IDENTIFICATION OF ABCG2/BCRP AS MAJOR CAUSE FOR GOUT

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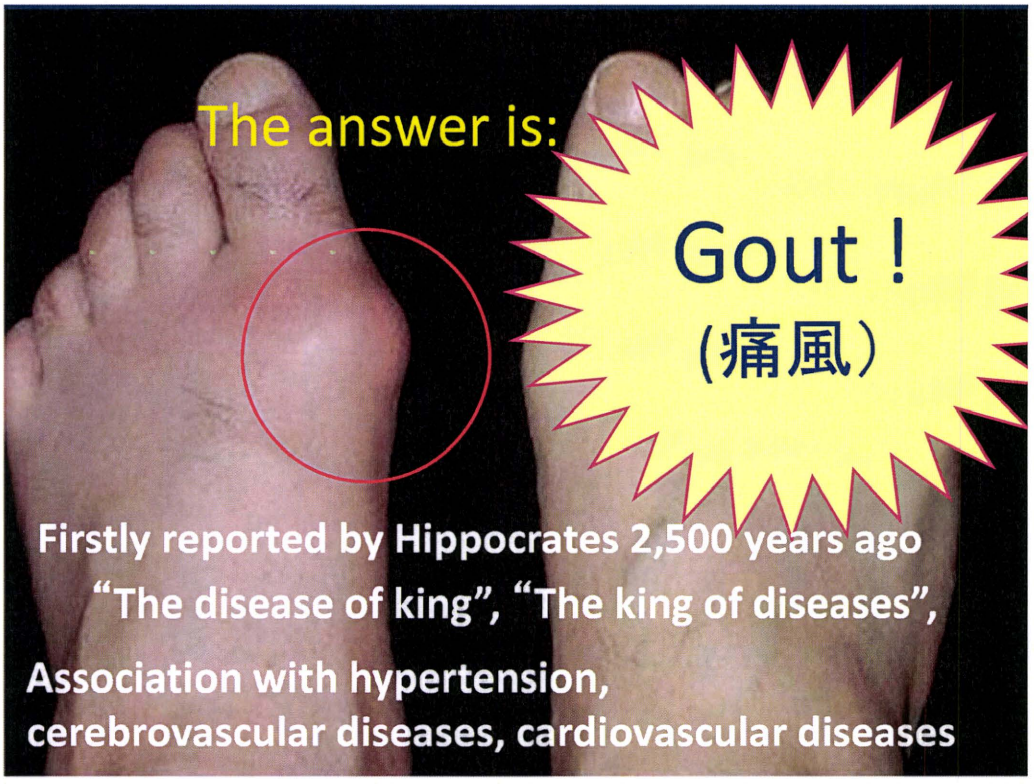
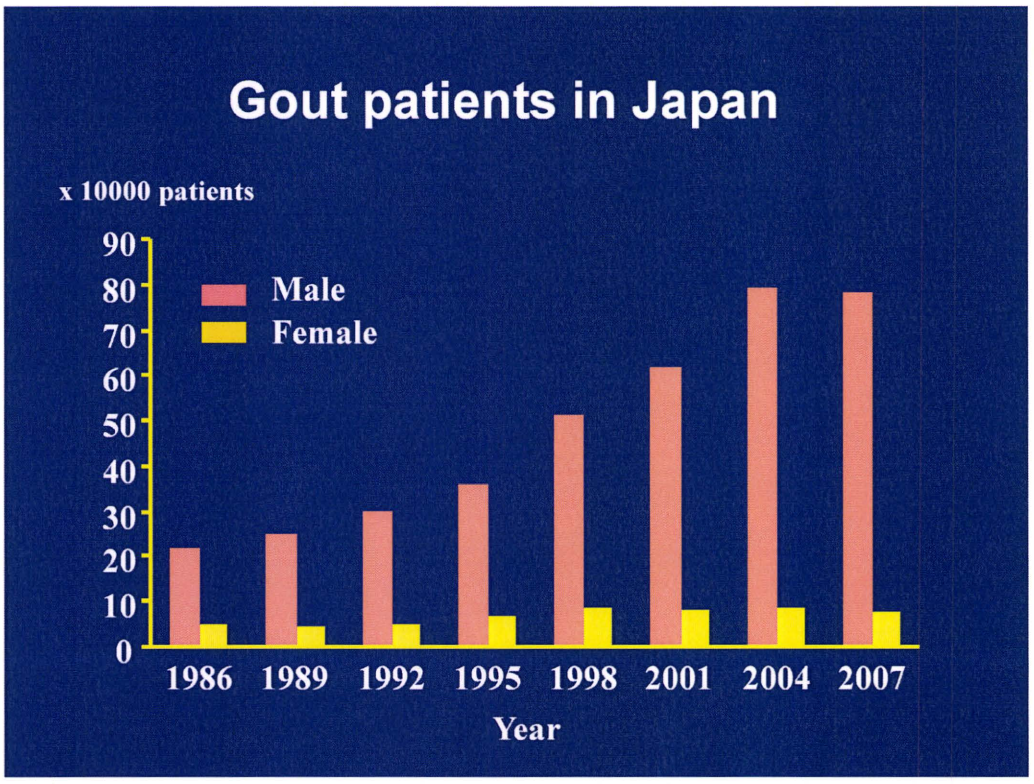
What's their common points?

- Alexander the Great
- Louis XIV
- Napoléon Bonaparte
- Johann Wolfgang von Goethe
- Leonardo da Vinci
- Michelangelo Buonarroti
- Sir Isaac Newton
- Charles Robert Darwin
- Martin Luther ...and many


The answer is:
Gout !
(痛風)
Firstly reported by Hippocrates 2,500 years ago
"The disease of king", "The king of diseases",
Association with hypertension,
cerebrovascular diseases, cardiovascular diseases


Gout patients in Japan
x 10000 patients
90
80
70
60
50
40
30
20
10
0
Male
Female
1986
1989
1992
1995
1998
2001
2004
2007
Year

1. Identification of a major causative gene for gout

Urate excretion transporter
ABCG2/BCRP

Matsuo et al. ***Science Transl. Med.*** 2009, Nov.

2. Identification of genes for renal hypouricemia

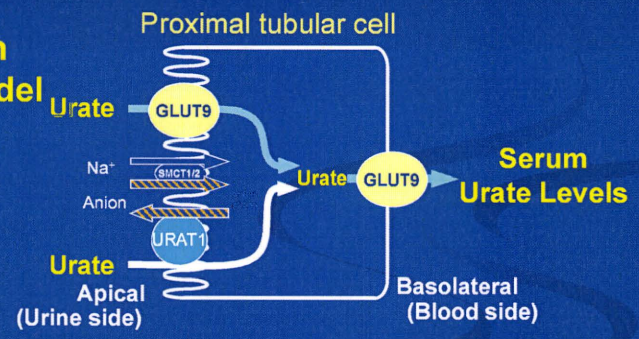
Renal urate reabsorption transporter
URAT1 Enomoto et al. ***Nature***. 2002.

GLUT9 Matsuo et al. ***Am J Hum Genet***. 2008.

