The rs2231142 Variant of the *ABCG2* Gene is Associated with Uric Acid Levels and Gout among Japanese

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Running title: *ABCG2*, urate and gout in Japanese

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Abstract

Objectives. Recent genome-wide association and functional studies have shown that the *ABCG2* gene encodes for a urate transporter, and a common causal *ABCG2* variant, rs2231142, leads to elevated uric acid levels and prevalent gout among whites and blacks. We examined whether this finding is observed in a Japanese population, since Asians have a high reported prevalence of the T risk allele.

Methods. A total of 3,923 Japanese from the Circulatory Risk in Communities Study aged 40-90 were genotyped for rs2231142. Associations of the rs2231142 variant with serum uric acid levels and prevalence of gout and hyperuricemia were examined.

Results. The frequency of the T risk allele was 31% in this Japanese sample. Multivariable adjusted mean uric acid levels were 7-9μmol/L higher for TG and TT than GG carriers (p-additive=0.0006). The multivariable adjusted odds ratio of prevalent gout was 1.37(95% confidence interval: 0.68-2.76) for TG and 4.37(1.98-9.62) for TT compared with the GG carriers (p-additive=0.001). When evaluating the combined outcome of hyperuricemia and gout, the respective odds ratios were 1.40(1.04-1.87) for TG and 1.88(1.23-2.89) for TT carriers. The population attributable risk was 29% for gout and 19% for gout and/or hyperuricemia.

Conclusions. The association of the causal *ABCG2* rs2231142 variant with uric acid levels and gout was confirmed in a sample of Japanese ancestry. Our study underscores the importance of this common causal variant in a population with a high risk allele frequency, especially as more Japanese adopt a Western lifestyle with a concomitant increase in mean serum uric acid levels. (250 words / 250 limits)

Introduction.

Hyperuricemia is a key risk factor for gout and has been described as associated with cardiovascular risk factors and diseases such as hypertension, diabetes, metabolic syndrome, stroke and coronary heart disease [1]. Recently, Dehghan and colleagues conducted population-based genome-wide association studies and identified three genetic loci associated with increased serum uric acid concentration and prevalence of gout [2]. The most significant association at one of the loci was observed for a missense single nucleotide polymorphism (SNP), rs2231142 in the *ABCG2* gene. Each copy of the T allele at rs2231142 was associated with gout in both white [odds ratio (95% confidence interval) =1.74 (1.51-1.99)] and black [1.71 (1.06-2.77)] study participants. In a subsequent functional study it was shown that ABCG2 is a urate transporter that mediates urate excretion in the kidney, and that the rs2231142 T allele encodes for a reduced-function mutation leading to reduced ability to excrete uric acid [3]. Interestingly, the risk allele is three times more frequent in the Japanese compared to the European-ancestry HapMap samples, but so far, the association of this locus with serum uric acid or prevalent gout has rarely examined in other races/ethnicities than whites and blacks. We therefore examined whether this association replicates in Japan, where prevalence of gout has rapidly increased

since the 1960s [4].

Methods.

Ethics statement

This study was approved by the Institutional Review Board of the University of Tsukuba. All participants whose data were analyzed in this study provided written informed consent for the collection of samples and subsequent analysis.

Study population and measurement of exposure and outcomes

The Circulatory Risk in Communities Study (CIRCS) is an ongoing dynamic community cohort study involving 5 communities in Japan. The rs2231142 variant in *ABCG2* (Q141K) was genotyped in participants in community surveys in 2001 in Kyowa and in 2003 in Ikawa, as a part of the CIRCS. A total of 4,418 participants, 40 years or over, in these Japanese farming communities provided written informed consent for DNA analysis (consent rate =98%). The genotype was determined by the Intercalator mediated Fluorescence Resonance Energy Transfer probe method using *Taq* DNA polymerase (rTaq; Toyobo, Osaka, Japan) at the Tsuruga Laboratory of Toyobo Gene Analysis Co., Ltd (Osaka, Japan). Detailed methods for genotyping were described elsewhere [5]. The designed probe was 5'-AGAGAAAACTTACAGTTCTCAGCAG-3' with Texas Red-end, specific to G

