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ARTICLE

Variants of *ALPK1* with *ABCG2*, *SLC2A9*, and *SLC22A12* increased the positive predictive value for gout

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Abstract

We investigated the interactions of *ALPK1* variants and the loci of *ABCG2*, *SLC2A9*, and *SLC22A12* on gout risk. We conducted two case–control studies. Participants were recruited from hospitals ($n = 410$; 104 gout cases and 306 controls) and communities ($n = 678$; 373 gout cases and 305 controls) in Taiwan. The genotypes of *ALPK1* (rs11726117 M861T, rs231247 R1084R, and rs231253 3′ UTR), *ABCG2* (rs2231142 Q141K and rs2231137 V12M), *SLC2A9* (rs3733591 R265H and rs1014290), and *SLC22A12* (rs3825016 H86H, rs11231825 H142H, and rs475688) were genotyped. Under a recessive model, the joint effects of *ALPK1* variants and the SNPs rs2231142 of *ABCG2*, rs1014290 of *SLC2A9*, or rs475688 and rs3825016 of *SLC22A12* were associated with gout. The rs11726117 [CC] of *ALPK1* and rs2231142 [TT] of *ABCG2* with the sequential addition of the rs1014290 [AA] of *SLC2A9* and rs3825016 [CC] of *SLC22A12* were associated with gout risk (odds ratio (OR): 13.01, 15.11, and 55.00 and positive predictive value (PPV): 56%, 69%, and 99% in the Han group, respectively; OR: 3.76, 5.78, and 12.30 and PPV: 74%, 80%, and 81% in the aboriginal group, respectively). Combined exposure to the four high-risk genotypes of *ALPK1* and the uric-acid-related loci of *ABCG2*, *SLC2A9*, and *SLC22A12* was associated with an increased gout risk and a high PPV for gout.

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Introduction

Gout is a common and treatable form of inflammatory arthritis [1]; it is a complex disease involving the metabolic, renal, cardiovascular, and immunological systems [1–3]. A central pathological feature of gout is the chronic deposition of monosodium urate crystals; in severe form, this deposition can be visualized as the presence of urate, tophus, and bone erosion on a dual-energy computed tomography scan [1, 4]. Although target uric acid levels (<6 mg/dL) can be achieved through the dose titration of available oral urate-lowering agents in most patients, whether lower targets are beneficial for all patients remains unclear [1, 5]. In Taiwan, there were 1,458,569 prevalent and 56,595 incident cases of gout with a prevalence of 6.24% and an incidence rate of 2.74 per 1000 person-years in 2010 [6]. The prevalence of gout in the Taiwanese population is ~1.6 times that in the Caucasian population (e.g., the prevalence was 3.9% in the United States [7]). Despite the availability of urate-lowering therapies in Taiwan, the prevalence of gout remains higher than that in other countries [6].

Hyperuricemia is a key risk factor for the pathogenesis of gout; however, only 20% of patients with hyperuricemia

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develop gout [8]. Furthermore, urate-lowering therapy fails in 3% of patients because of refractoriness, contraindication, or intolerance [5]. Many uncertainties remain, and the understanding of the pathogenesis of gout, such as genetic effects, is incomplete [1]. Three urate transporters in renal proximal tubule epithelial cells—*ABCG2*, *SLC2A9*, and *SLC22A12*—play crucial roles in the regulation of serum uric acid, and their dysfunction causes urate transport disorders such as gout [9–11]. *ABCG2* (4q22.1) is a well-studied hyperuricemic and gout-susceptible gene, a secretory urate transporter in the intestine and kidneys [12]. *SLC2A9* (4p16.1) is expressed in renal epithelial cells (urate reabsorption), hepatocytes, the intestine, peripheral leukocytes, and articular cartilage [10, 13, 14]. *SLC22A12* (11q13.1) has been related to decreased fractional urate excretion, uric acid levels, and gout risk [15–17]. Additive composite *ABCG2*, *SLC2A9*, and *SLC22A12* scores of high-risk alleles with alcohol use were shown to modulate the risks of asymptomatic hyperuricemia, gout, and tophaceous gout [18].

