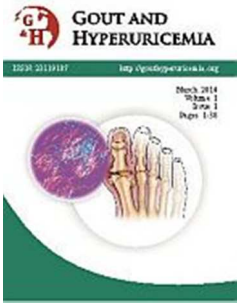


# Polymorphisms in insulin-like growth factor 1 receptorand PDZ domain containing 1 are associated with phenotype gout in Chinese Han males

| | |
|---|---|
| Journal: | *Gout and Hyperuricemia* |
| Manuscript ID | GH-2015-0022.R1 |
| Manuscript Type: | Original Article |
| Date Submitted by the Author: | 08-Nov-2015 |
| Complete List of Authors: | Wang, Xiaomin<br>Li, Xinde<br>Liu, Shiguo<br>Zhou, Zhaowei<br>Miao, Zhimin<br>Chen, Ying<br>Li, Changgui |
| Keywords: | gout, SNPs, rs6598541, rs7224610, rs1967017 |
| | |

# Polymorphisms in insulin-like growth factor 1 receptorand PDZ domain containing 1 are associated with phenotype gout in Chinese Han males

Xiaomin Wang[1,*], Xinde Li[1,2,*], Shiguo Liu[3], Zhaowei Zhou[1], Zhimin Miao[1], Ying Chen[1], Changgui Li[1,2]

1 Shandong Gout Clinical Medical Center, Qingdao 266003, China.

2 Shandong Provincial Key Laboratory of Metabolic Disease, The Affiliated Hospital ofQingdao University, Qingdao 266003, China.

3 Prenatal diagnosis center, The Affiliated Hospital of Qingdao University, Qingdao, 266003, China.

*These authors contributed equally to this work.

Correspondence to:

Changgui Li, MD

The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266003, China

Email: lichanggui@medmail.com.cn

Tel.: +86 10 82912019;

Fax: +86 10 82912015;

**Abstract**

**Objectives**: Three single nucleotide polymorphisms (SNPs), rs6598541 in insulin-like growth factor 1 receptor(*IGF1R*) gene, rs7224610 in hepatic leukemia factor (*HLF*) gene and rs1967017 in PDZ domain containing 1(*PDZK1*) gene, were proved to be significantly associated with the risk of hyperuricemia and phenotype gout in European and Polynesia descent. However, evidence for association with gout in Chinese is adequate. Thus, we conduct this association study to identify whether variants of these three loci affected the susceptibility to gout in Chinese Han males.

**Methods**: 932 gout patients and 1,124healthy controls were recruited for this study. The final results were obtained by sequenator. Association with gout was tested by logistic regression with false discovery rate adjusted and genotype-phenotype analysis was also conducted.

**Results**: Rs6598541 and rs1967017 showed a significant difference in allele frequencies between gout and control groups. Only rs1967017 showed a significant difference in genotype (rs6598541, P=0.033, OR=1.12 by allele level; P=0.099 by genotype level; rs1967017, P=0.009, OR=1.43 by allele level; P=0.015 by genotype level). Whereas rs7224610 was not replicated (rs7224610, P=0.990, OR=1.03 by allele level; P=0.802 by genotype level). With T-allele carriers of rs1967017 showing an increased risk of gout (OR=1.43, 95%CI=1.12-1.82), TT had a higher risk possibility as the recessive genotype (P=0.024, OR=1.33, 95%CI=1.08-1.65).

**Conclusion**: We validated rs6598541 and rs1967017 to be associated with gout in Chinese Han male population and unrelated with uric acid level. It may cause gout by

other ways. This study provides the new possible targets for diagnosis and treatment of gouty arthritis.

