by RACK1 suppression. It is possible that RACK1 is also involved in other unknown resistance mechanisms (Huang et al., 2008).

In conclusion, RACK1 regulated the expression and localization of ABCG2 in a post-transcriptional manner, suggesting that RACK1 affects the ABCG2 transport activity. Because RACK1 also regulates ABCB4 in a manner similar to that of ABCG2 but not ABCB1, it is possible that RACK1 plays a selective, functional role in the regulation of some ABC transporters.

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ORIGINAL ARTICLE

Polymorphisms in *NRXN3*, *TFAP2B*, *MSRA*, *LYPLAL1*, *FTO* and *MC4R* and their effect on visceral fat area in the Japanese population

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The predominant risk factor of metabolic syndrome is intra-abdominal fat accumulation, which is determined by waist circumference and waist–hip ratio measurements and visceral fat area (VFA) that is measured by computed tomography (CT). There is evidence that waist circumference and waist–hip ratio in the Caucasian population are associated with variations in several genes, including neurexin 3 (*NRXN3*), transcription factor AP-2β (*TFAP2B*), methionine sulfoxide reductase A (*MSRA*), lysophospholipase-like-1 (*LYPLAL1*), fat mass and obesity associated (*FTO*) and melanocortin 4 receptor (*MC4R*) genes. To investigate the relationship between VFA and subcutaneous fat area (SFA) and these genes in the recruited Japanese population, we genotyped 8 single-nucleotide polymorphisms (SNPs) in these 6 genes from 1228 subjects. Multiple regression analysis revealed that gender, age, and rs1558902 and rs1421085 genotypes (additive model) in *FTO* were significantly associated with body mass index (BMI; *P*=0.0039 and 0.0039, respectively), SFA (*P*=0.0027 and 0.0023, respectively) and VFA (*P*=0.045 and 0.040, respectively). However, SNPs in other genes, namely, *NRXN3*, *TFAP2B*, *MSRA*, *LYPLAL1* and *MC4R* were not significantly associated with BMI, SFA or VFA. Our data suggest that some SNPs, which were identified in genome-wide studies in the Caucasians, also confer susceptibility to fat distribution in the Japanese subjects.
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Keywords: fat mass and obesity associated (*FTO*); lysophospholipase-like 1 (*LYPLAL1*); melanocortin 4 receptor (*MC4R*); methionine sulfoxide reductase A (*MSRA*); neurexin 3 (*NRXN3*); subcutaneous fat area; transcription factor AP-2-β (*TFAP2B*); visceral fat area

INTRODUCTION

Metabolic syndrome is a common clinical phenotype that is manifested as concurrent metabolic abnormalities, including central obesity, glucose intolerance, dyslipidemia and hypertension.[1] Because several other definitions also exist,[2] the adequacy of this concept remains debatable. Recently, metabolic syndrome has attracted considerable interest because of increasing the number of patients. Although the pathogenesis of metabolic syndrome is not fully understood, the predominant underlying risk factor is considered to be visceral obesity due to an atherogenic diet and physical inactivity, in addition to certain genetic factors.[1,2] Adipose tissue, especially the visceral fat, secretes various adipocytokines. An increase in the adipose

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Table 1 Clinical characteristics of the subjects

	Men (n=518)	*Women (n=710)*	*Total (n=1228)*
Age (years)	49.7 ± 11.9	52.1 ± 11.3	51.1 ± 11.6
BMI (kg $^{-2}$)	30.3 ± 6.2	28.2 ± 5.3	29.1 ± 5.8
VFA (cm^2)	160.9 ± 66.8	102.5 ± 54.5	127.1 ± 66.5
SFA (cm^2)	213.0 ± 110.8	246.4 ± 98.6	232.3 ± 105.2

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; VFA, visceral fat area.
Data are shown as the mean ± s.d.

tissue mass leads to an alteration in the plasma adipocytokine level, resulting in dyslipidemia, hypertension and insulin resistance.[3,4] Intra-abdominal fat accumulation (central adiposity) is determined in terms of the waist circumference and waist–hip ratio measurements and visceral fat area (VFA) that is measured by computed tomography (CT).[1,5,6] Recently, two genome-wide association studies had been conducted to identify the loci that were linked with waist circumference or waist–hip ratio.[7,8]

In this study, we investigated the association of single-nucleotide polymorphisms (SNPs) in neurexin 3 (*NRXN3*), transcription factor AP-2β (*TFAP2B*), methionine sulfoxide reductase A (*MSRA*), lysophospholipase-like-1 (*LYPLAL1*), fat mass and obesity associated (*FTO*) and melanocortin 4 receptor (*MC4R*) genes with VFA and subcutaneous fat area (SFA) that were determined by CT.

MATERIALS AND METHODS

Study subjects

We recruited 1228 Japanese subjects from outpatient clinics after they agreed to undergo CT examinations (supine position) that were performed to determine the VFA and SFA values at the level of the umbilicus (L4–L5). Both VFA and SFA were calculated using the FatScan software program (N2system, Osaka, Japan).[9] The clinical characteristics of the patients are summarized in Table 1.

All subjects provided their informed written consent, and the protocol was approved by the ethics committee of each institution and by that of RIKEN.

DNA extraction and SNP genotyping

Using Genomix (Talent Srl, Trieste, Italy), genomic DNA was extracted from blood samples collected from each subject. We constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for rs1558902 and rs1421085 in *FTO*, rs489693 and rs17700144 in *MC4R*, rs1046997 in *NRXN3*, rs987237 in *TFAP2B*, rs782622 (rs545854) in *MSRA* and rs2605100 in *LYPLAL1*. The SNPs were genotyped using Invader assays as described previously.[10] The success rate of this assays was >99.0%.

Statistical analysis

Differences in the quantitative clinical data between case and control groups were tested by the Mann–Whitney *U*-test. Differences in the quantities of clinical data between the different genotypes were assessed by the Kruskal–Wallis test. We coded genotypes as 0, 1 or 2 depending on the number of copies of the risk alleles. Multiple linear regression analysis was performed to test the independent effect of risk alleles on body mass index (BMI), VFA or SFA by considering the effects of other variables (age and gender), which were assumed to be independent of the effect of the SNPs. The significance of the association between an independent variable and dependent variable was determined using a *t*-test. Hardy–Weinberg equilibrium was assessed using the χ^2-test.[11]

RESULTS

BMI, VFA and SFA are known to be affected by gender, and an association between rs2605100 in *LYPLAL1* and the waist–hip ratio has been reported only in women.[7] Therefore, we first compared the anthropometric parameters (BMI, VFA and SFA) among the different genotypes in the men and women. One SNP—rs10146997 in *NRXN3*—was found to be monomorphic, as reported in the HapMap database. Two SNPs (rs1558902 and rs1421085) in *FTO* were significantly associated with BMI in women (Table 2). The association of rs489693 and rs17700144 in *MC4R* with BMI has been reported in the Caucasian population;[7,8] however, such association was not observed in our study. The SNPs in *TFAP2B*, *MSRA* and *LYPLAL1* were not associated with BMI, which is a finding consistent with that of Lindgren *et al*.[7] No SNPs in the five genes were associated with VFA in either men or women (Table 3). Although rs489693 was marginally associated with VFA in men, the risk allele was different from that reported in the Caucasian population.[7] Two SNPs (rs1558902 and rs1421085) in *FTO* were significantly associated with SFA both in men (P=0.034 and 0.040, respectively) and women (P=0.010 and 0.0083, respectively) (Table 4). SNPs in other genes were not significantly associated with SFA. All SNPs were found to exhibit Hardy–Weinberg equilibrium ($P>0.10$).

