

ORIGINAL ARTICLE

Multiple common and rare variants of *ABCG2* cause gout

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ABSTRACT

Objective Previous studies have suggested an association between gout susceptibility and common dysfunctional variants in ATP-binding cassette transporter subfamily G member 2/breast cancer resistance protein (*ABCG2/BCRP*), including rs72552713 (Q126X) and rs2231142 (Q141K). However, the association of rare *ABCG2* variants with gout is unknown. Therefore, we investigated the effects of rare *ABCG2* variants on gout susceptibility in this study.

Methods We sequenced the exons of *ABCG2* in 480 patients with gout and 480 healthy controls (Japanese males). We also performed functional analyses of non-synonymous variants of *ABCG2* and analysed the correlation between urate transport function and scores from the protein prediction algorithms (Sorting Intolerant from Tolerant (SIFT) and Polymorphism Phenotyping v2 (PolyPhen-2)). Stratified association analyses and multivariate logistic regression analysis were performed to evaluate the effects of rare and common *ABCG2* variants on gout susceptibility.

Results We identified 3 common and 19 rare non-synonymous variants of *ABCG2*. SIFT scores were significantly correlated with the urate transport function, although some *ABCG2* variants showed inconsistent scores. When the effects of common variants were removed by stratified association analysis, the rare variants of *ABCG2* were associated with a significantly increased risk of gout (OR=3.2, $p=6.4\times 10^{-3}$). Multivariate logistic regression analysis revealed that the size effect of these rare *ABCG2* variants (OR=2.7, $p=3.0\times 10^{-3}$) was similar to that of the common variants, Q126X (OR=3.4, $p=3.2\times 10^{-6}$) and Q141K (OR=2.3, $p=2.7\times 10^{-16}$).

Conclusions This study revealed that multiple common and rare variants of *ABCG2* are independently associated with gout. These results could support both the 'Common Disease, Common Variant' and 'Common Disease, Multiple Rare Variant' hypotheses for the association between *ABCG2* and gout susceptibility.

INTRODUCTION

Gout is the most common form of inflammatory arthritis and is caused by hyperuricaemia. Many previous studies have indicated that

Key messages

What is already known about this subject?

- Common dysfunctional variants (Q126X and Q141K) of *ABCG2* are risk factors for gout/hyperuricaemia, which supports the 'Common Disease, Common Variant (CDCV)' hypothesis.

What does this study add?

- Multiple common and rare variants of *ABCG2* are independently associated with gout.
- This study supports both the 'CDCV' and 'Common Disease, Multiple Rare Variant' hypotheses for the association between *ABCG2* and gout susceptibility.

How might this impact on clinical practice?

- These findings indicate that genotyping the rare variants of *ABCG2* along with its common variants (Q126X and Q141K) is essential for evaluating the individual risk for gout.

dysfunctional variants (rs72552713 (Q126X) and rs2231142 (Q141K)) of the gene encoding ATP-binding cassette transporter subfamily G member 2/breast cancer resistance protein (*ABCG2/BCRP*) increase the risk of gout¹⁻³ and hyperuricaemia.¹⁻⁴ Approximately 80% of Japanese patients with gout have been reported to possess either the Q126X or Q141K variant of *ABCG2*,¹ and these variants increased the risk of gout conferring an OR of more than 3.^{1,3} Thus, the effects of common *ABCG2* variants on gout susceptibility are very strong, whereas any effect of rare *ABCG2* variants is still unknown.

In this study, we first identified common and rare non-synonymous variants of *ABCG2* by target exon sequencing of genomes from Japanese male patients with gout and healthy controls. Second, we evaluated the non-synonymous variants using three different protein



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prediction algorithms. Third, we performed molecular analyses of the urate transport function and evaluated the correlation between the functional analyses and the scores from the protein prediction algorithms. Finally, we performed association analyses between the *ABCG2* variants and gout susceptibility.