allele. Serum uric acid and other health information were also collected [6]. Uric acid was measured by the uricase/peroxidase methods at the Osaka Medical Center for Health Science and Promotion (Osaka, Japan). History/treatment of gout and treatment of hyperuricemia were ascertained face-to-face by trained interviewers. Gout was defined if participants answered that they had experienced gout; had been diagnosed gout by physicians; or were under treatment for gout. Hyperuricemia was defined if they had a uric acid concentration of ≥415.9 μmol/L (7.0 mg/dL) based on blood testing; or answered that they were under treatment for hyperuricemia at interview. If they had had a uric acid concentration of ≥415.9 μmol/L, based on records from previous annual checkups, the interview confirmed whether or not the participants were now under treatment for hyperuricemia. We excluded persons with unsuccessful genotyping (n=1), missing DNA (n=70), and missing uric acid data (n=424). Ultimately, 3,923 participants were included in this study.

Statistical analyses

We used analysis of covariance for calculation of multivariable adjusted mean values of serum uric acid across genotypes, and multivariable adjusted logistic regression models for

calculation of odds ratios and 95% confidence intervals. We evaluated an additive genetic model (ie, genotypes were coded as 0 for GG, 1 for TG and 2 for TT genotypes) and also compared the TG and TT genotypes separately to the reference genotype GG; the Hosmer-Lemeshow test showed that the logistic regression models fit the data well for these analyses. To test for significance, we assumed an additive genetic model analogous to previously reported analyses [2]. We did not find a significant difference in the variance in uric acid concentration across the genotype groups using the Levene's test ($p=0.35$). Although the distribution of uric acid concentration was not statistically normal (Kolmogorov-Smirnov $p<0.01$), we did not log-transform the values to permit comparability with the discovery report [2]. The distribution was virtually quasi-normal, and the log-transformation did not improve the normality. Covariates were selected to match covariates used in the discovery report [2], ie, age, sex, BMI (kg/m^2), alcohol consumption (g/day), hypertension treatment and community. Population attributable risk fraction (%), which represents the percentage of gout that might be due to rs2231142, was calculated as $[(P_{TG})(PR_{TG}-1)+(P_{TT})(PR_{TT}-1)] / [1+(P_{TG})(PR_{TG}-1)+(P_{TT})(PR_{TT}-1)] * 100$, where P_{TG} or P_{TT} corresponds to the proportion of individuals exposed to TG or TT genotype among those without gout, and PR_{TG} or PR_{TT} is the respective unadjusted

prevalence ratio of gout/hyperuricemia. We used SAS version 9.1.3 Service Pack 4 (SAS Institute Inc., Cary, NC) for analyses. All probability values for statistical tests were two-tailed, and $p<0.05$ was regarded as statistically significant.

Results.

The genotype distribution conformed to Hardy-Weinberg equilibrium ($p \geq 0.54$). The frequency of the risk allele (T) was 31% in this Japanese sample, much higher than that reported for whites (11-12%) and blacks (3%) by Dehghan et al. [2] (Table 1). Conversely, mean uric acid concentration was much lower in Japanese (282.1μmol/L) than reported for whites (315.2-350.9μmol/L) and blacks (374.7μmol/L). Age, body mass index (BMI), alcohol drinking and antihypertensive medication use did not differ substantially across the three genotype categories (Table 2).

As shown in Table 3, mean uric acid concentration was 7-9μmol/L higher for TG and TT than GG carriers after adjustment for age, sex, community, BMI, alcohol consumption, and antihypertensive treatment (p-additive = 0.0006). While this association was linear in women, uric acid levels were lower among TT than TG carriers in men. The R^2 for uric acid concentration was 0.279 in this multivariate model without *ABCG2* and 0.281 with *ABCG2* genotype.

