estrone-3-sulfate (ES), a typical substrate of ABCG2, is inhibited by urate as well as AZT and ES. ATP-dependent transport of urate was then detected in ABCG2-expressing vesicles but not in control vesicles. Kinetic analysis revealed that ABCG2 is a high-capacity urate transporter that maintained its function even under high-urate concentration. The calculated parameters of ABCG2-mediated transport of urate were a Km of 8.24 ± 1.44 mM and a Vmax of 6.96 ± 0.89 nmol/min per mg of protein. Moreover, the quantitative trait locus (QTL) analysis performed in 739 Japanese individuals revealed that a dysfunctional variant of ABCG2 increased SUA as the number of minor alleles of the variant increased ($p = 6.60 \times 10^{-5}$). Because ABCG2 is expressed on the apical membrane in several tissues, including kidney, intestine, and liver, these findings indicate that ABCG2, a high-capacity urate exporter, has a physiological role of urate homeostasis in the human body through both renal and extrarenal urate excretion.

Keywords High-capacity urate exporter; ABC transporter; ABCG2/BCRP; hyperuricemia/gout; urate handling

INTRODUCTION

The ATP-binding cassette (ABC), subfamily G, member 2, *ABCG2/BCRP* is reported to locate in a gout-susceptibility locus on chromosome 4q,[1] and is recently identified to relate to serum uric acid (SUA) by genome-wide association studies.[2] ABCG2 is also known to play a role in xenobiotics excretion as well as in offering resistance to anticancer drugs.[3] Because ABCG2 transports nucleotide analogs that are structurally similar to urate, and is an exporter with polymorphic reduced functionality variants,[4] we then hypothesized that ABCG2 might be a urate exporter that can affect SUA levels.

MATERIALS AND METHODS

Genetic Analysis

All procedures were performed in accordance with the standards of the institutional ethical committees involved in this project. After written informed consent had been given by each participant, genomic DNA was extracted from blood samples. Mutation analysis of *ABCG2* gene was performed for 90 Japanese hyperuricemia patients. For quantitative trait locus (QTL) analysis of SUA concentrations, genotyping of Q141K in 739 Japanese individuals was performed. Calculations of statistical analysis were performed with the software R.

Functional Analysis

Wild-type ABCG2 complementary DNA was inserted into the *Nhe* I and *Apa* I sites of pcDNA3.1(+) vector plasmid (Invitrogen, Carlsbad, CA, USA), with a myc-tag sequence attached at the 5′ end. To prepare membrane vesicles, HEK293 cells were transiently transfected with an expression vector for ABCG2 or an empty vector by FuGENE6 (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instructions. Forty-eight hours

later, cells were harvested and the membrane vesicles were isolated with a standard method described previously.[4] The study of [^{3}H]ES (500 nM) and [^{14}C]urate (28 μM) uptake was performed as reported previously.[5] Transport experiments with high concentrations of urate were performed under alkaline conditions, and nonlinear regression analysis of the ABCG2-mediated transport of urate was used to calculate kinetic parameters. With the site-directed mutagenesis technique, we constructed mutants of ABCG2 (V12M, Q126X, and Q141K), which were used for urate transport analysis, on the expression vector for ABCG2.

Materials

[^{3}H]ES was purchased from (PerkinElmer Life Science, Boston, MA, USA), [^{14}C]Uric acid from (American Radiolabeled Chemicals, St Louis, MO, USA), and unlabeled ES, AZT, and ATP from (Sigma Chemicals, St. Louis, MO, USA). Unlabeled uric acid was purchased from (Nacalai Tesque, Kyoto, Japan). All other chemicals used were commercially available and of reagent grade.

RESULTS

ABCG2 as a High-Capacity Urate Transporter

With membrane vesicles, we examined the inhibitory effect of urate on ABCG2-mediated transport of its typical substrate estrone-3-sulfate (ES) labeled with ^{3}H. Although urate required a higher concentration than did unlabeled ES to inhibit [^{3}H]ES transport via ABCG2, the potency of urate was similar to that of the previously reported substrate, 3′-azido-3′-deoxythymidine (AZT).[6] Transport assays showed ATP-dependent transport of urate in ABCG2-expressing vesicles but not in control vesicles. In

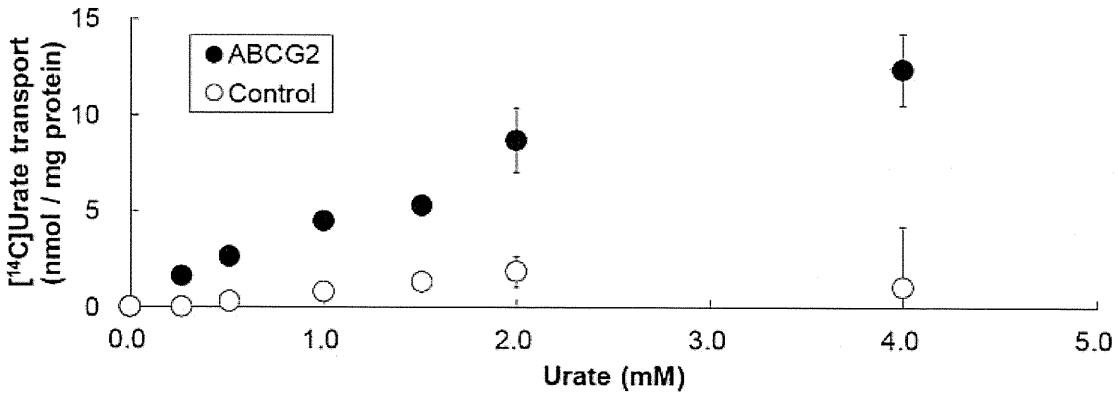

FIGURE 1 High-capacity urate transport via ABCG2. ATP-dependent [^{14}C]urate transport via ABCG2 was detected at the indicated urate concentrations. Results are expressed as means ± S.D.

kinetic analysis, ABCG2 mediated the high-capacity transport of urate, maintaining its function even under high urate conditions (Figure 1). The calculated parameters of ABCG2-mediated urate transport were a Km of 8.24 ± 1.44 mM and a V_{max} (maximum velocity) of 6.96 ± 0.89 nmol/min per mg of protein (Figure 1). The calculated Km value exceeded the highest concentration in the experimental condition, which is due to the low-solubility limitation of urate, a property related to the monosodium urate crystal formation in gout patients. Our data indicate that ABCG2 could play a physiological role as a high-capacity urate exporter.

Common Variants of *ABCG2* Found in Hyperuricemia Patients

For assessment of the physiological importance of *ABCG2* variants in patients, we then performed mutation analysis of all coding regions and intron–exon boundaries of the *ABCG2* gene in 90 Japanese hyperuricemia patients. We found the following six nonsynonymous mutations: V12M, Q126X, Q141K, G268R, S441N, and F506SfsX4, and the first three mutations are SNPs. Maekawa et al.[7] reported that these SNPs are quite common in the Japanese population, and allele frequencies for them are 31.9% for Q141K, 19.2% for V12M, and 2.8% for Q126X, respectively. Using Hardy–Weinberg equilibrium and these data on a Japanese population reported by Maekawa et al.,[7] the frequencies of Japanese individuals with these minor alleles were estimated to be 53.6% for Q141K, 34.7% for V12M, and 5.5% for Q126X, respectively.[8]

To clarify how *ABCG2* SNPs affect function, the urate transport capacity of these variants was examined in comparison with that of wild-type ABCG2. ATP-dependent transport of urate was reduced by approximately half (46.7%) in Q141K and was nearly eliminated in Q126X (Figure 2A).