METHODS

Patients and controls

This study was approved by the institutions' Ethical Committees (National Defense Medical College, National Institute of Genetics and Nagoya University). All protocols were in accordance with the Declaration of Helsinki, and written informed consent was obtained from all participants. For the study, 480 Japanese males with gout were recruited from the Ryougoku East Gate Clinic (Tokyo, Japan). All patients were clinically diagnosed with primary gout according to the criteria established by the American College of Rheumatology.⁵ Patients with inherited metabolic disorders, including Lesch-Nyhan syndrome, were excluded from this study. As the control group, 480 healthy Japanese males without hyperuricaemia (serum uric acid (SUA) levels >7.0 mg/dL) or history of gout were recruited from the participants of the Shizuoka Study, which is a part of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study).⁶ The mean age (\pm SD) of the case and control groups was 46.2 years (\pm 9.8) and 52.3 years (\pm 7.9), respectively, and their mean body mass index (\pm SD) was 25.3 kg/m² (\pm 3.7) and 23.2 kg/m² (\pm 2.6), respectively.

Targeted sequencing

Genomic DNA was extracted from the whole peripheral blood cells of the participants.⁷ We performed targeted exon sequencing of *ABCG2* with a pool and capture method described in a previous study.⁸ Briefly, the extracted DNA was quantified using the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA) on FilterMax F5 Multi-Mode Microplate Readers (Molecular Devices, Sunnyvale, California, USA). Twenty nanogram of DNA was simultaneously fragmented and ligated with adapters using the SureSelect QXT Library Prep Kit (Agilent Technologies, Santa Clara, California, USA). The 96 fragmented libraries with distinct indexed adapters were pooled in equimolar amounts. Target enrichment was then performed using the SeqCap EZ choice system (Roche Diagnostics, Tokyo, Japan). A DNA probe set complementary to the target region was designed using NimbleDesign (<https://design.nimblegen.com>). The libraries were sequenced on an Illumina HiSeq 2500 platform in rapid run mode with 2 \times 150 bp paired-end modules (Illumina).

Variant calling and annotation

The generated sequences were aligned to a human reference genome (hg19) using BWA⁹ and converted to the BAM format for subsequent analysis using SAMtools.¹⁰ The aligned reads were processed using Picard tools (

broadinstitute.github.io/picard) for removing the PCR duplicates. Local realignment and base quality recalibration were implemented using GATK.^{11 12} Genetic variations, including single nucleotide variants (SNVs) and short insertions and deletions (indels), were determined using HaplotypeCaller walker of GATK.^{11 12} Functional annotation of the identified variants was implemented using ANNOVAR.¹³

Functional analysis

To estimate the functions of the *ABCG2* variants, we used the protein prediction algorithms, Sorting Intolerant from Tolerant (SIFT)¹⁴ and Polymorphism Phenotyping v2 (PolyPhen-2).¹⁵ For PolyPhen-2, both PolyPhen-2 HumVar and PolyPhen-2 HumDiv¹⁵ were used. PolyPhen-2 HumVar is designed for distinguishing mutations with drastic effects from abundant mildly deleterious alleles, whereas PolyPhen-2 HumDiv is targeting rare alleles at loci potentially involved in complex phenotypes.¹⁶ To evaluate these protein algorithms, we performed functional analysis of *ABCG2*-mediated urate transport for previously identified variants (N208S, N299S, E311K, L447V, S486N and V516M) in our laboratories as well as reported variants whose vesicles were available (V12M, Q141K, V178I, G268R, P269S, S441N, G462R, V508I and A634V). We analysed the correlation between the urate transport function and the scores from these protein prediction algorithms. In addition, we performed functional analysis of one frameshift deletion (F506SfsX4) and four nonsense variants (Q126X, E334X, R575X and C608X).

