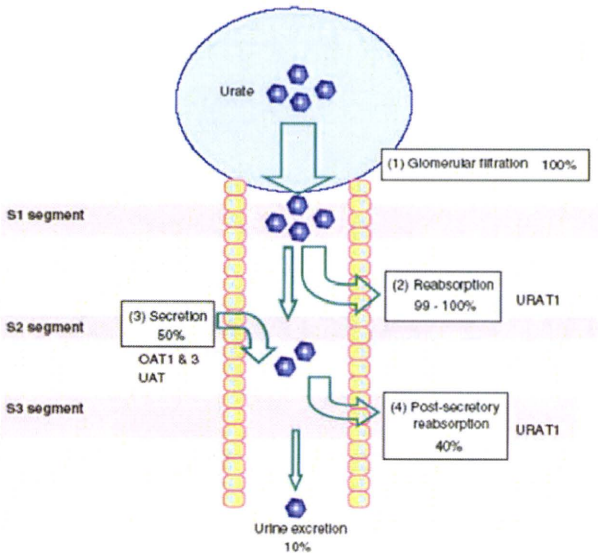
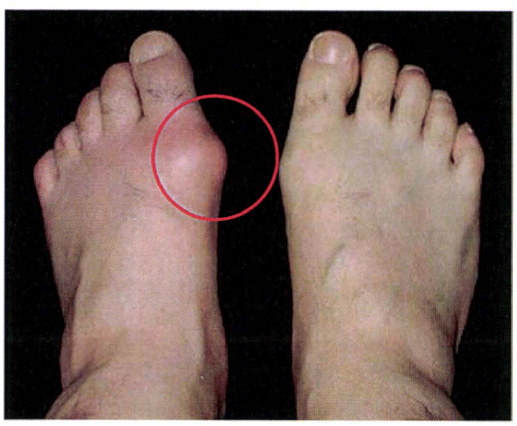
腎臓における尿酸動態の4−コンポーネントモデル

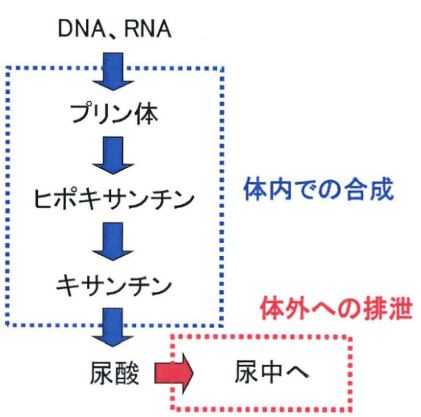


Enomoto et al., Clin Exp Nephrol (2005)

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
○痛風における関節炎は、関節包内に析出した尿酸の結晶に対する炎症反応である
○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
○高尿酸血症は、尿酸量の体内合成量の上昇または排泄量の低下により生じる





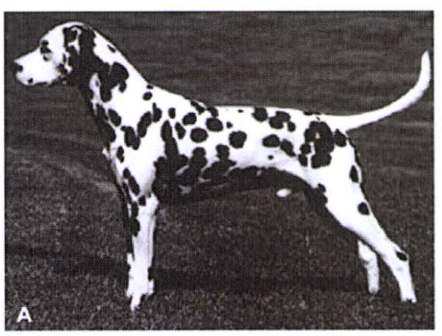
痛風の原因は？

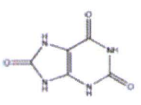
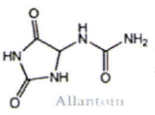
食べ過ぎ、飲み過ぎ
・・・生活習慣のみ？