Compared with the GG genotype, the multivariable adjusted odds ratio of gout was 1.37 (95% confidence interval: 0.68-2.76) for TG and 4.37 (1.98-9.62) for TT carriers [p-additive =0.001, odds ratio =2.03 (1.33-3.12) per T allele] (Table 3). When we combined

hyperuricemia (defined as hyperuricemia treatment and/or uric acid≥415.9μmol/L) with gout as one outcome, the respective odds ratios were 1.40 (1.04-1.87) for TG and 1.88 (1.23-2.89) for TT carriers [p-additive =0.002, odds ratio =1.38 (1.13-1.68) per T allele]. Of note, the recessive genetic model provided a better fit than the additive model for gout as indicated by a lower Akaike's Information Criterion (p-recessive=0.0002).

The population attributable fraction for rs2231142 was 29% for gout and 19% for gout and/or hyperuricemia.

Discussion

Our study confirms the results by Dehghan et al. [2], a subsequent meta-analysis of European ancestry subjects [7], and some other case-control studies including those from Japan [8] and China [9], reporting an association of rs2231142 with serum uric acid levels and gout. In addition, it is the first study of rs2231142 in a community-based setting of Asian ancestry, a Japanese sample. Our finding is consistent with the recently established causality of the rs2231142 variant [3,8].

Although the prevalence of the risk allele was higher among Japanese (31%) than whites (11-12%) and blacks (3%), serum uric acid levels and gout prevalence were much lower in this Japanese sample. Traditionally, gout was rarely observed among Japanese before the 1960's. Since then, the prevalence of gout has rapidly increased [4], presumably because of rapid changes in lifestyle after World War II. This phenomenon is commonly observed with complex diseases, where adverse environmental exposures are thought to act on genetically susceptible individuals. Our findings may suggest a potential gene-environment interaction in relation to gout/uric acid resulting in an increased incidence of gout. That is, some factors that are part of a traditional Japanese lifestyle may protect from gout despite the high prevalence of the risk allele among

Japanese. This hypothesis is in line with the fact that US Asians have almost three times the prevalence of diagnosed gout than US whites [10], since US Asians might be exposed to both a high prevalence of the risk allele and US lifestyle.

A limitation of this study is that we used the information of gout ascertained by a face-to-face interview. To our knowledge, no study has reported sensitivity, specificity or positive predictive value for interview in the diagnosis of gout based on a golden standard of clinical diagnosis. Another limitation is the moderate statistical power (approximately 50-60%); since we found a positive association, this would not have affected the results materially. Third, the usage of the odds ratio might not be appropriate as an approximate of relative risk in cross-sectional study, because gout and hyperuricemia are not scarce in general Japanese population, although rarer than in the United States or Europe (Table 1). However, when we used an unadjusted prevalence ratio instead, the results did not change materially. Finally, we found in men that the uric acid level was lower among TT than TG carriers; this was due probably to small sample size in the TT stratum (n=158).

A recent functional and association study of Japanese [8] has identified another causal SNP of *ABCG2*, Q126X, with less frequency of minor allele (1.8% among control), but stronger effect on gout/hyperuricemia than presently-analyzed *ABCG2* (Q141K). A

combination of these two SNP may better predict gout/hyperuricemia in Japanese, which needs to be replicated in community-based samples.

In conclusion, the T allele at rs2231142 in *ABCG2* was associated with higher serum uric acid levels and gout prevalence among Japanese, consistent with the previous results from whites and blacks. The high frequency of this established causal variant among Japanese may have important implications for gout prevalence as more Japanese adopt a Western lifestyle.

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A Conflict of Interest Statement

No Conflict of Interest has been declared by the authors.

Key Messages

A newly identified common causal *ABCG2* variant, rs2231142, has been reported to lead to elevated uric acid levels and prevalent gout among whites and blacks. We examined whether this finding is observed in a Japanese population who had a high prevalence of the risk allele.