Common Variant of *ABCG2* Increases SUA Levels in Humans

With the high-frequency dysfunctional variant Q141K, QTL analysis of SUA was performed in a random sample of 739 Japanese individuals. The analysis revealed a significant increase in SUA as the number of minor alleles of Q141K increased ($p = 6.60 \times 10^{-5}$), and the corrected p value is 2.02×10^{-6} when adjusted for sex (Figure 2B). A significant increase in SUA was also observed in both male ($p = .0144$) and female subjects ($p = .0137$). Unlike SUA, Q141K had no significant association with other clinical characteristics such as age, body mass index, or sex. These findings indicate that ABCG2 plays an important role in the regulation of SUA in vivo and that there could be great interindividual differences in this function because of its common polymorphic nature.

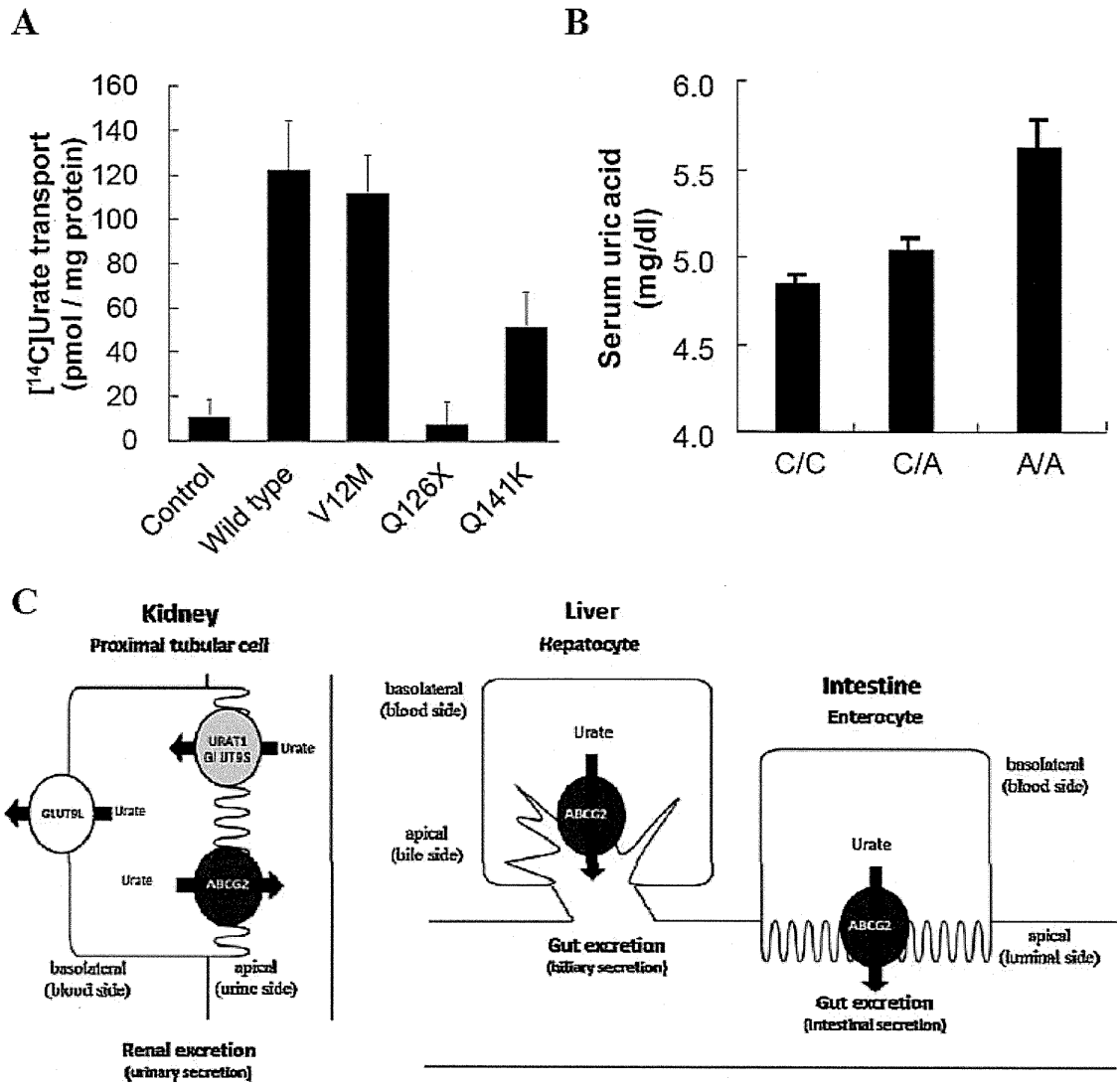

FIGURE 2 ABCG2 has a physiological role to lower serum uric acid levels in humans. (A) Urate transport analysis of mutated ABCG2. Results are expressed as means ± S.D. (B) QTL analysis of *ABCG2* Q141K and serum uric acid levels. "C/C," "C/A," and "A/A" indicate wild-type subjects, heterozygous mutation carriers, and homozygous mutation carriers of Q141K, respectively. Results are expressed as means ± S.E. (C) Physiological model of urate excretion in humans.

DISCUSSION

We identified that *ABCG2* gene encodes a high-capacity urate secretion transporter with dysfunctional common variants. We also demonstrated that Q141K, a dysfunctional variant of *ABCG2*, significantly affects human SUA levels. Our findings will serve to elucidate the physiological role of ABCG2.

ABCG2 is also known as breast cancer resistance protein (BCRP) and belongs to the ABC transporter superfamily. Together with P-glycoprotein (ABCB1) and multidrug resistance-associated proteins (ABCCs), ABCG2 is a well-known multispecific transporter that is expressed on the plasma membrane and mediates cellular extrusion of various compounds in an ATP-dependent manner. Clinical studies on the pharmacokinetics, efficacy, and

toxicity of the substrate xenobiotics of ABCG2, such as rosuvastatin,[9] imatinib,[10] and gefitinib,[11] have been reported because of its polymorphic nature in humans. Besides its ability to export porphyrins,[12] regulation of SUA through urate secretion may be an essential physiological role of ABCG2 in humans. Generally, because humans lack the uric acid degrading enzyme uricase, SUA concentrations in humans are higher than those in most other mammals. Because the uric acid metabolism in humans is considerably different from that in mice, the study of patients with loss-of-function mutations in *ABCG2* can provide advantages over *Abcg2* gene-deficient mouse models and help to analyze the physiological roles of ABCG2-mediated urate transport in humans. Two-thirds of the uric acid in the human body is normally excreted through the kidney, whereas one-third gains entrance to the gut where this acid is decomposed. In the human kidney, urate is bidirectionally reabsorbed and secreted via urate transporters. We previously identified transporters for renal urate reabsorption, URAT1 and GLUT9.[13, 14] Expression of urate exporter ABCG2 has been reported on the apical side of the proximal tubular cells in human kidneys,[15] and on the apical sides of enterocytes and hepatocytes in the human intestine and liver.[16] These findings could suggest important roles of ABCG2 in not only renal urate excretion but also gut urate excretion via intestinal and biliary secretion in humans (Figure 2C). Cellular localization of ABCG2, including its polarized localization in a porcine kidney-derived LLC-PK1 cells, reportedly may be regulated by signaling pathways such as Akt phosphorylation.[17–19] Therefore, regulation of the cellular ABCG2 localization would enable change in urate excretion in humans and lead to the discovery of a novel therapeutic method for gout and hyperuricemia.

Another study reported the urate efflux via ABCG2 expressed on oocytes,[20] consistent with our findings using the ABCG2-expressing vesicle system.[8] Functional studies using a vesicle system also enabled us to show the ATP dependence and urate concentration dependence of ABCG2-mediated urate transport. We also showed the relation between the protein expression levels and the ABCG2-mediated urate transport function,[8] as demonstrated in our previous study on ABCG2-mediated ES transport.[4] Collectively, our comprehensive analysis shows that ABCG2 is a high-capacity urate exporter and that dysfunctional variants of *ABCG2* increase SUA levels in humans. Our findings will serve to clarify the physiological role of ABCG2 as a urate excreter in humans and the molecular pathogenesis of hyperuricemia.