遺伝的要因もあるのでは？

まれな先天性疾患以外は
わかっていなかった・・・

ダルメシアンは尿酸代謝に異常を有し、**尿路結石**を発症しやすい

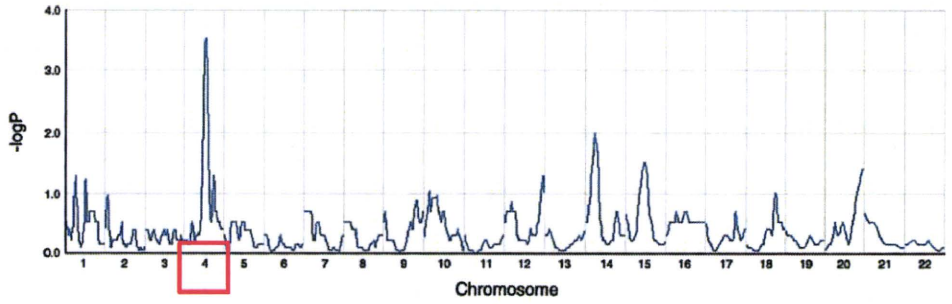


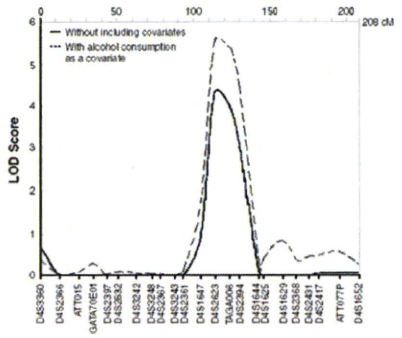
尿酸  ウリカーゼ  アラントイン

Allantoin

Bannasch et al., PLoS Genet (2008)

Genomewide scan for gout in taiwanese aborigines reveals linkage to chromosome 4q25.

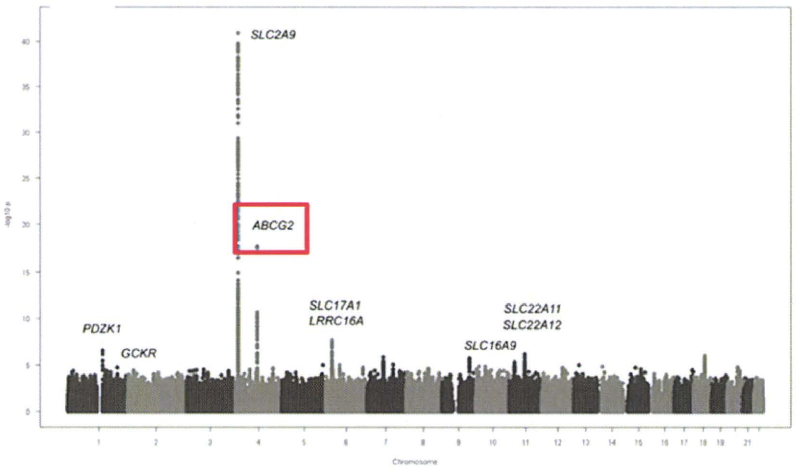




Cheng et al.,
Am J Hum Genet (2004)

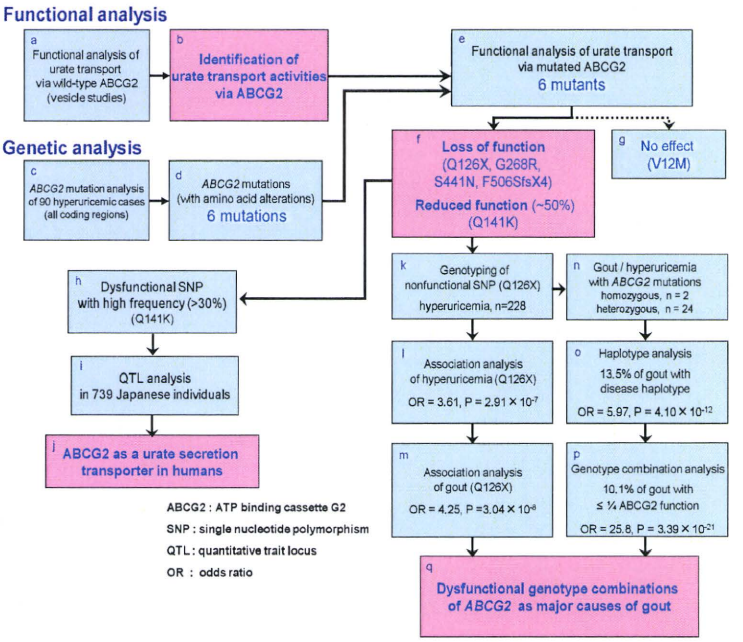
ゲノムワイド関連解析(GWAS)

