

## Common variant of *ALPK1* is not associated with gout: a replication study

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**Abstract** Gout is one of the most kinds of common inflammatory arthritis as a consequence of hyperuricemia. Alpha-protein kinase 1 (*ALPK1*) gene locates in a gout-susceptibility locus on chromosome 4q21–31, and encodes ALPK1 protein which plays a pivotal role in the phosphorylation of myosin 1. In the previous genetic study of Taiwanese populations, 3 single nucleotide polymorphisms (SNPs), rs11726117, rs231247 and rs231253, in *ALPK1* gene were reported to have a significant association with gout. However, no replication study has been performed to confirm this association. Therefore, we first conducted a replication study with clinically defined gout patients in a

different population. Linkage disequilibrium (LD) analyzes of the 3 SNPs in *ALPK1* revealed that these SNPs are in strong LD in a Japanese population. Among the 3 SNPs of *ALPK1*, rs11726117 (M861T) is the only missense SNP. Therefore, rs11726117 was genotyped in a Japanese population of 903 clinically defined gout cases and 1,302 controls, and was evaluated for a possible association with gout. The minor allele frequencies of rs11726117 were 0.26 and 0.25 in the case and control groups, respectively. The association analysis has not detected a significant association between rs11726117 and gout susceptibility in a Japanese population ( $p = 0.44$ ). Because *ABCG2*, a major causative gene for gout, also locates in the gout-susceptibility locus on chromosome 4q, these findings suggest that among genes in a gout-susceptibility locus, not *ALPK1* but *ABCG2* could be important as a gout-susceptible gene.

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### Introduction

Gout, a multifactorial disease, is characterized by acute inflammatory arthritis which induces severe painful attacks. Gout is caused as a consequence of hyperuricemia. Previous genetic studies have revealed that gout has associations with various genes such as ATP-binding cassette transporter, subfamily G, member 2 (*ABCG2/BCRP*) [1–4], monocarboxylate transporter 9 (*MCT9/SLC16A9*) [5], organic anion transporter 4 (*OAT4/SLC22A11*) [6], leucine-rich repeat-containing 16 A (*LRRC16A/CARMIL*) [7], and alpha-protein kinase 1 (*ALPK1*) [8].

*ALPK1* is thought to play a pivotal role in the phosphorylation of myosin 1 and the apical trafficking of raft-

associated sucrose–isomaltase [9]. In the previous study of Taiwanese Han and Taiwan aborigines, Ko et al. [8] reported that 3 single nucleotide polymorphisms (SNPs), rs11726117, rs231247 and rs231253, in *ALPK1* gene are associated with gout. However, no replication study has been performed to confirm the association between *ALPK1* and gout.

In the present study, we therefore investigated the association between gout and *ALPK1* with Japanese gout cases and controls.

## Materials and methods

### Study participants

As cases, 903 male Japanese patients with primary gout were collected from the outpatients of Midorigaoka Hospital (Osaka, Japan), Kyoto Industrial Health Association (Kyoto, Japan) and Jikei University Hospital (Tokyo, Japan). Gout diagnoses were obtained according to the criteria established by the American College of Rheumatology [10]. For controls, 1,302 male Japanese individuals were collected from the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) [11]. Exclusion criteria for the controls were high serum uric acid (SUA) levels ( $>7.0$  mg/dl) and any gout history. The mean ages with standard deviation of case and control groups were  $55.2 \pm 12.9$  and  $52.7 \pm 8.4$  years old, respectively, and their respective mean body-mass index was  $24.7 \pm 3.3$  and  $23.2 \pm 2.8$  kg/m<sup>2</sup>. In this study, all subjects provided written informed consent. This study was approved by the institutional ethical committees, and all procedures involved in this study were performed in accordance with the Declaration of Helsinki.

### Linkage disequilibrium analysis

Using the Phase III HapMap JPT (Japanese in Tokyo) data [12], linkage disequilibrium analyzes have been performed among rs11726117, rs231247 and rs231253 with software R (version 3.1.0) (<http://www.r-project.org/>) with package GenABEL.

### Genotyping

Genomic DNA was extracted from whole peripheral blood cells [13]. Genotyping of rs11726117 was performed by the TaqMan method (Life Technologies Corporation, Carlsbad, CA, USA) with a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) [14, 15]. To confirm their genotypes, more than 30 samples were subjected to direct sequencing with the following primers: forward 5'-ACCCTTCTGGCCTCATAATAC-3', and reverse 5'-CTTTACAACCATTAAGGTCCATC-3'. DNA sequencing analysis was performed with a 3130xl Genetic Analyzer (Life Technologies Corporation) [15].

The  $\chi^2$  test was used for association analysis with SPSS v.22.0J (IBM Japan Inc., Tokyo, Japan).