Next, we attempted to perform multiple linear regression analysis by using BMI, VFA and SFA as the dependent variables, and with age, gender or genotype as an explanatory variable. We transformed genotypes to 0, 1 or 2 depending on the number of copies of the risk alleles. The A-allele of rs1558902 and the C-allele of rs1421085 in *FTO* were significantly associated with increases in BMI (P=0.0039 and 0.0039, respectively), VFA (P=0.045 and 0.040, respectively) and SFA (P=0.0027 and 0.0023, respectively) even after age and gender were included in the model (Supplementary Tables 1, 2, and 3). Multiple linear regression analysis showed that the SNPs of other genes were not significantly associated with BMI, VFA and SFA.

We also conducted power analysis of linear regression (additive model) with a significance level of 0.05, considering the effect size of the parameters. For rs1558902, the estimated effect sizes per allele (regression coefficients) for VFA and SFA were 5.8 and 14.4 cm^2, respectively (Supplementary Tables 2 and 3). The power of our statistical test was calculated on the basis of these estimated effect sizes and by performing 10 000 simulations. When the allele frequency was assumed to be 0.2, the power was estimated to be 0.32 for VFA and 0.81 for SFA; however, when the allele frequency was assumed to be 0.1, the respective powers were estimated to be 0.20 and 0.56.

DISCUSSION

The most important risk factor for metabolic syndrome is visceral fat obesity. According to the criteria released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005,[5] metabolic syndrome is defined by the presence of two or more abnormalities (dyslipidemia, impaired glucose tolerance or diabetes and hypertension), in addition to visceral fat obesity. The cutoff points for visceral fat obesity (waist circumference, 85 cm in men and 90 cm in women) are based on the cutoff point for VFA (100 cm^2) that is determined by CT.[5,6] Visceral fat mass measurement by CT is more precise than that derived from BMI or waist circumference measurements. Furthermore, for predicting metabolic risk-factor clustering, VFA is superior to waist circumference or BMI.[12] Therefore, we examined the association of VFA and SFA with the SNPs related to waist circumference and waist–hip ratio, and identified in a genome-wide study.[7,8] We found that the SNPs in *FTO* were significantly associated with BMI, as we have previously reported.[13] We also found that these SNPs were associated with VFA and SFA; however, the association between these SNPs and VFA was marginal because VFA was not significantly different among the genotypes in men. We did not find any association between other SNPs and BMI, VFA or SFA. These findings may be due to the low power of this study. Therefore, further studies with more subjects should be conducted to conclude that SNPs in genes other than *FTO* are not associated with VFA or SFA.

Table 2 Comparison of BMI among the different genotypes

						BMI (kgm^{-2})			
SNP ID	*Closest gene*	*Allele1/allele2*	*Risk allele*	*Gender*	*Genotype*	*11*	*12*	*22*	*P-value*
rs1558902	*FTO*	A/T	A	Men	25/190/302	30.3±5.1	30.9±5.9	29.9±6.4	0.058
				Women	39/239/430	29.6±6.7	28.8±5.6	27.7±5.0	0.012
				Total	64/429/732	29.9±6.1	29.7±5.8	28.6±5.7	0.00021
rs1421085	*FTO*	C/T	C	Men	25/189/304	30.3±5.1	30.9±5.9	30.0±6.4	0.076
				Women	39/238/431	29.6±6.7	28.8±5.6	27.7±5.0	0.0093
				Total	64/427/735	29.9±6.1	29.7±5.8	28.6±5.7	0.00022
rs489693	*MC4R*	C/A	A	Men	308/184/26	30.8±6.9	29.5±4.6	30.2±6.6	0.24
				Women	422/238/50	28.4±5.2	27.8±5.5	28.3±6.2	0.13
				Total	730/422/76	29.4±6.1	28.5±5.2	28.9±6.4	0.045
rs17700144	*MC4R*	A/G	A	Men	0/30/488	—	30.7±5.6	30.3±6.2	0.48
				Women	2/42/662	25.0, 26.2	27.1±3.8	28.3±5.4	0.34
				Total	2/72/1150	25.0, 26.2	28.6±4.9	29.1±5.9	0.62
rs987237	*TFAP2B*	A/G	G	Men	330/168/19	30.2±6.2	30.6±6.1	30.1±6.3	0.56
				Women	434/242/32	28.2±5.5	28.0±5.2	28.8±4.7	0.47
				Total	764/410/51	29.1±5.9	29.1±5.7	29.3±5.4	0.80
rs7826222	*MSRA*	C/G	C	Men	77/235/205	30.7±5.1	30.5±5.9	30.0±6.9	0.22
				Women	117/321/271	28.0±5.3	28.2±4.8	28.2±5.9	0.68
				Total	194/556/476	29.1±5.4	29.2±5.4	29.0±6.4	0.44
rs2605100	*LYPLAL1*	A/G	G	Men	22/145/351	30.5±5.2	30.9±6.6	30.1±6.1	0.23
				Women	14/200/495	26.6±4.7	28.7±6.0	28.0±5.0	0.24
				Total	36/345/846	29.0±5.3	29.6±6.3	28.9±5.6	0.16

Abbreviations: BMI, body mass index; SNP, single-nucleotide polymorphism.
P-values were analyzed using the Kruskal–Wallis test. *P*values for 12 vs 22 at rs17700144 were analyzed using the Mann–Whitney *U*-test. Data are presented as the mean±s.d.