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IDENTIFICATION OF *ABCG2* DYSFUNCTION AS A MAJOR FACTOR CONTRIBUTING TO GOUT

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□ *The ATP-binding cassette, subfamily G, member 2 gene ABCG2/BCRP locates in a gout-susceptibility locus (MIM 138900) on chromosome 4q. Recent genome-wide association studies also showed that the ABCG2 gene relates to serum uric acid levels and gout. Since ABCG2 is also known as a transporter of nucleotide analogs that are structurally similar to urate, and is an exporter that has common polymorphic reduced functionality variants, ABCG2 could be a urate secretion transporter and a gene causing gout. To find candidate mutations in ABCG2, we performed a mutation analysis of the ABCG2 gene in 90 Japanese patients with hyperuricemia and found six non-synonymous mutations. Among the variants, ATP-dependent urate transport was reduced or eliminated in five variants, and two out of the five variants (Q126X and Q141K) were frequently detected in patients. Haplotype frequency analysis revealed that there is no simultaneous presence of Q126X and Q141K in one haplotype. As Q126X and Q141K are a nonfunctional and half-functional haplotype, respectively, their genotype combinations are divided into four estimated functional groups. The association study with 161 male gout patients and 865 male controls showed that all of those who had dysfunctional ABCG2 had an increased risk of gout, and that a remarkable risk was observed in those with ≤1/4 function (OR, 25.8; 95% CI, 10.3–64.6; $p = 3.39 \times 10^{-21}$). In 2,150 Japanese individuals, the frequency of those with dysfunctional ABCG2 was more than 50%. Our function-based clinicogenetic analysis identified the combinations of dysfunctional variants of ABCG2 as a major contributing factor in Japanese patients with gout.*

Keywords ABC transporter; hyperuricemia/gout; genome-wide association study (GWAS); dysfunctional SNP; common variant

INTRODUCTION

Gout based on hyperuricemia is a common disease with a genetic predisposition.[1] *ABCG2* locates in a gout-susceptibility locus on chromosome 4q, which was demonstrated by a genome-wide linkage study of gout.[2] Recent genome-wide association studies (GWAS) also identified *ABCG2* to relate to serum uric acid (SUA) and gout.[3] Besides its transport of nucleotide analogs[4] that are structurally similar to urate, we have reported that *ABCG2* is an exporter with polymorphic reduced functionality variants. We also found that *ABCG2* is a urate exporter and its common variants reduce the transport function. Subsequently, we hypothesized that common variants of *ABCG2* are contributing factors in Japanese gout patients.

MATERIALS AND METHODS

Genetic Analysis

All procedures were performed with the standards of the institutional ethical committees involved in this project and according to the Declaration of Helsinki. After each participant had given written consent, genomic DNA was extracted from whole peripheral blood samples. A mutation analysis of all coding regions and intron–exon boundaries of the *ABCG2* gene was performed for 90 Japanese hyperuricemia patients. For association studies, we additionally genotyped 228 Japanese male hyperuricemia cases (including

161 gout cases) as well as 871 Japanese male controls (SUA $\leq$ 7.0 mg/dl). All gout patients were clinically diagnosed with primary gout. Individuals whose SUA concentration was more than 8.0 mg/dl were selected as hyperuricemia cases. To investigate the frequency of ABCG2 dysfunction, genotyping was also performed in a random sample of 2,150 Japanese individuals.

Functional Analysis

In order to prepare membrane vesicles, HEK293 cells were transiently transfected with an expression vector for ABCG2 or an empty vector by FuGENE6 (Roche Diagnostics) according to the manufacturer's instructions. After 48 hr, cells were harvested and the membrane vesicles were isolated with a standard method. Using the site-directed mutagenesis technique, we constructed mutants of ABCG2 (V12M, Q126X, Q141K, G268R, S441N, and F506SfsX4), which were used for urate transport analysis, on the expression vector for ABCG2. Vesicles prepared from HEK293 cells expressing the wild-type or variants of ABCG2 were incubated with ^{14}C-labeled urate (28 μM) with or without ATP. The amount of ^{14}C-labeled urate was measured after 5 min.

[^{14}C]uric acid was purchased from American Radiolabeled Chemicals. All other chemicals used were commercially available and are of reagent grade.

Statistical Analysis

All calculations of statistical analysis were performed with the software R. The differences in the clinical covariates between the genotypes of the single-nucleotide polymorphisms (SNPs) of *ABCG2* were compared with the Mann–Whitney and Kruskall–Wallis tests. Regression analysis was used to obtain corrected p values. The χ^2 test and Fisher's exact test were used to compare the difference in genotype frequencies and allele frequencies between the case and control samples. A Haplotype estimation was performed with the EM algorithm.

RESULTS

ABCG2 Variants in Hyperuricemia Patients

In order to find common *ABCG2* variants in gout patients, we performed a mutation analysis of all coding regions and intron–exon boundaries of the *ABCG2* gene in 90 Japanese patients with hyperuricemia. The following six non-synonymous mutations, V12M, Q126X, Q141K, G268R, S441N, and F506SfsX4, were found (Figure 1A), and the first three mutations were SNPs. Maekawa et al.[5] reported that allele frequencies for these SNPs, which are quite common in the Japanese population, were 31.9% for Q141K, 19.2% for

