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.

Keywords Gouty arthritis · Uric acid · Urate · ABCG2/BCRP · Gout-susceptibility locus

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 ± 12.9 and 52.7 ± 8.4 years old, respectively, and their respective mean body-mass index was 24.7 ± 3.3 and 23.2 ± 2.8 kg/m^2. 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'-ACCCATTGGCGCTCATATACT-3', and reverse 5'-CTTTACAACCATTAGGTCCATC-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

| A1 | A2 | Taiwanese Han | Taiwan aborigines | HapMap JPT |
|----|----|---------------|-------------------|------------|
|----|----|----------------|-------------------|------------|
|    |    | A1/A1 | A1/A2 | A2/A2 | MAF | A1/A1 | A1/A2 | A2/A2 | MAF | A1/A1 | A1/A2 | A2/A2 | MAF | $r^2$ | $D^a$ |
|----|----|-------|-------|-------|-----|-------|-------|-------|-----|-------|-------|-------|-----|-----|-----|
| 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

* The major allele was referred to A1 and the minor allele as A2

*b* Data from reference 8

*c* Data from the Phase III HapMap JPT (Japanese in Tokyo)

*d* Results of linkage disequilibrium analysis between rs11726117 and rs231247, or between rs11726117 and rs231253

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Common variant of ALPK1 is not associated

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

| Genotype | C/C | C/T | T/T | p value | MAF | p 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

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 I [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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