Table 3 Comparison of VFA among the different genotypes

						VFA (cm^2)			
SNP ID	*Closest gene*	*Allele1/allele2*	*Risk allele*	*Gender*	*Genotype*	*11*	*12*	*22*	*P-value*
rs1558902	*FTO*	A/T	A	Men	25/190/302	163.2±52.1	166.9±67.5	157.1±67.4	0.22
				Women	39/239/430	105.8±69.3	106.2±53.1	100.1±53.7	0.23
				Total	64/429/732	128.2±68.7	133.0±67.0	123.6±66.0	0.039
rs1421085	*FTO*	C/T	C	Men	25/189/304	163.2±52.1	167.2±67.6	156.8±67.2	0.18
				Women	39/238/431	105.8±69.3	106.1±53.2	100.0±53.4	0.25
				Total	64/427/735	128.2±68.7	133.1±67.2	123.5±65.7	0.037
rs489693	*MC4R*	C/A	A	Men	308/184/26	166.7±67.2	154.7±66.2	135.5±56.7	0.047
				Women	422/238/50	105.6±55.1	95.5±51.2	109.1±61.8	0.089
				Total	730/422/76	131.4±67.6	121.3±65.2	118.2±61.0	0.035
rs17700144	*MC4R*	A/G	A	Men	0/30/488	—	161.5±62.4	160.9±67.1	0.87
				Women	2/42/662	68.3, 90.2	104.8±50.8	102.4±54.8	0.60
				Total	2/72/1150	68.3, 90.2	128.4±62.2	127.2±66.9	0.69
rs987237	*TFAP2B*	A/G	G	Men	330/168/19	158.4±65.7	169.0±69.1	132.6±56.7	0.06
				Women	434/242/32	102.7±53.4	101.3±55.7	109.4±61.5	0.71
				Total	764/410/51	126.7±65.2	129.0±69.9	118.0±60.3	0.65
rs7826222	*MSRA*	C/G	C	Men	77/235/205	161.7±65.6	162.9±66.6	158.5±67.7	0.80
				Women	117/321/271	105.4±57.1	103.3±54.8	100.5±53.1	0.74
				Total	194/556/476	127.7±66.5	128.5±66.9	125.4±66.3	0.71
rs2605100	*LYPLAL1*	A/G	G	Men	22/145/351	153.2±65.4	167.9±74.5	158.5±63.4	0.51
				Women	14/200/495	94.1±41.1	101.7±53.0	103.1±55.5	0.92
				Total	36/345/846	130.2±63.6	129.5±70.9	126.1±64.9	0.86

Abbreviations: VFA, visceral fat area; SNP, single-nucleotide polymorphism.
P-values were analyzed using the Kruskal–Wallis test. *P*-values for 12 vs 22 at rs17700144 were analyzed using the Mann–Whitney *U*-test. Data are presented as the mean±s.d.

SNPs in *FTO* were associated with SFA and VFA. Therefore, some of the SNPs, which were identified by genome-wide association studies in the Caucasian population, were also found to confer susceptibility to body fat distribution in the Japanese subjects. On the other hand, rs10146997 in *NRXN3* was found to be monomorphic in our subjects, and we could not find associations between SNPs in *MC4R*, *TFAP2B*,

Table 4 Comparison of SFA among the different genotypes

						SFA (cm^2)			
SNP ID	*Closest gene*	*Allele1/allele2*	*Risk allele*	*Gender*	*Genotype*	*11*	*12*	*22*	*P-value*
rs1558902	*FTO*	A/T	A	Men	25/190/302	215.9±110.5	223.0±104.4	206.2±114.7	0.034
				Women	39/239/430	279.6±129.9	255.9±96.9	238.5±95.6	0.010
				Total	64/429/732	254.3±125.7	241.3±101.5	225.2±105.1	0.0024
rs1421085	*FTO*	C/T	C	Men	25/189/304	215.9±110.5	223.1±104.6	206.4±114.4	0.040
				Women	39/238/431	279.6±129.9	256.2±96.9	238.4±95.6	0.0083
				Total	64/427/735	254.3±125.7	241.6±101.6	225.1±104.9	0.0021
rs489693	*MC4R*	C/A	A	Men	308/184/26	220.8±114.4	199.7±97.0	214.0±149.6	0.16
				Women	422/238/50	250.0±95.2	242.8±106.6	232.9±86.8	0.29
				Total	730/422/76	237.7±104.6	223.9±104.6	226.4±111.7	0.051
rs17700144	*MC4R*	A/G	A	Men	0/30/488	—	227.6±119.8	212.1±110.3	0.34
				Women	2/42/662	179.3, 180.0	222.4±76.3	248.6±99.9	0.15
				Total	2/72/1150	179.3, 180.0	224.5±96.1	233.1±105.9	0.67
rs987237	*TFAP2B*	A/G	G	Men	330/168/19	207.8±106.8	225.0±119.6	195.0±96.8	0.20
				Women	434/242/32	251.5±102.6	237.8±92.1	246.2±88.7	0.38
				Total	764/410/51	232.6±106.6	232.6±104.3	227.1±94.2	0.96
rs7826222	*MSRA*	C/G	C	Men	77/235/205	230.0±119.7	211.9±109.2	207.9±109.3	0.44
				Women	117/321/271	241.9±93.0	247.1±91.3	247.9±109.0	0.65
				Total	194/556/476	237.1±104.3	232.3±100.7	230.7±110.8	0.52
rs2605100	*LYPLAL1*	A/G	G	Men	22/145/351	217.3±106.5	216.2±112.5	211.4±110.7	0.66
				Women	14/200/495	224.2±94.0	257.5±108.6	242.8±94.2	0.17
				Total	36/345/846	220.0±100.5	240.1±112.0	229.7±102.5	0.21

Abbreviations: SFA, subcutaneous fat area; SNP, single-nucleotide polymorphism.
P-values were analyzed using the Kruskal–Wallis test. *P*-values for 12 vs 22 at rs17700144 were analyzed using the Mann–Whitney *U*-test. Data are presented as the mean±s.d.

MSRA or *LYPLAL1* and VFA or SFA. Thus, there is a possibility that genetic susceptibility to body fat distribution is likely to differ among various ethnic groups.

rs1558902 and rs1421085 in *FTO* were in linkage disequilibrium with rs9939609, which is associated with obesity and type II diabetes.[14–16] Concentrations of circulating adipocytokines are affected by the accumulation of adipose tissue, especially visceral adipose tissue. Therefore, rs1558902 and rs1421085 probably affect subcutaneous and visceral fat accumulation, leading to the development of type II diabetes by altered adipocytokine secretion. The precise mechanism of how *FTO* affects adipose tissue accumulation is not clear yet; however, there is evidence that *FTO* is involved in the development of obesity. *FTO* is ubiquitously expressed, and a strong expression is observed in the arcuate, paraventricular, dorsomedial and ventromedial nuclei, which are critical energy-regulation sites.[16–18] *FTO* also exists in the nucleus and is reported to be a member of the Fe(II) and 2-oxoglutarate-dependent oxygenase superfamily.[17,19] *FTO*-deficient and dominant-negative mutant *FTO* mice have shown reduced fat mass and increased energy expenditure.[20,21] These reports indicate that FTO has an important role in energy homeostasis by regulating energy expenditure. Although the effects of the SNPs rs1558902 and rs1421085 on gene expression need to be elucidated, variations in *FTO* probably affect subcutaneous and visceral fat accumulation.

In summary, we showed that rs1558902 and rs1421085 in *FTO* may be associated with SFA and VFA in the Japanese population. In our study, rs489693 and rs17700144 in *MC4R*, rs1046997 in *NRXN3*, rs987237 in *TFAP2B*, rs782622 (rs545854) in *MSRA* and rs2605100 in *LYPLAL1* were not associated with SFA or VFA.

