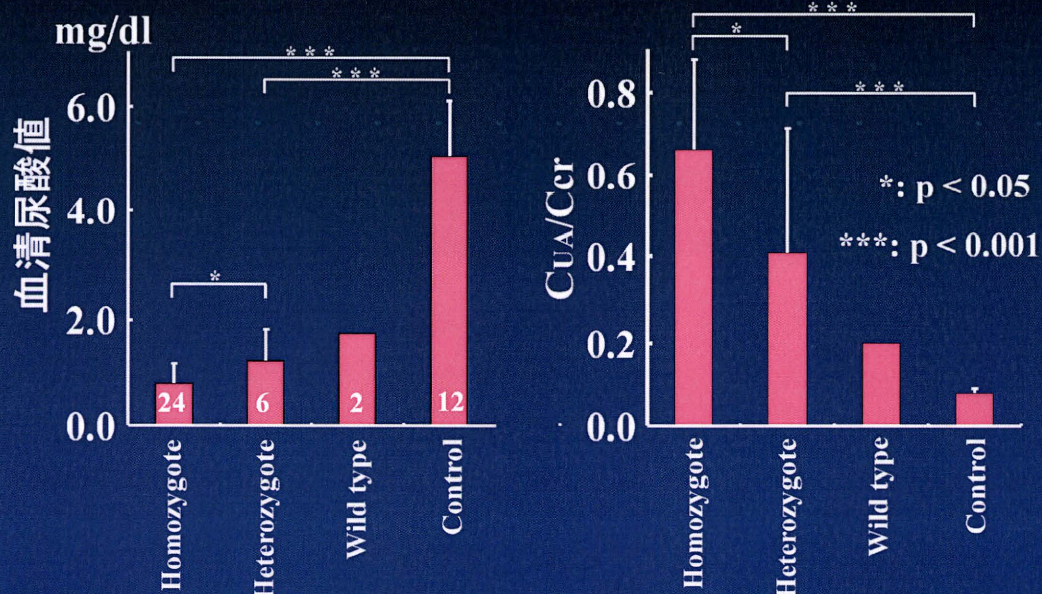
臨床像

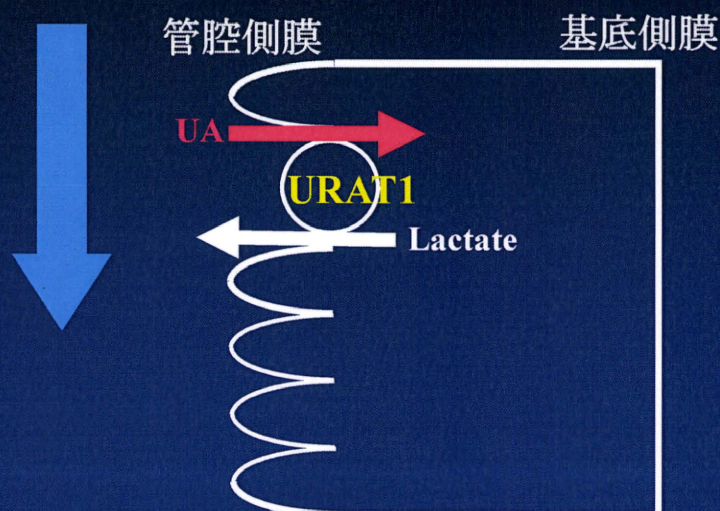
運動後急性腎不全の既往:9.4%
尿路結石:12.5%

腎性低尿酸血症患者の尿酸動態

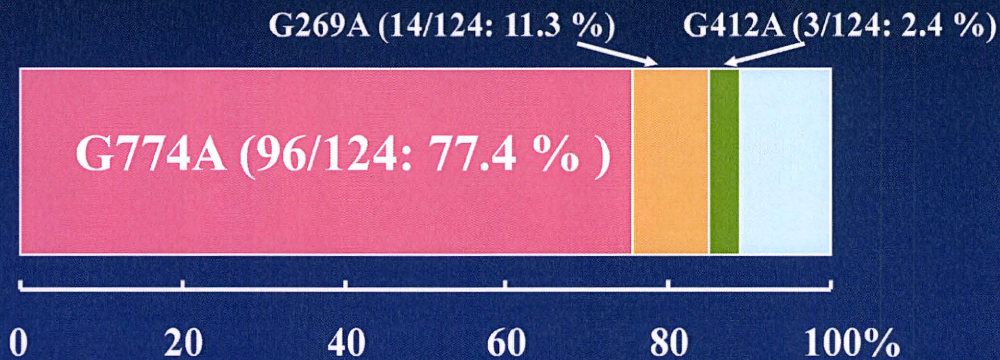
血清尿酸値:	0.93 ± 0.49 mg/dl	(n=32)
CUA :	68.3 ± 31.6 ml/min	(n=30)
CUA/Ccr:	0.584 ± 0.264	(n=32)
尿中尿酸排泄量:	704.6 ± 233.0 mg/day	(n=31)




URAT1のGenotypeによる血清尿酸値とCUA/Ccr
mg/dl
血清尿酸値
6.0
4.0
2.0
0.0
24
6
2
12
Homozygote
Heterozygote
Wild type
Control
CUA/Ccr
0.8
0.6
0.4
0.2
0.0
*: p < 0.05
***: p < 0.001
Ichida et al. J Am Soc Nephrol 15, 2004


近位尿細管の尿酸トランスポーター
管腔側膜
基底側膜
UA
URAT1
Lactate

URAT1遺伝子変異におけるG774Aの頻度



日本人のURAT1遺伝子におけるG774Aの Allele 頻度

2.37% (総数 1875人 : 吹田市)

Iwai N et al. Kidney Int 66, 2004

2.30% (総数 980人 :健常者)

Taniguchi A et al. Arthritis Rheum 52, 2005

なぜ日本人にG774Aの頻度が高いのか?