**Key words**: Gout; SNPs;rs6598541;rs7224610;rs1967017

**Short title: Polymorphisms in IGF1R and PDZK1 are associated with phenotype gout in Chinese Han males**

## Introduction

Gout is a urate crystal-induced arthritis resultingfromabnormal purine metabolism and/or the reduction of systematic uric acid excretion. With the improvement of living standards, the incidence and prevalence of gout increase year by year[1]. Compared with 1997, the prevalence of gout in the UK increased by 63.9% in 2012, the incidence rate increased by 29.6%[2]. This trend is more pronounced in China, especially in coastal areas. Investigation in five coastal areas of Shandong province in 2008 showed that the prevalence for hyperuricemia and gout in the studied populations was 13.19% and 1.14%[3]. Due to no way to cure gout completely, patients endure great agony when experiencing an acute attack of gout, and, they have to bear the economic burden lifelong[4].

In addition to environmental factors such as high-purine diet, drinking, obesity and dyslipidemias, genetic factors play an important role in the pathomechanism of gout and hyperuricemia (HUA)[5]. It is worthwhile to identify genetic factors to improve hyperuricemia and gout etiologic diagnosis. With the rapid development of high-throughput and chip technology, especially genome-wide association studies (GWAS). GWAS is an analysis comparing the allele frequencies of all available (or a whole GENOME representative set of polymorphic markers in unrelated patients with a specific symptom or disease condition, and those of healthy controls to identify markers associated with a specific disease or condition. In 2007, solute carrier family 2 (facilitated glucose transporter), member 9 (*SLC2A9)*SNP rs6855911 was suggested associated with serum uric acid level by Genome-wide scans in population samples

from Sardinia and Chianti[6]. Epidemiological studies have shown that there are significant ethnic and gender specificity in genetic susceptibility for gout and HUA[7,8]. In the following year, GWAS were done in the Framingham cohort and in the Rotterdam cohort, reporting rs16890979 in *SLC2A9* and rs2231142 in ATP-binding cassette, sub-family G, member 2 (*ABCG2*) and rs1165205 in *SLC17A3*were association with uric acid concentration and risk of gout[9]. Subsequent years, GWAs have also identified multiple loci of solute carrier family 22, member 11*SLC22A11*), solute carrier family 22, member 12(*SLC22A12*), solute carrier family 17, member 1*SLC17A1*), solute carrier family 16, member 9*SLC16A9*), glucokinaseregulato(*GCKR*), ras responsive element binding protein 1*RREB1*), inhibin, beta C*INHBC*), leucine rich repeat containing 16A (*RRC16A*), WD repeat domain 1 (*WDR1*), tripartite motif containing 46(*TRIM46*), scm-like with four mbt domains 1 (*SFMBT1*), transmembrane protein 171(*TMEM171*), vascular endothelial growth factor A (*VEGFA*), bromodomain adjacent to zinc finger domain, 1B (*BAZ1B*), protein kinase, AMP-activated, gamma 2 non-catalytic subunit (*PRKAG2*), stanniocalcin 1 (*STC1*), hepatocyte nuclear factor 4, gamma (*HNF4G*), ataxin 2 (*ATXN2*), ubiquitin-conjugating enzyme E2Q family member 2 (*UBE2Q2*), insulin-like growth factor 1 receptor(*IGF1R*), nuclear factor of activated T-cells 5, tonicity-responsive (*NFAT5*), v-maf avian musculoaponeuroticfibrosarcoma oncogene homolog *(MAF*), hepatic leukemia factor (*HLF*), beta-1,3-N-acetylglucosaminyltransferase 4 (*B3GNT4*), activin A receptor, type IB (*ACVR1B*) and protein phosphatase 1, regulatory subunit 12B

(*PPP1R12B*)associated with uric acid level in different ethnic[10,11].