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COMMENTARY

Commentary to 'A remark on rare variants'

Takahiro Nakamura

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The article entitled 'A remark on rare variants' by Oexle[1] is an interesting report in which the author describes an approach to the study of common diseases caused by multiple rare variants. The article contains three parts. The first part is a discussion of the concept of population attributable risk (PAR) proposed by Bodmer and Bonilla.[2] PAR is a kind of index that represents the contribution of a variant to the onset of the disease. For calculating the cumulative genetic effect of rare variants, an approximate expression for estimating PAR is presented. However, the explanation of PAR in the original article is difficult to understand because it does not go into enough detail. The author explained the process of obtaining the equations described in the original article. The second part is a discussion of the efficiency of genetic analysis for rare variants with strong effect size. The powers of both affected sib-pair analysis and transmission/disequilibrium test (TDT) were calculated based on the methods of Risch and Merikangas.[3] The author illustrated that affected sib-pair analysis is more sensitive to a decrease in frequency or effect size than TDT. The third part proposes a disease model based on Kimura's infinite sites model. The author derived a simple relationship between the variant's selection coefficient and its effect size. The number of contributing genetic variants can be estimated by this model. Finally, TDT was applied to the disease model. The author derived the required sample size for the test.

The first two parts are very informative and contribute to the understanding of original articles. Moreover, I find the disease model proposed in the third part very intriguing.

Recently, many genetic variants that cause multifactorial disease have been detected by genome-wide association study (GWAS). To increase the power of GWAS, high-frequency single-nucleotide polymorphisms (SNPs) are used as markers. This means that common variants with low odds ratio (OR) can be detected by GWAS. However, it is also possible to discover rare variants with high OR by sequencing around the positive markers. For example, the association between *ABCG2* and serum uric acid levels was identified by GWAS.[4] The functional variant Q141K in *ABCG2* increases the serum uric acid level. We searched for the causative SNPs of gout and hyperuricemia in the *ABCG2* gene. The common variant Q141K was associated with gout (minor allele frequency (MAF)=0.32; OR=2.23). Moreover, the rare variant Q126X was detected as a disease-related SNP (MAF=0.03; OR=4.25). Functional analysis showed that Q141K reduced activity by half and that Q126X was associated with no activity.[5]

Discoveries such as these may increase in the future. However, there may be many rare causative SNPs that cannot be detected by GWAS. Genetic researchers are accordingly interested in the question of how many causative variants exist with a particular OR and MAF. The mathematical model proposed in the third part may give an approximate answer to this question. The proposed model contains complicated equations; in particular, there are many equations about selection parameter s. However, the parameter s was integrated out and two parameters remained: relative fertility and affection rate of the disease. These two parameters alone are required to estimate the number of the causative variants. The model therefore seems to be simple.

The simulation study for the power of TDT was performed using estimates of the number of causative SNPs in the third part. The estimated number can be applied not only for a power calculation of TDT but also for a power calculation of an association study using chi-square test. Therefore, the model can help in study design. Cumulative PAR, which represents the summary genetic effect of particular variants, can also be calculated by the estimate. Moreover, if we can obtain a more exact estimate of the number of causative SNPs with some genetic effect, the heritability of the disease may be estimated.

This model is based on the assumption that relative fertility and affection rate determine the ORs and the frequencies for the variants. Moreover, it is assumed that the variants associated with disease are under selection, even if the disease onset is late. These two assumptions seem not to be robust. Therefore, evidence that this model represents the real structure of genetic disease is required for genetic researchers to use the model confidently.

One method for confirming the validity of the model is to check the inheritance of the variants. The author claims that disease susceptibility genes may be associated with a selective disadvantage even if the average onset is late. If this assumption is true, the rare variants contributing to diseases are not inherited with a probability of 1/2. It appears that the fitness of the model for the real genetic architecture could be verified by investigating the probability of inheritance of the disease-related variant, genetic effect, frequency and relative fertility.

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The real structure of genetic disease would not appear as simple as described in the article. However, for the purpose of theoretical study it is useful to start from the simplest case. I hope that this article serves as the basis from which to develop research on rare causative variants.

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栄養・食品機能とトランスポーター

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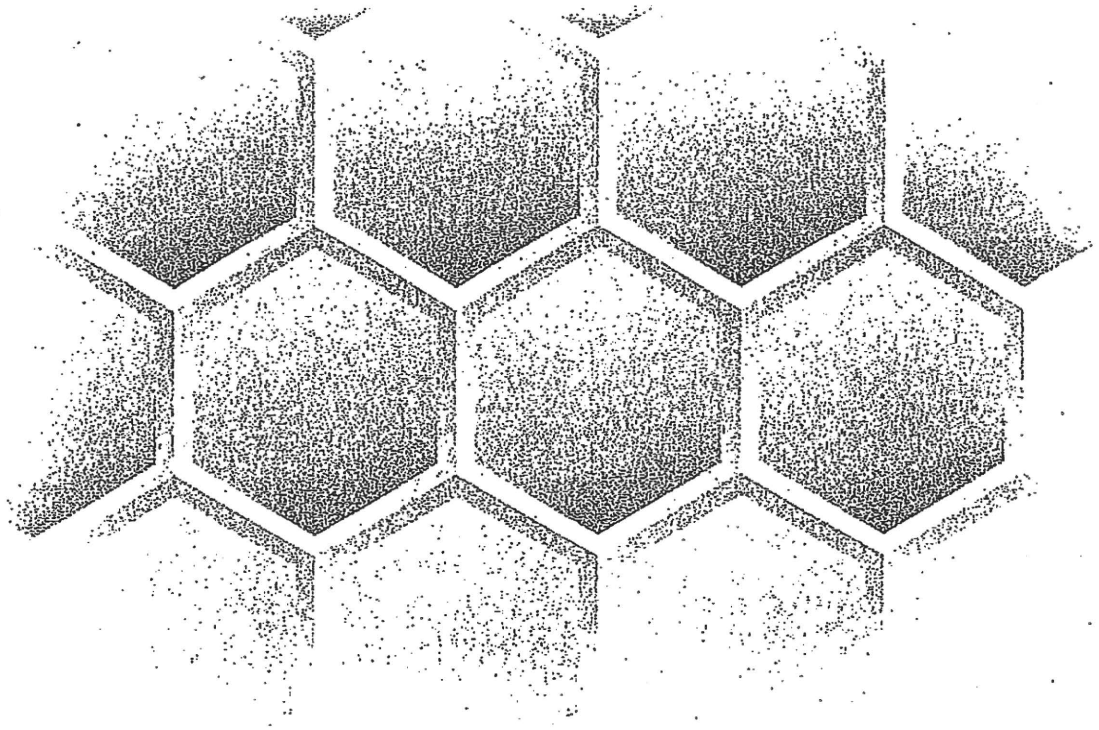

建帛社
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第7章　尿酸のトランスポーター

松尾　洋孝*

1．はじめに

高尿酸血症は，性・年齢を問わず，血漿中の尿酸溶解濃度である7.0 mg/dLを正常上限としてそれを超えるものと定義される生活習慣病の1つである。高尿酸血症は突発的な激しい関節痛を生じる痛風の直接的な原因であり，高血圧[1,2]，腎障害[3,4]，虚血性心疾患[5]，脳卒中[6,7]などの“common disease”（ありふれた疾患）[8]の要因となることも知られている。そのため，血清尿酸値の管理は，医学・栄養学を含む医療の現場において極めて重要であるといえる。