- 昔の変異が日本人に広がった.

または

- 腎性低尿酸血症が日本の環境に好都合であった.

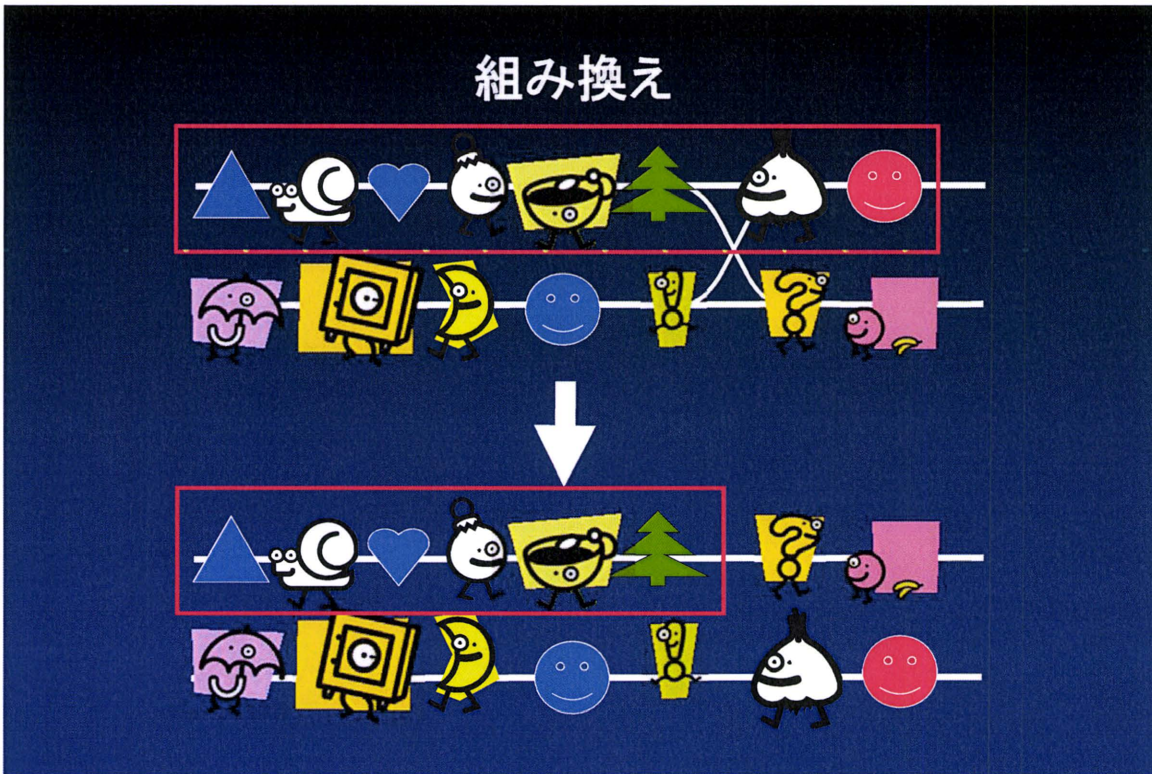
対象

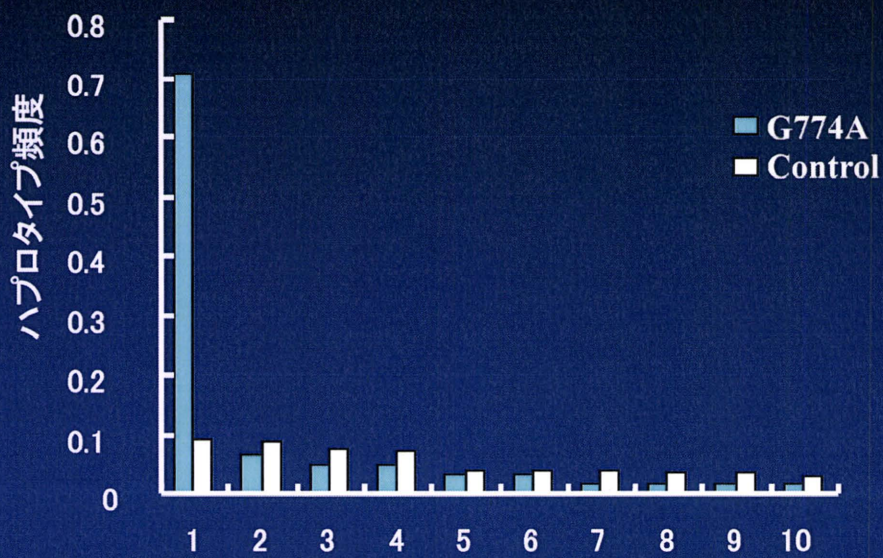
腎性低尿酸血症患者73例中、ホモ接合体として変異G774Aを持つ腎性低尿酸血症患者　31例

コントロール:　健常者49名

方法

G774A の5'側に650 kbまで8個のSNPs、3'側に 400 kbまで5個のSNPsを、JSNPsデータベース及びdb-SNPデータベースより選択した。今回、JST112418, JST079554, JST041197, JST041196, JST092410, JST091567, JST691566, JST074206, JST057661, JST074205, JST092399, JST161698, Rs506594を検討した。ハプロタイプ－質的表現型関連検定プログラム(PENHAPLO) を用いて、ハプロタイプ推定を行った。


組み換え


最高頻度から10個のハプロタイプの頻度分布
ハプロタイプ頻度
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
5
6
7
8
9
10
G774A
Control
Ichida K et al. Clin Genet 2008