SCIENTIFIC REPORTS

OPEN

SUBJECT AREAS:
MISCELLANEOUS RHEUMATIC DISEASES
RISK FACTORS

Received
28 April 2014

Accepted
21 May 2014

Published
9 June 2014

Common dysfunctional variants of *ABCG2* have stronger impact on hyperuricemia progression than typical environmental risk factors

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Gout/hyperuricemia is a common multifactorial disease having typical environmental risks. Recently, common dysfunctional variants of *ABCG2*, a urate exporter gene also known as *BCRP*, are revealed to be a major cause of gout/hyperuricemia. Here, we compared the influence of ABCG2 dysfunction on serum uric acid (SUA) levels with other typical risk factors in a cohort of 5,005 Japanese participants. ABCG2 dysfunction was observed in 53.3% of the population investigated, and its population-attributable risk percent (PAR%) for hyperuricemia was 29.2%, much higher than those of the other typical environmental risks, i.e. overweight/obesity (BMI ≥ 25.0; PAR% = 18.7%), heavy drinking (>196 g/week (male) or >98 g/week (female) of pure alcohol; PAR% = 15.4%), and aging (≥60 years old; PAR% = 5.74%). SUA significantly increased as the ABCG2 function decreased ($P = 5.99 \times 10^{-19}$). A regression analysis revealed that ABCG2 dysfunction had a stronger effect than other factors; a 25% decrease in ABCG2 function was equivalent to "an increase of BMI by 1.97-point" or "552.1 g/week alcohol intake as pure ethanol" in terms of ability to increase SUA. Therefore, ABCG2 dysfunction originating from common genetic variants has a much stronger impact on the progression of hyperuricemia than other familiar risks. Our study provides a better understanding of common genetic factors for common diseases.

Gout, which is characterized by acute arthritis, is a common disease as a consequence of hyperuricemia[1]. In addition to sex, several environmental factors are well-known typical risks of hyperuricemia and gout[2] such as obesity, alcohol consumption, and aging; all of which were first reported by Hippocrates 2,500 years ago[3] and confirmed by modern public health studies[4–6]. Recently, common dysfunctional variants in *ABCG2* gene (also known as *BCRP* gene) that encodes a high-capacity urate exporter were reported to be a major genetic

Table 1 | ABCG2 functions of participants

	Total		Male		Female	
Function of ABCG2	*N*	%	*N*	%	*N*	%
Full function	2,338	46.7	1,592	46.8	746	46.5
3/4 function (mild dysfunction)	1,971	39.4	1,332	39.2	639	39.8
1/2 function (moderate dysfunction)	619	12.4	424	12.5	195	12.2
≤1/4 function (severe dysfunction)	77	1.5	53	1.6	24	1.5
Total	5,005	100.0	3,401	100.0	1,604	100.0

cause of gout in both Caucasian[7] and Japanese[8,9] populations, and their pathophysiological involvement was also reported[10,11]. However, the influence of such genetic traits on serum uric acid (SUA) levels, especially as compared with other typical environmental risk factors, remains to be clarified. In the present study, we show that the dysfunctional *ABCG2* variants, the major genetic factors of SUA, have stronger effects on the risk of hyperuricemia progression than other typical environmental factors.