Factors that influence serum urate levels are candidate causal risk factors for gout because of elevated serum uric concentration as an independent risk factor for gout[5].Such loci were proofed associated with uric acid levels but not associated with gout, such as *IGF1R*, *PDZK1*,*HLF[10]*. *IGF1R* belongs to the insulin receptor family located in chromosome 15q26.3, with the function of cell growth and differentiation[12]. GWAS in Chinese descent from 12,218individuals found IGF1R was feeble associated with uric acid. There are no relevant reports with gout in previous days[13]. *PDZK1* is located in lq21, contacting with urate transporter[14]. GWAS in European descent found *PDZK1*1rs1471633 was associated with uric acid, but could not gain the significantly evidence about gout. It was confirmed in 8,340 cases and 5,820 cases of Indian origin in Africa lineage again. Anna Kottgen found that *HLF* SNP associated with uric acid in 140,000 Europeans by whole genome sequencing, but no correlated with gout[15].3,451 Chinese Han population were genotyped, the result shown that HLF has no significant correlation with uric acid. The crowd also got the same results in Japan[13]. Recently, A J Phipps-Green filtrated1,536 gout cases and 2,645 healthy controls from New Zealand Europeans and Polynesians to test the twenty-eight genetic loci associated with serum uric acid levels which have been found by GWAS[10]. As a result,*igf1r* shown correlated with gout in European populations, while got the opposite result in the Polynesian, a meta-analysis got a positive result. A JPhipps-Green could not get statistically significant results that *PDZK1*was associated with gout in a separate group until they

did a meta-analysis in all participants of European and Polynesians. *HLF* showed a significant correlation with gout only in participants with higher polynesian ancestry.

Rs6598541, rs7224610 and rs1967017 polymorphisms were first reported associated with primary gout, but has not yet been reported in Chinese Han population. In this study, we aimed to assess the association of three SUA correlated SNPs in Chinese Han males.

## Methods

### *Subjects*

Epidemiological investigation shown that main population suffering from gout are mostly in older men and incidence rate ratio of gout approximately 18: 1 between men and women[8].We have not collected enough material about female cases. We enrolled 932 male gout patients (mean age, 49.80±13.27) and 1,124 male gout-free controls (mean age, 58.52±14.21) from the affiliated Hospital of Qingdao University (Qingdao, Shandong, China). All samples were derived from similar genetic background with Chinese Han male ancestry and resided in Shandong or nearby regions. Gout patients were explicitly diagnosed based on the criteria published by the American college of Rheumatology in 1977[16].The control group recalled that they and their family members have neither got gout attack nor had the experience of uric acid escalated. Participants suffering from cancer, liver damage, kidney disease or other coexisting severe diseases were excluded. Anthropometric and biochemical traits correlated with uric acid including systolic pressure, diastolic pressure, fasting

plasma glucose, triglycerides, total cholesterol, urea nitrogen, uric acid, creatinine, aspartate aminotransferase (AST),alanine aminotransferase (ALT) were measured in all participants. Body mass index (BMI) was calculated as weight (kg)/height ($m^2$), andwaist–hip ratio (WHR) was calculated as waistline (cm)/ hipline (cm). Clinical characteristics were obtained such as hypertension that systolic blood pressure ≥140mmHg or diastolic blood pressure ≥90 mmHg or receiving anti-hypertensive medication with a previous history of hypertension, diabetes that fasting blood glucose ≥7.0 mmol/l (126 mg/dl) or non-fasting blood ≥11.1 mmol/l (200 mg/dl) or under treatment of diabetes, and obesity that BMI ≥30 kg/$m^2$. In addition, tophi were also recorded. This study abidedby the principle of the Helsinki Declaration II and got the approval of the Ethics Committee of Affiliated Hospital of Qingdao University with all participants providing written informed consent.

*DNA analysis*

Genomic DNA was extracted from peripheral leukocytes by conventional methods. Extracted DNA was confirmed and quantified with a NanoDrop 1000 Spectrophotometer. For the genotyping of these SNPs, PCR amplification was performed. The primer sequences were following which designed using Primer 3.0 online: rs6598541forward, 5'-TGGGCTTAGTCACTGTGTGG-3'and reverse, 5'-TGGCTACCTCGTGTTCCTTT-3'; rs7224610forward,5'-CCCCCTTCCCCTTGTAAATA-3', reverse, 5'-GGGGGTAGATGCCTTATGGT-3'; rs1967017forward, 5'-CCCTGTCCTGACACTTGGTT-3', reverse,

5'-AGGCGACTGTGTACTCTGCAT-3'. 3% agarose gel electrophoresis was performed to separate the PCR products. Finally, detected by sequencing, genotyping results obtained.

