Independent effects of ADH1B and ALDH2 common dysfunctional variants on gout risk

Masayuki Sakiyama1,2, Hirotaka Matsuo1, Airi Akashi1, Seiko Shimizu1, Toshio Higashino3, Makoto Kawaguchi3, Akiyoshi Nakayama1, Mariko Naito3, Sayo Kawai3, Hiroshi Nakashima4, Yutaka Sakurai6, Kimiyoshi Ichida5, Toru Shimizu6, Hiroshi Ooyama7 & Nariyoshi Shinomiya1

Gout is caused by hyperuricemia, with alcohol consumption being an established risk factor. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are crucial enzymes for alcohol metabolism. We recently performed a genome-wide association study of gout and a subsequent fine-mapping study which identified rs671 of ALDH2 as a gout locus. However, the association between gout and common variants of ADH1B has hitherto remained unreported, prompting us to investigate the association between gout and common dysfunctional variants of ADH1B (rs1229984) and ALDH2 (rs671). We used 1,048 clinically defined gout cases and 1,334 controls of Japanese male. The “His carrier” (His/His or His/Arg) of rs1229984 (His48Arg) of ADH1B significantly increased gout risk (P = 4.3 × 10−4, odds ratio = 1.76), as did the “non-Lys carrier (Glu/Glu)” of rs671 (Glu504Lys) of ALDH2. Furthermore, common variants of ADH1B and ALDH2 are independently associated with gout. Our findings likewise suggest that genotyping these variants can be useful for the evaluation of gout risk.

Gout is an increasingly common disease resulting from hyperuricemia, which causes acute arthritis. Several genes have been reported to be associated with gout1–5. Some urate transporter genes, such as ABCG26–8, SLC2A93, 4, SLC17A13, 9 and SLC22A1210, have major effects on the progression of gout/hyperuricemia. Certain environmental factors appear also to be risk factors for gout/hyperuricemia, of which alcohol consumption is one of the best known. Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase (ADH), and acetaldehyde is further metabolized to acetate by aldehyde dehydrogenase (ALDH)11. These processes crucially depend on ADH1B and ALDH2, respectively (Fig. 1). We recently performed a genome-wide association study (GWAS) of gout4 followed by a fine-mapping study12 that identified rs671 (Glu504Lys) of ALDH2 as a gout locus12. On the other hand, to our knowledge, the association between gout and common variants of ADH1B has not hitherto been reported.

Additionally, there are no association analysis reports between gout and common variants of ADH1B and ALDH2 that include adjustment for alcohol consumption. We therefore performed an association analysis between gout and a common dysfunctional variant of ADH1B, rs1229984 (His48Arg). We further investigated the effects of alcohol consumption on the association between gout and common variants of ADH1B and ALDH2.

Results

Association analysis between gout and common variants of ADH1B and ALDH2. We performed genotyping of rs1229984 (His48Arg) of ADH1B using 1,048 clinically defined gout cases and 1,334 controls of Japanese male (Table 1). The results are shown in Table 2 and Supplementary Table S1. The call rate for rs1229984 was 98.4%: this variant in the control group was in Hardy-Weinberg equilibrium (P > 0.05). The common

References

1Department of Integrative Physiology and Bio-Nano Medicine, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama, 359-8513, Japan. 2Department of Dermatology, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama, 359-8513, Japan. 3Department of Preventive Medicine, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi, 466-8550, Japan. 4Department of Preventive Medicine and Public Health, National Defense Medical College, 3-2 Namiki, Tokorozawa, Saitama, 359-8513, Japan. 5Department of Pathophysiology, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo, 192-0392, Japan. 6Kyoto Industrial Health Association, 67 Kitatsuboi-cho, Nishinokyo, Nakagyo-ku, Kyoto, 604-8472, Japan. 7Ryougoku East Gate Clinic, 3-21-1 Ryougoku, Sumida-ku, Tokyo, 130-0026, Japan. Masayuki Sakiyama and Hirotaka Matsuo contributed equally to this work. Correspondence and requests for materials should be addressed to H.M. (email: hmatsuo@ndmc.ac.jp)
Figure 1. Ethanol oxidation by ADH and ALDH. Ethanol is oxidized to acetaldehyde by alcohol dehydrogenase (ADH), and acetaldehyde is further metabolized to acetate by aldehyde dehydrogenase (ALDH).

Table 1. Clinical characteristics of participants. Plus-minus values are means ± SD. *Participants who consumed alcohol at least once a month were classified as drinkers.