Results

Genetic risk factor of hyperuricemia in the population. Participants of this study were 5,005 Japanese individuals (Supplementary Table S1) including 831 hyperuricemia patients. Based on the previous studies[8,11], all of the participants were divided into four groups by the combination of common dysfunctional variants of *ABCG2*, non-functional Q126X (rs72552713) and half-functional Q141K (rs2231142), as follows: full function (normal function), 3/4 function (mild dysfunction), 1/2 function (moderate dysfunction) and ≤1/4 function (severe dysfunction) (see Supplementary Figure S1 and Table S2). ABCG2 dysfunction (≤3/4 function) was observed in 53.3% of the total population investigated without obvious difference between sexes (Table 1). We then calculated the population-attributable risk percent (PAR%) of ABCG2 dysfunction for hyperuricemia, which indicates the percentage of hyperuricemic patients originated from ABCG2 dysfunction in the population. Also, PAR% of other typical risks including sex (male), aging (≥60 years old), overweight/obesity (BMI ≥ 25.0), and heavy drinking (more than 196 g/week of pure alcohol for male, and more than 98 g/week of pure alcohol for female)[12,13] was calculated. PAR% of ABCG2 dysfunction for hyperuricemia was 29.2% (95% CI, 22.7–35.5) with a risk ratio (RR) of 1.77 (95% CI, 1.55–2.03; $P = 6.83 \times 10^{-18}$) (Fig. 1), which was much higher than those of other environmental factors, i.e. overweight/obesity (PAR% = 18.7% [95% CI, 14.9–22.6]; RR = 2.01 [95% CI, 1.78–2.28; $P = 1.52 \times 10^{-27}$]), heavy drinking (PAR% = 15.4% [95% CI, 11.5–19.2]; RR = 1.79 [95% CI, 1.57–2.04; $P = 4.03 \times 10^{-18}$]), and aging (PAR% = 5.74% [95% CI, 2.27–9.29]; RR = 1.28 [95% CI, 1.11–1.47; $P = 5.81 \times 10^{-4}$]), although sex difference has the strongest effect (PAR% = 91.7% [95% CI, 88.3–94.9]; RR = 17.3 [95% CI, 11.40–26.38; $P = 5.22 \times 10^{-88}$]). Each dysfunctional group of ABCG2 has PAR% with significant RRs as shown in Fig. 1 (PAR% = 18.0% [95% CI, 12.8–23.2]; RR = 1.64 [95% CI, 1.42–1.90; $P = 5.61 \times 10^{-12}$] for mild dysfunctional group; PAR% = 10.1% [95% CI, 7.36–13.0]; RR = 2.16 [95% CI, 1.81–2.57; $P = 1.61 \times 10^{-17}$] for moderate dysfunctional group; and PAR% = 1.1% [95% CI, 0.194–2.05]; RR = 1.99 [95% CI, 1.31–3.02; $P = 2.13 \times 10^{-3}$] for severe dysfunctional group).

Effect size of ABCG2 dysfunction on SUA. To evaluate the effect size on SUA by each factor, 4,857 individuals, who received no treatment for gout and/or hyperuricemia, were selected from 5,005 participants, and further regression analysis was performed. As shown in Fig. 2 and Supplementary Table S2, SUA was trending upward both in males and females as ABCG2 function decreased. A regression analysis was performed to examine the significance of the effect size of ABCG2 dysfunction as well as other typical factors, which revealed that SUA was significantly affected by both ABCG2 dysfunction and typical risk factors (Table 2). The effect size on SUA, i.e. regression coefficient (β) by a 25% decrease in ABCG2 dysfunction was a gain of 0.193 mg/dl, whereas the effect of other environmental factors were as follows: 1.46 mg/dl between sexes, 4.0×10^{-3} mg/dl by a year-old in age, 0.098 mg/dl by a point of BMI, and 3.5×10^{-4} mg/dl by a gram per week of pure alcohol in alcohol consumption. The ratio of regression coefficients (β_{ABCG2}/β: effect size on SUA by a 25% decrease in ABCG2 dysfunction vs. by each risk factor) showed that ABCG2 dysfunction had a stronger effect than other environmental factors; a 25% decrease in ABCG2 function

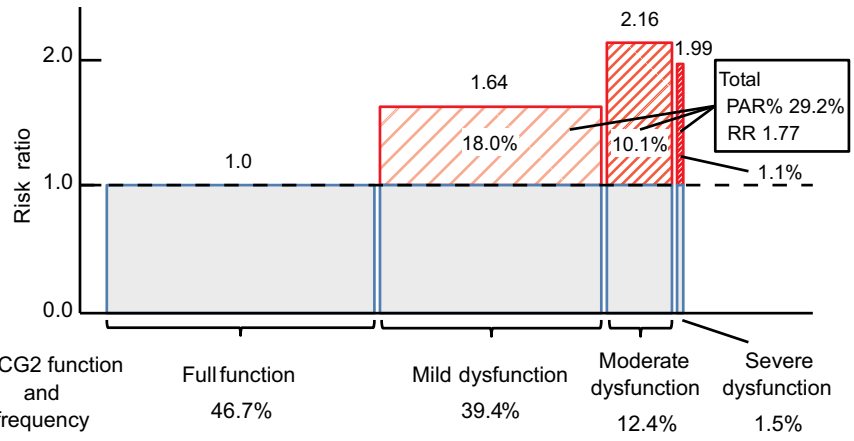

Figure 1 | Population-attributable risk percent (PAR%) of ABCG2 dysfunction for hyperuricemia in 5,005 participants. For the boxes, the red shaded area means PAR% of ABCG2 dysfunction; the width represents the frequency of ABCG2 dysfunction in the population, and the height shows the risk ratio.