## Results

In the previous genetic analysis of the Taiwanese populations by Ko et al. [8], the genotype distributions are very similar among the 3 SNPs (rs11726117, rs231247 and rs231253) of *ALPK1* (Table 1). Therefore, we hypothesized that these SNPs are in linkage disequilibrium. To confirm this hypothesis, the HapMap JPT data have been analyzed. According to the hypothesis, the 3 SNPs were in strong linkage disequilibrium ( $r^2 \geq 0.99$ ; Table 1) among the Japanese population in HapMap data, indicating that

**Table 1** Minor allele frequencies and linkage disequilibrium of 3 SNPs of *ALPK1* gene

	A1 <sup>a</sup>	A2 <sup>a</sup>	Taiwanese Han <sup>b</sup>				Taiwan aborigines <sup>b</sup>				HapMap JPT <sup>c</sup>					
			A1/A1	A1/A2	A2/A2	MAF	A1/A1	A1/A2	A2/A2	MAF	A1/A1	A1/A2	A2/A2	MAF	$r^2$ <sup>d</sup>	$D'$ <sup>d</sup>
rs231247	G	A	204	167	36	0.29	225	414	201	0.49	59	46	8	0.27	0.99	1
rs231253	G	C	215	164	28	0.27	223	416	201	0.49	59	46	8	0.27	0.99	1
rs11726117	C	T	209	168	30	0.28	244	396	200	0.47	57	47	8	0.28	–	–

MAF Minor allele frequency

<sup>a</sup> The major allele was referred to A1 and the minor allele as A2

<sup>b</sup> Data from reference 8

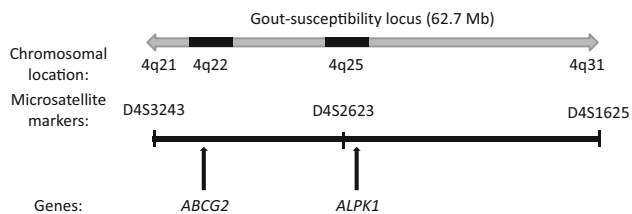
<sup>c</sup> Data from the Phase III HapMap JPT (Japanese in Tokyo)

<sup>d</sup> Results of linkage disequilibrium analysis between rs11726117 and rs231247, or between rs11726117 and rs231253

**Table 2** Association analysis of rs11726117 of *ALPK1* gene in gout cases and controls

	Genotype				Allele frequency mode			
	C/C	C/T	T/T	<i>p</i> value	MAF	<i>p</i> value	OR	95 % CI
Case	487	338	66	0.75	0.26	0.44	1.05	0.92–1.21
Control	706	465	86	–	0.25	–	Ref	–

MAF minor allele frequency, OR odds ratio, CI confidence interval, Ref reference



**Fig. 1** The locations of *ALPK1* and *ABCG2* in the gout-susceptibility locus. Gout-susceptibility locus was previously identified between D4S3243 and D4S1625 on chromosome 4q21–31. Both *ALPK1* and *ABCG2* locate in this locus

minor alleles of the 3 SNPs exist in one haplotype. Therefore, in this study, rs11726117 was genotyped to reveal its association with gout, because only this SNP is the nonsynonymous mutation (M861T) among these SNPs; rs231247 is a synonymous mutation (R1084R) and rs231253 is in the 3' untranslated region (3' UTR).

Table 2 shows the genotyping result of rs11726117 for 903 gout patients and 1,302 controls. The call rate was 97.4 %. The frequencies of genotypes were in Hardy–Weinberg equilibrium ( $p = 0.43$ ).

As compared with the control group, the genotype distribution of rs11726117 (C/C, C/T or T/T) in the case group was not significantly different ( $p = 0.75$ ; Table 2).

The minor allele (T) frequencies of the variant were 0.26 and 0.25 in case and control groups, respectively, indicating that rs11726117 is a common missense mutation. The association analysis has not detected a significant association between rs11726117 and gout susceptibility in the allele frequency mode ( $p = 0.44$ ; Table 2).

## Discussion

*ALPK1* gene locates in a gout-susceptibility locus (between microsatellite markers 4DS3243 and 4DS1625) on chromosome 4q21–31 [16]. In the Taiwanese populations, *ALPK1* was previously reported to be associated with gout susceptibility [8].

*ALPK1* belongs to the alpha-kinase family and plays a role in the phosphorylation of myosin 1 [9]. A recent genome-wide association study (GWAS) revealed the possible relationship between *ALPK1* SNPs and chronic

kidney disease (CKD) [17]. As hyperuricemia is highly correlated with CKD risk [18, 19], together with the renal expression of *ALPK1* [17], *ALPK1* could be a possible susceptible gene for gout/hyperuricemia.