ALPK1 (4q25) may contribute to the inflammatory process associated with the development of chronic kidney disease and gout, a mechanism related to nephrotoxicity [19, 20]. In previous studies, we suggested that ALPK1 phosphorylates myosin IIA modulating TNF-α trafficking in gout flares and found that colchicine treatment did not affect ALPK1 [21]; we revealed that *ALPK1* variants can effectively interfere with microRNA target recognition and modulate the mRNA expression in gout patients [19]. In our previous transgenic mice study, increased expression of ALPK1 reduced the expression of URAT1 (encoded by *SLC22A12*), a potential repressor of URAT1 protein expression, suggesting that *ALPK1* prevents urate reuptake through *SLC22A12* and that *ALPK1* is negatively associated with gout [22]. Therefore, the variation caused by the inflammatory process in *ALPK1* function might be an important genetic checkpoint for gout risk, particularly in association with the effects of urate transporter genes.

We hypothesized that the gout-susceptible gene *ALPK1* and the uric-acid-related loci of *ABCG2*, *SLC2A9*, and *SLC22A12* can mediate interactions contributing to gout risk. This study investigated the epistasis or joint effects of *ALPK1* and the genes of *ABCG2*, *SLC2A9*, and *SLC22A12* on gout risk in Taiwan Han and aboriginal groups.

Materials and methods

Study participants

We conducted two case–control studies, as described previously [18, 19, 23]. A total of 410 Taiwan Han men were recruited through hospitals, of which 306 were controls with normal uric acid levels and 104 were patients with gout. In addition, 678 Taiwan aborigines were recruited from communities, of which 305 were control subjects with normal urate levels and 373 were patients with gout. Patients received a diagnosis of gout on the basis of the criteria provided by the 1977 American Rheumatism Association classification [24]. The study was approved by the ethical committee of participating hospitals and written informed consent was obtained from each participant.

Genotypes

DNA (Venous whole blood) was extracted using QIAGEN Gentra Puregene Blood Kit. In our previous studies, *ALPK1* variants and *ABCG2*, *SLC2A9*, and *SLC22A12* loci related to gout [16, 18, 19, 25]. Therefore, the genotypes of *ALPK1* (rs11726117 M861T, rs231247 R1084R, and rs231253 3′ UTR), *ABCG2* (rs2231142 Q141K and rs2231137 V12M), *SLC2A9* (rs3733591 R265H and rs1014290), and *SLC22A12* (rs475688) were genotyped. In addition, the single-nucleotide polymorphisms (SNPs) rs3825016 H86H and rs11231825 H142H of *SLC22A12* were genotyped; these SNPs were previously associated with decreased renal uric acid excretion and hyperuricemia in a German population [15].

Statistical analysis

Descriptive results were analyzed between gout and control groups using *t*-tests and chi-square tests, as appropriate. Genetic models, including the dominant and recessive models of inheritance, were estimated for the control and gout groups by using the chi-square test with one or two degrees of freedom. Under a recessive genetic model, the association of the joint effects of *ALPK1* variants and *ABCG2*, *SLC2A9*, or *SLC22A12* variants on gout risk was evaluated using a logistic regression model. Adjusted ORs were calculated after adjusted for covariates, such as age, body mass index, total cholesterol, triglycerides, and creatinine levels, hypertension, and alcohol use in the Han group and adjustment for age, sex, glutamic pyruvic transaminase (GPT), glutamate oxaloacetate transaminase (GOT), total cholesterol, and creatinine levels, family history, hypertension, and alcohol use in the aboriginal group. We investigated the epistatic association with gout by introducing an interaction term—*ALPK1* × *ABCG2*, *ALPK1* × *SLC2A9*, or *ALPK1* × *SLC22A12*—in addition to *ALPK1* and *ABCG2*, *ALPK1* and *SLC2A9*, or *ALPK1* and *SLC22A12*, to the model after adjustment for covariates. The attributable fraction (AF) was estimated using the following equation: (OR − 1)/OR; the AF in all gout patients in the population (population AF (PAF)) was estimated using the following equation: exposure frequency in patients × (OR − 1)/OR [26]. The positive predictive value (PPV) is the probability that a person with a positive