Functional assays were performed to determine the urate transport activity of each *ABCG2* variant, as described in previous studies.^{1 17} Briefly, using site-directed mutagenesis, vectors expressing the different *ABCG2* variants were generated from a myc-*ABCG2* wild-type (WT)/pcDNA3.1(+) plasmid that was prepared in our previous study.¹ Plasma membrane vesicles were isolated from human embryonic kidney-derived cells that were transiently transfected with the vectors expressing the *ABCG2* variants using a standard method¹⁸ or with empty vector as a control using general lipofection methods.¹⁹ The isolated membrane vesicles were stored at -80°C until use. Expression of *ABCG2* protein in the membrane vesicles was examined by immunoblotting with an anti-myc antibody (see online supplementary figure S1) according to our previous studies.^{1 17} Then, using a rapid filtration technique,¹⁷ the [¹⁴C]-urate transport assay was performed for the *ABCG2*-expressing or control membrane vesicles. Based on the radioactivity detected in the membrane vesicles, the urate transport activity was calculated based on the formula; incorporated clearance ($\mu\text{L}/\text{mg protein}/\text{min}$) = incorporated urate level (DPM/mg protein/min) / urate level in the incubation mixture (DPM/ μL). By subtracting the urate transport activity in the absence of ATP from that in the presence of ATP, ATP-dependent urate transport activity was also determined.

Statistical analysis

Statistical analyses were performed using SPSS v.22.0J (IBM Japan, Tokyo, Japan).

We selected missense and nonsense SNVs and indels in the exons of *ABCG2* based on the DNA reference sequence NM_004827 for association analyses and excluded synonymous SNVs in *ABCG2* exons as well as variants in introns or untranslated regions. Rare variants and common variants were defined when the minor allele frequencies (MAF) were <1% and ≥1%, respectively. MAF in the Japanese population was determined based on the Japanese in Tokyo (JPT) population from the 1000 Genomes Project data (<http://www.internationalgenome.org/>).²⁰ We analysed multiple rare non-synonymous variants using the Collapsing method.²¹ If a rare allele was present at any of the variant sites, an individual was regarded as a rare variant carrier and as a non-carrier otherwise.²¹ Since the effects of common dysfunctional variants of *ABCG2*, Q126X and Q141K, on gout susceptibility were very strong,¹ we performed stratified association analyses between rare non-synonymous variants of *ABCG2* and gout susceptibility by its common variants. The χ^2 test was used for association analyses. Furthermore, multivariate logistic regression analysis was performed between the rare non-synonymous variants and the common variants (Q126X and Q141K) of *ABCG2*. The dominant model of the Collapsing method²¹ was applied for rare variants of *ABCG2*, whereas the additive codominant model was applied for the common variants.

RESULTS

Details of all the non-synonymous variants of *ABCG2* found in the cases and the control samples by targeted exon sequencing are shown in [table 1](#). We identified 3 common and 19 rare non-synonymous variants of *ABCG2*. Genotype counts of the common non-synonymous variants (V12M, Q126X and Q141K) are shown in online supplementary table S1. For the identified rare variants, we observed 33 carriers among patients with gout and 18 carriers among the controls ([table 1](#)); no participants were found to be homozygous for rare variants.

Results of the functional analysis of 19 non-synonymous variants of *ABCG2* are shown in [figure 1A](#) and [table 2](#). The ATP-dependent urate transport activity was almost completely eliminated in several missense variants (S441N, G462R, F208S, G268R, S486N and V516M), nonsense variants (Q126X, R575X and E334X) and a frameshift deletion (F506SfsX4) of *ABCG2* ([figure 1A](#)). In contrast, V12M and P269S variants did not show altered urate transport activity. The other rare variants addressed in this study exhibited lower transport activity as compared with WT. Based on the relationship between the protein level and urate transport activity of each *ABCG2* variant (see online supplementary figures S1 and S2), the diminished function of each *ABCG2* variant could primarily depend on the quantitative changes in the *ABCG2* protein on the plasma membrane. On the other

hand, several variants (L447V, N299S, S486N, V516M and S441N) would affect the intrinsic ability of *ABCG2* as a urate transporter. Of note, the quantitative changes must have been due to the presence of each variant because the membrane vesicles were prepared by using certain procedure established in previous reports.^{1,18} In addition, previous reports showed that three variants (Q126X, S441N and F506SfsX4) disrupt the transport activity of *ABCG2*, whereas other three variants (V12M, A149P and P269S) have little effect on the transport activity.^{1,18}