However, the present study detected no significant association between *ALPK1* and gout. This may be partly due to the difference of the investigated population. In addition, we previously reported that *ABCG2*, which also locates in a gout-susceptibility locus on chromosome 4q21–31 (Fig. 1), is strongly associated with gout [2, 20]. Taken together, these findings suggest that among genes in a gout-susceptibility locus, not *ALPK1* but *ABCG2* is important as a susceptible gene for gout (Fig. 1). Although further studies of *ALPK1* are necessary to reveal the relationship between *ALPK1* SNPs and gout, our study at least revealed that rs11726117 of *ALPK1* is not a strong genetic risk for gout.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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## References

- Woodward OM, Köttgen A, Coresh J, Boerwinkle E, Guggino WB, Köttgen M. Identification of a urate transporter, *ABCG2*, with a common functional polymorphism causing gout. *Proc Natl Acad Sci USA*. 2009;106(25):10338–42.
- Matsuo H, Takada T, Ichida K, Nakamura T, Nakayama A, Ikebuchi Y, et al. Common defects of *ABCG2*, a high-capacity urate exporter, cause gout: a function-based genetic analysis in a Japanese population. *Sci Transl Med*. 2009;1(5):5ra11.

3. Ichida K, Matsuo H, Takada T, Nakayama A, Murakami K, Shimizu T, et al. Decreased extra-renal urate excretion is a common cause of hyperuricemia. *Nat Commun*. 2012;3:764.
4. Matsuo H, Nakayama A, Sakiyama M, Chiba T, Shimizu S, Kawamura Y, et al. *ABCG2* dysfunction causes hyperuricemia due to both renal urate underexcretion and renal urate overload. *Sci Rep*. 2014;4:3755.
5. Nakayama A, Matsuo H, Shimizu T, Ogata H, Takada Y, Nakashima H, et al. A common missense variant of monocarboxylate transporter 9 (*MCT9/SLC16A9*) gene is associated with renal overload gout, but not with all gout susceptibility. *Hum Cell*. 2013;26(4):133–6.
6. Sakiyama M, Matsuo H, Shimizu S, Nakashima H, Nakayama A, Chiba T, et al. A common variant of organic anion transporter 4 (*OAT4/SLC22A11*) gene is associated with renal underexcretion type gout. *Drug Metab Pharmacokinet*. 2014;29(2):208–10.
7. Sakiyama M, Matsuo H, Shimizu S, Chiba T, Nakayama A, Takada Y, et al. A common variant of leucine-rich repeat-containing 16A (*LRRC16A*) gene is associated with gout susceptibility. *Hum Cell*. 2014;27(1):1–4.
8. Ko AM, Tu HP, Liu TT, Chang JG, Yuo CY, Chiang SL, et al. *ALPK1* genetic regulation and risk in relation to gout. *Int J Epidemiol*. 2013;42(2):466–74.
9. Heine M, Cramm-Behrens CI, Ansari A, Chu HP, Ryazanov AG, Naim HY, et al. Alpha-kinase 1, a new component in apical protein transport. *J Biol Chem*. 2005;280(27):25637–43.
10. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yu TF. Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum*. 1977;20(3):895–900.
11. Hamajima N. J-MICC Study Group. The Japan multi-institutional collaborative cohort study (J-MICC Study) to detect gene-environment interactions for cancer. *Asian Pac J Cancer Prev*. 2007;8(2):317–23.
12. International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426(6968):789–96.
13. Matsuo H, Chiba T, Nagamori S, Nakayama A, Domoto H, Phetdee K, et al. Mutations in glucose transporter 9 gene *SLC2A9* cause renal hypouricemia. *Am J Hum Genet*. 2008;83(6):744–51.
14. Daimon M, Ji G, Saitoh T, Oizumi T, Tominaga M, Nakamura T, et al. Large-scale search of SNPs for type 2 DM susceptibility genes in a Japanese population. *Biochem Biophys Res Commun*. 2003;302(4):751–8.
15. Sakiyama M, Matsuo H, Chiba T, Nakayama A, Nakamura T, Shimizu S et al. Common variants of *cGKII/PRKG2* are not associated with gout susceptibility. *J Rheumatol*. 2014;41(7):1395–7.
16. Cheng LS, Chiang SL, Tu HP, Chang SJ, Wang TN, Ko AM, et al. Genomewide scan for gout in Taiwanese aborigines reveals linkage to chromosome 4q25. *Am J Hum Genet*. 2004;75(3):498–503.
17. Yamada Y, Nishida T, Ichihara S, Kato K, Fujimaki T, Oguri M, et al. Identification of chromosome 3q28 and *ALPK1* as susceptibility loci for chronic kidney disease in Japanese individuals by a genome-wide association study. *J Med Genet*. 2013;50(6):410–8.
18. Iseki K, Oshiro S, Tozawa M, Iseki C, Ikemiya Y, Takishita S. Significance of hyperuricemia on the early detection of renal failure in a cohort of screened subjects. *Hypertens Res*. 2001;24(6):691–7.
19. Tomita M, Mizuno S, Yamanaka H, Hosoda Y, Sakuma K, Matuoka Y, et al. Does hyperuricemia affect mortality? A prospective cohort study of Japanese male workers. *J Epidemiol*. 2000;10(6):403–9.
20. Matsuo H, Ichida K, Takada T, Nakayama A, Nakashima H, Nakamura T, et al. Common dysfunctional variants in *ABCG2* are a major cause of early-onset gout. *Sci Rep*. 2013;3:2014.