Table 1 Characteristics of the study participants

	Han group		*P*-value	Aboriginal group		*P*-value
	Gout	Controls		Gout	Controls	
n	104	306		373	305	
Age (SD), years	52.8 (13.7)	55.9 (14.6)	0.0631	50.1 (14.7)	55.9 (15.7)	<0.0001
Age of onset (SD), years	45.2 (12.3)	—		39.9 (15.0)	—	—
Duration of gout (SD), years	8.2 (6.3)	—		10.0 (8.0)	—	—
Tophi patients, *n* (%)	51 (49.0)			152(40.8)		
GPT (SD), U/L	—	—		36.4 (29.7)	27.5 (18.8)	<0.0001
GOT (SD), U/L	—	—		31.5 (26.5)	22.1 (17.8)	<0.0001
Systolic pressure (SD), mm Hg	136 (18.0)	131.7 (19.0)	0.0603	137.8 (21.0)	130.7 (21.6)	<0.0001
Diastolic pressure (SD), mm Hg	85.6 (13.3)	82.7 (11.4)	0.0511	89.2 (15.3)	81.1 (12.2)	<0.0001
Body mass index (SD), kg/m^2	26.0 (4.0)	24.2 (3.4)	<0.0001	26.3 (4.3)	26.4 (4.0)	0.5890
Total cholesterol (SD), mg/dL	210.7 (48.1)	188.4 (36.7)	<0.0001	186.8 (48.5)	184.2 (52.9)	0.5007
Triglycerides (SD), mg/dL	225.2 (121.4)	128.9 (100.3)	<0.0001	261.2 (279.1)	171.6 (299.6)	<0.0001
Creatinine (SD), mg/dL	1.4 (0.4)	1.2 (0.2)	<0.0001	1.1 (0.2)	1.0 (0.2)	<0.0001
Uric acid (SD), mg/dL	8.9 (1.8)	5.6 (0.9)	<0.0001	9.5 (2.4)	5.3 (0.9)	<0.0001
Men, *n*%	104 (100.0)	306 (100.0)	1.0000	289 (77.5)	131 (43.0)	<0.0001
Hypertension, *n* (%)	32 (30.8)	48 (15.7)	0.0008	166 (44.5)	96 (31.5)	0.0005
Family history, *n* (%)	—	—		86 (23.1)	18 (5.9)	<0.0001
Alcohol use, *n* (%)						
Non-drinker	69 (66.4)	237 (77.5)		103 (27.6)	172 (56.4)	
Former drinkers	15 (14.2)	15 (5.0)		68 (18.2)	25 (8.2)	
Current drinker	20 (19.2)	54 (17.7)	0.0041	202 (54.2)	108 (35.4)	<0.0001

Data of continuous and categorical variables were analyzed using the *t*-test and chi-square test

— not calculated, *SD* standard deviation, *GPT* glutamic pyruvic transaminase, *GOT,* glutamate oxaloacetate transaminase

screening result (denoted as carrying risk genotypes) has the disease (denoted as gout). By using Bayes' theorem, we estimated the PPV by using the following equation: ([number of patients with gout and carrying high-risk genotypes] + [number of individuals without gout and carrying high-risk genotypes])/(total number of individuals carrying high-risk genotypes). The Breslow–Day statistic has not been generalized for this type of k × R × C table and can be used only for k × 2 × 2 tables. The GENMOD procedure can derive the homogeneity statistic in this situation with pooled data. Therefore, the PROC CATMOD general test of homogeneity was used to examine whether the distribution between the four SNPs and gout was the same or different in different ancestry groups. The handling of data and the investigation of associations were estimated using SAS software 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

The descriptive results of the study participants are listed in Table 1. In the Taiwan Han group, the patients with gout were younger; had higher total cholesterol, triglycerides, creatinine, and uric acid levels; had higher body mass index; and had a higher proportion of hypertension and alcohol use than did the control participants. In the Taiwan aboriginal group, the patients with gout were younger; had higher GPT, GOT, total cholesterol, triglycerides, creatinine, and uric acid levels; and again had a higher proportion of hypertension and alcohol use than did the control participants.

Genetic analysis

A recessive model of inheritance was more appropriate than a dominant model of inheritance because some cells with a wild-type genotype had a small size (Supplementary Tables 1 and 2). Additionally, the variants rs3733591 and rs1014290 of *SLC2A9* were not associated with gout in the aboriginal group (Supplementary Table 2). The effect of the SNP rs475688 of *SLC22A12* was limited to the Han participants and that of the SNP rs3825016 of *SLC22A12* was limited to the aboriginal participants with an increased risk of gout in both groups.