尿酸トランスポーターは，細胞膜に存在して血清尿酸値を調節する分子であり，腎臓において尿酸の再吸収に働く分子と，尿酸の排泄に働く分子がある。尿酸再吸収トランスポーターの輸送機能を阻害することで，痛風や高尿酸血症の治療薬であるベンズブロマロン（ユリノーム®）は薬効を示すことがわかっている。また，尿酸排泄トランスポーター遺伝子の個人差が，痛風や高尿酸血症の主要病因であることが最近明らかになった[9]。

本章では尿酸トランスポーターの視点からみた血清尿酸値の生理学的な調節機能と，その機能不全による病態についても解説し，トランスポーター研究の成果から生活習慣病の克服が可能となりつつある現状についても概説する。

＊　防衛医科大学校分子生体制御学講座

2．ヒトにおける尿酸動態の特徴

（1）尿酸動態の種差

医学・食品栄養学における血清尿酸値管理を考えるうえでは，まず，ヒトにおける尿酸動態の特徴を理解する必要がある。ヒトを含む霊長類の一部では尿酸分解酵素であるウリカーゼが欠損しているため，ウリカーゼの機能が保たれているマウスのような他の多くの哺乳類と比較すると，尿酸値は高値を示すことが知られている[10,11]。また，ウリカーゼの欠損のため，ヒトにおいて尿酸はプリン代謝の最終代謝産物となり，腎臓や腸管から排泄される[12]。したがって，ヒトにおける尿酸の代謝，輸送動態やその異常に起因する疾患については，ノックアウトマウスなどのモデル動物を用いた解析が困難であることが多く，ヒトを対象とした解析，特に，ヒトの疾患における臨床遺伝学的解析とそれに基づく分子機能解析は不可欠である[13]。

尿酸は抗酸化作用が強いため，マウスなどの他の哺乳類に比べて，ヒトが長寿である一因は血清尿酸値が高いことにあるとも考えられている[14,15]。すなわち，尿酸にはヒトの健康維持において抗酸化作用による良い面と，痛風や心血管病のリスクとなるような悪い面の両面性が認められる。このことは，適正な血清尿酸値管理を考えるうえでも重要である。

（2）ヒトの尿酸輸送機構とトランスポーター病

上記のような尿酸動態の特徴のために，ヒトにおける尿酸トランスポーターの同定は，ヒトゲノムの解読後に初めて成功したが，ゲノムワイド解析などのポストゲノム研究が進展するまでは，その後の第2，第3の尿酸トランスポーターの同定も長らくなされていなかった。これまでに　ヒトの疾患における臨床遺伝学的解析とそれに基づく分子機能解析から，尿酸トランスポーターの機能不全に起因するトランスポーター病の存在が明らかとなり，ヒトにおける生

表７－１　ヒトの尿酸トランスポーターとトランスポーター病

尿酸トランスポーター	生理機能（尿酸輸送）	遺伝子座位	機能不全によるトランスポーター病
URAT1/SLC22A12	腎近位尿細管における尿酸再吸収	11q13	腎性低尿酸血症１型 (RHUC1, renal hypouricemia type 1)
GLUT9/SLC2A9	腎近位尿細管における尿酸再吸収	4p16-p15.3	腎性低尿酸血症２型 (RHUC2, renal hypouricemia type 2)
ABCG2/BCRP	尿酸排泄	4q22	痛風（gout）*

＊痛風は単一遺伝子疾患ではないが，主要病因遺伝子として *ABCG2* 遺伝子が同定されている。

理学的な尿酸輸送の役割を担う尿酸トランスポーターが同定されてきた（表７－１）。Urate transporter 1（*URAT1/SLC22A12*）と Glucose transporter 9（*GLUT9/SLC2A9*）は，腎性低尿酸血症の病因遺伝子であることが同定され[16,17]，その腎近位尿細管における発現パターンから，生理学的には尿酸再吸収の役割を共同して担うこと[16,17]がわかってきた（図７－１）。すなわち，URAT1 または GLUT9 のいずれかの機能障害（尿酸再吸収障害）は血清尿酸値が低い状態，つまり腎性低尿酸血症を来す（図７－１）。腎性低尿酸血症は単一遺伝子病と考えられており，合併症としての尿路結石や運動後急性腎不全[18,19]が臨床上の問題となる。

URAT1 と GLUT9 はそれぞれ有機アニオントランスポーター（SLC22）のファミリーとグルコーストランスポーター（SLC2）のファミリーに属し，いずれも ATP 依存性の ABC トランスポーターではなく，solute carrier（SLC）トランスポーターである。尿酸と構造の類似性が低いグルコースのトランスポーターのファミリーから，生理学的に重要な尿酸再吸収トランスポーター GLUT9 が同定されたことは意外なことであり非常に興味深いことであった。このように，ヒトを対象とする臨床遺伝学的解析とそれに基づく分子機能解析からは，従来の常識にとらわれない新規の発見が期待できる。

ATP-binding cassette（ABC）transporter G2（ABCG2/BCRP）は，ABC トランスポーターの１種であり，ATP 依存性の基質の排出作用が特徴である。

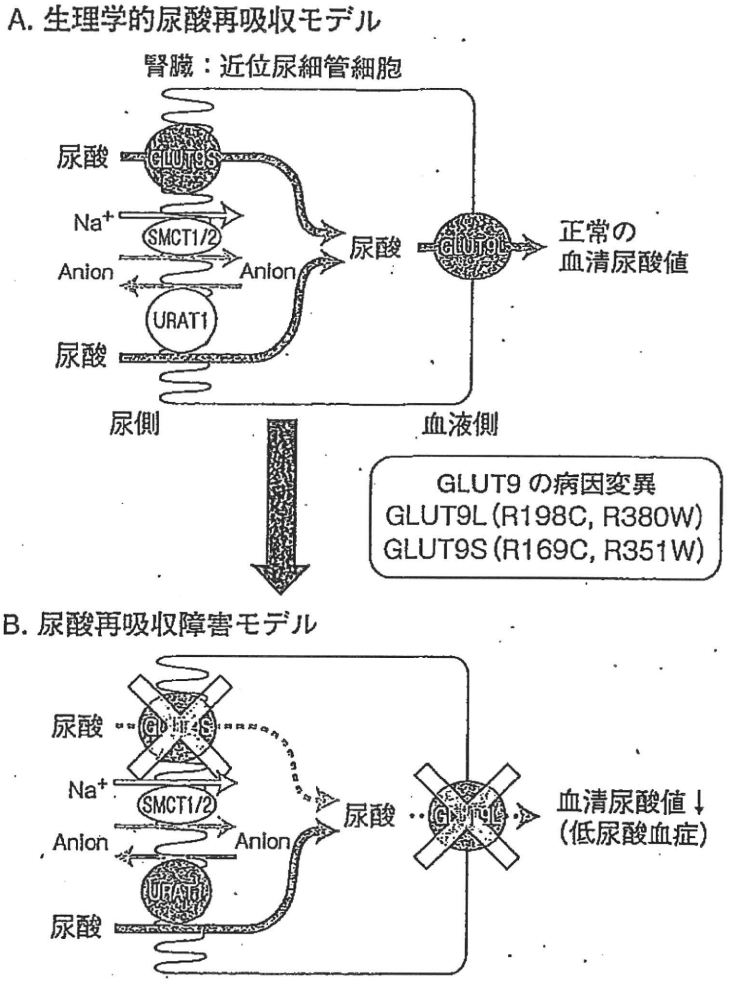

図7－1　ヒト腎臓における尿酸再吸収の分子機構[17]