→ **Therapeutic targets for hyperuricemia and gout**

- Renal urate transporters
  →URAT1 and GLUT9
  (Enomoto A, et al. ***Nature***, 2002. &
  Matsuo H, et al. ***Am J Hum Genet***, 2008)

Urate reabsportion
:Physiological model



⇒Another candidate for urate transporter:ABCG2
(Cheng LS, et al. ***Am J Hum Genet***, 2004
Dehghan A, et al. ***Lancet***, 2008)

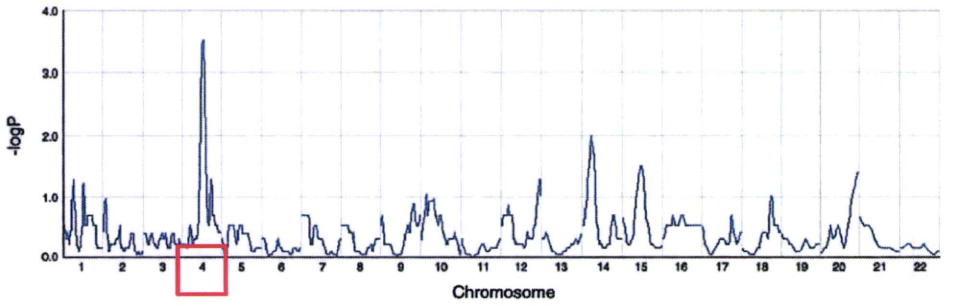
What's the cause of gout!?

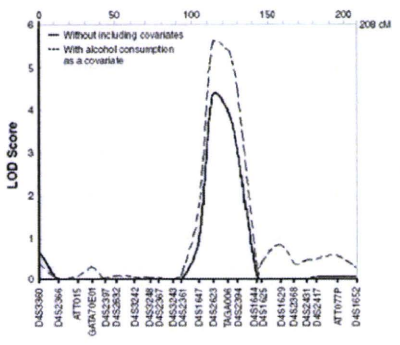
Life style (Food, Alcohol) ?

Only environmental factors?

There should be genetic factors.

However, only rare genetic causes are identified.
(Mendelian disorders with abnormal metabolism)



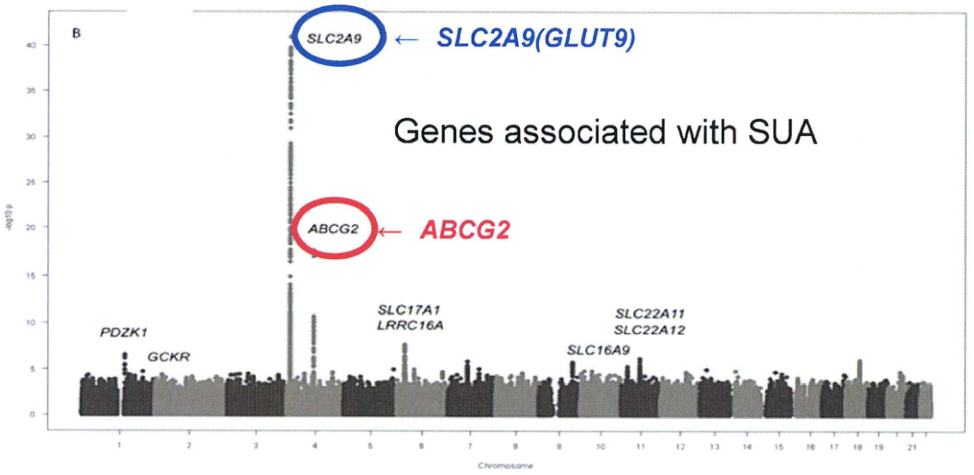


Genome-wide linkage study of Gout (Ch4q) ABCG2

c.f. **Genome-wide association study (GWAS)**

Cheng et al.,
Am J Hum Genet (2004)

Genome-wide association study (GWAS) of serum uric acid levels (SUA)



Kolz M, et al. ***PLoS Genet.*** 2009.
Dehghan A, et al. ***Lancet***, 2008.

Method

To identify a major causative gene of gout, we performed "molecular function-based genetic analysis."

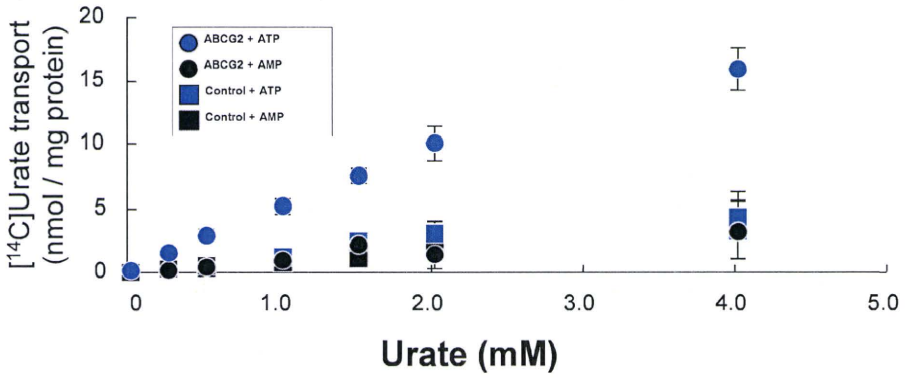
Functional analysis

- **Urate transport assay via ABCG2** (vesicle assay)
- **Transport assay of ABCG2 mutants** (6 mutants)

Genetic analysis

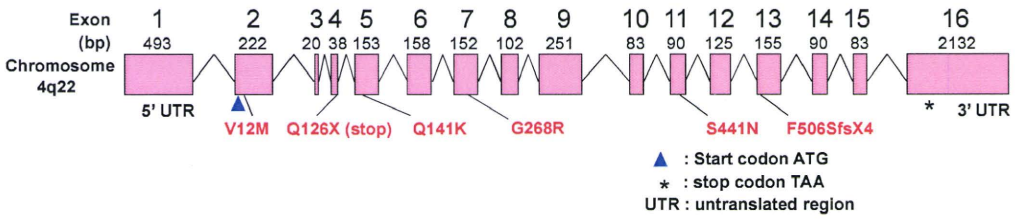
- **Mutation analysis**; Re-sequencing of 90 hyperuricemia patients
- **QTL (Quantative trait locus) analysis** (739 Japanese)
- **Additional genotyping of dysfunctional SNPs** (228 patients)
- **Haplotype frequency analysis**
- **Further association analysis** (genotype combination analysis)

ABCG2 is identified as a high-capacity urate exporter



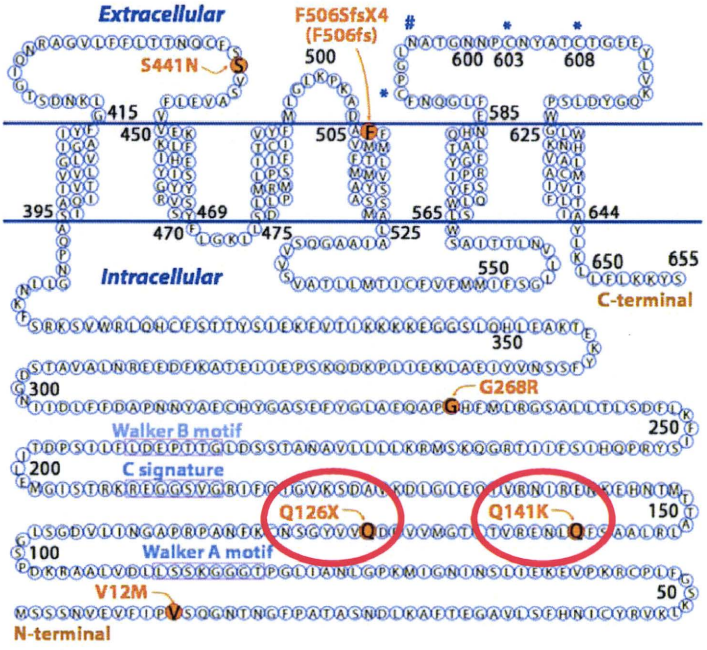
Matsuo *et al.* ***Science Transl Med***, 2009.