*Statistical analysis*

We tested the Hardy-Weinberg equilibrium before all the analysis. The U-test was used to evaluate the difference of demographic and clinical data between case and control group.Allelic and genotypic frequencies were contrasted by the $\chi^2$ test, and odds ratios were calculated by multiple logistic regression analysis. The P values and odds ratios were adjusted for the demographic and clinical data which have been confirmed the significant difference by the U-test above. K-W test was used for genotype – phenotype analysis. P values less than 0.05 was considered significant.

**Results**

*Clinical characteristics*

The demographic and clinical data of the participants are shown in Table 1. Analysis showed that there were significant differences in age, BMI, diastolic blood pressure, blood glucose, triglycerides, total cholesterol, blood urea nitrogen, uric acid, creatinine and ALT between the patient group and the control group ($P<0.001$). It is worth mentioning that 17.27%of patients accompany with tophi.

*Allelic and genotypic frequencies analysis*

Those three SNPs were consistent with the Hardy-Weinberg Law test wholly ($P>0.05$).The results of the analysis of allele and genotype frequencies was displayed

in Table 2. The allele frequencies analysis of SNP rs6598541 and rs1967017 showed significant difference between two goroups, and that have nothing to do with uric acid level ($P^{*}_{rs6598541}$=0.029,$P_{rs6598541}$=0.003;$P^{*}_{rs1967017}$=$P_{rs1967017}$=0.009). SNP rs6598541 analysis showed that A is the risk allele of gout and the allele frequencies in cases and controls were 0.51 and 0.47. The difference was significant in two groups (P=0.033, OR=1.23, 95%CI=1.05-1.44). Even though genotypic frequencies analysis showed significant differences (AA/AG Vs GG: P=0.019) between twogroups, difference missed after bonferroni-correction been did (AA Vs AG Vs GG: P=0.099; AA/AG Vs GG: P=0.056) (P=0.099). We ascertained a statistically significant difference between the T and C alleles of rs1967017 (P=0.009, OR=1.43, 95%CI=1.12-1.82). The T allele frequency was 0.89 and 0.86 in cases and controls respectively, indicating a decreased risk of gout associated with the C allele. In the genotype analysis of rs1967017 we got the information which carriers of at least one C allele had a decreased risk of gout (P=0.024, OR=1.33, 95% CI=1.08-1.65). Both allele and genotype frequencies of rs7224620 were no significant differences between the control group and the patient group.

*Genotype–phenotype analysis*

The analysis of genotype–phenotype showed in Table 3. Patients carryingCC of rs1967017 had a lower WHR than patients carrying CT+TT (0.90 ±0.03 Vs 0.93 ±0.06 and 0.93 ±0.04, P=0.025). The carriers of TT phenotype of rs1967017 had a lower ratio than carriers of CC+ TT in Hypertension group (320/679 Vs 96/164 and 9/13, P=0.004). There were no significant differences in other aspects of additional

between patients and controls.

**Discussion**

This study was performed to assess the association of three SUA correlated SNPs first identified in Europeans and Polynesia descent with phenotype gout in Han Chinese males. Rs6598541is located in the intron region of NM_000875.4, which is defined as homo sapiens *IGF1R*. *IGF1R* belongs to the insulin receptor family located in chromosome15q26.3, containing about 315000bp of 21 exons and 20 introns[12]. This receptor binds insulin-like growth factor with a high affinity. It has tyrosine kinase activity, mainly concerned with cell growth and differentiation. Due to the cross-linking, there are certain functions of regulating metabolic activity. IGF binds with α subunit of the IGF1R, causing a conformational change in β subunit, and thereby facilitating the autophosphorylation of *IGF-1R*. This structural change activates phosphatidylinositol 3-kinase within the cytoplasm (PI3K), and then participates in intracellular second messenger pathway by expression the regulation of gene in the nucleus. The bind of *IGF1R* and *IGF1* bear on the regulation of glucose homeostasis and promote glucose metabolism[17]. It is maybe the cofactors of insulin resistance and impaired glucose tolerance. It is noteworthy that insulin resistance connected with hyperuricemia and gout. Study about the polymorphisms of IGF1R is mostly associated with the pathogenesis of cancer. YYang[18]found that the genetic susceptibility of rs1976667 and rs2684788 in *IGF1R* loci significantly associated withidiopathic short stature. Winder found *IGF1R* rs2272037 was closely related to