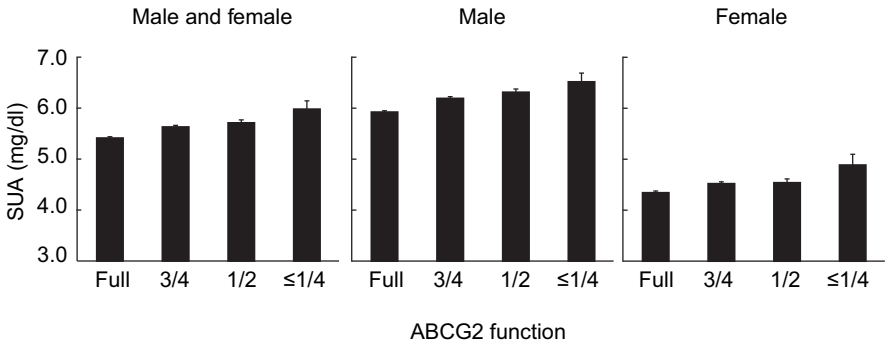

Figure 2 | Serum uric acid (SUA) levels according to each ABCG2 function. All bars show mean ± s.e.m.

showed an effect equivalent to "an increase of BMI by 1.97-point," "552.1 g/week alcohol intake as pure ethanol," or "47.6 years aging" in terms of ability to increase SUA levels.

Discussion

Our study revealed that ABCG2 dysfunction originating from common genetic variants has a much stronger impact on the progression of hyperuricemia than other familiar risk factors except sex. To our knowledge, this is the first study to report that common genetic variants of a common disease showed a stronger effect than typical environmental factors.

ABCG2, also known as a drug exporter BCRP, is expressed on the epithelial cells of small intestine[14] and renal tubules[15]. We have previously shown that ABCG2 is a high-capacity urate transporter which physiologically excretes urate for the regulation of SUA[8,10]. We also found that *ABCG2* has two common dysfunctional variants: a nonsense variant Q126X and a missense variant Q141K[8]. Functional analyses revealed that Q126X is a nonfunctional variant and Q141K is a half-functional variant due to the halved ABCG2 expression on the membrane[8]. Since haplotype frequency analyses demonstrated no simultaneous presence of the minor alleles of Q126X and Q141K in one haplotype, the combination of nonfunctional variant Q126X and half-functional variant Q141K makes it possible to estimate dysfunctional levels of ABCG2[8,10] (Supplementary Figure S1 and Table S2). In the present study, any ABCG2 dysfunction was proved to be commonly observed in the Japanese population (53.3%). Such a high-frequency dysfunction of ABCG2 implies the importance of ABCG2 as a risk factor for these common diseases, hyperuricemia and gout, and therefore indicates the usefulness of screening high-risk individuals.

PAR% of ABCG2 dysfunction was 29.2% and much higher than those of other typical risk factors, including overweight/obesity, heavy drinking, and aging. This result indicates that about 30% of hyperuricemia patients in the Japanese population originate from ABCG2 dysfunction, and other environmental factors did not show such impact. As shown in Fig. 1, RRs of hyperuricemia increased as the ABCG2 function decreased. Lower RR in severe ABCG2 dysfunction as compared with that in moderate dysfunction may be ascribed to the relatively few hyperuricemia patients with severe dysfunction (n = 18). As for the relationship between SNPs and the risk of hyperuricemia, Woodward *et al.* so far reported that the PAR% of Q141K for gout was 10% in Caucasians[7], and Yamagishi *et al.* indicated that PAR% for gout and/or hyperuricemia was 19% in Japanese[16]. Ours is the first report to show the PAR% of ABCG2 dysfunction using the combination of Q126X and Q141K for functional evaluation. It is reasonable that the PAR% of Q141K in Caucasians would be lower than that in Japanese, because the minor allele frequency in Caucasians (0.11 according to Woodward *et al.*[7]) is lower than those of Japanese (0.31 by Yamagishi *et al.*[16] and 0.29 in the present study). When we re-calculated PAR% according to the definition of hyperuricemia in Yamagishi *et al.*[16] (SUA ≥ 7.0 mg/dl), the resulting PAR% of Q141K was 22.2%, which is comparable to that in Yamagishi *et al.*[16]. In the present study, we defined hyperuricemia as SUA >7.0 mg/dl[17] and obtained the PAR% of Q141K and Q126X as 23.5% and 2.6%, respectively. Moreover, the results imply that our approach using the combination of the two variants (PAR% = 29.2%) is more effective and useful than using each variant.