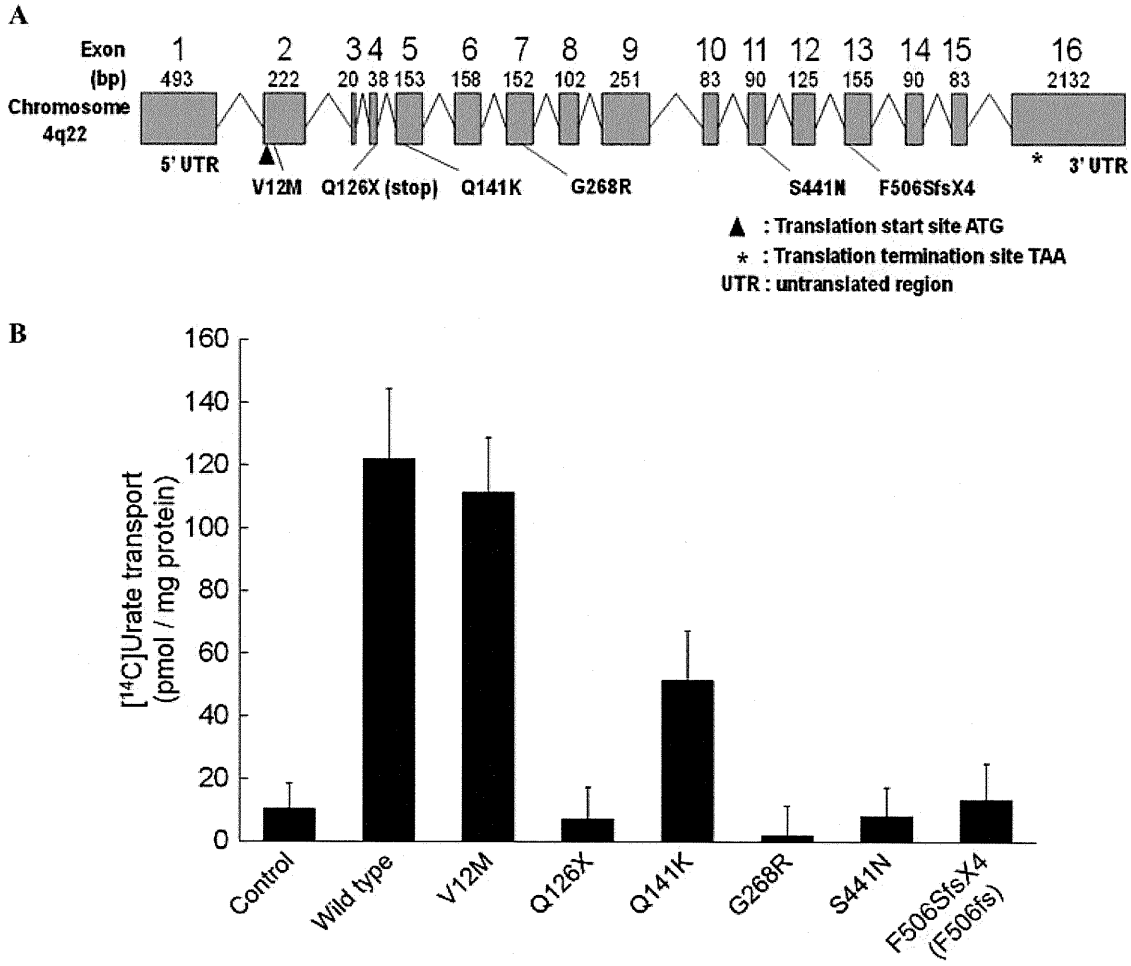

FIGURE 1 Non-synonymous *ABCG2* mutations in hyperuricemia patients. (A) Genomic structure and six non-synonymous mutation sites of the human *ABCG2* gene. (B) ATP-dependent urate transport via ABCG2 mutants. Results are expressed as means ± SD.

V12M, and 2.8% for Q126X. With the Hardy–Weinberg equilibrium and the data reported by Maekawa et al. of the Japanese population,[5] we calculated estimates of the minor allele frequencies of Japanese individuals to be 53.6% for Q141K, 34.7% for V12M, and 5.5% for Q126X.

The subsequent urate transport assay with the six variant proteins was examined and compared with that of wild-type ABCG2. The ATP-dependent transport of urate was reduced by approximately half (46.7%) in Q141K and was nearly eliminated in Q126X, G268R, S441N, and F506SfsX4 mutants (Figure 1B). The V12M variant did not show any changes in urate transport relative to wild-type ABCG2.

Additional genotyping of *ABCG2* SNPs was performed for 228 Japanese men with hyperuricemia (including 161 men with gout) and identified Q126X homozygous ($N = 2$) and heterozygous ($N = 24$) mutations. Two patients with Q126X homozygous mutations showed very high SUA (>10 mg/dl) before they were treated for hyperuricemia. A total of 871

TABLE 1 Association analysis of *ABCG2* genotype combination in gout patients

	Genotype		Number				
Estimated transport	Q126X	Q141K	Gout	Control	*p* value	OR*	95% CI*
≤1/4 function	**T**/**T** **T**/C	C/C **A**/C	16	8	3.39×10^{-21}	25.8	10.3–64.6
1/2 function	**T**/C C/C	C/C **A**/**A**	37	110	2.23×10^{-9}	4.34	2.61–7.24
3/4 function	C/C	**A**/C	72	308	2.29×10^{-7}	3.02	1.96–4.65
Full function	C/C	C/C	34	439		1.00	

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

Japanese men (SUA ≤ 7.0 mg/dl) were then assigned to controls and genotyped for association studies. The association study showed that the risk of hyperuricemia was increased by Q126X (odds ratio [OR], 3.61; 95% confidence interval [CI], 2.14–6.08; $p = 2.91 \times 10^{-7}$). There were Q126X homozygous ($N = 1$) and heterozygous ($N = 21$) mutations among the 161 patients with gout, which revealed that Q126X dramatically increased gout risk (OR, 4.25; 95% CI, 2.44–7.38; $p = 3.04 \times 10^{-8}$). The half-functional SNP Q141K also increased gout (OR, 2.23; 95% CI, 1.75–2.87; $p = 5.54 \times 10^{-11}$). The haplotype frequency analysis revealed no simultaneous presence of the minor alleles of Q126X and Q141K in one haplotype. The haplotype with Q126X was present in up to 13.5% of gout patients, and markedly increased gout risk (OR, 5.97; 95% CI, 3.39–10.51; $p = 4.10 \times 10^{-12}$) compared with non-risk haplotypes. Our data also revealed enrichment of the Q126X minor allele in gout or hyperuricemia patients relative to normouricemic subjects (SUA ≤ 7.0 mg/dl). Thus, the Q126X mutation of the *ABCG2* gene is identified as a major contributing factor in Japanese gout patients. Together, these findings suggest that nonfunctional variants of ABCG2, such as Q126X, essentially block urate excretion and cause gout. Our findings showed that V12M is exclusively assigned to a non-risk haplotype and that the V12M variant does not exhibit altered urate transport activity (Figure 1B). These findings may help explain why V12M decreases gout risk (OR, 0.68; 95% CI, 0.49–0.94; $p = 0.02$). Because the nonfunctional variant Q126X and half-functional variant Q141K are assigned to different risk haplotypes, their genotype combinations are divided into four functional groups on the basis of the estimated ABCG2 transport functions, i.e., full function, 3/4 function, 1/2 function, and ≤1/4 function (Table 1). Gout risk of "3/4 ABCG2 transport function" and "1/2 function" was increased with an OR of 3.02 (95% CI, 1.96–4.65; $p = 2.29 \times 10^{-7}$) and 4.34 (95% CI, 2.61–7.24; $p = 2.23 \times 10^{-9}$), respectively. In genotype combinations of ≤1/4 function, a remarkable increase in gout risk

was observed (OR, 25.8; 95% CI, 10.3–64.6; $p = 3.39 \times 10^{-21}$) (Table 1). Up to 10.1% of gout patients had these genotypes, while only 0.9% of Japanese males (SUA $\leq$ 7.0 mg/dl) have the same genotype combinations. In addition, genotype combinations of full function are detected in 50.8% of the normouricemic subjects but only in 21.4% of gout patients. Among 2,150 individuals, ABCG2 of 3/4 function (39.8%), 1/2 function (12.9%), and $\leq$1/4 function (1.3%) were found. These data mean that more than a half of Japanese individuals (54.0%) have some ABCG2 dysfunction. These findings suggested that combinations of nonfunctional and partially functional variants are important for the development of gout, thereby providing evidence for a common disease attributed to common nonfunctional variants and their combinations.

DISCUSSION

We identified nonfunctional mutations of *ABCG2*, a high-capacity urate exporter, in more than 10% of Japanese gout patients. We also demonstrated that the nonfunctional *ABCG2* variant Q126X is assigned to the identical haplotype, which increases gout risk, conferring an OR of 5.97. Importantly, we found that some genotype combinations remarkably decrease ABCG2 function ($\leq$25% of wild type) and markedly increase gout risk, conferring an OR of 25.8. Our findings indicate that dysfunctional genotype combinations of *ABCG2* are major contributing factors in Japanese gout patients.

Most genetic findings on gout have been derived from rare Mendelian disorders,[2] such as rare familial diseases with symptoms of abnormal uric acid metabolism and hypoxanthine guanine phosphoribosyltransferase deficiency, including Lesch–Nyhan syndrome. Our approach reported here revealed that nonfunctional variants of *ABCG2*, such as Q126X, essentially block urate excretion and cause gout. The disease haplotype with the *ABCG2* Q126X mutation (OR, 5.97; 95% CI, 3.39–10.51; $p = 4.10 \times 10^{-12}$) is present in up to 13.5% of primary gout patients in our population, suggesting that Q126X may well be a major causative mutation for gout. We also found that 10.1% of gout patients have genotype combinations in *ABCG2* that decreased the ABCG2 transport function ($\leq$25% of control), which increased the gout risk remarkably (OR, 25.8; 95% CI, 10.3–64.6; $p = 3.39 \times 10^{-21}$). Thus, the more severe the ABCG2 transport dysfunction, the more likely the individual is to develop gout.