Table 2. Association analysis between gout and two common variants of ADH1B and ALDH2. Abbreviations: SNP = single nucleotide polymorphism; OR = odds ratio; CI = confidence interval; ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase. †The ORs were calculated per allele model. ‡The genotyping results of rs671 are obtained from our previous report 12.

Table 3. Logistic regression analysis of the association between gout and ALDH2 rs671 genotype. Odds ratios (ORs) are shown as an adjusted for alcohol consumption, BMI, and age (Table 2).
of ALDH2 significantly decrease the risk of gout (P = 3.8 × 10^{-19} and 4.8 × 10^{-17}; OR = 0.45 and 0.41, respectively) as compared with the G/G (Glu/Glu) genotype; however, there is no significant difference in effect sizes on gout between A/G (Lys/Glu) and A/A (Lys/Lys) genotypes (P = 0.71), and the OR is close to 1.00 (OR = 1.09; 95% CI: 0.75–1.62). Based on these results and enzyme activity,6 the “non-Lys carrier (Glu/Glu)” (high tolerance for alcohol) vs. “Lys carrier (Lys/Glu or Lys/Lys)” (low tolerance for alcohol) model was used for the following analysis. We also performed a multivariate logistic regression analysis that included alcohol consumption in the model because ALDH2 genotypes were significantly associated with the proportion of non-drinkers (P = 2.5 × 10^{-83}; 93.5% for A/A, 32.2% for A/G and 6.3% for G/G; Supplementary Table S2) and alcohol consumption in controls (P = 2.0 × 10^{-3}; 6.8 g/week for A/A, 91.2 g/week for A/G and 231.0 g/week for G/G; Supplementary Table S2). The association between gout and rs671 of ALDH2 remained significant even after adjustment for alcohol consumption (P = 4.3 × 10^{-11}; OR = 1.92; 95% CI: 1.60–2.31: Supplementary Table S3). Contrary to the result for ADH1B, this association was still significant in the analysis conducted in both non-drinkers only and drinkers only, and the direction of OR and the effect size were similar to those obtained in the analysis conducted in all participants (P = 0.021 and 7.2 × 10^{-11}; OR = 1.93 and 1.92; 95% CI: 1.12–3.33 and 1.58–2.34, respectively: Table 3).

**Gout risk due to combination of the ADH1B and ALDH2 genotypes.** Next, we investigated the combined effects on gout of the common variants of ADH1B (rs1229984) and ALDH2 (rs671). Based on enzyme activity,13–15, the “His carrier (His+−)” vs. “non-His carrier (His−)” model was selected for the association analysis between gout and rs1229984 (His48Arg) of ADH1B. The association analysis between gout and rs671 (Glu504Lys) of ALDH2, we adopted the “non-Lys carrier (Lys−)” vs. “Lys carrier (Lys+)” model as described in our previous paper.12 Individuals whose combination of rs1229984 and rs671 is “His−/Lys−”, “His+−/Lys−” or “His+/Lys−” were subject to a significantly lower risk of gout (P = 3.0 × 10^{-3}, 2.9 × 10^{-3} and 8.7 × 10^{-2}, respectively) than the other group (“His+/Lys−”), as shown in Table 4. Furthermore, although the 95% CIs overlap each other, the OR of “His−/Lys−” (OR = 0.36; 95% CI: 0.18–0.71) is lower than those of “His+/Lys−” and “His+/Lys+” (OR = 0.44 and 0.42; 95% CI: 0.25–0.75 and 0.36–0.51, respectively).

| Gene | SNP | Genotype | Amino acid | All participants | Only drinkers | Only non-drinkers |
|------|-----|----------|------------|-----------------|--------------|------------------|
|      |     |          |            | Gout cases Controls | P value | OR (95%CI) | Gout cases Controls | P value | OR (95%CI) | Gout cases Controls | P value | OR (95%CI) |
| ADH1B | rs1229984 | A/A or A/G | His carrier | 991 1,249 | 4.3 × 10^{-14} | 1.76 (1.15–2.69) | 877 941 | 0.013 | 1.77 (1.13–2.78) | 114 299 | 0.24 | 2.48 (0.55–11.2) |
|       |     | G/G non-His carrier | 32 71 | — | Reference | 30 57 | — | Reference | 2 13 | — | Reference |
| ALDH2 | rs671 | G/G non-Lys carrier | 729 670 | 2.9 × 10^{-11} | 2.27 (1.92–2.69) | 703 625 | 7.2 × 10^{-11} | 1.92 (1.58–2.34) | 26 40 | 0.021 | 1.93 (1.12–3.33) |
|       |     | A/A or A/G Lys carrier | 318 664 | — | Reference | 226 386 | — | Reference | 92 273 | — | Reference |