Accordingly, common dysfunctional ABCG2 in the population and high PAR%, imply the importance of *ABCG2* genotyping for the screening of high-risk individuals for hyperuricemia/gout.

Subsequent regression analysis revealed that ABCG2 dysfunction defined by the combination of Q126X and Q141K significantly increased SUA, while previous studies showed the association of SUA and only Q141K[7,8]. The effect size, or regression coefficient (β), of a 25% decrease in ABCG2 function for SUA was 0.193 mg/dl, and mean SUA of participants who have severe dysfunction (≤1/4 function) and full function are 5.98 mg/dl and 5.41 mg/dl, respectively, a difference of 0.57 mg/dl (Supplementary Table S2). Hyperuricemia (SUA > 7.0 mg/dl) is more common in males due to the SUA-lowering effect by female hormone. Considering that the difference of SUA between sexes is approximately 1.5 mg/dl, the effect of ABCG2 function is strong enough as a factor in SUA

Table 2 | Effect of ABCG2 dysfunction and other risk factors on SUA levels in 4,857 individuals

Risk factors	β‡ (regression coefficient)	95% CI	*P* value	β_{ABCG2}/β (ratio of regression coefficients)
ABCG2 function*	0.193	0.150–0.235	5.99×10^{-19}	1.00
Sex†	1.46	1.38–1.53	2.34×10^{-296}	0.13
Age, years	4.0×10^{-3}	4.5×10^{-4}–7.6×10^{-3}	0.028	47.6
BMI, kg/m²	0.098	0.087–0.108	1.29×10^{-68}	1.97
Alcohol consumption, g/week of pure alcohol	3.5×10^{-4}	1.7×10^{-4}–5.3×10^{-4}	1.77×10^{-4}	552.1

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).
*Calculation for ABCG2 function was conducted for full function as 1, 3/4 function (mild dysfunction) as 2, 1/2 function (moderate dysfunction) as 3, and ≤1/4 function (severe dysfunction) as 4.
†Calculation for sex was conducted for female as 1 and male as 2.
‡"β" indicates the increase of SUA (mg/dl) per unit of each risk factor. The ratio of regression coefficients (β_{ABCG2}/β) was calculated from the β of ABCG2 function divided by that of each risk factor, showing an effect equivalent to a 25% decrease in ABCG2 function in terms of ability to increase SUA levels.

increase. The effect of age (4.0×10^{-3} mg/dl), BMI (0.098 mg/dl), or alcohol consumption (3.5×10^{-4} mg/dl) was a significant factor in the increase of SUA, but we found that the effect of ABCG2 dysfunction was stronger than those of the typical environmental factors.

The ratios of regression coefficient (β_{ABCG2}/β) for BMI and alcohol consumption were 1.97 and 552.1, respectively. This indicates that a decrease of 25% in ABCG2 function had the power to raise SUA levels comparable to "gaining a body weight of 5.7 kg for a 170 cm-tall man," or "drinking 1.7 L of whiskey every week." Although both obesity/overweight and drinking alcohol are especially targeted as the first step for assessment of gout/hyperuricemia in guidelines[17–19], genotyping of ABCG2 is revealed to be essential for the risk estimation of gout/hyperuricemia.

Our results in the present study show that genetic factor *ABCG2* should be considered to be one of the common risks of hyperuricemia/gout, which is stronger than other typical environmental risks. Since ABCG2 dysfunction can be estimated easily by genotyping only two variants[8–11], our findings will help to recognize a trait of hyperuricemia at a very early stage and to assist prevention and treatment for hyperuricemia and ultimately for gout.

Methods

Study participants. All procedures involved in this study were approved by the institutional ethical committees (National Defense Medical College and Nagoya University), and were performed in accordance with the Declaration of Helsinki. All of the 5,005 Japanese individuals in this study were recruited from Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study)[20]. Written informed consent was obtained from all subjects. Hyperuricemia was defined as the SUA level that exceeds 7.0 mg/dl (=416.36 μmol/l). Alcohol consumption was calculated from the participants' written questionnaires as shown in Supplementary Table S3. Among the 5,005 participants, those who were under treatment for or had past histories of gout/hyperuricemia were excluded; then multiple regression analysis was performed for 4,857 individuals to evaluate the relationship among SUA levels, ABCG2 dysfunction, and other risk factors.