Chen et al.[2] previously identified a gout susceptibility locus on chromosome 4q by a genome-wide linkage study performed with 21 multiplex pedigrees with gout from an aboriginal tribe in Taiwan. Because the Pacific Austronesian population, including Taiwanese aborigines, has a remarkably high prevalence of gout and hyperuricemia, there may be a founder effect across the Pacific region. As the *ABCG2* gene locates on chromosome 4q, *ABCG2* could be a major causative gene for those in other Pacific regions as

well as for Japanese gout patients. Dehghan et al.[3] reported the association of several genetic loci, including those of *GLUT9* and *ABCG2*, with SUA in a GWAS. They also found that Q141K was associated with self-reported gout in Caucasians (OR, 1.74; 95% CI, 1.51–1.99), which is consistent with our findings from clinically diagnosed Japanese gout patients. A meta-analysis of GWAS in European descent also revealed that the nine loci, including *GLUT9* and *ABCG2*, influence SUA, which confirms the findings of Dehghan et al.[3] The authors[6] and Woodward et al.[7] independently found a urate transport ability via *ABCG2* to transport urate and characterized the effects of dysfunctional variant Q141K using different methods. In our study, we also identified several nonfunctional *ABCG2* mutations including Q126X which shows stronger effects on gout development than Q141K did in a previous study (OR < 2.0).[3] There is a possibility that these dysfunctional mutations, including Q126X, are specific to the Japanese or Asian population, or alternatively, there may be nonfunctional mutations in the *ABCG2* gene that increase the incidence of gout in Caucasians and other groups. To clarify the presence of such nonfunctional mutations, further experiments in non-Japanese gout patients are warranted. Our findings also show that a function-based clinicogenetic (FBCG) analysis is useful to pinpoint truly causal variants, especially dysfunctional variants, including nonfunctional variants, and to clarify the molecular pathogenesis of common diseases such as gout and hyperuricemia.

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ORIGINAL ARTICLE

Genetic variations in the *CYP17A1* and *NT5C2* genes are associated with a reduction in visceral and subcutaneous fat areas in Japanese women

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Visceral fat accumulation has an important role in increasing the morbidity and mortality rates, by increasing the risk of developing several metabolic disorders, such as type 2 diabetes, dyslipidemia and hypertension. New genetic loci that are associated with increased systolic and diastolic blood pressures have been identified by genome-wide association studies in Caucasian populations. This study investigates whether single nucleotide polymorphisms (SNPs) that confer susceptibility to high blood pressure are also associated with visceral fat obesity. We genotyped 1279 Japanese subjects (556 men and 723 women) who underwent computed tomography for measuring the visceral fat area (VFA) and subcutaneous fat area (SFA) at the following SNPs: *FGF5* rs16998073, *CACNB2* rs11014166, *C10orf107* rs1530440, *CYP17A1* rs1004467, *NT5C2* rs11191548, *PLEKHA7* rs381815, *ATP2B1* rs2681472 and rs2681492, *ARID3B* rs6495112, *CSK* rs1378942, *PLCD3* rs12946454, and *ZNF652* rs16948048. In an additive model, risk alleles of the *CYP17A1* rs1004467 and *NT5C2* rs11191548 were found to be significantly associated with reduced SFA (P=0.00011 and 0.0016, respectively). When the analysis was performed separately in men and women, significant associations of rs1004467 (additive model) and rs11191548 (recessive model) with reduced VFA (P=0.0018 and 0.0022, respectively) and SFA (P=0.00039 and 0.00059, respectively) were observed in women, but not in men. Our results suggest that polymorphisms in the *CYP17A1* and *NT5C2* genes influence a reduction in both visceral and subcutaneous fat mass in Japanese women.
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Keywords: computed tomography; *CYP17A1*; Japanese subjects; *NT5C2*; sexual dimorphism; subcutaneous fat area; visceral fat area

INTRODUCTION

Metabolic syndrome is a combination of multiple risk factors, including central obesity, impaired glucose tolerance, dyslipidemia and hypertension, which increases cardiovascular disease morbidity and mortality.[1] Several studies have indicated that the intra-abdominal adipose tissue has a central role in metabolic syndrome, as the

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accumulated visceral adipose tissue leads to alterations in the plasma levels of adipocytokines, resulting in the development of dyslipidemia, hypertension and insulin resistance.[2,3] Intra-abdominal fat accumulation (central adiposity) is determined by waist circumference, waist–hip ratio, biological impedance or the visceral fat area (VFA) measured using computed tomography.[1,4,5] There is abundant evidence that body fat distribution is influenced by genetic loci.[6–8] Individual variation in waist–hip ratio is heritable, with heritability estimates ranging from 22 to 61%. Recent genome-wide association studies (GWAS) showed that genetic loci were associated with waist circumference and waist–hip ratio in the Caucasian population.[9,10] We previously reported that the rs1558902 and rs1421085 genotypes of the fat mass- and obesity-associated gene (*FTO*) were significantly associated with VFA, as well as with the subcutaneous fat area (SFA) and body mass index (BMI) in the Japanese population.[11]

Recent progress in GWAS has increased the number of known genetic susceptibility loci for obesity.[12–16] We investigated the association between the single nucleotide polymorphisms (SNPs) underlying susceptibility to obesity and fat distribution (as determined by computed tomography), and found that rs7498665 in the SH2B adaptor protein 1 (*SH2B1*) gene was associated with VFA, uncovering the genetic background of central obesity.[17]

GWAS, and meta-analysis of GWAS, have identified various disease-associated genetic variations.[18] Hypertension is one of the risk factors of metabolic syndrome and is considerably related to central obesity. Obesity-associated allele of rs1558902 and rs1421085 in the *FTO* gene were associated with hypertension, but not that of rs7498665 in the *SH2B1* gene in the Japanese population.[19] The genetic variations associated with hypertension have been identified by GWAS.[20,21] In this study, we investigate whether the recently reported hypertension-related loci are also associated with VFA, which is another important factor responsible for metabolic syndrome.

MATERIALS AND METHODS

Study subjects

We enrolled 1279 Japanese subjects from outpatient clinics; these patients agreed to undergo computed tomography testing (in the supine position) to determine VFA and SFA values at the umbilical level (L4–L5), as previously reported.[17] Both VFA and SFA values were calculated using the FatScan software program (N2system, Osaka, Japan).[22] The patients visited the hospitals to undergo treatment for obesity and/or metabolic abnormalities, such as hypertension, dyslipidemia and type 2 diabetes. Patients with secondary obesity and obesity-related hereditary disorders were excluded from this study. Patients with disease (such as cancer, and renal, heart and hepatic failure), or under treatment (such as corticosteroid and chemotherapy) that strongly affects body weight, were also excluded. Athletes were also excluded from this study. Clinical data were recorded at the first visit to the hospital. The clinical characteristics of the subjects are summarized in Table 1. Metabolic syndrome and metabolic abnormalities were diagnosed according to the criteria released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005.[4,5] Written informed consent was obtained from each subject, and the protocol was approved by the ethics committee of each institution and by that of Kyoto University.

DNA extraction and SNP genotyping

Genomic DNA was extracted from the blood samples collected from each subject using the Genomix kit (Talent Srl, Trieste, Italy). We selected 12 SNPs that were previously identified as susceptibility loci for hypertension by GWAS in Caucasian populations,[20,21] and constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for each. The 12 selected SNPs were as follows: rs16998073 in the fibroblast growth factor 5 (*FGF5*) gene; rs11014166 in the calcium channel, voltage-dependent, β-2 subunit (*CACNB2*) gene; rs1530440 in the chromosome 10 open reading frame 107 (*C10orf107*) gene; rs1004467 in the cytochrome P450, family 17, subfamily A, polypeptide 1 (*CYP17A1*) gene; rs11191548 in the 5′-nucleotidase, cytosolic II (*NT5C2*) gene; rs381815 in the pleckstrin homology domain containing, family A member 7 (*PLEKHA7*) gene; rs2681472 and rs2681492 in the ATPase, Ca^{2+} transporting, plasma membrane 1 (*ATP2B1*) gene; rs6495112 in the AT-rich interactive domain 3B (BRIGHT-like) (*ARID3B*) gene; rs1378942 in the c-src tyrosine kinase (*CSK*) gene; rs12946454 in the phospholipase C, delta 3 (*PLCD3*) gene; and rs16948048 in the zinc finger protein 652 (*ZNF652*) gene. The SNPs were genotyped using Invader assays, as previously described.[23] The success rate of these assays was >99.0%.