shorter survival of metastatic colorectal cancer, rs2016347and rs2229765 of *IGF1R*has nothing to do with metastatic colorectal cancer. Another study found that rs2272037 associated with gliomas[19]. Rs6598541 in *IGF1R* been confirmed associated with hyperuricemia in China and Europe[13,15]. There are no relevant reports that *IGF1R* SNP rs6598541 was associated with gout until A J Phipps-Greendetected it in European [10]. Our experiments also confirmed rs6598541 associated with gout in Chinese Han people, and as long as carrying the A allele increases the risk of suffering from gout. But it becomes not statistically significant after we did bonferronicorrection. We should not take arbitrary attitude to deny the existence of such a possibility, there may be transformation when we increase the sample size.

*PDZK1* is a scaffold protein 4 PDZ domain located in lq2, belong to the sodium hydrogen exchanger regulatory factor (NHERF) family, also known as *NHERF3*. The skeleminPDZK1 encodes expressed in the apical membrane of the kidney proximal small pieces, liver, small intestine, adrenal cortex, etc. It plays an important role in Ion transport in the body, the cellular localization of some proteins, cell proliferation, differentiation, cell-cell interactions, tumor resistance and lipoprotein metabolism. *PDZK1* contacts with urate transporter 1(URAT1), Organic anion transporter (OAT4) and type 1 sodium-dependent phosphate transporter (NPT1) via the class I PDZ motif, which had been proven as uric acid transporters [14]. For instance, URAT1 and sodium-coupled monocarboxylate transporter (SMCT) binding *PDZK1* can induce efficient delivery of the substrate, and actuate URAT1 to mediate reabsorption of urate

by SMCT. *PDZK1* interacts with protein products, such as *ABCG2, SLC22A13, SLC5A8, SLC5A12, SLC22A11, SLCl7A1 and SLCl7A3*, known as channel proteins and also exist gene mutations associated uric acid concentrations[20,21]. There were also have studies suggested that L-1βmediated joint inflammation anddecrease levels of *PDZK1* mRNA and protein significantly[22,23]. Yuzo Takada provides proof which rs129861 in *PDZK1* related with uric acid but no significant correlation with gout in Japanese[24]. Melanie Kolz also found rs1797052 and rs1298954 of *PDZK1* polymorphism associated with serum uric acid in a total of 2439 participants in Vis land and Korcula island and three cities of Split[25]. GWAS in European descent found that rs1471633 SNP is located in *PDZK1* genes and it is associated with uric acid, but could not gain the significantly evidence about gout. It was confirmed in 8,340 cases and 5,820 cases of Indian origin in Africa lineage again [15]. A J Phipps-Green recently assessed the association of PDZK1 rs1967017 with gout in European and Polynesian group. They could not get statistically significant results in a separate group until they did a meta-analysis in all participants of European and Polynesians[10]. We increased the sample size in our study, including 932 Chinese male patients with gout and 1024 Chinese male healthy people. We found that the rs1967017 has significantly correlation with gout in Chinese Han men, and T allele is a risk factor of gout incidence as recessive manner even after correction. And, the patients of TT phenotype of rs1967017 had a lower ratio than patients of CC+ TT in Hypertension group.

*HLF* is protein-coding gene located in 17q22, belong to the Bzip family and PAR

(proline and acid rich amino) subfamily of transcriptional regulatory proteins. Rs7224610 pertains to variation of HLF intron region. Anna Kottgen found that HLF rs7224610 associated with uric acid in 140,000 Europeans by whole genome sequencing, but no correlated with gout [15]. Binyao Yang genotyped 3,451 Chinese Han population shown that rs7224610 has no significant correlation with uric acid. The crowd also got the same results with the population in Japan[13]. A J Phipps-Green study found that *HLF* rs7224610 has a correlation with gout neither in European nor in all participants of Polynesian, even meta-analysis was done in all the populations of two groups. While they stratified analysis in higher Polynesian ancestry participants found rs7224610 locus has a significant correlation with gout (gender and age corrected) [10]. It was most likely that rs1967017 related to susceptibility to gout has racial specificity. We also failed to confirm rs7224610 associated with gout in Chinese Han male in our study.