尿酸値の変動に関連する遺伝子: *SLC2A9 (GLUT9), ABCG2*

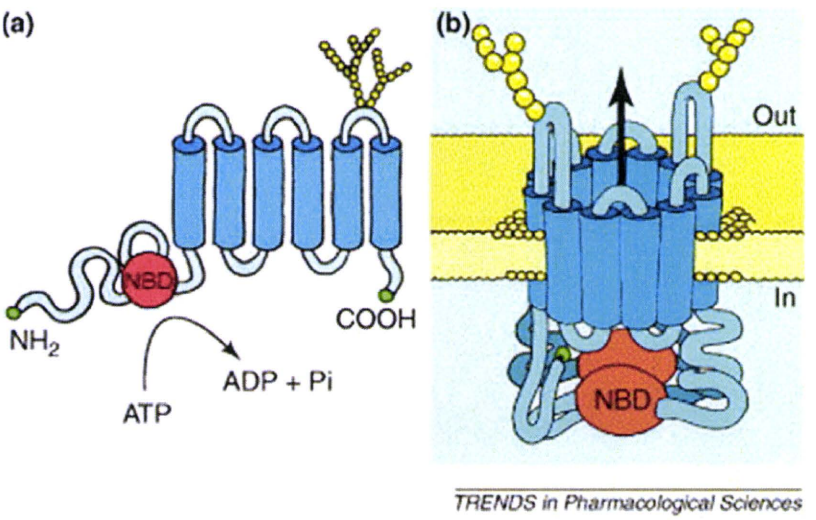


Kolz, et al., PLoS Genet (2009)
Dehghan, et al., Lancet (2008)

Flowchart for molecular-function-based clinicogenetic analysis of gout with ABCG2 polymorphic variants

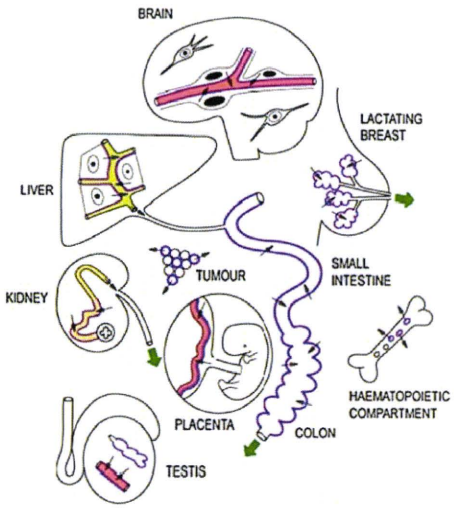


ABCG2/BCRPの構造モデル



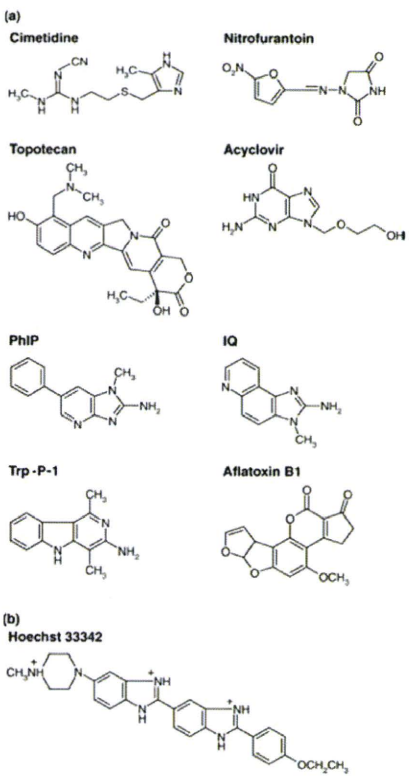
van Herwaarden et al., Trends Pharmacol Sci (2006)