Genetic analysis. Genomic DNA was extracted from whole peripheral blood cells[21]. Genotyping of the two variants in *ABCG2* gene, Q126X and Q141K, was performed with a LightCycler 480 (Roche Diagnostics) by high resolution melting (HRM) analysis[22]. To confirm the genotypes, more than one hundred samples were subjected to direct sequencing. DNA sequencing analysis was performed with a 3130xl Genetic Analyzer (Applied Biosystems)[23]. The MAFs of Q126X and Q141K were 0.025 and 0.294, respectively, and both variants were in Hardy-Weinberg equilibrium ($P > 0.05$). ABCG2 function was estimated from the genotype combination as shown in Supplementary Figure S1 and Table S2.

Statistical analysis. For all calculations in the statistical analysis, a software R (version 3.0.2) (http://www.r-project.org/) and a software SPSS v.17.0J (IBM Japan Inc., Tokyo, Japan) were used. The PAR% of ABCG2 dysfunction and other typical risk factors for hyperuricemia was calculated from the following equation:

$$\mathrm{PAR\%} = [\{(N_{HUA,Risk}/N_{Risk} - N_{HUA,NonRisk}/N_{NonRisk}) \times (N_{Risk}/N_{All})\}/(N_{HUA}/N_{All})] \times 100$$

("$N_{HUA,Risk}$" and "$N_{HUA,NonRisk}$" indicate the numbers of hyperuricemia patients in the risk group and non-risk group, respectively. "N_{Risk}" and "$N_{NonRisk}$" represent the numbers of individuals in the risk group and non-risk group, respectively. "N_{HUA}" and "N_{All}" mean the number of all hyperuricemia patients and all participants, respectively.)

For the robustness of statistics, random resampling methods with computer simulation are often applied[24,25]. In this study, to evaluate the 95% CI of PAR%, the bootstrap method[25] was used for random resampling of all participants' data set with replacement for 10,000 times.

The linear regression analysis was conducted with the model in which ABCG2 dysfunction, sex, age, BMI, and alcohol consumption were included.

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Acknowledgments

We would like to thank all the participants involved in this study. We also thank K. Gotanda, H. Amaki, Y. Morimoto, N. Katsuta, C. Okada, H. Inoue, H. Ogata, S. Tatsukawa, and Y. Tanahashi (National Defense Medical College, Tokorozawa, Japan), M. Naito and N. Hamajima (Nagoya University, Nagoya, Japan) for collecting samples and genetic analysis. This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (including the Grant-in-Aid for Scientific Research on Innovative Areas "Genome Science"), the Ministry of Health, Labour and Welfare of Japan, the Ministry of Defense of Japan, the Japan Society for the Promotion of Science, the Takeda Science Foundation, the AstraZeneca VRI Research Grant, the Kawano Masanori Memorial Foundation for Promotion of Pediatrics, and the Gout Research Foundation of Japan.

Author contributions

A.N., H.M. and N.S. conceived and designed this study. H. Nakaoka, T.N., H. Nakashima, Y. Sakurai and K.I. assisted with research design. K.W., S.K., R.O. and T. Tamura collected samples and analyzed clinical data. H.M., Y.T., Y.O., M.S., S.S., Y.K., T.C., J.A., Y. Shichijo and A.A. performed genetic analysis. A.N., H. Nakaoka, T.N. and H. Nakashima performed statistical analysis. A.N., H.M., H. Nakaoka, T.N., H. Nakashima analyzed data. T. Takada,

H.S., T.H., Y. Sakurai and K.I. provided intellectual input and assisted with the preparation of the manuscript. A.N., H.M. and N.S. wrote the manuscript. A.N. and H.M. contributed equally to this work.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: There is potential competing interest: H.M., T.N., T. Takada, K.I. and N.S. have a patent pending based on the work reported in this paper. The other authors declare that they have no conflict of interest.

How to cite this article: Nakayama, A. *et al.* Common dysfunctional variants of *ABCG2* have stronger impact on hyperuricemia progression than typical environmental risk factors. *Sci. Rep.* **4**, 5227; DOI:10.1038/srep05227 (2014).