ABCG2の分子機能としては，これまでは抗がん剤などの薬剤の排出作用[20, 21]のほか，内因性基質としてはポルフィリンの排出作用が知られていた[22]。*ABCG2*は，痛風・高尿酸血症の主要病因遺伝子であることが同定され[9]，その発現パターンから生理学的には尿や糞便中への尿酸排泄の役割を担うことが示唆された（図7－2）[9]。すなわち，ABCG2の機能障害（尿酸排泄障害）は血清尿酸値が高い状態，つまり高尿酸血症とその続発症である痛風を引き起こす主要な病因である（図7－2）。痛風および高尿酸血症は，いわゆる生活習慣病としても重要であり，多因子疾患あるいは「ありふれた疾患“common disease”」の1つである。

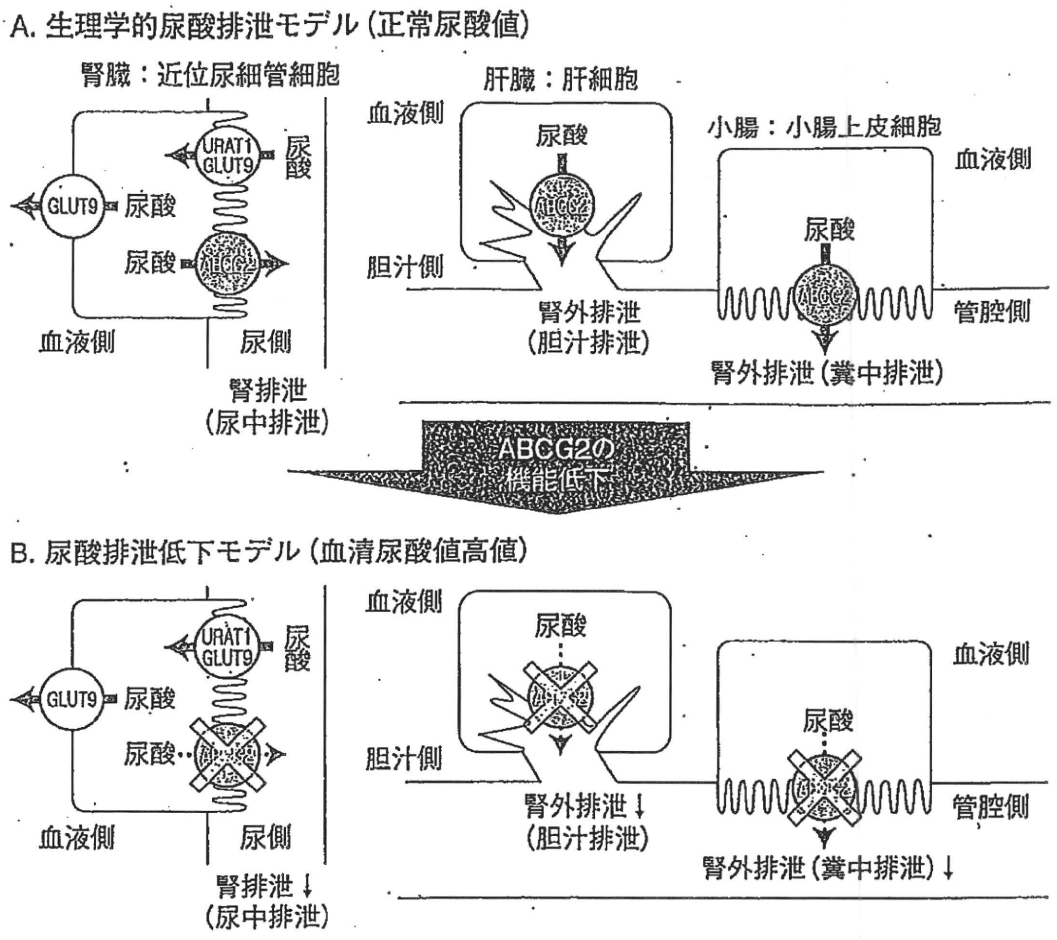

図７－２　ヒト腎臓および腸管からの尿酸排泄機構[9)]

A：ABCG2 の分子機能に基づく臨床遺伝学的解析により，生理学的な尿酸排泄機構を提案することができた。ABCG2 の局在は，ヒトの腎臓，肝臓および小腸の免疫組織化学的解析の報告に基づいて示した。B：ABCG2 機能低下型変異による血清尿酸値の上昇から，その病態における尿酸排泄低下の分子機序が解明された。

3．尿酸再吸収トランスポーター

(1) URAT1/SLC22A12

ヒトの血清尿酸値を調節する遺伝子として最初に同定されたのは，*URAT1/SLC22A12* 遺伝子であった。*URAT1* 遺伝子は，解読されたばかりのゲノム情報概要版を用いた相同性解析により，有機アニオントランスポー

ター遺伝子 *OAT4/SLC22A11* と相同性を持つ遺伝子として2002年に発見された[16]。URAT1は12回膜貫通型の膜タンパク質で，腎臓特異的に発現する。また，近位尿細管の管腔側に局在する尿酸再吸収トランスポーターであり，痛風や高尿酸血症の治療薬であるベンズブロマロンの分子標的であることもあわせて報告された[16]。

このようなURAT1の生理学的な機能の解明は，*URAT1/SLC22A12* が腎性低尿酸血症の最初の病因遺伝子として同定されたことに基づいており，自衛隊熊本病院の症例[23]を対象とした遺伝子解析により証明された[16]。*URAT1/SLC22A12* 遺伝子における腎性低尿酸血症の病因変異としては，W258X (G774A) 変異が日本人の症例で最も多い (74.1%)[24]。このW258X変異は，URAT1タンパク質の258番目のアミノ酸であるトリプトファン (W) が終止コドン (X) となるようなナンセンス変異と呼ばれる遺伝子変異であり，URAT1の分子機能が完全に消失することがわかっている。W258X変異は日本人において頻度の高い一塩基多型 (single nucleotide polymorphism, SNP) であり，健常人のアレル頻度は2.30～2.37%[25,26]と報告されている。このことは，染色体100本 (50人相当) あたり2本程度にW258X変異を認めるということを意味している。日本人の腎性低尿酸血症ではそのほとんどに *URAT1* の変異が認められるが，一部に *URAT1* の変異を認めない症例が存在することも報告されており[24,27]，*URAT1* 以外の腎性低尿酸血症の病因遺伝子が存在することが示唆されていた。