ABCG2/BCRPの体内分布



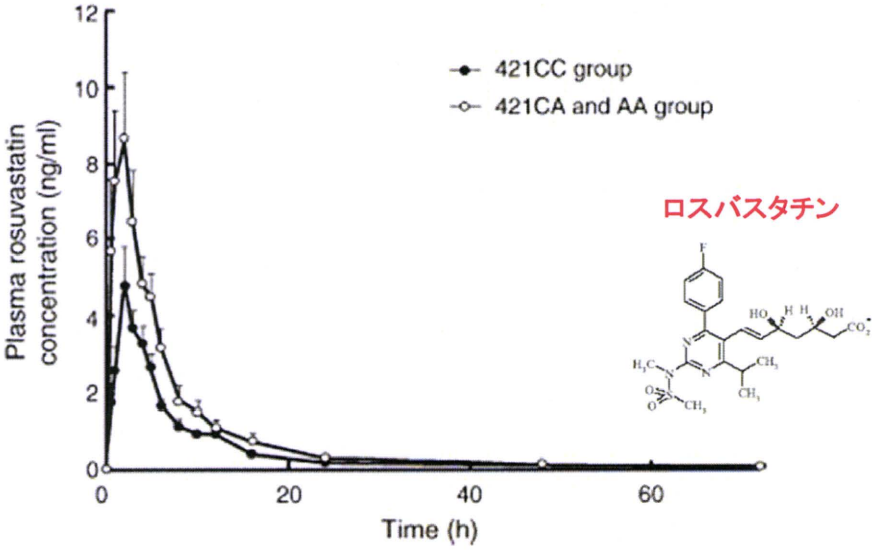
Vlaming et al., Adv Drug Deliv Rev (2009)

ABCG2/BCRP基質の構造式



van Herwaarden et al., Trends Pharmacol Sci (2006)