Table 1 Clinical characteristics of the subjects

	Men	*Women*	*Total*
n	556	723	1279
Age (years)	49.4±12.2	52.2±11.3	51.0±11.8
BMI (kg m^{-2})	30.2±6.1	28.1±5.3	29.0±5.8
VFA (cm^2)	155.3±67.7	99.8±53.6	123.9±66.1
SFA (cm^2)	206.7±108.6	241.6±97.2	226.5±103.7
Waist circumference (cm)	97.5±11.3	91.8±10.3	94.2±11.1
Prevalence of metabolic disease			
Dyslipidemia	293 (53%)	244 (34%)	537 (42%)
Hypertension	379 (68%)	452 (63%)	831 (65%)
Impaired fasting glucose	177 (32%)	176 (24%)	353 (28%)
Metabolic syndrome	248 (45%)	162 (22%)	410 (32%)

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

Statistical analysis

For the additive model, we coded the genotypes as 0, 1 or 2 depending on the number of copies of the risk alleles. For the recessive model, homozygosity with the risk allele was coded as 1 and the others were coded as 0. Risk alleles refer to the hypertension-associated alleles, according to previous reports.[20,21] Multiple linear regression analyses were performed to test the independent effect of the risk alleles on BMI, VFA and SFA, by taking into account the effects of other variables (that is, age and gender) that were assumed to be independent of the effect of each SNP. The values of BMI, VFA and SFA were logarithmically transformed before performing the multiple linear regression analysis. Differences in the quantities of anthropometric parameters among the different genotypes were assessed by the analysis of covariance, by taking into account the effects of other variables (that is, age and/or institute). Hardy–Weinberg equilibrium was assessed using the χ^2-test.[24] To test SNP×SNP epistasis, we used a linear regression model for each SNP1 and SNP2, and fit the model in the form of $Y=\beta_0+\beta_1\times\mathrm{SNP1}+\beta_2\times\mathrm{SNP2}+\beta_3\times\mathrm{SNP1}\times\mathrm{SNP2}+\beta_4\times\mathrm{age}+\beta_5\times\mathrm{gender}$. Although we collected the samples at the region of Hondo (Kanto, Kinki, Chugoku and Kyushu; Supplementary Table 1), we performed Wright's F-statistics[25] to evaluate the difference in the population structures of our sample using randomly selected 31 SNPs. We divided our samples into two groups (SFA >208 cm^2 and ⩽208 cm^2). Median of SFA (208 cm^2) was used as a cut-off value. The results indicated that the population structure of the two groups were almost the same in view of a very small F_{ST} value between both the groups (mean F_{ST}=0.00023). Statistical analysis was performed using R software (http://www.r-project.org/). *P*-values were assessed with a Bonferroni correction and $P<0.0042$ (0.05/12) was considered statistically significant.

RESULTS

The clinical characteristics and genotypes of the subjects are shown in Tables 1 and 2, respectively. All the SNPs were in Hardy–Weinberg equilibrium and the minor allele frequencies did not diverge from those reported in the HapMap database. The BMI, VFA and SFA values for each SNP genotype are reported in Table 3. Multiple linear regression analyses of the anthropometric parameters with respect to the 12 analyzed SNPs are shown in Table 4. The A-allele of rs1004467

Table 2 Genotypic characteristics of the subjects

SNP ID	*CHR*	*Position (Build 36.3)*	*Nearby gene*	*Allele 1/2*	*BP-associated allele*	*Genotype*	*HWE P-value*
rs16998073	4	81 403 365	*FGF5*	T/A	T	120/514/644	0.24
rs11014166	10	18 748 804	*CACNB2*	T/A	A	4/124/1151	0.73
rs1530440	10	63 194 597	*C10orf107*	T/C	C	30/296/953	0.22
rs1004467	10	104 584 497	*CYP17A1*	A/G	A	559/567/153	0.62
rs11191548	10	104 836 168	*NT5C2*	T/C	T	675/504/100	0.66
rs381815	11	16 858 844	*PLEKHA7*	C/T	T	842/381/56	0.13
rs2681472	12	88 533 090	*ATP2B1*	A/G	A	546/562/171	0.17
rs2681492	12	88 537 220	*ATP2B1*	C/T	T	168/561/549	0.19
rs6495112	15	72 619 851	*ARID3B*	A/C	A	530/575/173	0.39
rs1378942	15	72 864 420	*CSK*	A/C	C	49/410/817	0.78
rs12946454	17	40 563 647	*PLCD3*	T/A	T	34/343/901	0.84
rs16948048	17	44 795 465	*ZNF652*	G/A	G	18/326/935	0.08

Abbreviations: BP, blood pressure; CHR, chromosome; HWE, Hardy–Weinberg equilibrium.

Table 3 Mean BMI, VFA and SFA for 12 blood pressure risk variants

		Mean ± s.d.								
		BMI (kg m^{-2})			*VFA (cm^2)*			*SFA (cm^2)*		
		Genotype			*Genotype*			*Genotype*		
SNP ID	*Nearby gene*	*11*	*12*	*22*	*11*	*12*	*22*	*11*	*12*	*22*
rs16998073	*FGF5*	28.8 ± 4.7	29.0 ± 5.8	29.0 ± 6.0	126.2 ± 66.1	121.6 ± 66.5	125.3 ± 65.9	227.4 ± 98.3	224.5 ± 111.0	227.7 ± 98.7
rs11014166	*CACNB2*	27.0 ± 2.7	29.6 ± 6.1	28.9 ± 5.8	123.4 ± 82.2	136.7 ± 68.2	122.5 ± 65.8	178.6 ± 35.8	233.7 ± 106.4	225.8 ± 103.6
rs1530440	*C10orf107*	30.8 ± 6.5	28.6 ± 5.4	29.1 ± 5.9	129.8 ± 63.1	120.2 ± 66.4	124.9 ± 66.2	236.4 ± 119.2	223.0 ± 91.4	227.2 ± 106.9
rs1004467	*CYP17A1*	28.4 ± 5.6	29.5 ± 6.1	29.4 ± 5.2	117.5 ± 64.9	130.6 ± 68.5	122.5 ± 59.3	215.5 ± 92.7	231.4 ± 111.5	247.9 ± 107.9
rs11191548	*NT5C2*	28.6 ± 5.8	29.5 ± 5.9	28.9 ± 5.1	119.2 ± 65.3	130.9 ± 68.6	120.5 ± 55.7	217.2 ± 96.0	238.5 ± 113.2	228.1 ± 98.8
rs381815	*PLEKHA7*	29.2 ± 5.9	28.7 ± 5.7	27.8 ± 4.3	124.1 ± 64.2	124.3 ± 71.5	117.9 ± 55.9	229.4 ± 105.9	221.5 ± 101.6	215.3 ± 83.1
rs2681472	*ATP2B1*	29.2 ± 5.8	28.8 ± 5.4	29.0 ± 7.0	127.1 ± 67.5	121.5 ± 64.8	121.8 ± 65.8	227.4 ± 100.4	223.5 ± 100.3	233.1 ± 123.6
rs2681492	*ATP2B1*	29.0 ± 7.1	28.7 ± 5.2	29.3 ± 5.9	121.9 ± 66.3	121.4 ± 64.8	127.0 ± 67.4	234.6 ± 123.9	221.9 ± 98.3	228.7 ± 102.3
rs6495112	*ARID3B*	28.9 ± 5.8	29.0 ± 5.7	29.3 ± 6.2	122.5 ± 63.4	125.0 ± 69.2	124.9 ± 64.3	223.7 ± 106.5	229.5 ± 102.6	225.1 ± 99.2
rs1378942	*CSK*	28.0 ± 4.2	28.9 ± 6.1	29.1 ± 5.7	110.0 ± 63.3	121.8 ± 62.4	125.6 ± 67.6	222.9 ± 84.5	225.9 ± 104.3	227.0 ± 104.7
rs12946454	*PLCD3*	30.1 ± 8.2	28.6 ± 5.0	29.1 ± 5.9	137.2 ± 80.0	123.0 ± 67.4	123.7 ± 65.1	254.4 ± 105.0	216.4 ± 93.7	229.2 ± 107.0
rs16948048	*ZNF652*	28.1 ± 2.8	29.4 ± 5.9	28.9 ± 5.8	128.6 ± 73.7	124.8 ± 65.9	123.5 ± 66.1	215.0 ± 60.6	227.0 ± 96.3	226.5 ± 106.9