(2) GLUT9/SLC2A9

ヒトゲノム情報の解読後，ゲノムワイド関連解析 (genome-wide association study, GWAS) による疾患関連遺伝子の探索が盛んに行われるようになった (GWASの詳細については後述)。血清尿酸値にかかわるGWASも，2007年以降，複数のグループにより実施され，尿酸値の変動に関与する遺伝子として *GLUT9/SLC2A9* が報告された[28-31]。これにより，GLUT9がヒトにおいて生理学的に重要な尿酸トランスポーターの候補であることが示された (表7－

2)。GLUT9は12回膜貫通型の膜タンパク質であり，前述のようにグルコーストランスポーターのファミリーに属する分子である。

Vitartらは，GLUT9が尿酸を輸送することをGWASの報告の際に初めて記載し，さらに，その輸送特性（K_m値，890 μM）についても明らかにした[30]。また，Vitartらは，GLUT9の機能がベンズブロマロンにより（URAT1と比べて弱く）抑制されることも報告している[30]。GLUT9による尿酸輸送能

表7－2　血清尿酸値を対象とした主なゲノムワイド関連解析（GWAS）

年	著者	対象数	対象	遺伝子	文献
2007	Li et al.	4,731人 [1,301人]	イタリア人 Sardinia [イタリア人 Chianti]	*GLUT9/SLC2A9, PJA2*	28
2008	Döring et al.	1,644人 [4,162人] [4,066人] [1,719人]	ドイツ人 Augsberg [ドイツ人 Augsberg] [ドイツ人 Pomerania] [オーストリア人 Salzburg]	*GLUT9/SLC2A9*	29
2008	Vitart et al.	986人 [708人]	クロアチア人 [イギリス人 Orkney島]	*GLUT9/SLC2A9*	30
2008	McArdle et al.	868人	ドイツ系アメリカ人 Amish	*GLUT9/SLC2A9*	31
2008	Dehghan et al.	7,699人 4,148人 11,024人 3,843人	ヨーロッパ系白人 オランダ人系 Rotterdam アメリカ人白人 アメリカ人黒人	*GLUT9/SLC2A9, ABCG2, SLC17A3-SLC17A1-SLC17A4*	32
2009	Kolz et al.	28,141人	ヨーロッパ人（メタ解析）	*GLUT9/SLC2A9, ABCG2, SLC17A3-SLC17A1-SLC17A4, URAT1/SLC22A12, OAT4/SLC22A11, MCT9/SLC16A9, PDZK1, GCKR, LRRC16A-SCGN*	33
2010	Kamatani et al.	14,700人	日本人	*URAT1/SLC22A12, GLUT9/SLC2A9, ABCG2, LRP2*	34

注　[　] は replication study の対象を示す。（文献13より引用；改変）

は，その後の報告でも確認され[17, 35, 36]，従来，主要な輸送基質と考えられていたD-グルコース，D-フルクトースなどの糖輸送活性よりも尿酸輸送活性の方が数十倍高いことが示されている[35]。

*GLUT9/SLC2A9*が尿酸値の変動に関与することに加えて，過去の報告で近位尿細管におけるGLUT9の発現が示されていることから，*GLUT9/SLC2A9*遺伝子が腎性低尿酸血症の第2の病因遺伝子である可能性が示唆されていた。筆者らは過去10年間にわたる85万セットの健康診断データを有する海上自衛隊の健康診断データベースを活用することにより，十分な症例数を確保したうえで，*GLUT9*遺伝子を対象とした低尿酸血症の臨床遺伝学的解析を実施した。その詳細は他の総説に記したが[13]，この解析により腎性低尿酸血症を来す2つの機能消失型のミスセンス変異（アミノ酸置換を来たす遺伝子変異）としてR198CとR380Wを見いだすことができ（図7－1），かつGLUT9がその生理学的機能として，ヒトの近位尿細管における尿酸の再吸収という役割を担っていることを示すことができた（図7－1A）[17]。このほか，低尿酸血症の1例に*GLUT9*のP412R変異を認めたという報告[36]があるが，報告された機能変化の程度が小さいこと[36]，および機能解析の結果が別のグループにより再現できていないこと[17]から，今後の検討が必要とされている[37]。

腎性低尿酸血症の病因変異として見いだされた*GLUT9*遺伝子の2つの変異（R198C，R380W）は，ともに膜貫通部位近傍の細胞内ループの中に存在している。また，塩基性アミノ酸のアルギニンから中性アミノ酸への置換が起きることにより，プラスの電荷の消失が生じている[17]。興味深いことに上記の2つの*GLUT9*遺伝子の変異は，GLUT1欠損症候群（GLUT1 deficiency syndrome, GLUT1DS）で認められるGlucose transporter 1（*GLUT1/SLC2A1*）遺伝子の病因変異（R153CとR333W）[38, 39]と完全に相同なアミノ酸残基の変異であった[17]。GLUT1欠損症候群は，脳内へのグルコース供給が低下するためにてんかん発作や知能発育障害を来たす遺伝性の神経疾患である。これらの*GLUT1*および*GLUT9*遺伝子に共通する病因変異部位のアルギニンは，

GLUT family で保存されたモチーフの中に存在し，哺乳類のみならず，細菌，酵母，植物の糖のトランスポーターに共通したコンセンサスパターンである sugar transport proteins signature の中に位置する[17]。

GLUT1 の R333 を含む配列については，膜貫通部位をつなぎとめるアンカーの1つとして重要な役割を担うことが Sato らにより示されており，このモチーフにおける3つのアルギニン残基を中性アミノ酸に置換することで，この細胞内ループが前後の膜貫通部位とともに，細胞外に飛び出ることが報告されている[40]。GLUT9 においては，相同のモチーフ中に認められるアルギニン残基は2つのみであり，かつ R380 は sugar transport proteins signature の中でも最も良く保存されているため，細胞質内アンカーとして膜トポロジーの維持に重要な役割を担っていると考えられる。したがって，R380 を含む膜貫通部位の保存されたアルギニン残基の置換を来す変異は，正電荷消失によるアンカー機能不全を来し，このことが尿酸輸送機能の消失につながる主要なメカニズムの1つであると考えられる[17,41]。

(3) 腎性低尿酸血症1型および2型

腎性低尿酸血症の新規病因遺伝子 *GLUT9* の同定により，既知病因遺伝子である *URAT1* 変異によるものが「腎性低尿酸血症1型」(RHUC1, renal hypouricemia type 1, MIM 220150)，*GLUT9* 変異によるものが「腎性低尿酸血症2型」(RHUC2, renal hypouricemia type 2, MIM 612076) と初めて分類されるようになった[13]。その後，*GLUT9* のホモ病因変異を認める海外の複数の症例が報告され[42]，筆者らの報告[17]が支持された。さらに，「腎性低尿酸血症1型」と同様に「腎性低尿酸血症2型」においても，運動後急性腎不全や尿路結石の合併があることが併せて示された[42]。これまでの解析により，*URAT1* および *GLUT9* の両遺伝子に変異を認めない腎性低尿酸血症例が存在することも確認されており，未知の病因遺伝子異常による「腎性低尿酸血症3型」(RHUC3, renal hypouricemia type 3) の存在が示唆されている[13]。また，*GLUT9* 遺伝子の SNP と痛風の関連も複数の施設の症例対照研究におい