The scores from SIFT were significantly correlated with the urate transport function ([figure 1B](#); $r=0.57$, $p=0.026$). On the other hand, the scores of PolyPhen-2 were not significantly correlated; these scores tended to decrease as the urate transport function increased ([figure 1C,D](#); $r=-0.42$, $p=0.12$ in PolyPhen-2 HumVar; $r=-0.46$, $p=0.089$ in PolyPhen-2 HumDiv). These correlations suggested that the protein prediction algorithms were useful for predicting changes in the transport function of *ABCG2*; however, some variants of *ABCG2* showed inconsistent scores ([table 2](#); P269S and S441N). Therefore, we determined the effects of *ABCG2* variants using the following protocol: (1) when functional data were available, we used the results of urate transport analysis and (2) when functional data were not available, we used the scores from SIFT and PolyPhen-2. V12M and P269S variants were excluded from these analyses because they did not significantly decrease urate transport activity. The V12M variant did not show a significant association with gout susceptibility in the multivariate logistic regression analysis with the other common variants, Q126X and Q141K ([table 3](#)). In addition, the A149P variant was also excluded because it was not likely to cause functional changes, according to the scores of SIFT (1), PolyPhen-2 HumVar (0) and PolyPhen-2 HumDiv (0) (see online supplementary table S2). It was shown in a previous study through functional analysis that the A149P variant did not significantly affect the drug transport activity of *ABCG2*.¹⁸

To evaluate the effects of rare variants by removing the effects of the common variants, stratified association analyses were performed, as shown in [table 4](#). When the gout susceptibility was analysed for samples without Q126X or Q141K, rare non-synonymous variants of *ABCG2* were found to increase the gout risk conferring OR to 3.2 ($p=6.4\times 10^{-3}$, [table 4](#)). In the multivariate logistic regression analysis as well, rare non-synonymous variants of *ABCG2* were found to be associated with gout susceptibility independent of the Q126X and Q141K variants ([table 5](#)). In addition, the effect size of rare variants of *ABCG2* (OR=2.7, 95% CI 1.4 to 5.2, $p=3.0\times 10^{-3}$) was similar to that of common variants Q126X (OR=3.4, 95% CI 2.0 to 5.6, $p=3.1\times 10^{-6}$) and Q141K (OR=2.3, 95% CI 1.9 to 2.9, $p=2.7\times 10^{-16}$) ([table 5](#)). Thus, our findings indicated that multiple common and rare variants of *ABCG2* are strongly and independently associated with gout susceptibility.

Table 1 Non-synonymous variants of *ABCG2* found in this study

Type of variant	rs number	Position*	Change in DNA sequence†	AA change	Case (n)‡	MAF in case (%)	Control (n)‡	MAF in control (%)
Common variant	rs2231137	89061114	G34A	V12M	118	12.3	180	18.8
	rs72552713	89052957	C376T	Q126X	55	5.73	23	2.40
	rs2231142	89052323	C421A	Q141K	325	33.9	218	22.7
Rare variant		89052998	C335T	P112L	6	0.625	4	0.417
	rs149106245	89052361	A383T	D128V	2	0.208	1	0.104
	rs201006821	89052299	G445C	A149P	0	0	1	0.104
	rs199753603	89052289	T455C	M152T	1	0.104	0	0
		89052255	G489C	R163S	1	0.104	0	0
	rs746311704	89042944	G532A	V178I	1	0.104	1	0.104
	rs200190472	89039366	C736T	R246X	1	0.104	2	0.208
	rs34678167	89039297	C805T	P269S	3	0.313	2	0.208
		89039275	A827G	Y276C	0	0	1	0.104
	rs750972998	89034567	1079_1081delAGA	K360del	2	0.208	1	0.104
		89022427	G1322A	S441N	3	0.313	0	0
	rs752408502	89020584	G1384A	G462R	1	0.104	0	0
	rs192169063	89020503	T1465C	F489L	8	0.833	3	0.312
	rs868217328	89018737	1515delC	F506SfsX4	3	0.313	0	0
		89018730	G1522A	V508I	2	0.208	0	0
	rs548254708	89016686	C1723T	R575X	0	0	2	0.208
rs200933122	89013532	T1822C	C608R	2	0.208	0	0	
rs748531218	89013495	A1859G	D620G	1	0.104	0	0	
	89013453	C1901T	A634V	1	0.104	0	0	
Total of rare variant carriers§				33		18		
Total of participants					480		480	

For all rare variants, there were only heterozygous and no homozygous participants.