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single nucleotide polymorphism; VFA, visceral fat area.
11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2. Allele 1 and allele 2 of each SNP is indicated in Table 2.

in the *CYP17A1* gene was significantly associated with reduced BMI (P=0.0018). The other SNPs were not significantly associated with BMI. No SNP was significantly associated with VFA. The A-allele of rs1004467 in the *CYP17A1* and the T-allele of rs11191548 in the *NT5C2* gene were significantly associated with reduced SFA. These SNPs are in linkage disequilibrium, as reported in the HapMap database (D'=0.98, r^2=0.71), and the A-allele of rs1004467 and T-allele of rs11191548 are reported to be risk alleles for increased blood pressure.[20,21]

BMI, VFA and SFA are known to be affected by gender; therefore, we compared rs1004467 and rs11191548 alleles with anthropometric parameters (BMI, VFA and SFA) in men and women independently (Table 5). Associations of both SNPs with VFA (P=0.0018 and P=0.0043) and SFA (P=0.00039 and P=0.0021) in women were significant, except the association of T-allele of rs11191548 with VFA. The VFA and SFA values of the rs11191548 genotype suggest that the recessive model would be the best-fitted model both in men and women. By using the recessive model, results revealed significant associations of the rs11191548 genotype with VFA (P=0.0022) and SFA in women (P=0.00059). These SNPs did not show any association with VFA or SFA in men, suggesting that they exhibit sexual dimorphism, as has been suggested in a recent report.[26] As both rs1004467 and rs11191548 were associated with a reduction in both VFA and SFA, we examined the association of these SNPs with total fat area. The SNPs were significantly associated with total fat area (P=0.00012 at rs1004467, P=0.00052 at rs11191548 in additive model) in women, but not in men, suggesting that risk allele for high blood pressure of these SNPs are associated with reduced adiposity in women. The very small mean F_{ST} value (0.00023) indicated no population structure in our subjects. As we collected the samples from nine institutes in four regions of Japan (Supplementary Table 1), we tested multiple linear regression analysis with age and institute as explanatory variables in men and women. Very similar results were observed. In additive model, significant associations of the

Table 4 Relationship between blood pressure-associated loci and adiposity measures

		BMI			*VFA*			*SFA*		
SNP ID	*Nearby gene*	β	*s.e.*	P-*value*	β	*s.e.*	P-*value*	β	*s.e.*	P-*value*
rs16998073	*FGF5*	−0.002	0.003	0.55	−0.003	0.010	0.78	−0.010	0.008	0.22
rs11014166	*CACNB2*	−0.005	0.007	0.48	−0.043	0.021	0.043	−0.008	0.017	0.63
rs1530440	*C10orf107*	−0.002	0.004	0.71	0.010	0.014	0.48	−0.005	0.011	0.64
rs1004467	*CYP17A1*	−0.010	0.003	**0.0018**	−0.022	0.010	0.027	−0.030	0.008	**0.00011**
rs11191548	*NT5C2*	−0.008	0.003	0.015	−0.019	0.011	0.078	−0.026	0.008	**0.0016**
rs381815	*PLEKHA7*	−0.007	0.004	0.046	−0.004	0.012	0.76	−0.015	0.009	0.10
rs2681472	*ATP2B1*	0.002	0.003	0.43	0.006	0.010	0.52	0.005	0.008	0.49
rs2681492	*ATP2B1*	0.003	0.003	0.34	0.006	0.010	0.54	0.006	0.008	0.40
rs6495112	*ARID3B*	−0.002	0.003	0.45	−0.004	0.010	0.65	−0.007	0.008	0.36
rs1378942	*CSK*	0.005	0.004	0.20	0.010	0.012	0.40	0.005	0.009	0.61
rs12946454	*PLCD3*	−0.003	0.004	0.39	0.009	0.013	0.50	−0.011	0.010	0.28
rs16948048	*ZNF652*	0.005	0.004	0.30	0.008	0.014	0.57	0.005	0.011	0.67

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single nucleotide polymorphism; VFA, visceral fat area.
Data were derived from a linear regression analysis. The values of BMI, VFA and SFA were logarithmically transformed. Logarithmically transformed BMI, VFA and SFA were adjusted for age and gender. Tested alleles are risk alleles of increased blood pressure.

Table 5 Relationship between rs1004467 and rs11191548, and adiposity in men and women

			Values at each genotype			*Additive model*		*Recessive model*	
SNP ID (gene)	*Phenotype*	*Gender*	*11*	*12*	*22*	β *(s.e.)*	P-*value*	β *(s.e.)*	P-*value*
rs1004467	*n*	Men	233	259	64				
(*CYP17A1*)		Women	326	308	89				
	BMI (kg m^{-2})	Men	29.7 ± 6.6	30.6 ± 5.9	30.1 ± 5.1	−0.011 (0.005)	0.029	−0.017 (0.006)	0.0085
		Women	27.6 ± 4.6	28.5 ± 6.0	28.9 ± 5.3	−0.010 (0.004)	0..017	−0.013 (0.006)	0.019
	VFA (cm^2)	Men	152.9 ± 67.7	160.6 ± 69.2	142.8 ± 59.9	0.004 (0.014)	0.78	−0.012 (0.019)	0.52
		Women	92.3 ± 49.2	105.4 ± 56.8	107.8 ± 54.7	−0.044 (0.014)	**0.0018**	−0.061 (0.019)	**0.0014**
	SFA (cm^2)	Men	198.6 ± 103.0	211.9 ± 113.8	215.4 ± 106.7	−0.028 (0.013)	0.037	−0.036 (0.018)	0.047
		Women	227.6 ± 82.7	248.0 ± 106.9	271.2 ± 103.3	−0.033 (0.009)	**0.00039**	−0.040 (0.013)	**0.0020**
rs11191548	*n*	Men	289	220	47				
(*NT5C2*)		Women	386	284	53				
	BMI (kg m^{-2})	Men	30.0 ± 6.8	30.6 ± 5.4	29.4 ± 5.4	−0.007 (0.005)	0.19	−0.013 (0.006)	0.049
		Women	27.6 ± 4.7	28.7 ± 6.1	28.5 ± 4.8	−0.010 (0.004)	0.021	−0.015 (0.006)	0.0080
	VFA (cm^2)	Men	153.8 ± 68.0	161.3 ± 69.3	137.2 ± 54.8	0.007 (0.014)	0.65	−0.008 (0.018)	0.65
		Women	93.3 ± 49.3	107.4 ± 58.1	105.8 ± 52.8	−0.043 (0.015)	0.0043	−0.059 (0.019)	**0.0022**
	SFA (cm^2)	Men	202.1 ± 107.5	214.3 ± 111.3	199.9 ± 102.5	−0.023 (0.014)	0.10	−0.035 (0.018)	0.048
		Women	228.5 ± 84.9	257.4 ± 111.1	253.0 ± 89.0	−0.031 (0.010)	**0.0021**	−0.044 (0.013)	**0.00059**