Table 2 The joint effects of *ALPK1* and *ABCG2*, *SLC2A9*, or *SLC22A12* on gout risk

	Gout versus controls		*P* for interaction
	Adjusted OR (95% CI)	Adjusted OR (95% CI)	
Han group			
ALPK1 and *ABCG2*	rs2231142 GG+ GT	rs2231142 TT	
rs11726117 TT+CT	1.00	9.67 (3.28–28.54)	
rs11726117 CC	3.12 (1.55–6.29)	12.71 (4.71–34.31)	0.2160
ALPK1 and *SLC2A9*	rs1014290 GG+AG	rs1014290 AA	
rs11726117 TT+CT	1.00	0.77 (0.29–2.02)	
rs11726117 CC	1.29 (0.63–2.66)	4.16 (1.96–8.85)	0.0187
ALPK1 and *SLC22A12*	rs3825016 TT+CT	rs3825016 CC	
rs11726117 TT+CT	1.00	0.97 (0.39–2.43)	
rs11726117 CC	2.53 (1.05–6.09)	2.01 (0.85–4.75)	0.7319
Aboriginal group			
ALPK1 and *ABCG2*	rs2231142 GG+GT	rs2231142 TT	
rs11726117 TT+CT	1.00	2.45 (1.43–4.18)	
rs11726117 CC	1.79 (1.15–2.79)	3.76 (1.84–7.71)	0.7485
ALPK1 and *SLC2A9*	rs1014290 GG+AG	rs1014290 AA	
rs11726117 TT+CT	1.00	1.42 (0.91–2.21)	
rs11726117 CC	1.90 (1.12–3.23)	2.19 (1.26–3.81)	0.5932
ALPK1 and *SLC22A12*	rs3825016 TT+CT	rs3825016 CC	
rs11726117 TT+CT	1.00	2.41 (1.47–3.96)	
rs11726117 CC	3.19 (1.46-7.00)	3.25 (1.87–5.65)	0.0615

Under a recessive genetic model, odds ratio (OR) with 95% confidence interval (CI) in parentheses was calculated after adjustment for age, body mass index, total cholesterol, triglycerides, creatinine levels, hypertension, and alcohol use by using a multiple logistic regression model in the Han group. OR with 95% CI in parentheses was calculated after adjustment for age, sex, GOT, GPT, total cholesterol, creatinine levels, family history, hypertension, and alcohol use in the aboriginal group

We investigated epistatic associations with gout by introducing an interaction term—*ALPK1* × *ABCG2*, *ALPK1* × *SLC2A9*, or *ALPK1* × *SLC22A12* variants—in addition to *ALPK1* and *ABCG2*, *ALPK1* and *SLC2A9*, or *ALPK1* and *SLC22A12* variants, to the model after adjustment for covariates

We evaluated whether combined exposure to *ALPK1* variants and the uric-acid-related loci of *ABCG2*, *SLC2A9*, or *SLC22A12* further increased the odds of gout development. The study participants in each case–control study were separately classified into four groups under a recessive model of inheritance (Supplementary Tables 3 and 4). From the univariate analysis, we found that the high-risk genotypes of *ALPK1* variants and *ABCG2* (rs2231142), *SLC2A9* (rs1014290), or *SLC22A12* (rs475688 or rs3825016) were strongly associated with gout. The joint effects of *ALPK1* variants and the uric-acid-related loci of *ABCG2*, *SLC2A9*, or *SLC22A12* were related to gout risk after adjustment for confounding variables (OR: 4.16–13.87 in the Han group and 1.77–4.23 in the aboriginal group; Table 2, Supplementary Tables 5 and 6). Specifically, the results revealed the supra-multiplicative epistasis effect of *ALPK1* variants and the SNP rs1014290 of *SLC2A9* on risk of gout (interaction $P \leq 0.0187$) in the Han group and the epistatic effect of *ALPK1* variants and the SNP rs3825016 of *SLC22A12* on risk of gout (interaction $P \leq 0.0084$) in the aboriginal group.