Appendix

CIRCS Investigators

The Circulatory Risk in Communities Study (CIRCS) is a collaborative study managed by the Osaka Medical Center for Health Science and Promotion, University of Tsukuba, Osaka University and Ehime University. The CIRCS investigators who contributed to this study are as follows: Kazumasa Yamagishi, Mitsumasa Umesawa, Kyoko Kirii, Choy-Lye Chei, Kimiko Yokota, and Minako Tabata, University of Tsukuba, Tsukuba; Hiroyasu Iso, Tetsuya Ohira, Hironori Imano, Renzhe Cui, and Satoyo Ikehara, Osaka University, Suita; Masamitsu Konishi, Yoshinori Ishikawa, Masakazu Nakamura MD, Akihiko Kitamura, Masahiko Kiyama, Takeo Okada, Kenji Maeda, Masatoshi Ido, Masakazu Nakamura PhD, Takashi Shimamoto, Minoru Iida and Yoshio Komachi, Osaka Medical Center for Health Science and Promotion, Osaka; Shinichi Sato, Chiba Prefectural Institute of Public Health, Chiba; Takeshi Tanigawa, Isao Saito, Katsutoshi Okada, and Susumu Sakurai, Ehime University, Toon; Masayuki Yao, Ranryoen Hospital, Ibaraki; Ai Ikeda and Hiroyuki Noda, Harvard School of Public Health, Boston, MA.

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Table 1. Comparison of the association between rs2231142 polymorphism and prevalent gout in community-based studies

Study	race/ethnicity	sample size	MAF	UA (μmol/L)	gout (%)	aOR* (95%CI)
Framingham	White	7,699	0.11	315.2	2.7	1.97 (1.49-2.59)
Rotterdam	White	4,148	0.12	321.2	3.3	1.71 (1.30-2.25)
ARIC	White	11,024	0.11	350.9	5.4	1.68 (1.38-2.04)
(White pooled)	White	22,871	-	-	-	1.74 (1.51-1.99)
ARIC	Black	3,843	0.03	374.7	8.8	1.71 (1.06-2.77)
CIRCS	Japanese	3,923	0.31	282.1	1.0	2.03 (1.33-3.12)

MAF: minor allele frequency (ie, T allele frequency); UA: uric acid; aOR: additive odds ratio; CI: confidence interval; ARIC: Atherosclerosis Risk in Communities; CIRCS: Circulatory Risk in Communities Study.

* aOR is the odds ratio for gout per copy increment of the T allele, adjusting for age, sex, body mass index, alcohol consumption, hypertension treatment, cohort status in Framingham, cohort and study center in ARIC and CIRCS.

Data other than CIRCS were derived from Dehgan *et al.* [2]

Table 2. Characteristics of study participants according to rs2231142 genotype, 3,923 Japanese men and women aged 40-90.

	rs2231142		
	GG	TG	TT
Men			
Participants (%)	706 (46)	670 (44)	158 (10)
Age, yrs	63.2 (10.3)	63.0 (10.5)	64.1 (9.2)
Body mass index, kg/m^2	23.4 (2.9)	23.7 (3.0)	23.7 (3.2)
Current drinker	495 (70.2)	490 (73.2)	107 (67.7)
Alcohol drinking (g/d)*	29.6 (16.1, 46.0)	29.6 (18.4, 46.0)	29.6 (18.7, 46.0)
Antihypertensive medication	212 (30.0)	186 (27.8)	53 (33.5)
Diabetes†	70 (9.9)	62 (9.3)	16 (10.1)
Uric acid, μmol/L	320.6 (71.2)	334.3 (76.6)	323.7 (77.8)
Gout‡	11 (1.6)	18 (2.7)	8 (5.1)
Gout and/or hyperuricemia§	78 (11)	107 (16)	26 (16.5)
Women			
Participants (%)	1155 (48)	1003 (42)	231 (10)
Age, yrs	61.1 (10.7)	61.4 (10.3)	62.8 (10.0)
Body mass index, kg/m^2	23.7 (3.4)	23.9 (3.5)	23.9 (3.1)
Current drinker	127 (11.0)	112 (11.2)	29 (12.6)
Alcohol drinking (g/d)*	6.6 (3.3-11.5)	6.6 (3.9-11.5)	4.9 (3.0-9.9)
Antihypertensive medication	322 (27.9)	281 (28.0)	72 (31.2)
Diabetes†	57 (4.9)	55 (5.5)	12 (5.2)
Uric acid, μmol/L	250.1 (62.7)	254.8 (59.3)	263.7 (65.9)
Gout‡	4 (0.3)	0 (0.0)	4 (1.7)
Gout and/or hyperuricemia§	16 (1.4)	10 (1.0)	9 (3.9)