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single nucleotide polymorphism; VFA, visceral fat area.
Values are shown as the mean ± s.d. Data were derived from a linear regression analysis. The values of BMI, VFA and SFA were logarithmically transformed. Logarithmically transformed BMI, VFA and SFA were adjusted for age. Tested alleles (allele1 at both SNPs) are risk alleles of increased blood pressure.

rs1004467 and rs11191548 genotype with VFA (P=0.0015 and 0.0011, respectively) and SFA (P=0.00021 and 0.00062, respectively) were observed in women (Supplementary Table 2). Statistical analysis using analysis of covariance indicated significant associations of the rs1004467 and rs11191548 genotype with VFA (P=0.0020 and 0.0015, respectively) and SFA (P=0.00033 and 0.00042, respectively) in women (Supplementary Table 2). As some diabetes medications have an effect on adiposity,[27] we performed the analysis excluding 147 type 2 diabetic patients treated with sulfonylureas, biguanides and thiazolidinediones We found the similar significant associations of the rs1004467 and rs11191548 genotype with VFA and SFA in women (Supplementary Table 3).

We have reported that rs1558902 in the *FTO* gene is associated with both VFA and SFA,[11] and that rs7498665 in the *SH2B1* gene is associated with VFA.[17] Thus, we examined SNP×SNP epistasis in men, women and all subjects. The combination of rs1004467 and rs7498665 exhibited no epistatic effect on VFA in men (P=0.43), women (P=0.86) or all subjects (P=0.76). The combination of rs1004467 and rs1558902 did not show epistatic effect on VFA in men (P=0.99), women (P=0.53) or all subjects (P=0.60), or on SFA in men (P=0.63), women (P=0.83) or all subjects (P=0.89).

Among the SNPs tested in this study, rs16998073 in the *FGF5* gene and rs11191548 in the *NT5C2* gene were associated with increased systolic blood pressure ($P<0.05$). Rs11191548 in the *NT5C2* gene were also associated with hypertension ($P<0.05$). We could replicate the association between blood pressure and the above two SNPs that were reported to be strongly associated with blood pressure in the Japanese population (Supplementary Table 4).[28]

DISCUSSION

In this study, we showed that the A-allele of rs1004467 in the *CYP17A1* and the T-allele of rs11191548 in the *NT5C2* gene were significantly associated with reduced VFA, SFA and total fat area in women. Association of T-allele of rs11191548 in the *NT5C2* gene with increased systolic blood pressure and hypertension was replicated in our sample, as reported previously.[28] Our hypothesis was that these risk alleles would be associated with increased VFA and/or SFA as increased adiposity is a risk for hypertension;[4,5] however, these alleles affected decreased adiposity. The associations between SNPs and increased blood pressure/hypertension were evaluated after being adjusted for BMI, age and gender. Thus, the SNPs associated with visceral fat obesity-related and gender-dependent hypertension would be excluded in the screening stage. Indeed, recent analysis has shown that genetic variation near insulin receptor substrate 1 (*IRS1*) is associated with reduced adiposity and an impaired metabolic profile.[29] Thus, it is likely that rs1004467 and rs11191548 are associated with reduced VFA and SFA, as well as with hypertension in women.

The SNPs rs1004467 and rs11191548 were not associated with BMI in men or women, as reported for rs2943650 near *IRS1*.[29] As BMI represents both fat and lean body mass, our observation suggests that these SNPs influence a reduction in VFA and SFA, or influence an increased percentage of lean body mass. The significant associations of rs1004467 and rs1191548 with reduced VFA and SFA were observed in women, but not in men. The rs1004467 SNP is located in the intron of the *CYP17A1* gene. CYP17A1 is involved in the biosynthesis of glucocorticoids, mineral corticoids, androgens and estrogens.[30] The rs1004467 risk allele may reflect differences in *CYP17A1* gene expression that alter the biosynthesis of steroid hormones, leading to hypertension and reduced adiposity in women. The region of linkage disequilibrium that includes rs1004467 and rs11191548 contains a couple of genes in addition to *CYP17A1*: *NT5C2*, arsenic (+3 oxidation state) methyltransferase (*AS3MT*) and cyclin M2 (*CNNM2*). NT5C2 is a cytosolic IMP/GMP selective 5′-nucleotidase and involved in nucleic acids or DNA synthesis.[31] CNNM2 (ancient conserved domain protein, ACDP2) is a transporter of magnesium, which is required for the catalytic activity of numerous metalloenzymes.[32] Thus, these genes would be important for metabolism in adipocyte hyperplasia and hypertrophy. Further investigation is warranted to elucidate the functional SNPs and susceptibility genes.

We have previously reported that *FTO* rs1558902 is associated with VFA and SFA, and that *SH2B1* rs7498665 is associated with VFA.[11,17] Epistasis, or gene–gene interaction, has recently received much attention in human genetics.[33] In this study, the effect of these SNPs on VFA and SFA was additive, and an epistatic effect was not observed.

In summary, we showed that *CYP17A1* rs1004467 and *NT5C2* rs11191548 SNPs are significantly associated with both reduced VFA and SFA in women. Our results suggest that the region encompassing *CYP17A1* to *NT5C2* has a role in reducing visceral and subcutaneous fat mass. However, these results require confirmation in other populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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5. 腎性低尿酸血症の遺伝学

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key words　renal hypouricemia, urate transporter, URAT1/SLC22A12, GLUT9/SLC2A9, ABCG2/BCRP, genome-wide association study

動　向

腎性低尿酸血症は，腎臓の近位尿細管における尿酸の再吸収不全に起因する尿酸トランスポーター病である．合併症として運動後急性腎不全や尿路結石が問題となる．腎性低尿酸血症は遺伝学的には1型と2型に分類されており，病因遺伝子はurate transporter 1（*URAT1/SLC22A12*）遺伝子およびglucose transporter 9（*GLUT9/SLC2A9*）遺伝子であることが，いずれも日本人の症例解析から明らかとなっている．URAT1およびGLUT9は，どちらも腎臓の近位尿細管における尿酸再吸収トランスポーターであり，これらの機能不全により，腎性低尿酸血症とその合併症が起きる（表1）．一方，尿酸排泄トランスポーター遺伝子*ABCG2/BCRP*（ATP-binding cassette transporter, subfamily G, member 2）は，高尿酸血症や痛風の主要病因遺伝子であり（表1），その機能低下により血清尿酸値が有意に上昇することがわかってきた．

URAT1，*GLUT9*，*ABCG2*の各遺伝子は，いずれも血清尿酸値のゲノムワイド関連解析genome-wide association study（GWAS）においてその関与が示されており（表2），ヒトの血清尿酸値の調節に重要な生理学的役割を担っている．これらの遺伝子のうち，*URAT1*遺伝子はヒトゲノム解読以降に初めて同定されたものであり，*GLUT9*および*ABCG2*の両遺伝子についてもヒトゲノム解読後のGWASが進展した時期に

表1　ヒトの尿酸トランスポーターと尿酸代謝関連疾患

尿酸トランスポーター	遺伝子座位	生理機能（尿酸輸送）	トランスポーター機能不全による尿酸代謝関連疾患
URAT1/SLC22A12	11q13	腎近位尿細管における尿酸再吸収	腎性低尿酸血症1型 (RHUC1, renal hypouricemia type 1)
GLUT9/SLC2A9	4p16-p15.3	腎近位尿細管における尿酸再吸収	腎性低尿酸血症2型 (RHUC2, renal hypouricemia type 2)
未同定	—	—	腎性低尿酸血症3型？ (RHUC3, renal hypouricemia type 3?)
ABCG2/BCRP	4q22	尿酸排泄	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．