Table 3. Effect of ADH1B and ALDH2 genotypes and alcohol consumption on gout susceptibility. Abbreviations: OR = odds ratio; CI = confidence interval; His = histidine; Lys = lysine. *Participants who consumed alcohol less than once a month were classified as non-drinkers. †The P values were calculated using logistic regression analysis. For rs1229984 (His48Arg), A/A (His/His) or A/G (His/Arg) genotype (His carrier, high tolerance for alcohol) is a risk, so the “His carrier” vs. “non-His carrier” model was used for the analysis of rs1229984. §For rs671 (Glu504Lys), G/G (Glu/Glu) genotype (non-Lys carrier, high tolerance for alcohol) is a risk, so the “non-Lys carrier” vs. “Lys carrier” model was used for rs671.

| rs1229984 (ADH1B) | rs671 (ALDH2) | Gout cases Controls | P value | OR (95% CI) |
|-------------------|--------------|-------------------|---------|-------------|
| His− | Lys+ | 12 30 | 3.0 × 10^{-3} | 0.36 (0.18–0.71) |
| His− | Lys− | 20 41 | 2.9 × 10^{-3} | 0.44 (0.25–0.75) |
| His+ | Lys+ | 297 628 | 8.7 × 10^{-22} | 0.42 (0.36–0.51) |
| His+ | Lys− | 693 621 | — | Reference |

Table 4. Gout risk due to combination of ADH1B and ALDH2 genotypes. Abbreviations: His = histidine; Lys = lysine; OR = odds ratio; CI = confidence interval. *In the analysis of rs1229984 (His48Arg), “His+” and “His−” mean His carrier (His/His or His/Arg) and non-His carrier (Arg/Arg), respectively. In the analysis of rs671 (Glu504Lys), “Lys+” and “Lys−” mean Lys carrier (Lys/Lys or Lys/Glu) and non-Lys carrier (Glu/Glu), respectively. We investigated the combined effects of rs1229984 and rs671 on gout as compared with “His+/Lys−”. The P value was calculated using logistic regression analysis.
Discussion

ADH1B and ALDH2 are crucial enzymes for alcohol metabolism, and it is already established that individual differences in these two enzymes’ activities are caused by common variants\(^{15}\). The functionally important variants for ADH1B are rs1229984 (His/48Arg) and rs2066702 (Arg370Cys)\(^{17-20}\). The allele frequencies of rs1229984 and rs2066702 of ADH1B differ among populations, according to the results of a previous paper\(^{17,21}\) and 1000 Genomes Phase 3\(^{22}\), rs1229984 is polymorphic in Europeans and East Asians, including Japanese, while it is monomorphic in Africans. On the other hand, rs2066702 is monomorphic in Europeans and East Asians but polymorphic in Africans. In this study, therefore, we genotyped rs1229984 with Japanese participants. Because the A/A (His/His) or A/G (His/Arg) genotype of rs1229984 has been reported to produce 40-fold faster ethanol oxidation than the G/G (Arg/Arg) genotype\(^{15}\), in the present study, we investigated not only the genotype model but also the “His carrier” vs. “non-His carrier” model for the analysis of rs1229984. Regarding the analysis of ALDH2, rs671 (Glu504Lys) is a noted functional variant\(^{16,22}\). The Lys allele of rs671 is common in East Asians, but quite rare in Europeans and Africans\(^{20,23}\). Individuals with heterozygotes (Lys/Glu) of rs671 have only 6.25% of the enzyme activity of those with normal ALDH2 (Glu/Glu), and those with homozygotes (Lys/Lys) show almost no activity\(^{16}\). We therefore adopted the “non-Lys carrier” vs. “Lys carrier” model for rs671 in the present study.

No reports on the association between gout and common variants of ADH1B have been published, although Yokoyama et al. recently reported that a common dysfunctional variant of ADH1B, rs1229984, is associated with serum uric acid (SUA) levels in male Japanese alcoholics\(^{24}\). In this study, for the first time, we revealed a significant association between a common dysfunctional variant of ADH1B (rs1229984) and gout (Table 2 and Supplementary Table S1).

We previously reported the association between gout and rs671 of ALDH2\(^{25}\). Other Japanese\(^{26}\) and Chinese\(^{27}\) studies have also indicated this association. However, in these studies\(^{2,24,25}\), alcohol consumption was not taken into consideration, even though rs671 is associated with alcohol consumption (Supplementary Table S2). Thus, we first investigated the association between gout and rs671 of ALDH2 including alcohol consumption in the model. The common dysfunctional variant of ALDH2, rs671, also showed a significant association with gout, even after adjustment for alcohol consumption (Supplementary Table S3) and even in non-drinkers or in drinkers (Table 3). On the other hand, although the association between gout and rs1229984 of ADH1B was still significant even after adjustment for alcohol consumption (Supplementary Table S3) and in drinkers (Table 3), this association was not significant in non-drinkers (Table 3). Because the sample size of non-drinkers was relatively small, further studies are necessary to clarify the effects of alcohol consumption on the association between gout and common variants of ADH1B and ALDH2.