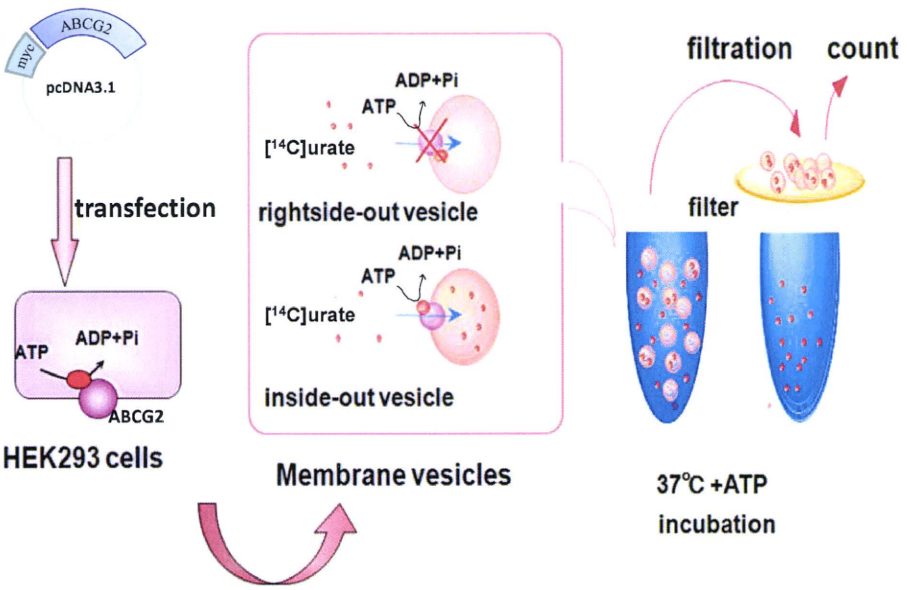
ABCG2/BCRPの遺伝子多型とロスバスタチンの血中濃度推移



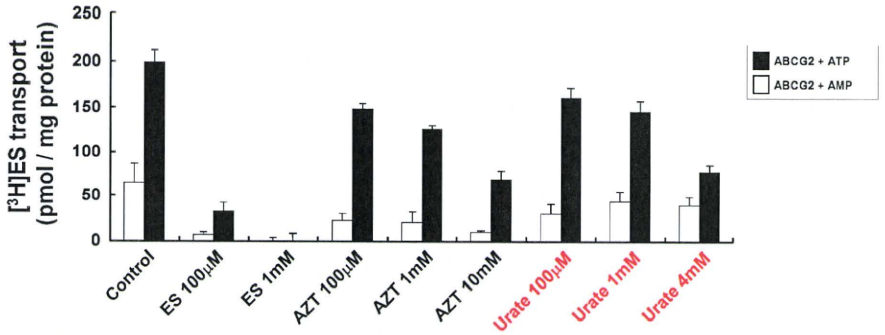
ロスバスタチン

Zhang et al., Clinica Chimica Acta (2006)

Experimental protocol for vesicle transport assays



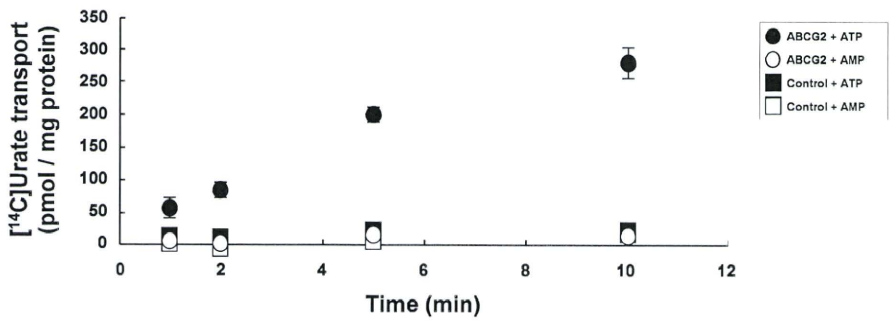
Urate inhibits ABCG2-mediated transport



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. AZT, 3'-azido-3'-deoxythymidine. Results are expressed as means ± S.D.

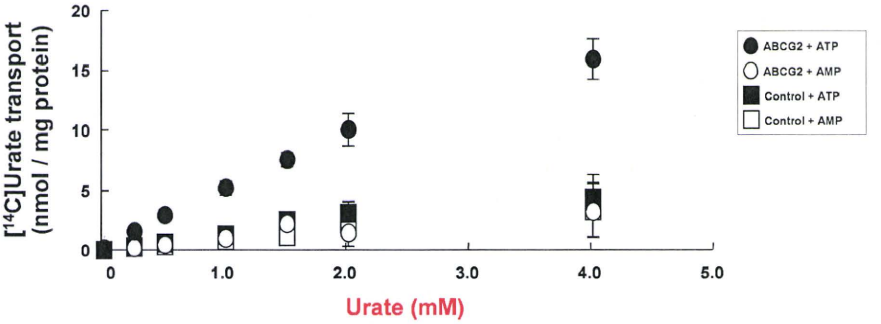
Although urate required a higher concentration than did unlabeled ES to inhibit [^{3}H]ES transport via ABCG2, the potency of urate was similar to that of the previously reported substrate, AZT.

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.

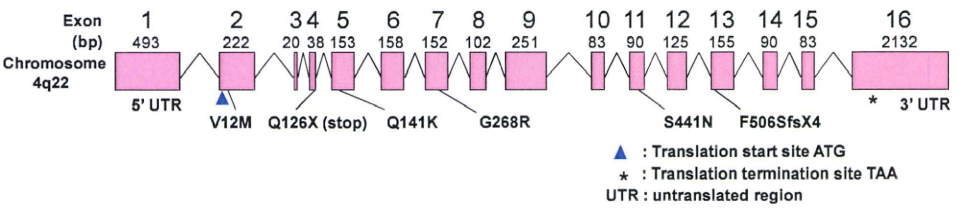
ABCG2 transports urate with high capacity