Genomic structure of human *ABCG2* gene and mutation sites

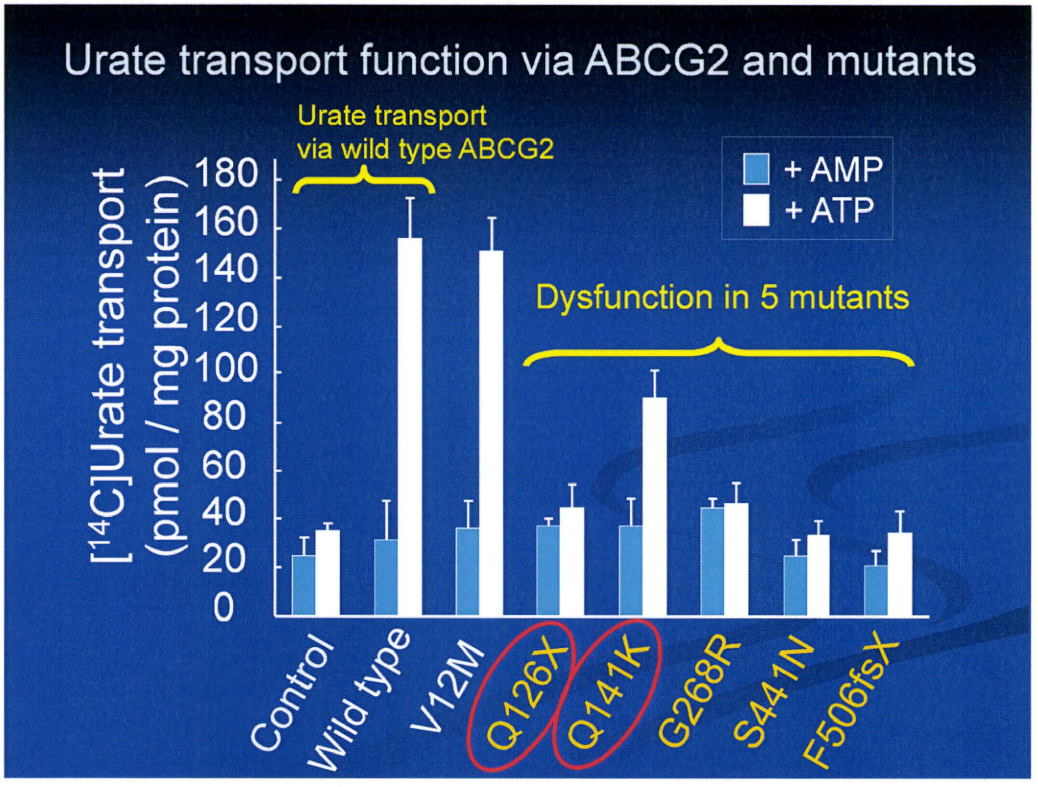


We performed mutational analysis of all coding regions and intron–exon boundaries of the ABCG2 gene in 90 hyperuricemia patients.

Topology model of ABCG2 and mutation sites

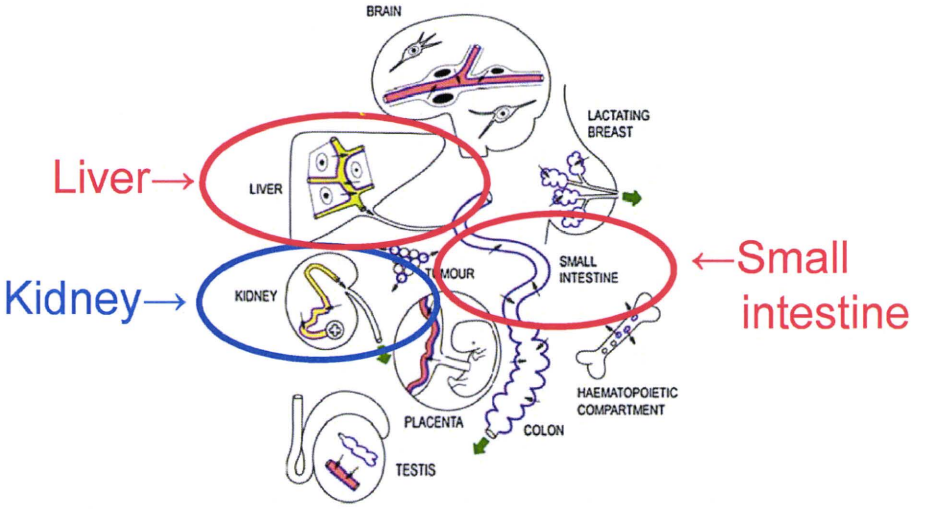


Urate transport function via ABCG2 and mutants



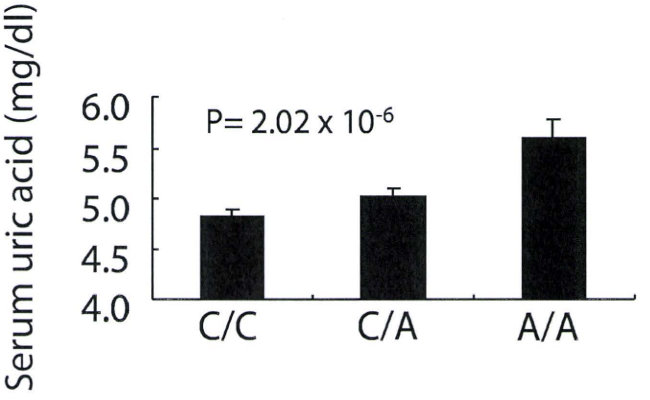
25th JSSX Annual Meeting (Tokyo), Oct 8, 2010