As shown in Tables 3 and 4, combined exposure to the high-risk genotypes *ALPK1*, *ABCG2*, *SLC2A9*, and *SLC22A12* was associated with an increased risk of gout and an increased PPV for gout in both of the groups. We estimated the joint effects on gout risk of the rs11726117 [CC] of *ALPK1* and rs2231142 [TT] of *ABCG2* with the sequential addition of rs1014290 [AA] of *SLC2A9* and rs3825016 [CC] of *SLC22A12*. Our results revealed that the patients carrying high-risk genotypes had a strong association with the odds of gout risk (Han group—OR: 13.01, 15.11, and 55.00 and PPV: 56, 69, and 99%; aboriginal group—OR: 3.76, 5.78, and 12.30 and PPV: 74, 80, and 81%). The four high-risk genotypes of *ALPK1* (rs11726117 [CC]), *ABCG2* (rs2231142 [TT]), *SLC2A9* (rs1014290 [AA]), and *SLC22A12* (rs475688 [CC]) and gout could be explained by the exposure of the Han participants to the four high-risk genotypes (OR: 33.91; PPV: 80%; Supplementary Table 7); however, these genotypes were not significant in the aboriginal participants.

In the pooled analysis, we found that the patients carrying the four high-risk genotypes rs11726117 [CC],

Table 3 The joint effects of *ALPK1* and *ABCG2*, *SLC2A9*, and *SLC22A12* on gout risk in Han group

	Gout n=104	Controls n=306	Adjusted OR (95% CI)	*P*-value	AF-Exp	PPV
Two SNPs						
ALPK1 rs11726117 and *ABCG2* rs2231142						
TT+CT and GG+ GT	17 (16.3)	129 (42.2)	1.00			
CC and TT	19 (18.3)	15 (4.9)	13.01 (4.80-35.2)	<0.0001	92.31%	0.56 (0.38–0.73)
Three SNPs						
ALPK1 rs11726117, *ABCG2* rs2231142 and *SLC2A9* rs1014290						
TT+CT, GG+ GT, and GG+ AG	12 (11.5)	84 (27.5)	1.00			
CC, TT, and AA	11 (10.6)	5 (1.6)	15.11 (3.95–57.83)	<0.0001	93.38%	0.69 (0.41–0.89)
Four SNPs						
ALPK1 rs11726117, *ABCG2* rs2231142, *SLC2A9* rs1014290 and *SLC22A12* rs3825016						
TT+CT, GG+ GT, GG+ AG, and TT+ CT	8 (7.7)	27 (8.8)	1.00			
CC, TT, AA, and CC	8 (7.7)	0 (0.0)	55.00 (2.87-1055.10)[a]	<0.0001	98.18%	0.99 (0.63–0.99)

OR with 95% CI in parentheses was calculated after adjustment for covariates by using a multiple logistic regression model in the Han group

SNPs single-nucleotide polymorphisms, *AF-Exp ([OR − 1]/OR)* attributable fraction among exposed gout cases, *PPV* positive predictive value for gout

[a]Crude OR with 95% CI was calculated using Haldane's modification, which adds 0.5 in all cells to accommodate possible zero counts

rs2231142 [TT], rs1014290 [AA], and rs3825016 [CC] had high odds of gout development (OR: 14.99, 95% CI: 4.76–47.24; PPV: 85%, 95% CI: 0.69–95; Supplementary Table 8), and the considerable PAF for gout was 5.69%. We also observed that the patients carrying the four high-risk genotypes rs11726117 [CC], rs2231142 [TT], rs1014290 [AA], and rs475688 [CC] had a strong association with gout risk (OR: 7.56, 95% CI: 2.24–25.56). Additionally, we analyzed the data for each variable including four gene variants related to gout; the results show in Supplementary Tables 5 and 6, and 9–11. We also analyzed the data after adjusted uric acid in the logistic regression model; the results were not significant associated with gout risk (Supplementary Tables 5 and 6). Interestingly, patients carrying the four high-risk genotypes adjusted covariates and uric acid had a significant association with gout risk in the pooled analysis (OR: 5.97, 95% CI: 1.03–34.43; Supplementary Table 8), suggesting the results may be explained the causal effects on gout occurrence.

Discussion

This study indicates the gene–gene interactions of *ALPK1* and the urate transporter genes *ABCG2*, *SLC2A9*, and *SLC22A12* on gout risk in two groups in Taiwan. The individuals carrying the additive composite four high-risk genotypes *ALPK1*, *ABCG2*, *SLC2A9*, and *SLC22A12* had an increased risk of gout (OR ≥ 12.30) and a high PPV (PPV ≥ 81%) for gout.