Data are mean (SD) or n (percentage) unless indicated otherwise.

*Median (1st and 3rd quartile) values among drinkers presented.

†Diabetes was defined as fasting blood glucose≥7.0mmol/L (126mg/dL) or non -fasting blood glucose≥11.1mmol/L (200mg/dL), and/or treatment of diabetes.

‡Gout was defined as a history of symptoms and/or past or present treatment of gout.

§ Hyperuricemia was defined as uric acid of≥415.9 μmol/L (7.0 mg/dL) , and/or treatment of hyperuricemia.

Table 3. Mean serum uric acid concentration (standard error), prevalence of gout and hyperuricemia according to *ABCG2* rs2231142 genotype, 3,923 Japanese men and women aged 40-90.

	rs2231142			
	GG	TG	TT	p for additive model†
Uric acid concentration (μmol/L)				
Total, n	1,861	1,673	389	
Age, sex and community-adjusted	277.6 (1.6)	286.0 (1.6)	287.1 (3.4)	0.0002
Multivariable adjusted*	278.2 (1.5)	285.5 (1.6)	286.8 (3.3)	0.0006
Men, n	706	670	158	
Age and community-adjusted	320.6 (2.8)	334.1 (2.9)	324.4 (5.9)	0.04
Multivariable adjusted*	321.2 (2.7)	333.7 (2.8)	324.0 (5.7)	0.05
Women, n	1,155	1,003	231	
Age and community-adjusted	250.1 (1.8)	254.9 (1.9)	263.2 (4.0)	0.002
Multivariable adjusted*	250.6 (1.7)	254.4 (1.9)	262.9 (3.9)	0.004
Gout‡, n (%)	15 (1%)	18 (1%)	12 (3%)	
Age, sex and community-adjusted OR	1.0	1.34	4.18	0.002
(95% confidence interval)		(0.67-2.68)	(1.92-9.10)	
Multivariable-adjusted OR*	1.0	1.37	4.37	0.001
(95% confidence interval)		(0.68-2.76)	(1.98-9.62)	
Gout‡ and/or Hyperuricemia§, n (%)	94 (5%)	117 (7%)	35 (9%)	
Age, sex and community-adjusted OR	1.0	1.40	1.90	0.001
(95% confidence interval)		(1.05-1.87)	(1.25-2.90)	
Multivariable-adjusted OR*	1.0	1.40	1.88	0.002
(95% confidence interval)		(1.04-1.87)	(1.23-2.89)	

* Further adjusted for body mass index (kg/m^2), daily alcohol consumption (g/day) and antihypertensive medication use.

† Assigning 0 for GG, 1 for TG, and 2 for TT genotypes.

‡Gout was defined as a history of symptoms and/or past or present treatment of gout.

§ Hyperuricemia was defined as uric acid of≥415.9 μmol/L (7.0 mg/dL), and/or treatment of

hyperuricemia.

Persons missing covariates were eliminated from the multivariable adjusted analyses (n=9 excluded).