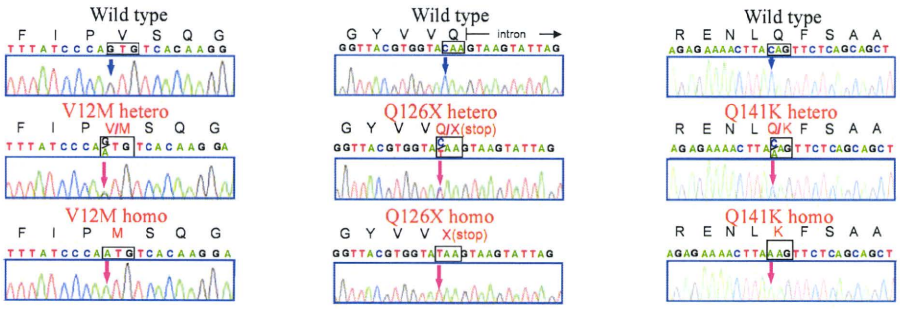
Concentration dependence of ABCG2-mediated transport of [^{14}C]urate was detected with 5-minute incubation. Results are expressed as means ± S.D.

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.

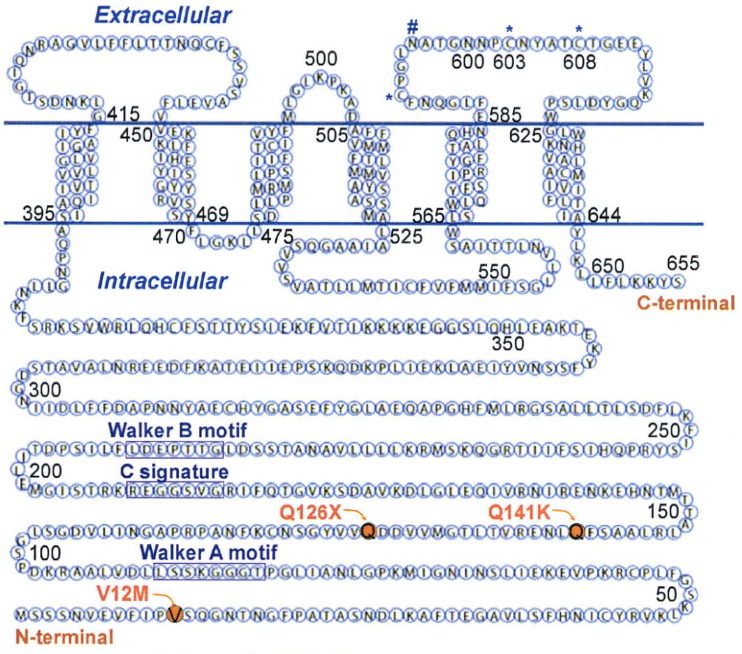
Genomic structure and mutation sites of human *ABCG2* gene



Results of sequence analysis of *ABCG2* gene



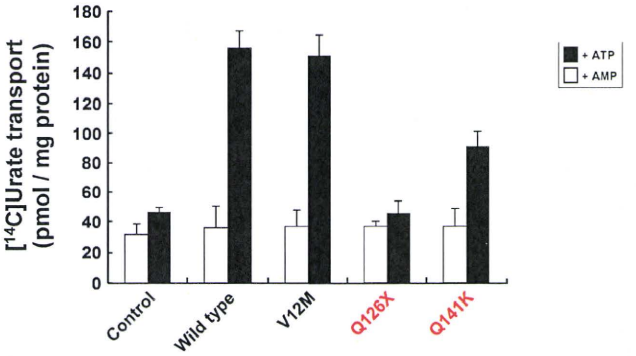
Topological model of ABCG2



#: N-linked glycosylation site (N596)

*: cysteine residues for disulfide bonds (C592, C603 and C608)

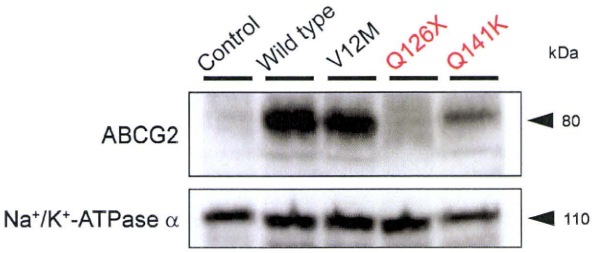
Urate transport analysis of mutated ABCG2



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.