Tissue distribution of urate transporter ABCG2

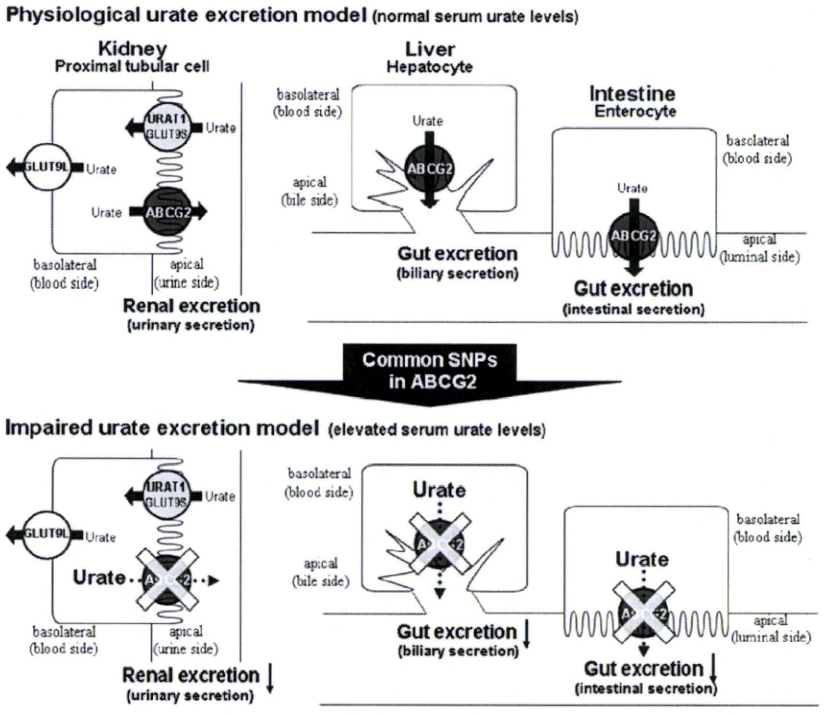


Vlaming et al., Adv Drug Deliv Rev (2009)

QTL (Quantative trait locus) analysis of a dysfunctional variant of *ABCG2* in 739 Japanese individuals

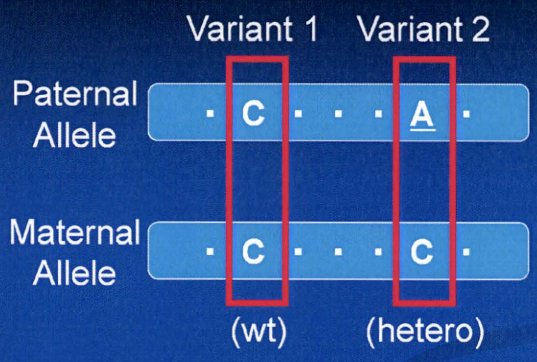


→ ABCG2 dysfunction increases serum uric acid levels!



Proposed model for urate excretion in humans

Haplotype Frequency Analysis



in *ABCG2*...

Variant			Frequency		P-value	OR	95% CI
V12M	Q126X	Q141K	Gout	Control			
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	-	-	-

⇒Only one variant (Q126X or Q141K) exists in one haplotype.

Estimated Transport Function of ABCG2

1. One or no variant in one haplotype
2. Q126X → non-functional
   Q141K → half-functional

⇒Patterns and functions are calculable!

Table. ABCG2 function estimated from uniparental haplotypes

	Uniparental Haplotype 1	Uniparental Haplotype 2	Estimated Function	In Total
1	None	None	(100%+100%)/2	100%
2	None	Q141K only	(100%+50%)/2	75%
3	Q141K only	Q141K only	(50%+50%)/2	50%
4	Q126X only	Q141K only	(0%+50%)/2	25%

ABCG2 dysfunction and gout risk

Estimated transport	Genotype Q126X	Genotype Q141K	Number Gout	Number Control	P-value	OR*	95% CI*
≤ 1/4 function	**T**/**T**	C/C	16	8	3.39×10^{-21}	25.8	10.3-64.6
	T/C	**A**/C					
1/2 function	**T**/C	C/C	37	110	2.23×10^{-9}	4.34	2.61-7.24
	C/C	**A**/**A**					
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.

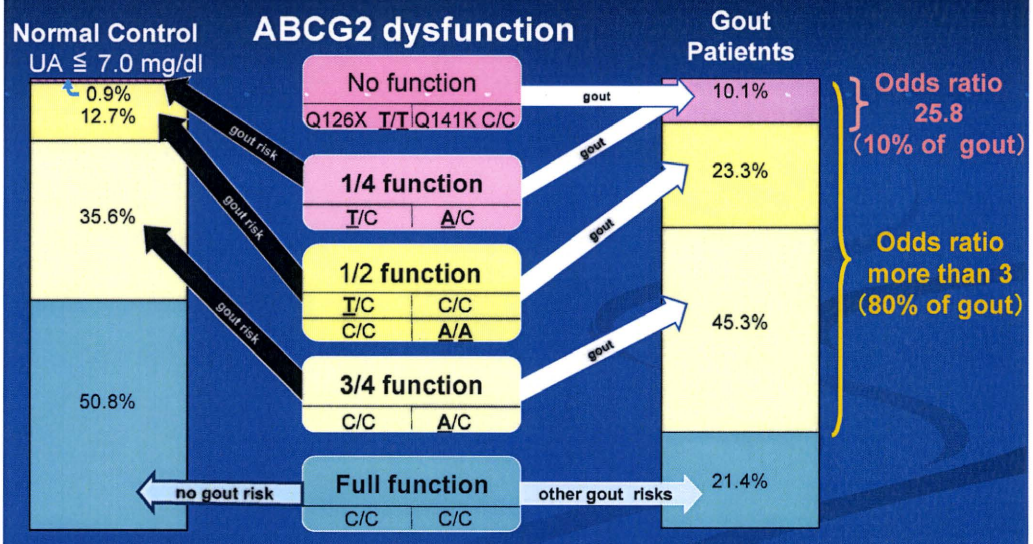
⇒ABCG2 dysfunction increases gout risk.

Odds ratio: more than 3

(maximum, 25.8)

Matsuo *et al.* ***Science Transl Med***, 2009.

Relationship between ABCG2 function and gout



Matsuo *et al.* ***Science Transl Med***, 2009.

Conclusion 1

ABCG2

A high capacity urate exporter

A major causative gene for gout/hyperuricemia

(Matsuo *et al.* ***Science Transl Med***, 2009.)

1. Identification of a major causative gene for gout

Urate excretion transporter
ABCG2/BCRP

Matsuo et al. ***Science Transl. Med.*** 2009, Nov.

2. Identification of genes for renal hypouricemia

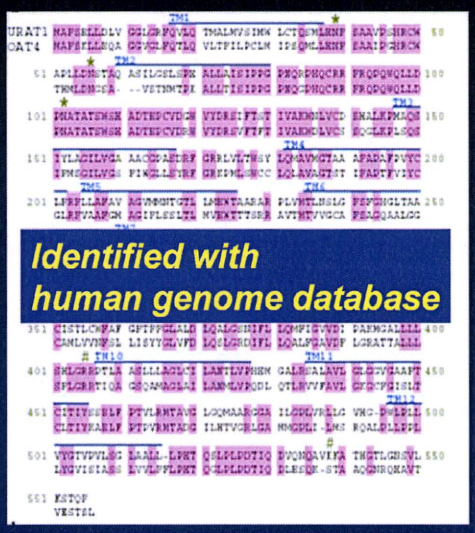
Renal urate reabsorption transporter

URAT1 Enomoto et al. ***Nature***. 2002.

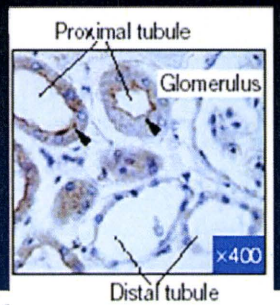
GLUT9 Matsuo et al. ***Am J Hum Genet***. 2008.