*Positions refer to the GRCh37 assembly.

†Nucleotide numbering is based on the DNA reference sequence NM_004827.

‡Summary count of participants with homozygous or heterozygous variants.

§Count of participants with one or more rare variants.

AA, amino acid; *ABCG2*, ATP-binding cassette transporter subfamily G member 2; MAF, minor allele frequency.

DISCUSSION

Common dysfunctional variants (Q126X and Q141K) of *ABCG2*, a urate transporter gene, have been shown to be strongly associated with gout susceptibility^{1,2} and age of onset of gout.³ Q126X, a common variant in the Japanese population, is a rare variant in Caucasian and African-American populations, whereas Q141K is a common variant in all these populations.²² The MAF of Q126X and Q141K are 0.024 and 0.322, respectively in the Japanese population (JPT from the 1000 Genomes Project).²⁰

ABCG2 excretes urate from the intestine and kidney, and its dysfunction causes 'extrarenal urate underexcretion type'²³ and/or 'renal urate underexcretion type'²⁴ gout. *ABCG2*-mediated intestinal urate excretion was also discovered in humans recently after investigation of haemodialysis and acute gastroenteritis patients.²⁵

It was also shown that increased SUA could be a useful marker of intestinal impairment, because increased SUA of gastroenteritis patients was explained both by dehydration and by impaired intestinal epithelium which excretes urate via *ABCG2*.²⁵ In addition, *ABCG2* variants have been shown to have stronger effects on the risk of hyperuricaemia than major environmental risk factors such as obesity and heavy drinking.⁴

In recent genome-wide association studies (GWASs) of clinically defined gout,^{26–28} the *ABCG2* locus showed the most significant association with gout susceptibility. The *ABCG2* locus was also the most significantly detected locus in GWASs of gout in Caucasian populations.^{29,30} These findings indicate that common variants of *ABCG2* are extremely important in gout pathogenesis.

In addition to Q126X and Q141K, V12M is another common non-synonymous variant of *ABCG2*.¹ A

