A common variant of *MAF/c-MAF*, transcriptional factor gene in the kidney, is associated with gout susceptibility

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Abstract Gout is a multifactorial disease characterized by acute inflammatory arthritis, and it is caused as a consequence of hyperuricemia. A recent meta-analysis of genome-wide association studies has newly identified the relationship between serum uric acid (SUA) levels and rs889472, a single nucleotide polymorphism of musculoaponeurotic fibrosarcoma oncogene (*MAF/c-MAF*). However, it remained unclear whether rs889472 is associated with gout susceptibility. In the present study, we investigate the association between *c-MAF* rs889472 and gout in Japanese male population. We genotyped 625 male patients who were clinically

diagnosed as gout and 1221 male control subjects without hyperuricemia or a history of gout by TaqMan method. As a result, the major allele (C), which reportedly increases SUA levels, had a higher frequency in the gout cases (58.8%) than in the controls (55.0%). A logistic regression analysis showed a significant association between rs889472 and gout ($p = 0.029$, odds ratio = 1.17; 95% confidence interval 1.02–1.34). C-MAF is reported as a pivotal transcriptional factor in the development and differentiation of renal proximal tubular cells. Because urate is mainly regulated in renal proximal tubular cells, c-MAF may have an important role in urate regulation in the kidney and influence not only SUA but also gout susceptibility. Our finding shows that rs889472 of *c-MAF* is associated with gout susceptibility.

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Introduction

Gout is a multifactorial disease characterized by acute inflammatory arthritis, and it is caused as a consequence of hyperuricemia. Previous genetic analyses have shown that gout susceptibility and/or serum uric acid (SUA) levels are associated with single-nucleotide polymorphisms (SNPs) of several genes including urate transporter genes [1–7] such as ATP-binding cassette transporter, subfamily G, member 2 (*ABCG2/BCRP*); glucose transporter 9 (*GLUT9/SLC2A9*); and urate transporter 1 (*URAT1/SLC22A12*). A recent meta-analysis of genome-wide association studies on kidney function-related traits in East Asian population [1] has identified the relationship between SUA levels and rs889472, a SNP of musculoaponeurotic fibrosarcoma oncogene (*MAF/c-MAF*) which is one of the proto-oncogenes. However, the effect of

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rs889472 on gout susceptibility has not been investigated to date. In the present study, we investigated the association between *c-MAF* rs889472 and gout susceptibility in clinically defined patients with gout and in control subjects.

Materials and methods

Study participants

This study was approved by the institution's Ethical Committee (National Defense Medical College). All the protocols were in accordance with the Declaration of Helsinki, and written informed consent was obtained from all the participants. This study included 625 Japanese male patients with gout, who were clinically diagnosed according to the criteria established by the American College of Rheumatology [9], and who were selected from outpatients of Ryougoku East Gate Clinic (Tokyo, Japan). In addition, this study included 1221 Japanese men with SUA levels of ≤ 7.0 mg/dl and without a history of gout as control subjects. The control subjects were selected from participants in the Shizuoka area in the Japan Multi-Institutional Collaborative Cohort Study (J-MICC Study) [10].

Genotyping

Genomic DNA was extracted from whole peripheral blood cells [11]. *C-MAF* rs889472 was genotyped using TaqMan method (Thermo Fisher Scientific Inc., Waltham, MA, USA) with a LightCycler 480 (Roche Diagnostics, Mannheim, Germany) [12].

Statistical analysis

Statistical analyses were performed using SPSS v.22.0J (IBM Japan Inc., Tokyo, Japan). Chi-square analysis was used for the testing for Hardy–Weinberg equilibrium. Association analysis was performed using the univariate logistic regression. The clinical data (age, body mass index, and serum uric acid level) of the patients and control subjects were compared using independent sample *t* test. A *p* value < 0.05 was considered statistically significant.

Results

Clinical information of the patients with gout and of control subjects is shown in Table 1. Genotyping results of *c-MAF* rs889472 are shown in Table 2. The call rate for rs889472 was 98.8%. Genotype frequency followed the Hardy–Weinberg equilibrium in control subjects ($p = 0.21$). The major allele (C), which increases SUA levels, had a

Table 1 Characteristics of patients with gout and of control subjects included in the present study

	Patients	Controls
Number of participants	625	1221
Age (years)	45.4 $\pm$ 10.2	53.3 $\pm$ 8.6
Body mass index (kg/m ²)	25.4 $\pm$ 3.7	23.3 $\pm$ 2.7
Serum uric acid (mg/dl)	8.4 $\pm$ 1.1	5.7 $\pm$ 0.9

Values are expressed as mean $\pm$ SD

higher frequency in the gout cases (58.8%) than in the controls (55.0%). Univariate logistic regression analysis showed a significant association between rs889472 and gout ($p = 0.029$, odds ratio (OR) = 1.17; 95% confidence interval (CI) 1.02–1.34).

Discussion

C-MAF is a cellular homolog of the viral oncogene v-maf that is recognized in the genome of the avian transforming retrovirus [13]. *C-MAF* is a member of large MAF protein subfamily which is a subgroup of MAF family together with MAFA, MAFB, and NRL [14, 15]. Similar to other Maf family proteins, mouse c-Maf has a basic region leucine zipper (bZIP) structure and forms a homodimer or heterodimer with other compatible bZIP proteins, including other large Maf proteins, c-Fos, and c-Jun [14]. Dimerized large Maf proteins bind to DNA and act as transcriptional factors that are essential for the regulation of cell differentiation in several organs such as the brain, eye, kidney, and pancreas [14, 15]. *C-MAF* is expressed in the proximal tubules of human [15] and mouse [16] fetal kidney. Renal proximal tubular cells of *c-Maf*-knockout mice show significantly smaller cytoplasmic volume than those of wild-type mice [16]. Compound heterozygote *C-Maf*-mutant mice (Maf/R291Q) can develop marked tubular nephritis, with dilated tubules containing eosinophil casts [17]. These findings suggest that c-MAF is a pivotal transcriptional factor in the development and differentiation of renal proximal tubular cells, and affects the structure and the function of them.

Uric acid is mainly excreted in the urine. Renal proximal tubules reabsorb and secrete uric acid through urate transporters after it was freely filtered at the glomerulus [3]. The following are urate transporters on proximal tubular cells: (1) urate reabsorption transporters URAT1 [4] (in the apical membrane) and GLUT9 [5] (in the basolateral membranes) and (2) urate excretion transporters ABCG2 [6, 7] and type 1 sodium-dependent phosphate transporter (NPT1/SLC17A1) [8] (in the apical membrane). Approximately, 10% of uric acid filtered at the glomerulus is excreted by the action of these urate transporters [3]. Because c-MAF functions as a

Table 2 Association between gout and the *MAF/c-MAF* SNP, rs889472

	Number	Genotypes ^a			RAF	<i>p</i> value	OR (95% CI)
		C/C	C/A	A/A			
Case	625	218	298	108	0.59	0.029	1.17 (1.02–1.34)
Control	1221	373	572	254	0.55		

CI confidence interval, OR odds ratio, RAF risk allele frequency

Association analysis was performed using the univariate logistic regression

^a“C” is a risk allele

transcriptional factor in renal proximal tubular cells, it may affect urate regulation in the kidney and influence not only SUA but also gout susceptibility.

In conclusion, we observed that rs889472, a common variant of *C-MAF*, was associated with gout susceptibility. This association may be because of individual differences in the functions of *c-MAF* as a transcriptional factor in renal proximal tubular cells. Our finding shows that rs889472 of *c-MAF* is associated with gout susceptibility.

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Compliance with ethical standards

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

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