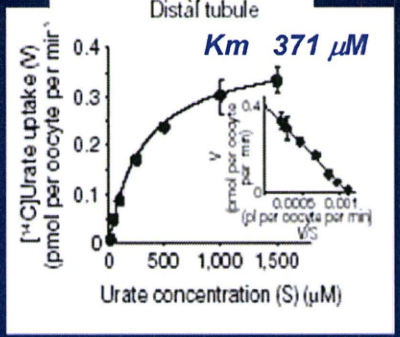
→ **Therapeutic targets for hyperuricemia and gout**

Identification of a renal urate transporter URAT1



Identified with human genome database

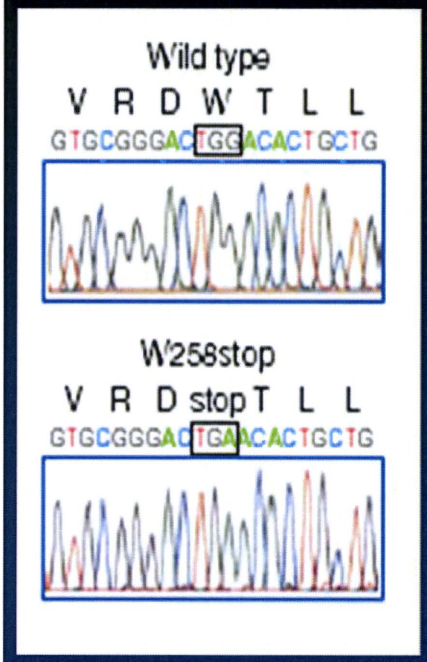




Enomoto A et al. Nature 417: 447-452, 2002

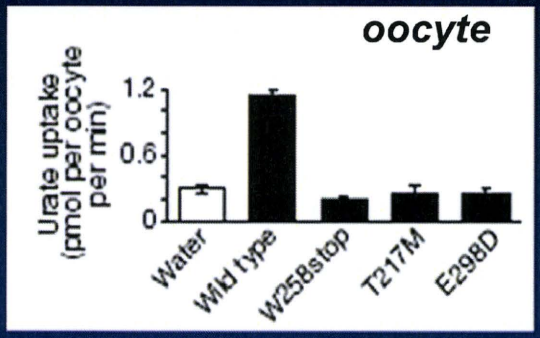
Urate transport via URAT1

URAT1 is a causative gene for hypouricemia



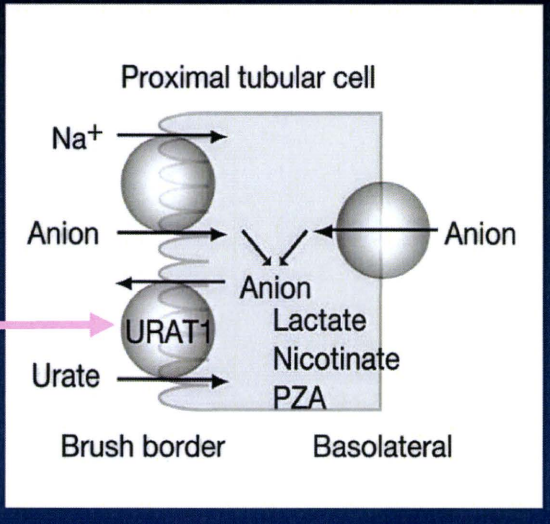
Hypouricemia patients (JSDF hospital)

Kikuchi Y et al. Clin Nephrol 53: 467-472, 2000



Enomoto A et al. Nature 417: 447-452, 2002

The physiological role of URAT1 : Renal urate reabsorption transporter



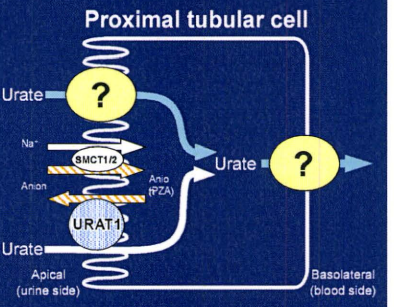
Target for uricosuric agent
Benzbromarone
Probenecid

Enomoto A et al. Nature 417: 447-452, 2002

Renal hypouricemia

URAT1 (*SLC22A12*)
regulates serum urate levels.
a causative gene for renal hypouricemia type 1
(Enomoto, *et al. Nature* 2002)

Another type of renal hypouricemia?
Presence of another urate transporter?

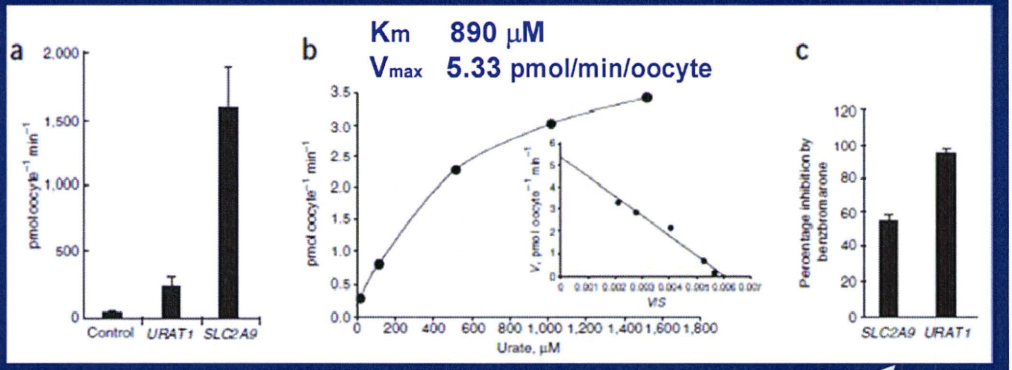


GLUT9 (*SLC2A9*) ; the most significant association with serum urate levels.
(**GWAS** studies; Li, *et al. PLoS. Genet.* 2007;
Döring, *et al. Nat. Genet.* 2008;
Vitart, *et al. Nat. Genet.* 2008)

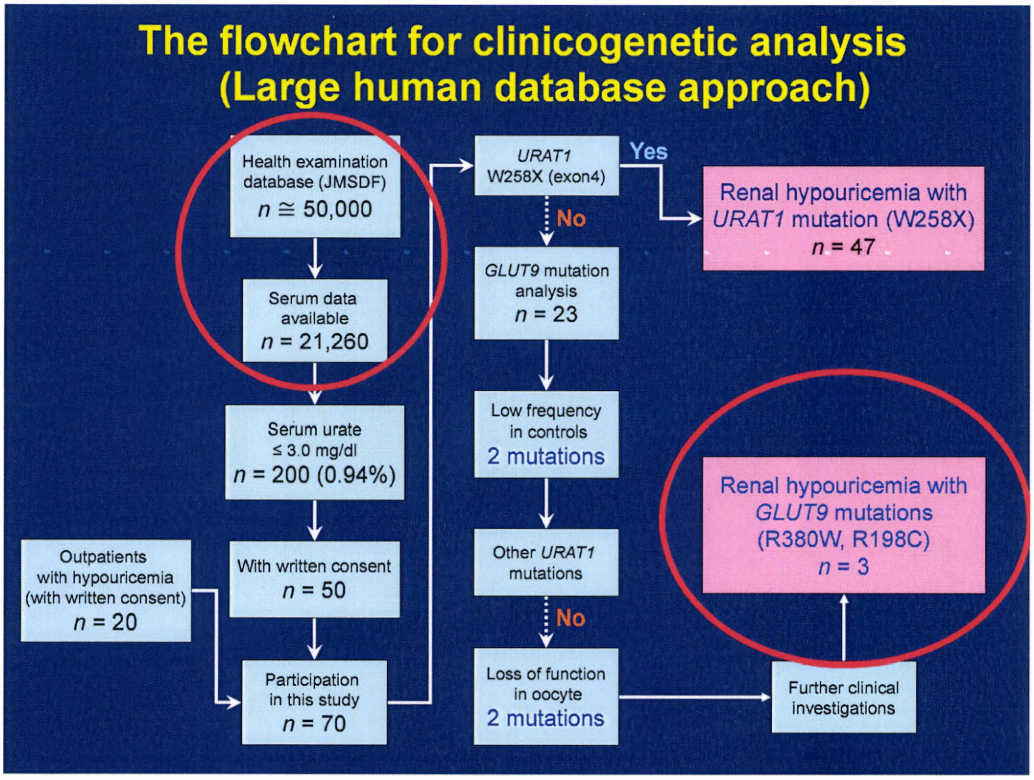
A candidate gene for hypouricemia?