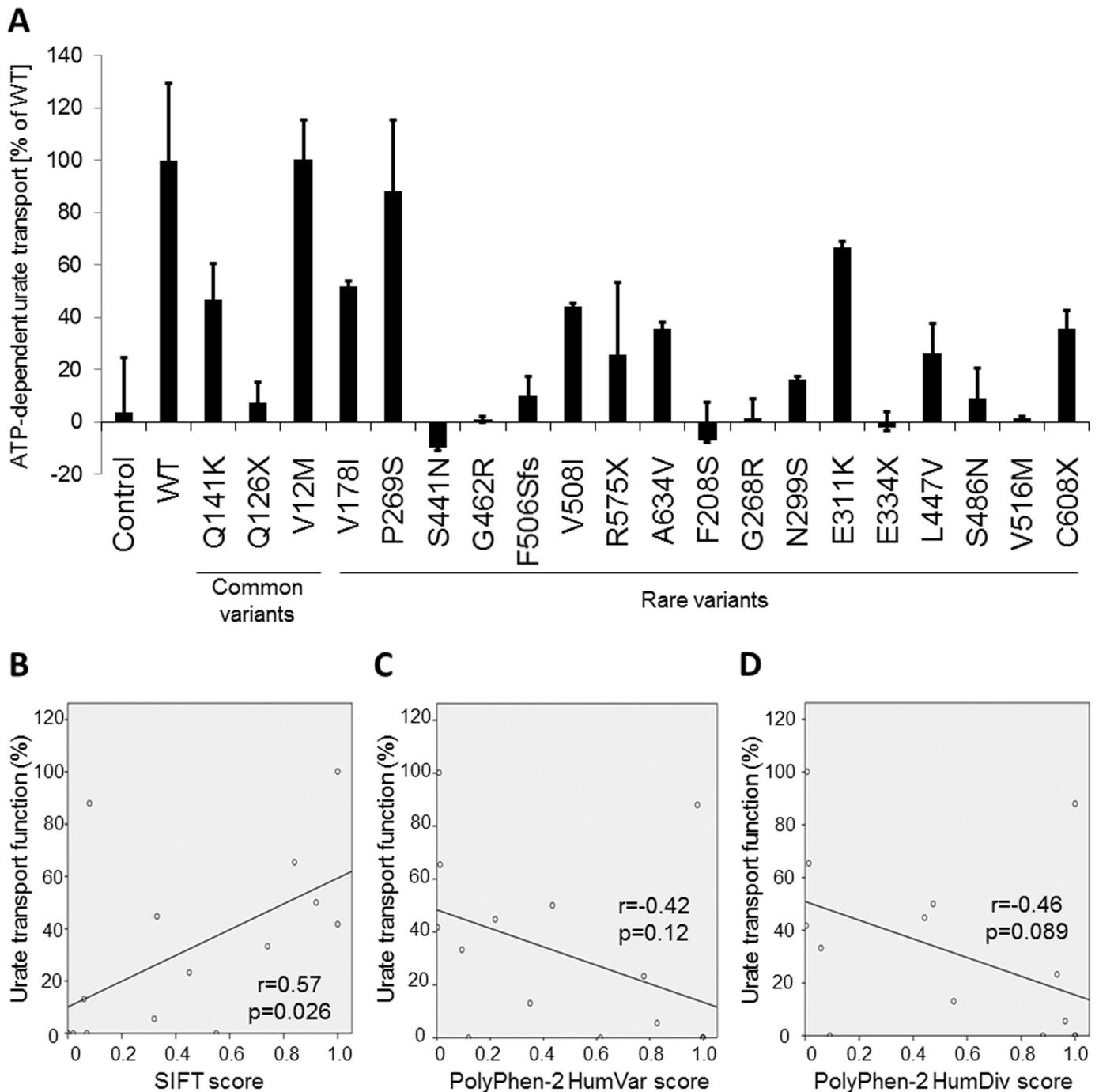


Figure 1 Functional analyses of ATP-binding cassette transporter subfamily G member 2 (ABCG2) variants. (A) Data from ATP-dependent urate transport analyses of ABCG2 variants are presented as the mean function (%) relative to the activity of the wild type (WT) ABCG2 transporter. Transport function was almost completely abolished in several missense variants (S441N, G462R, F208S, G268R, S486N and V516M) and two nonsense variants (Q126X and E334X) of ABCG2. Transport function was also diminished in F506SfsX4 (F506Sfs), a frameshift deletion of ABCG2. In contrast, no remarkable changes in urate transport activity were observed in the V12M and P269S variants. (B) Sorting Intolerant from Tolerant (SIFT) scores were significantly correlated ($r=0.57$, $p=0.026$) with the results of the urate transport analyses. (C and D) The PolyPhen-2 HumVar and HumDiv scores showed non-significant correlation, although these scores showed a tendency toward decrease urate transport function ($r=-0.42$, $p=0.12$ and $r=-0.46$, $p=0.089$, respectively), based on the results of the functional analyses.

haplotype frequency analysis in a previous study showed that the minor alleles of V12M, Q126X and Q141K were not simultaneously present in one haplotype.^{1 23 24} In other words, these three variants are in complete linkage disequilibrium. Therefore, the minor allele of V12M

seemingly has a protective effect on gout susceptibility, although it does not have an actual effect. In this study, we performed both univariate and multivariate logistic regression analyses of gout susceptibility in common variants of ABCG2 (V12M, Q126X and Q141K). Indeed,