It appears that alcohol intake elevates SUA level by increasing urate production\(^{26,27}\) and decreasing renal urate excretion\(^{28}\). Ethanol is oxidized to acetate mainly by ADH1B and ALDH2 (Fig. 1). When acetate is further metabolized to acetaldehyde, aminotransferase A, adenosine triphosphate (ATP) hydrolyzes to adenosine monophosphate (AMP), which is ultimately metabolized to urate. Thus, alcohol consumption could increase urate by enhancing hydrolysis from ATP to AMP\(^{27}\). Furthermore, the “His+/Lys−” genotype combination causes faster ethanol and acetaldehyde elimination and may accelerate the increase in ATP degradation, which further elevates SUA\(^{23}\). This may be one of the reasons why “His+/Lys−” tends to have a stronger effect on gout than other genotype combinations, in spite of the 95% CIs overlapping each other (Table 4). It is also well known that alcohol consumption can increase lactate\(^{29}\) which is exchanged for urate via urate transporter 1 (URAT1/SLC22A12) in the human kidney\(^{30}\). Therefore, alcohol consumption could also increase the SUA level by enhancing the renal urate reabsorption via URAT1. Taking into consideration the factors mentioned above, alcohol consumption could increase the risk of gout susceptibility resulting from hyperuricemia. ADH1B and ALDH2 enzyme activities, which depend on the common variants, affect alcohol consumption behavior, and the genotyping of ADH1B and ALDH2 variants can be a surrogate for alcohol consumption in the estimation of risks for several diseases, including esophageal cancer, which were demonstrated by Mendelian randomization approaches\(^{31,32}\). Thus, we initially assumed that the associations between gout and common variants of ADH1B and ALDH2 would be accounted for by alcohol consumption. Contrary to this expectation, these associations were still significant even after adjustment for alcohol consumption (Supplementary Table S3), which indicates that common variants of ADH1B and ALDH2 can be associated with gout susceptibility through not only alcohol consumption but also other factors and/or mechanisms. However, the association of ADH1B was not significant in non-drinkers (Table 3). This study had several limitations in that we were able to use only the frequency data, not the quantity data, on alcohol consumption by gout cases. Similarly, the adjustment for alcohol consumption might not be sufficient because these alcohol-drinking data were self-reported, and it is difficult to obtain data on lifetime alcohol consumption. A further problem is that adjustment of the association between these genetic variants and gout for alcohol consumption could also lead to collider bias. It is similar that the adjustment for cigarettes smoked per day does not entirely mediate the relationship between genetic variants and lung cancer: this is most likely due to the fact that daily cigarette consumption does not accurately capture total tobacco exposure\(^{33}\). Therefore, from the point of view of alcohol consumption, further studies are necessary to be able to elucidate the association between gout and common variants of ADH1B and ALDH2.

In summary, our data show that common variants of ADH1B (rs1229984) and ALDH2 (rs671) are independently associated with gout, which indicates that the genotyping of rs1229984 and rs671 can be useful for the evaluation of gout risk.

Methods

Study participants. This study was approved by the institutions’ Ethical Committees (National Defense Medical College and Nagoya University). All procedures were performed in accordance with the Declaration of Helsinki, with written informed consent obtained from each subject. In this study, all the participants were Japanese males: the frequency of Japanese female gout patients is extremely low, at about only 1% of the entire population. Therefore, our data provide useful genetic information for the evaluation of gout risk. In this study, we provided detailed information on alcohol consumption behavior, including smoking habits and alcohol-related diseases, and we also provided detailed information on alcohol consumption behavior, including smoking habits and alcohol-related diseases. In this study, therefore, we genotyped rs1229984 with Japanese participants. Because the A/A (His/His) or A/G (His/Arg) genotype of rs1229984 has been reported to produce 40-fold faster ethanol oxidation than the G/G (Arg/Arg) genotype\(^{15}\), in the present study, we investigated not only the genotype model but also the “His carrier” vs. “non-His carrier” model for the analysis of rs1229984. Regarding the analysis of ALDH2, rs671 (Glu504Lys) is a noted functional variant\(^{16,22}\). The Lys allele of rs671 is common in East Asians, but quite rare in Europeans and Africans\(^{20,23}\). Individuals with heterozygotes (Lys/Glu) of rs671 have only 6.25% of the enzyme activity of those with normal ALDH2 (Glu/Glu), and those with homozygotes (Lys/Lys) show almost no activity\(^{16}\). We therefore adopted the “non-Lys carrier” vs. “Lys carrier” model for rs671 in the present study.