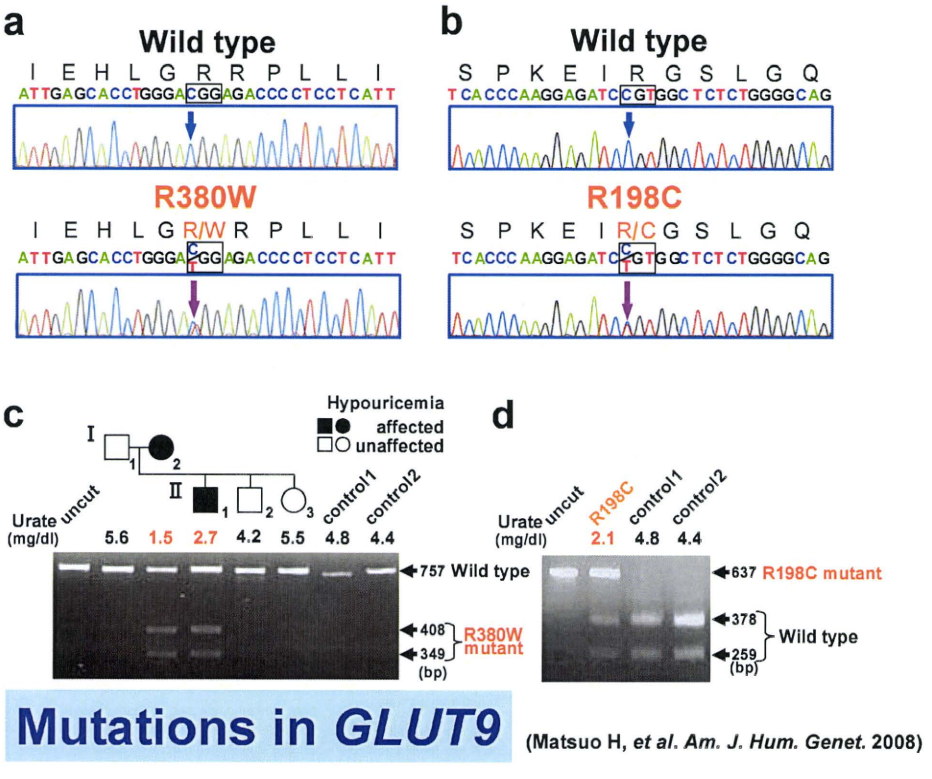
Vitart, *et al. Nat. Genet.* 2008, April

- **GWAS (Genome-wide association study)**
  ***GLUT9/SLC2A9* – serum urate levels**
- **Functional analysis**
  Urate transport via GLUT9/SLC2A9



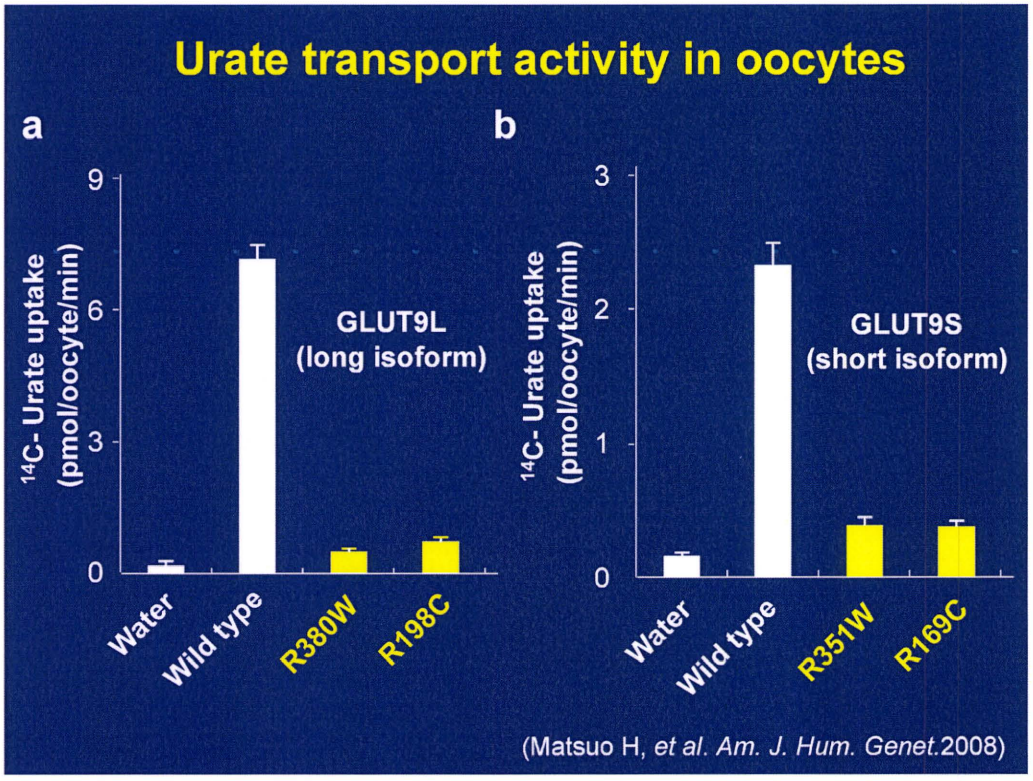
The effect of benzbromarone on GLUT9-mediated urate transport