The variation caused by the inflammatory process in *ALPK1* function might be an important genetic checkpoint for gout risk, particularly in association with the effects of urate transporter genes in the kidney and intestine. Gouty arthritis (e.g., tophus) is the response involves both innate and adaptive immune cells [1]. *ALPK1* has been linked to the inflammatory process and involved in monosodium urate monohydrate-induced inflammatory responses [19, 27]; it may also be a susceptibility gene for renal disease in patients with diabetes mellitus [20]. A study speculated that ALPK1 participates in the regulation of Golgi-derived TNF-α trafficking through myosin IIA phosphorylation and found that colchicine treatment did not affect ALPK1 [21].

ALPK1 expression reduced URAT1 expression [22]. *ALPK1* belongs to the atypical kinase group as implicated in epithelial cell polarity and exocytic vesicular transport toward the apical plasma membrane [28]. Gout patients have difficulty eliminating renal urate [29], whereas an elevated serum urate level is necessary but not sufficient for the pathogenesis of gout [30]. Uric acid is determined by its production and the net balance of reabsorption or secretion by the kidneys and intestine [31]. One study performed the immunohistochemical analysis of ALPK1 in the human kidneys [20]; ALPK1 immunoreactivity was detected in the renal tubular epithelial cells and urinary casts, and the findings showed diabetic glomerulosclerosis being strongly positive than normal renal. ALPK1 overexpression resulted in the upregulation of the expression of *SLC22A1* and *CST3*, both of which may play crucial roles related to renal excretion and tissue remodeling [20].

Table 4 The joint effects of *ALPK1* and *ABCG2*, *SLC2A9*, and *SLC22A12* on gout risk in aboriginal group

	Gout *n*=373	Controls n=305	Adjusted OR (95% CI)	*P*-value	AF-Exp	PPV
Two SNPs						
ALPK1 rs11726117 and *ABCG2* rs2231142						
TT+CT and GG+ GT	145 (38.9)	191 (62.6)	1.00			
CC and TT	42 (11.3)	15 (4.9)	3.76 (1.84–7.70)	0.0003	73.40%	0.74 (0.60–0.84)
Three SNPs						
ALPK1 rs11726117, *ABCG2* rs2231142 and *SLC2A9* rs1014290						
TT+CT, GG+ GT, and GG+ AG	73 (19.6)	104 (34.1)	1.00			
CC, TT, and AA	24 (6.4)	6 (2.0)	5.78 (1.92–17.44)	0.0018	82.70%	0.80 (0.61–0.92)
Four SNPs						
ALPK1 rs11726117, *ABCG2* rs2231142, *SLC2A9* rs1014290 and *SLC22A12* rs3825016						
TT+CT, GG+ GT, GG+ AG, and TT+ CT	15 (4.0)	43 (14.1)				
CC, TT, AA, and CC	21 (5.6)	5 (1.6)	12.30 (3.36–44.99)	0.0002	91.87%	0.81 (0.61–0.93)

OR with 95% CI in parentheses was calculated after adjustment for covariates by using a multiple logistic regression model in the aboriginal group

SNPs single-nucleotide polymorphisms, *AF-Exp ([OR − 1]/OR)* attributable fraction among exposed gout cases, *PPV* positive predictive value for gout

Recently, a signaling pathway study showed that *ALPK1* is a master regulator of innate immunity against both invasive and extracellular gram-negative bacteria [32]. In addition, an association study reported that intestinal microbiota (microbial index) differed between patients with gout and healthy controls, suggesting the intestinal microbiota metabolism in the mechanistic interrogation of gout [33]. Because *ABCG2* plays physiologically important roles in both renal and extrarenal (e.g., intestinal) urate excretion mechanisms [9], *ALPK1* might interact with *ABCG2* and be linked to gout occurrence. In the present study, compared with the joint effects of *ALPK1* variants and *SLC2A9* or *SLC22A12* variants, the joint effects of the high-risk genotypes of *ALPK1* variants and rs2231142 [TT] of *ABCG2* were highly associated with gout (adjusted OR ≥ 12.71 in the Han group and OR ≥ 3.76 in the aboriginal group). *SLC2A9* also plays crucial roles in both extrarenal (e.g., intestinal) and renal urate excretion mechanisms [13, 14]. A recent study demonstrated that mice deficient in Glut9 (encoded by *SLC2A9*) developed impaired enterocyte uric acid transport kinetics, the progression of hyperuricemia, and early onset metabolic syndrome [14]. By contrast, hypertension and hypercholesterolemia was reversed in *SLC2A9* knockout mice after treatment with allopurinol (a xanthine oxidase inhibitor) [14]. Our results showed that the high-risk genotypes rs11726117 [CC] of *ALPK1* and rs1014290 [AA] of *SLC2A9* were associated with gout in both Taiwan groups (OR: 4.16 and 2.19; Table 2); however, the SNP rs1014290 of *SLC2A9* was not associated with gout in the aboriginal group. *ALPK1* may relate to the control of intestinal homeostasis or renal by modulating the molecular activities of gene products, such as those of urate transporter genes (e.g., *ABCG2*, *SLC2A9*, and *SLC22A12*) taking place between the renal or intestinal epithelium, and the immune system.