Table 2 ATP-dependent urate transport activity and scores of protein prediction algorithms for each *ABCG2* variant

rs number	AA change	Transport function (%)	SIFT	PolyPhen-2 HumVar	PolyPhen-2 HumDiv
rs2231137	V12M*	100.2	1	0.008	0.007
rs72552713	Q126X*	3.6	N/A	N/A	N/A
rs2231142	Q141K*	44.8	0.33	0.219	0.442
rs746311704	V178I*	50.0	0.92	0.434	0.474
rs34678167	P269S*	88.0	0.08	0.978	1
	S441N*	0	0.55	0.119	0.091
rs752408502	G462R*	0	0.07	0.997	1
rs868217328	F506SfsX4*	6.6	N/A	N/A	N/A
	V508I*	41.8	1	0.002	0.003
rs548254708	R575X*	22.8	N/A	N/A	N/A
	A634V*	33.3	0.74	0.094	0.058
rs1061018	F208S	0	0	1	1
	G268R	0	0	1	1
	N299S	13.1	0.06	0.350	0.550
	E311K	65.4	0.84	0.012	0.013
rs3201997	E334X	0	N/A	N/A	N/A
	L447V	23.3	0.45	0.777	0.933
rs780310265	S486N	5.5	0.32	0.827	0.963
	V516M	0	0.02	0.614	0.881
	C608X	33.2	N/A	N/A	N/A

*These variants were detected through exonic sequencing analysis in this study. For rare variants, there were no homozygous participants. AA, amino acid; *ABCG2*, ATP-binding cassette transporter subfamily G member 2; N/A, not applicable; PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant from Tolerant.

V12M showed a significant association with gout susceptibility only in the univariate logistic regression analysis (see online supplementary table S3), although it was no more statistically significant after adjustment for Q126X and Q141K genotypes in the multivariate logistic regression analysis ($p=0.27$, table 3). This finding was also consistent with the results of the molecular functional analysis,¹ in which the V12M variant did not show decreased urate transport (figure 1A). These findings showed that V12M had no significant protective effects against gout susceptibility, as shown in table 3.

Molecular functional analysis of transporters is useful for *in vitro* quantitative assessment of functional changes caused by non-synonymous variants and for evaluating the scores from the protein prediction algorithms.

Table 3 Multivariate logistic regression analysis for gout susceptibility with three common variants of *ABCG2*

Variables	β	OR (95% CI)	p Value
V12M	-0.15	0.86 (0.66 to 1.1)	0.27
Q126X	1.1	3.0 (1.8 to 5.1)	2.3×10^{-5}
Q141K	0.78	2.2 (1.8 to 2.7)	5.7×10^{-13}

Each variant was adjusted by the other two variants in this analysis.
ABCG2, ATP-binding cassette transporter subfamily G member 2.

While functional analysis of the ABC transporters by a vesicle system is powerful, it takes considerable effort to analyse all the rare variants identified by targeted exon sequencing. Therefore, in this study, we performed functional analyses for half of the non-synonymous variants whose vesicles were available detected through the targeted exon sequencing ($n=11$, table 2) and previously identified rare variants ($n=9$, table 2). We then compared the results of the functional analyses with the scores from SIFT and PolyPhen-2. Based on these results, we predicted the functional changes of other missense variants ($n=9$) (see online supplementary table S2) with the protein prediction algorithms. We considered that it was reasonable to use molecular functional analyses and protein prediction algorithms in combination. We propose this as a suitable model for analysing rare variants of transporter genes to evaluate individual genetic risks for common diseases including gout.

Previous studies have indicated that common variants of *ABCG2* are risk factors for gout/hyperuricaemia.¹⁻⁴ These studies supported the 'Common Disease, Common Variant (CDCV)' hypothesis, which states that common genetic variants are the major contributors to genetic susceptibility to common diseases.^{31 32} In contrast, recent genetic studies have suggested that multiple rare variants also play important roles in several

Table 4 Stratified association between rare non-synonymous variants of *ABCG2* and gout susceptibility by common variants of *ABCG2*, Q126X and Q141K

Sample set	Case			Control			p Value	OR (95% CI)
	Number	Carrier*	Frequency (%)†	Number	Carrier*	Frequency (%)†		
All	480	30	6.3	480	15	3.1	0.022	2.1 (1.1 to 3.9)
Without Q126X	425	30	7.1	457	15	3.3	0.011	2.2 (1.2 to 4.2)
Without Q126X or Q141K	131	14	10.7	247	9	3.6	6.4×10 ⁻³	3.2 (1.3 to 7.5)

Only non-synonymous SNVs or indels with minor allele frequency less than 1% were considered rare non-synonymous variants in this analysis.