Western blot analysis of wild-type and mutated ABCG2

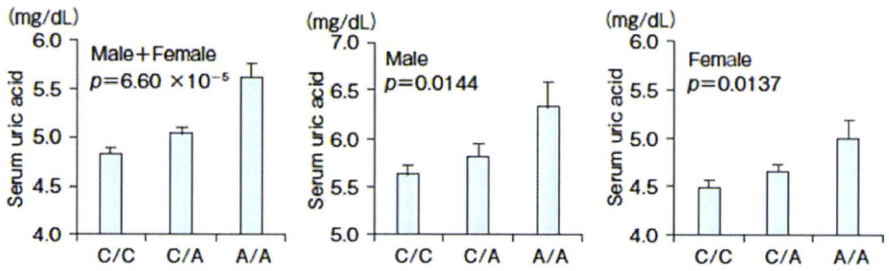


Western blot analysis showed a band of approximately 80 kDa in wild-type ABCG2. V12M showed a similar ~80 kDa band of almost the same density. Half-reduced expression in Q141K and no expression in Q126X were observed. As a loading control, the expression of Na^+/K^+-ATPase α was detected.

QTL analysis of ABCG2 Q141K and serum uric acid levels

量的形質座位（りょうてきけいしつざい、Quantitative trait locus、QTLと略す：量的形質遺伝子座ともいう）とは、量的形質がどのように生物に表現されるかに影響を与える染色体上のDNA領域のことである。

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.

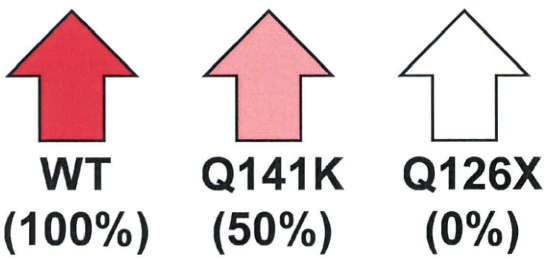
The analysis revealed that SUA significantly increased as the number of minor alleles of Q141K increased (p=6.60x10^{-5}). These findings indicate that ABCG2 controls SUA *in vivo*, and that there could be great inter-individual differences in this function because of its polymorphic nature.

日本薬剤学会第25年会（徳島）平成22年5月14日

Haplotype frequency analysis of variants

Allele			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	-	-	-

* 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.



薬学用語解説

社団法人 日本薬学会 The Pharmaceutical Society of Japan

Odds Ratio（オッズ比）

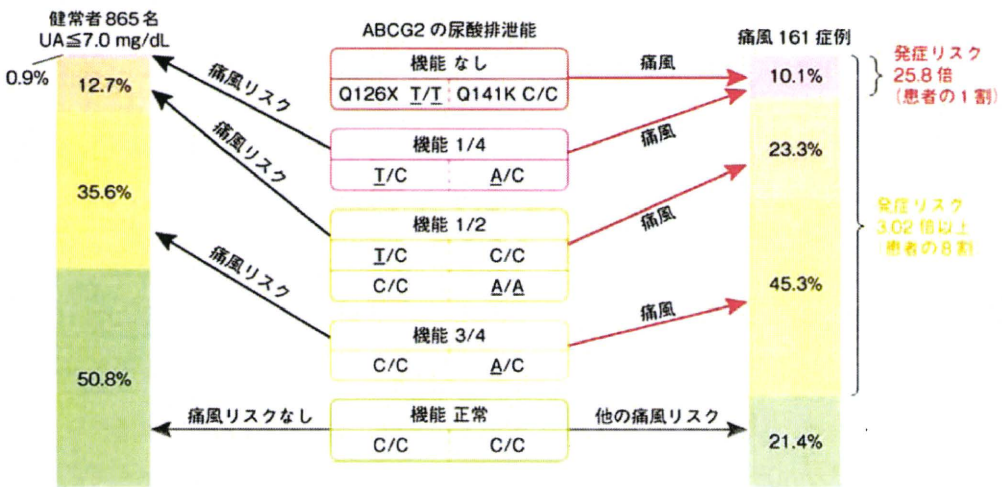
生命科学の分野において，ある疾患などへの罹りやすさを２つの群で比較して示す統計学的な尺度である．オッズ比が１とは，ある疾患への罹りやすさが両群で同じということであり，１より大きいとは，疾患への罹りやすさがある群でより高いことを意味する．逆に，オッズが１より小さいとは，ある群において疾患に罹りにくいことを意味する．例えば，ある多型が
疾患群１００名中の４０名で，
健常群１００名中の２０名で認められたとする．このオッズ比は，
（４０／６０）／（２０／８０）＝２．６７
となる．これは，ある多型において疾患群で出現するリスクが健常群に対して２．６７倍高いこととなる．