ALPK1 variants might result in the differential ability to effectively regulate *ABCG2*, *SLC2A9*, and *SLC22A12*, which might be related to urate homeostasis and gout occurrence. The *ABCG2*, *SLC2A9*, and *SLC22A12* in renal proximal tubule epithelial cells related to urate levels and gout occurrence [9, 10]. In our previous study, we reported that the high-risk allele scores of *ABCG2*, *SLC2A9*, and *SLC22A12* increased to gout risk (genetic risk score OR = 1.95) in Han [18]. In this study, we found that the joint effects of *ALPK1* and *ABCG2* with added *SLC2A9* and *SLC22A12* (rs475688 [only Han] or rs3825016) contributed to a higher risk of gout than did single-gene variants. Importantly, the Taiwan aboriginal participants had higher serum urate levels; the inflammatory gene of ALPK1 and urate-raising gene loci may be contributes to an increase in urate level and gout risk [30]. Our findings showed that the percentage of the patients with gout carrying the rs3825016 [CC] of *SLC22A12* was higher in the aborigines (76.9%) than in the Han subjects (54.8%). In addition, the frequencies of rs2231142 [TT] of *ABCG2* (34.6% and 32.2%, respectively), rs1014290 [AA] of *SLC2A9* (48.1% and 48.5%, respectively), or rs475688 [CC] of *SLC22A12* (42.3% and 42.1%, respectively) in the patients with gout were similar in both the Han and aboriginal groups (Supplementary Tables 1 and 2). These findings suggest that

ALPK1 prevents urate reuptake through the rs3825016 [CC] of *SLC22A12* and that *ALPK1* is negatively associated with gout risk, particularly in the aboriginal group (*P* for interaction ≤ 0.0084). This agreed with our previous transgenic mice study results, which revealed that ALPK1 overexpression reduced URAT1 protein expression in mouse kidneys [22]. Although the frequency of the SNP rs11726117 [CC] of *ALPK1* was lower in the aborigines (40.2%) than in the Han subjects (67.3%), the joint effects of the high-risk genotypes of *ALPK1* variants and rs2231142 [TT] of *ABCG2* were more strongly associated with risk of gout in the Han group (OR ≥ 12.71) than in the aboriginal group (OR ≥ 3.76). Thus, we suggest that *ALPK1* variants modulate *ABCG2*, *SLC2A9*, and *SLC22A12* in the differential ability to effective occurrence of gout in Taiwan populations. However, a pooled analysis indicated that the patients carrying the four high-risk genotypes *ALPK1* (rs11726117 [CC]), *ABCG2* (rs2231142 [TT]), *SLC2A9* (rs1014290 [AA]), and *SLC22A12* (rs3825016 [CC]) had a strong association with the odds of gout risk (OR: 14.99, PPV: 85%, and PAF: 5.69%).

In conclusion, this study indicated the joint effects of *ALPK1* and the genes *ABCG2*, *SLC2A9*, and *SLC22A12* on risk of gout. Our results revealed that the epistatic effect of *ALPK1* and *SLC2A9* or *SLC22A12* on gout risk differed between the Taiwan Han and aboriginal groups. The individuals carrying the four high-risk genotypes of *ALPK1*, *ABCG2*, *SLC2A9*, and *SLC22A12* were discovered to have an increased gout risk and high PPV for gout. These findings strongly support the hypothesis that the epistatic or joint effects of *ALPK1* and the loci of *ABCG2*, *SLC2A9*, and *SLC22A12* are key factors affecting the risk of gout, suggesting the development of personalized treatment for specific Taiwan populations for the prevention, prediction, and treatment of gout.

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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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