In result, we found rs6598541in *IGF1R* and rs1967017 near *PDZK1* associated with gout in this study, but there are still some limitations. As noted above, although we have a relatively large sample size, but still not entirely sufficient. For example, the risk of gout allele for rs6298541 is A allele with dominant inheritance, but would have no significant after getting Bonferroni correction. Perhaps statistical difference becomes even more significant after increasing sample size. The larger the sample size, the better to detect disease susceptibility genes. Secondly, we just chose Han Chinese male population surrounding Shandong in this study. It will be more persuasive to get verified in more different races. And, expanded research needs to

continue to explore the functional role of re6598541 and re1967017 in the pathogenesis of gout. Only enough follow-up studies to be done on the pathogenesis of gout we can have a more in-depth understanding, and diagnosis and treatment of gout may be a new development than before.

## Reference

1.Kuo CF, Grainge MJ, Zhang W, Doherty M. Global epidemiology of gout: prevalence, incidence and risk factors. Nat Rev Rheumatol. 2015.

2.Kuo CF, Grainge MJ, Mallen C, Zhang W, Doherty M. Rising burden of gout in the UK but continuing suboptimal management: a nationwide population study. Ann Rheum Dis. 2015; 74: 661-7.

3.Miao Z, Li C, Chen Y, et al. Dietary and lifestyle changes associated with high prevalence of hyperuricemia and gout in the Shandong coastal cities of Eastern China. J Rheumatol. 2008; 35: 1859-64.

4.Brook RA, Kleinman NL, Patel PA, et al. The economic burden of gout on an employed population. Curr Med Res Opin. 2006; 22: 1381-9.

5.Roddy E, Doherty M. Epidemiology of gout. Arthritis Res Ther. 2010; 12: 223.

6.Li S, Sanna S, Maschio A, et al. The GLUT9 gene is associated with serum uric acid levels in Sardinia and Chianti cohorts. PLoS Genet. 2007; 3: e194.

7.Wallace KL, Riedel AA, Joseph-Ridge N, Wortmann R. Increasing prevalence of gout and hyperuricemia over 10 years among older adults in a managed care population. J Rheumatol. 2004; 31: 1582-7.

8.Smith EU, Diaz-Torne C, Perez-Ruiz F, March LM. Epidemiology of gout: an update. Best Pract Res Clin Rheumatol. 2010; 24: 811-27.

9.Dehghan A, Kottgen A, Yang Q, et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. Lancet. 2008; 372: 1953-61.

10.Phipps-Green AJ, Merriman ME, Topless R, et al. Twenty-eight loci that influence serum urate levels: analysis of association with gout. Ann Rheum Dis. 2014.

11.Li C, Li Z, Liu S, et al. Genome-wide association analysis identifies three new risk loci for gout arthritis in Han Chinese. Nat Commun. 2015; 6: 7041.

12.Klammt J, Kiess W, Pfaffle R. IGF1R mutations as cause of SGA. Best Pract Res Clin Endocrinol Metab. 2011; 25: 191-206.

13.Yang B, Mo Z, Wu C, et al. A genome-wide association study identifies common variants influencing serum uric acid concentrations in a Chinese population. BMC Med Genomics. 2014; 7: 10.

14.Ichida K. What lies behind serum urate concentration? Insights from genetic and genomic studies. Genome Med. 2009; 1: 118.

15.Kottgen A, Albrecht E, Teumer A, et al. Genome-wide association analyses identify 18 new loci associated with serum urate concentrations. Nat Genet. 2013; 45: 145-54.

16.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: 895-900.