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In summary, our data show that common variants of ADH1B (rs1229984) and ALDH2 (rs671) are independently associated with gout, which indicates that the genotyping of rs1229984 and rs671 can be useful for the evaluation of gout risk.
population of gout patients that we analyzed. The gout cases comprised 1,048 patients assigned from Japanese male outpatients at the gout clinics of Kyoto Industrial Health Association (Kyoto, Japan) or Ryugoku East Gate Clinic (Tokyo, Japan). All patients were clinically diagnosed with primary gout according to the criteria established by the American College of Rheumatology. Patients with inherited metabolic disorders, including Lesch–Nyhan syndrome, were excluded. For the control group, 1,334 Japanese males with SUA levels of $\leq 7.0$ mg/dl and without a history of gout were recruited from the participants in the Shizuoka area in the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study)\textsuperscript{35,36}. Participants who consumed alcohol at least once a month were classified as drinkers. In the controls, the information on alcohol consumption was collected at the point of recruitment into the study. Meanwhile, in the gout cases, we used information on alcohol consumption at the point of gout onset. There is detailed information on alcohol consumption for the controls: we show and analyze the amount of alcohol consumption data for each genotype (Supplementary Table S2). On the other hand, the information on alcohol consumption in gout cases was limited to whether the subject is a drinker or non-drinker. Thus, in this study, the adjustment for alcohol consumption was performed using the classification of drinker or non-drinker. The details on the participants in this study are shown in Table 1.

**Genetic analysis.** Genomic DNA was extracted from whole peripheral blood cells\textsuperscript{37}. Genotyping of rs1229984 of ADH1B was performed using the TaqMan method (Thermo Fisher Scientific, Waltham, MA, USA) employing a LightCycler 480 (Roche Diagnostics, Mannheim, Germany)\textsuperscript{37} with minor modifications. The custom TaqMan assay probe was designed as follows: VIC-CTGTAGGATTCTGTCACACAG and FAM-GTGTAGGATCTGTCGCCACAG. Genotyping data on rs671 of ALDH2 was obtained from our previous study\textsuperscript{45}.

**Statistical analyses.** R-3.1.1 (http://www.r-project.org/) software was used for all calculations in the statistical analysis\textsuperscript{46}. The association analyses were examined using Fisher's exact test, Cochran-Armitage test, linear regression analysis and logistic regression analysis. All P values were two-tailed and P values of $< 0.05$ were regarded as statistically significant.

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**Acknowledgements**

We would like to thank all the participants for their generous involvement in this study. Our sincere gratitude also goes to the members of the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) Shizuoka Field for supporting the study. We are indebted to K. Gotanda, Y. Morimoto, M. Miyazawa, Y. Kawamura, T. Chiba, H. Inoue, M. Komatsu, R. Sugiyama and T. Nakamura at National Defense Medical College for genetic analysis and helpful discussions, and to A. Tokumasu and K. Ooyama at Ryougoku East Gate Clinic and K. Wakai and N. Hamajima at Nagoya University for sample collection. This study was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan including the MEXT Kakenhi Grant (Grant numbers 253023145 and 15K15227), the Ministry of Health, Labour and Welfare of Japan, the Ministry of Defense of Japan, the Japan Society for the Promotion of Science, the Kawano Masanori Memorial Foundation for Promotion of Pediatrics, and the Gout Research Foundation of Japan. The study was also supported by a JSPS Kakenhi Grant (Grant number 16H06277) and Grants-in-Aid for Scientific Research on Priority Areas (Grant number 17015018) and Innovative Areas (Grant numbers 221S0001 and 221S0002) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

**Author Contributions**

M.S. and H.M. conceived and designed this study. M.N., S.K., T.S. and H.O. collected samples and analyzed the clinical data. M.S., H.M., A.A., S.S., T.H., M.K. and A.N. performed genetic analyses. M.S., H.M. and H.N. performed statistical analyses. Y.S., K.I. and N.S. provided intellectual input and assisted with the preparation of the manuscript. M.S. and H.M. wrote the manuscript. M.S. and H.M. contributed equally to this work.

**Additional Information**

**Supplementary information** accompanies this paper at doi:10.1038/s41598-017-02528-z

**Competing Interests:** The authors declare that they have no competing interests.

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