て示されているが，その分子機構は明らかにされておらず，今後の研究の進展が期待される。

4．尿酸排泄トランスポーター

（1）ABCG2/BCRPと痛風・高尿酸血症

ABCG2は6回膜貫通型の膜タンパク質であり，ホモ2量体を形成するハーフタイプのABCトランスポーターである。ABCG2は抗がん剤などの薬剤の排出を司ることが知られていたが，内因性の基質としてはポルフィリン以外の報告はほとんどされていなかった。

血清尿酸値にかかわるGWASに先立ち，痛風を認める台湾の先住民21家系を対象としたゲノムワイド連鎖解析（genome-wide linkage analysis）から，第4染色体長腕に，痛風の病因遺伝子の候補領域が存在することが報告された[43]。common diseaseとしての痛風と遺伝子の関係について，全ゲノムを対象とした報告はこれが最初であった。なお，第4染色体長腕上の候補領域には，のちに痛風の主要な病因遺伝子として同定される*ABCG2/BCRP*が含まれていた。その後，血清尿酸値に関するGWASの結果，*ABCG2*を含む関連候補遺伝子が報告された（表7－2）[32-34]。*ABCG2*遺伝子については，高容量性のATP依存性尿酸排泄輸送体ABCG2をコードしており[9]，この遺伝子における輸送機能を低下させる変異が痛風と関連していることを，筆者ら[9]とWoodwardら[44]がそれぞれ独立に見いだし報告した。筆者らはさらに，*ABCG2*遺伝子における高い頻度のSNPであるQ126XおよびQ141Kの組み合わせが痛風の発症に重要であり，痛風の主要な病因であることを見いだした[9]。これらを詳しく調べたところ，①Q126X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（図7－3），②これらのSNPは同じハプロタイプ上にない（すなわち片親からは，Q126XとQ141Kの2種類のSNPが同時に遺伝することはない），という2つの結果から，③ヒ

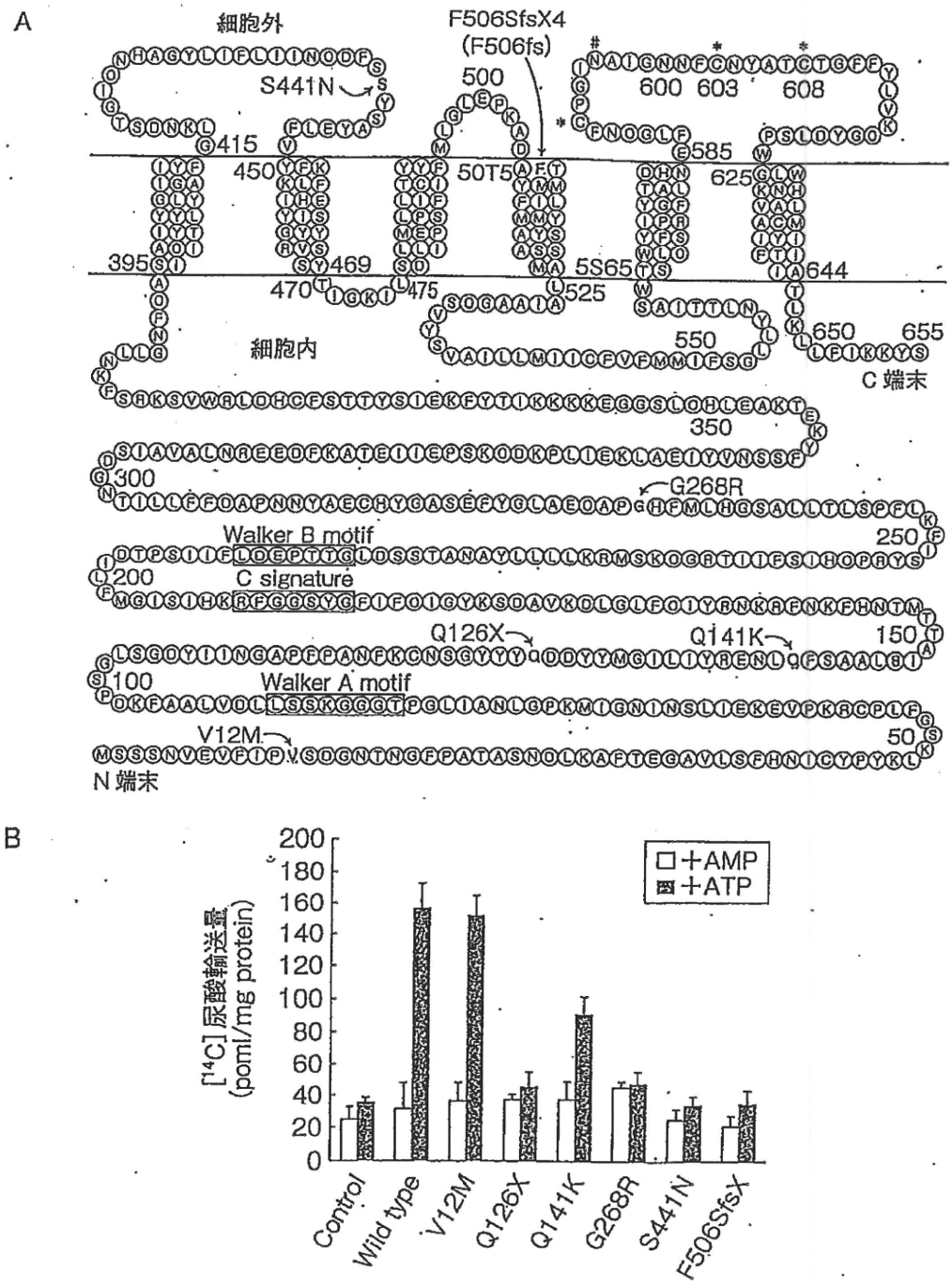

図7－3　ABCG2におけるアミノ酸置換を伴う変異

A：トポロジーモデルと変異部位。90人の高尿酸血症症例を対象としたリシークエンスにより認められた6つのアミノ酸置換を伴う遺伝子変異を示す。#：N結合型糖鎖結合部位（N596），＊：ジスルフィド結合の形成に必要なシステイン残基（C592, C603, C603）。B：変異体による尿酸輸送能の低下。野生型（wild type）または6つの変異体を発現させたHEK293細胞から調製した細胞膜小胞（ベシクル）を用いて，ATP存在下または非存在下における尿酸の輸送を検討した（平均値±標準偏差で表示）。（文献9より引用，改変）