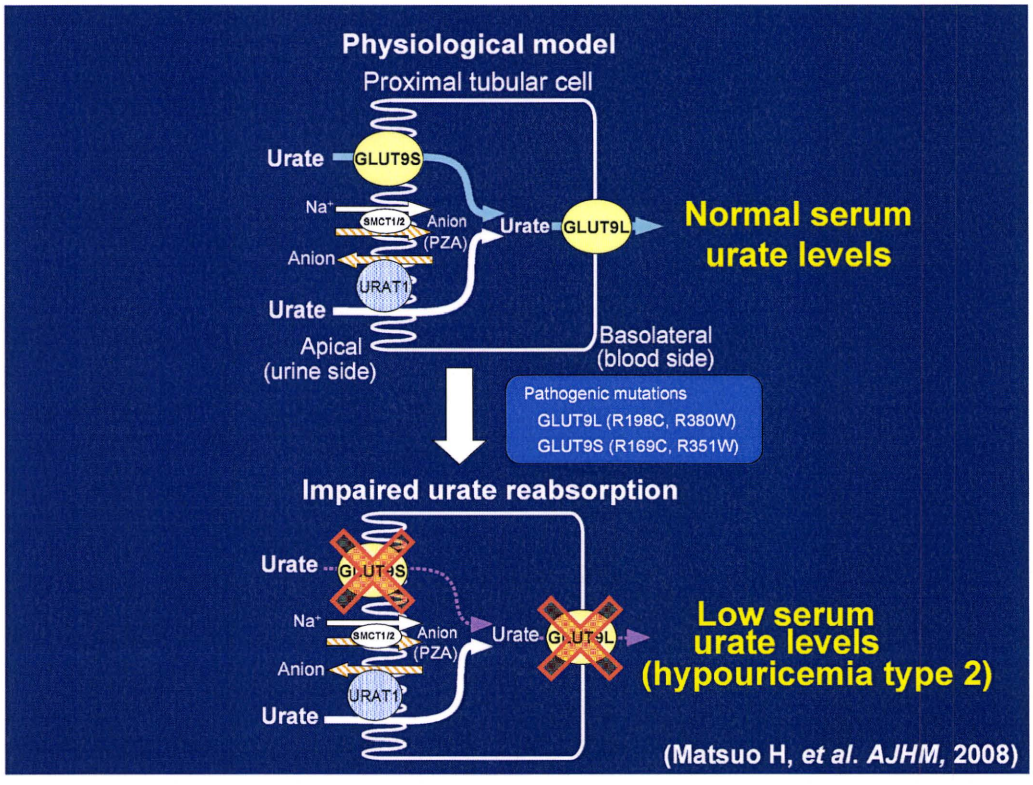




Mutations in *GLUT9*

(Matsuo H, *et al. Am. J. Hum. Genet.* 2008)


Urate transport activity in oocytes
a
b
^{14}C- Urate uptake (pmol/oocyte/min)
9
6
3
0
GLUT9L (long isoform)
Water
Wild type
R380W
R198C
3
2
1
0
GLUT9S (short isoform)
Water
Wild type
R351W
R169C
(Matsuo H, et al. Am. J. Hum. Genet.2008)


Physiological model
Proximal tubular cell
Urate
GLUT9S
Na+
SMCT1/2
Anion
(PZA)
Anion
URAT1
Urate
Apical
(urine side)
Urate
GLUT9L
Basolateral
(blood side)
Normal serum urate levels
Pathogenic mutations
GLUT9L (R198C, R380W)
GLUT9S (R169C, R351W)
Impaired urate reabsorption
Urate
GLUT9S
Na+
SMCT1/2
Anion
(PZA)
Anion
URAT1
Urate
Urate
GLUT9L
Low serum urate levels (hypouricemia type 2)
(Matsuo H, et al. AJHM, 2008)

Renal hypouricemia

Renal hypouricemia type 2

Symbol; RHUC2 (MIM 612076)
Gene; *GLUT9/SLC2A9*
chromosome 4p16-p15.3
(ref. Matsuo et al, *Am J Hum Genet*, 2008)

Renal hypouricemia type 1

Symbol; RHUC1 (MIM 220150)
Gene; *URAT1/SLC22A12*
chromosome 11q13
(ref. Enomoto et al, *Nature*, 2002)

Conclusion 2

URAT1 and GLUT9

Renal urate reabsorption transporters

Causative genes for hypouricemia type 1 and 2

(Enomoto et al, ***Nature***, 2002)
(Matsuo *et al.* ***Am J Hum Genet***, 2008.)

ABCG2

A high capacity urate exporter

A major causative gene for gout/hyperuricemia

(Matsuo *et al.* ***Science Transl Med***, 2009.)

RESEARCH ARTICLE

GENETICS

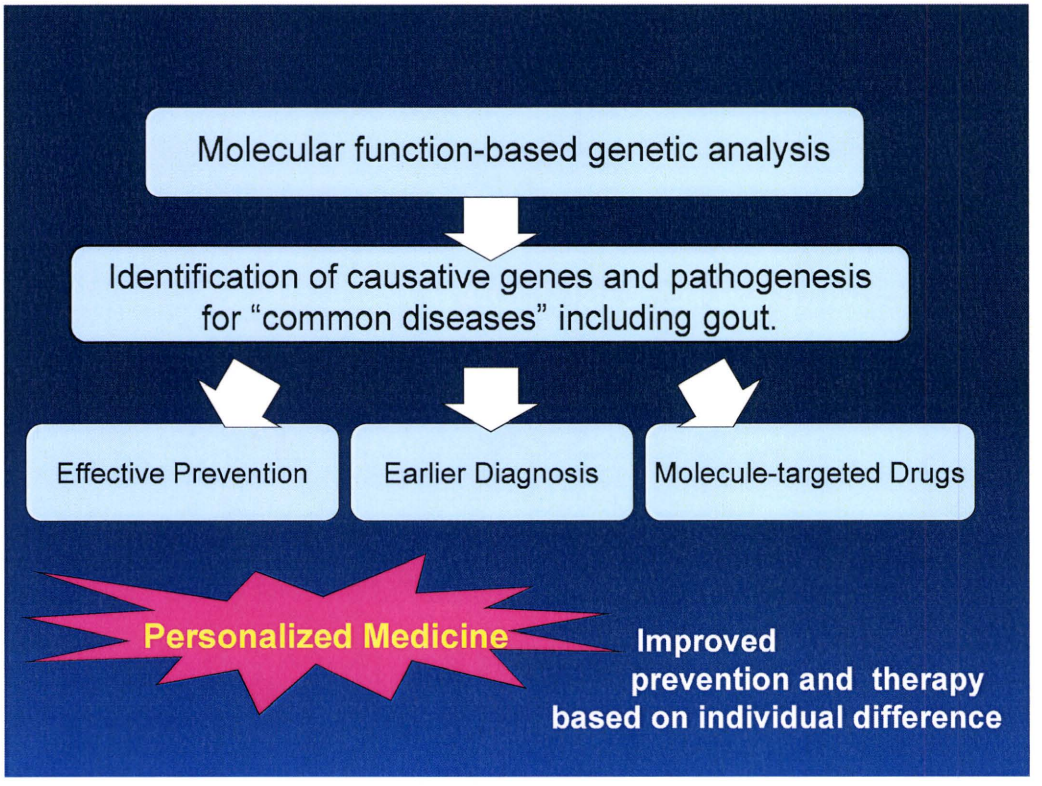
Common Defects of ABCG2, a High-Capacity Urate Exporter, Cause Gout: A Function-Based Genetic Analysis in a Japanese Population

Hirotaka Matsuo,[1]* Tappei Takada,[2] Kimiyoshi Ichida,[3,4] Takahiro Nakamura,[5,6] Akiyoshi Nakayama,[1,7] Yuki Ikebuchi,[2] Kousei Ito,[2] Yasuyoshi Kusanagi,[1] Toshinori Chiba,[1] Shin Tadokoro,[1] Yuzo Takada,[8] Yuji Oikawa,[9] Hiroki Inoue,[1] Koji Suzuki,[10] Rieko Okada,[11] Junichiro Nishiyama,[12] Hideharu Domoto,[13] Satoru Watanabe,[14] Masanori Fujita,[14] Yuji Morimoto,[1] Mariko Naito,[11] Kazuko Nishio,[11] Asahi Hishida,[11] Kenji Wakai,[11] Yatami Asai,[15] Kazuki Niwa,[9] Keiko Kamakura,[16] Shigeaki Nonoyama,[17] Yutaka Sakurai,[18] Tatsuo Hosoya,[4] Yoshikatsu Kanai,[19] Hiroshi Suzuki,[2] Nobuyuki Hamajima,[11] Nariyoshi Shinomiya[1]