Association analysis of *ABCG2* genotype combination in gout patients

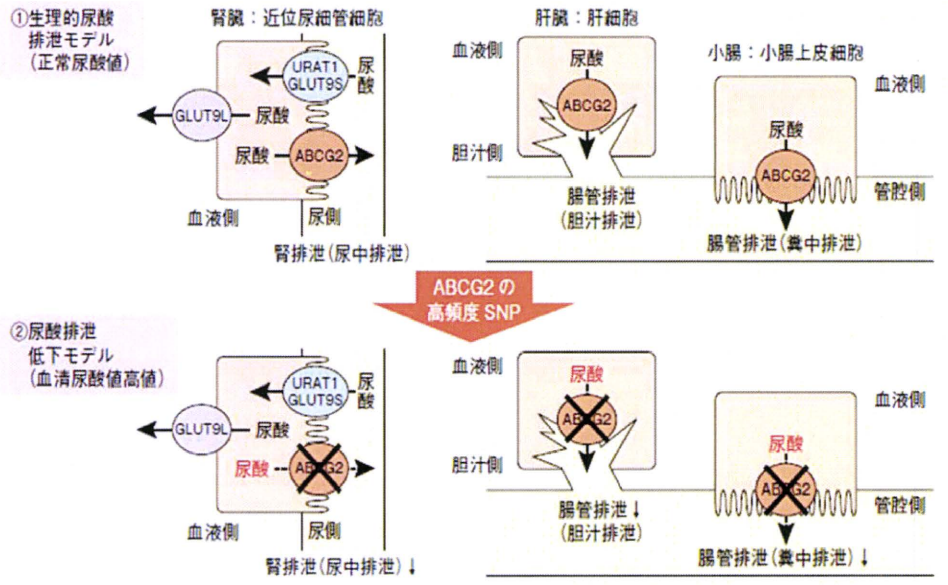
Estimated transport	Genotype Q126X	Genotype Q141K	Number Gout	Number Control	P-value	OR*	95% CI *
≦1/4 function	T/T	C/C	16	8	3.39×10^{-21}	25.8	10.3 - 64.6
	T/C	A/C					
1/2 function	T/C	C/C	37	110	2.23×10^{-9}	4.34	2.61 - 7.24
	C/C	A/A					
3/4 function	C/C	A/C	72	308	2.29×10^{-7}	3.02	1.96 - 4.65
full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

*OR = odds ratio; 95% CI = 95% confidence interval.
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.

Relation between ABCG2 transport dysfunction and gout



松尾, 高田, et al., 実験医学 (2010)



松尾，高田，et al.，実験医学(2010)

結論

○ABCG2は高容量性の尿酸排泄トランスポーターである

○ABCG2の遺伝子多型のうち、Q141Kは機能半減を、Q126Xは機能消失を生じる

●ABCG2の遺伝子多型の組み合わせによる機能低下の程度により、血中尿酸値および痛風発症リスクの上昇がもたらされる

⇒痛風・高尿酸血症病態のさらなる解明や
新たな予防法・治療薬の開発に繋がることが期待される

腎性低尿酸血症の全国的実態把握

平成22年度　総括研究報告書

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