Since the P268S variant of *ABCG2* did not decrease urate transport activity, it was excluded from this analysis.

The A149P variant of *ABCG2* was also excluded from this analysis due to its scores of SIFT and PolyPhen-2 as well as a previous report of functional analysis.¹⁸

*The number of carriers with rare non-synonymous variants of *ABCG2*.

†The percentage of cases or controls carrying rare non-synonymous variants of *ABCG2*.

ABCG2, ATP-binding cassette transporter subfamily G member 2; indels, short insertions and deletions; PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant from Tolerant; SNVs, single nucleotide variants.

complex genetic diseases such as early-onset myocardial infarction³³ and Alzheimer's disease,³⁴ which support the 'Common Disease, Multiple Rare Variant (CDMRV)' or 'Common Disease, Rare Variant (CDRV)' hypothesis.³² In this study, gene-based stratified association analyses revealed an association between rare non-synonymous variants of *ABCG2* and gout susceptibility. In addition, we used logistic regression analysis to evaluate the effect of both common and rare variants of *ABCG2* on gout susceptibility. Results of this study support the CDMRV hypothesis for *ABCG2* and gout, whereas many previous studies^{1-3,26,27} as well as this study have also supported the CDCV hypothesis. We found that multiple rare variants as well as common variants of *ABCG2* are independently associated with gout risk. These results further indicate that *ABCG2* is a key molecule in the pathogenesis of gout. Evaluating the risk associated with common and rare variants of individual genes could help in developing precision medicine or personalised genome medicine for common diseases such as gout. Our results showed

that genotyping the rare variants of *ABCG2* along with its common variants (Q126X and Q141K) is essential for evaluating the individual risk for gout. Although the rare variants of *ABCG2* showed highly significant association with gout susceptibility in this study, the number of the participants is small compared with other association analyses of rare genetic variants.^{33,34} Further studies are required in order to find other important rare variants of *ABCG2* and perform more detailed analyses.

In summary, our findings revealed that multiple common and rare variants of *ABCG2* could cause gout. Thus, this study could support both 'CDCV' and 'CDMRV' hypotheses, and we proposed the novel 'Common Disease, Multiple Common and Rare Variant' model for the association between *ABCG2* and gout.

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Table 5 Multivariate logistic regression analysis of gout susceptibility with rare and common variants of *ABCG2*

Variables	β	OR (95% CI)	p Value
Rare variant	0.99	2.7 (1.4 to 5.2)	3.0×10 ⁻³
Q126X	1.21	3.4 (2.0 to 5.6)	3.1×10 ⁻⁶
Q141K	0.85	2.3 (1.9 to 2.9)	2.7×10 ⁻¹⁶

Each variant was adjusted by the other two variants in this analysis.

Since the P268S variant of *ABCG2* did not decrease urate transport activity, it was excluded from this analysis.

The A149P variant of *ABCG2* was also excluded from this analysis due to its scores of SIFT and PolyPhen-2 as well as a previous report of functional analysis.¹⁸

ABCG2, ATP-binding cassette transporter subfamily G member 2; PolyPhen-2, Polymorphism Phenotyping v2; SIFT, Sorting Intolerant from Tolerant.

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Contributor THigashino, TT, HNakaoka and YToyoda contributed equally.

Contributors Thi, TT, HNakao, KH, NS and HMa conceived and designed this study. BS and KY assisted with research design. KW, RO and HO collected samples and analysed clinical data. Thi, HNakao, SS, MS, MK, AN, AA, YTa, YK, KY, KH, II, NS and HMa performed genetic analysis. TT, YTo, HMI and YI performed functional analysis. Thi, HNakao, HNakas, TN and HMa performed statistical analyses. BS, KY, KH, THo, KI, HS, TRM and NS provided intellectual input and assisted with the preparation of the manuscript. Thi, TT, HNakao, YTo and HMa wrote the manuscript. All authors have read and approved the final version of the manuscript.

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Competing interests TT, TN, KI, HS, NS and HMa have a patent pending based on the work reported in this paper.

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