(Published 4 November 2009; Volume 1 Issue 5 5ra11)

Gout based on hyperuricemia is a common disease with a genetic predisposition, which causes acute arthritis. The *ABCG2/BCRP* gene, located in a gout-susceptibility locus on chromosome 4q, has been identified by recent genome-wide association studies of serum uric acid concentrations and gout. Urate transport assays demonstrated that ABCG2 is a high-capacity urate secretion transporter. Sequencing of the *ABCG2* gene in 90 hyperuricemia patients revealed several nonfunctional *ABCG2* mutations, including Q126X. Quantitative trait locus analysis of 739 individuals showed that a common dysfunctional variant of *ABCG2*, Q141K, increases serum uric acid. Q126X is assigned to the different disease haplotype from Q141K and increases gout risk, conferring an odds ratio of 5.97. Furthermore, 10% of gout patients (16 out of 159 cases) had genotype combinations resulting in more than 75% reduction of ABCG2 function (odds ratio, 25.8). Our findings indicate that nonfunctional variants of *ABCG2* essentially block gut and renal urate excretion and cause gout.

Matsuo *et al.* ***Science Transl Med***, 2009 Nov.



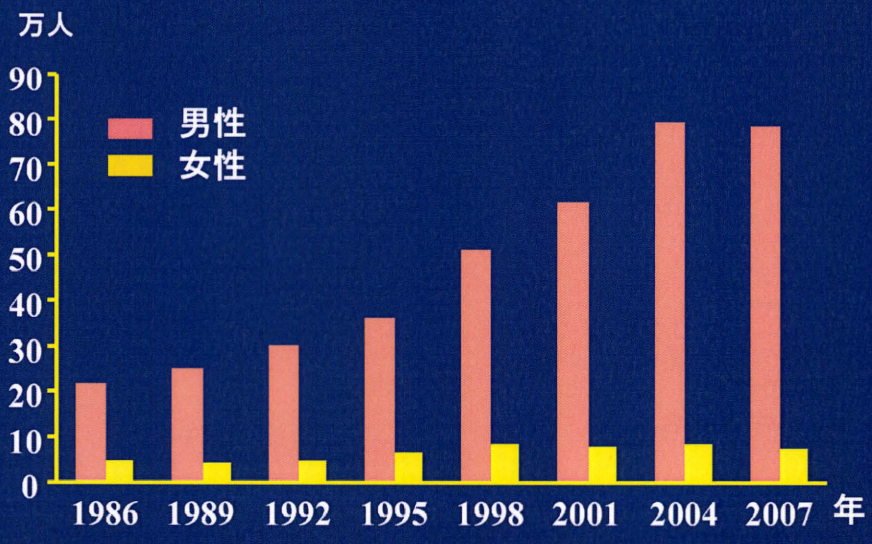
25th JSSX Annual Meeting (Tokyo), Oct 8, 2010

生活習慣病発症に関わる
尿酸トランスポーター
～ ゲノムワイド解析後の新展開 ～

松尾洋孝[1)]、高田龍平[2)]、市田公美[3)]、
鈴木洋史[2)]、四ノ宮成祥[1)]

1)防衛医大　分子生体制御学、2)東大病院　薬剤部、
3)東京薬科大　病態生理学

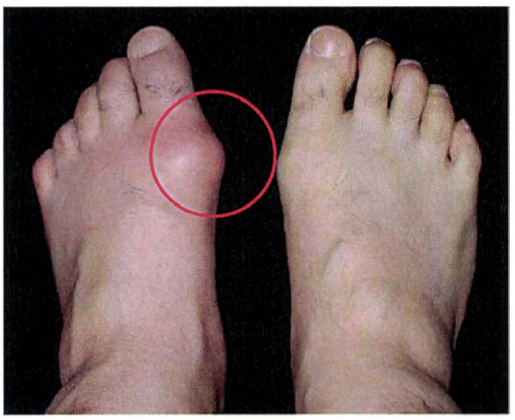
国民生活基礎調査による痛風患者数

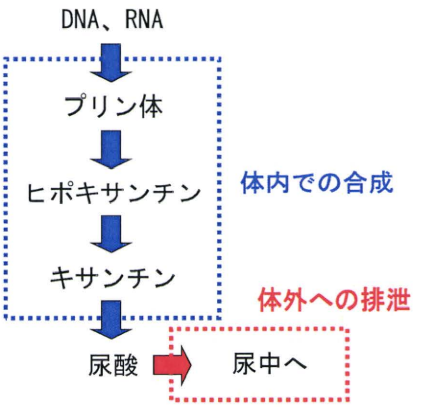


痛風および高尿酸血症について

○痛風は、高尿酸血症を原因とした関節炎を来す疾患である

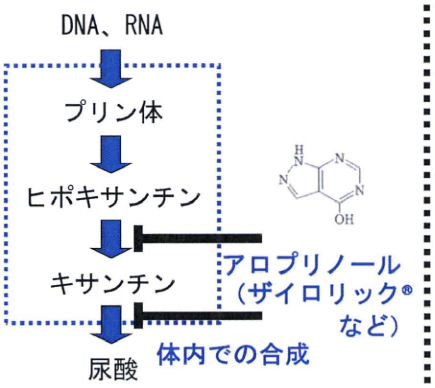
○現在、国内の痛風患者数は約30～50万人、
無症候性高尿酸血症（痛風予備群）は、約500万人と推計されている





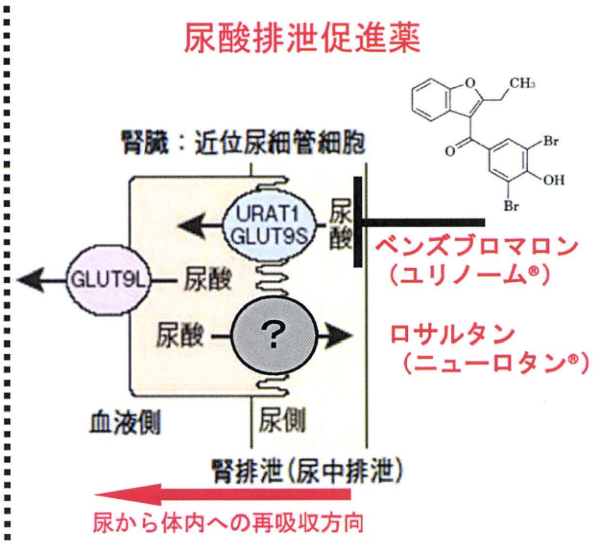
高尿酸血症治療薬の分類

尿酸産生抑制薬



○尿酸の産生を抑制し
血中尿酸値低下をもたらす

尿酸排泄促進薬



○尿からの尿酸の再吸収を阻害し
血中尿酸値低下をもたらす