17.Keyhanfar M, Booker GW, Whittaker J, Wallace JC, Forbes BE. Precise mapping of an IGF-I-binding site on the IGF-1R. Biochem J. 2007; 401: 269-77.

18.Yang Y, Huang H, Wang W, Yang L, Xie LL, Huang W. Association of insulin growth factor-1 receptor gene polymorphisms with genetic susceptibility to idiopathic short stature. Genet Mol Res. 2013; 12: 4768-79.

19.Winder T, Zhang W, Yang D, et al. Germline polymorphisms in genes involved in the IGF1 pathway predict efficacy of cetuximab in wild-type KRAS mCRC patients. Clin Cancer Res. 2010; 16: 5591-602.

20.Anzai N, Jutabha P, Amonpatumrat-Takahashi S, Sakurai H. Recent advances in renal urate transport: characterization of candidate transporters indicated by genome-wide association studies. Clin Exp Nephrol. 2012; 16: 89-95.

21.Gisler SM, Pribanic S, Bacic D, et al. PDZK1: I. a major scaffolder in brush borders of proximal tubular cells. Kidney Int. 2003; 64: 1733-45.

22.Joosten LA, Netea MG, Mylona E, et al. Engagement of fatty acids with Toll-like receptor 2 drives interleukin-1beta production via the ASC/caspase 1 pathway in monosodium urate monohydrate crystal-induced gouty arthritis. Arthritis Rheum. 2010; 62: 3237-48.

23.Lenzen H, Lunnemann M, Bleich A, Manns MP, Seidler U, Jorns A. Downregulation of the NHE3-binding PDZ-adaptor protein PDZK1 expression during cytokine-induced inflammation in interleukin-10-deficient mice. PLoS One. 2012; 7: e40657.

24.Takada Y, Matsuo H, Nakayama A, et al. Common variant of PDZK1, adaptor protein gene of urate transporters, is not associated with gout. J Rheumatol. 2014; 41: 2330-1.

25.Kolz M, Johnson T, Sanna S, et al. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009; 5: e1000504.

**Table 1. Clinical characteristic of the samples**

| Parameter | Gout patients (n=932) | Controls (n=1124) | P |
|---|---|---|---|
| Age (years) | 49.80 ± 13.27 | 58.52 ± 14.21 | **<0.001** |
| Tophi, n(%) | 161(17.27%) | - | - |
| Body mass index (kg/m$^2$) | 27.01 ± 3.67 | 24.40 ± 3.59 | **<0.001** |
| Waist–hip ratio | 0.92 ± 0.07 | 0.93 ± 0.05 | 0.133 |
| Systolic pressure (mmHg) | 135.00 ± 18.39 | 136.91 ± 20.16 | 0.088 |
| Diastolic pressure (mmHg) | 88.66 ± 12.17 | 84.19 ± 11.62 | **<0.001** |
| Blood glucose (mmol/L) | 6.16 ± 1.45 | 6.13 ± 1.84 | **<0.001** |
| Triglycerides (mmol/L) | 2.43 ± 1.82 | 1.39 ± 0.98 | **<0.001** |
| Total cholesterol (mmol/L) | 5.19 ± 1.15 | 5.45 ± 1.16 | **<0.001** |
| Urea nitrogen (mmol/L) | 5.38 ± 2.80 | 5.73 ± 1.53 | **<0.001** |
| Uric acid (μmo1/L) | 460.88 ± 119.90 | 281.69 ± 53.46 | **<0.001** |
| Creatinine (μmo1/L) | 91.01 ± 50.70 | 72.67 ± 15.39 | **<0.001** |
| Alanine aminotransferase | 24.51 ± 12.69 | 23.52 ± 11.42 | 0.732 |
| Aspartate aminotransferase | 33.43 ± 27.30 | 21.55 ± 12.91 | **<0.001** |

**Table 2.The allele- genotype frequencies between case and control.**

| SNP | Locus | Location (GRCh38.p2) | Chromosome | Risk allele | | Detail | | P* | P | OR [95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|
| rs6598541 | IGF1R | 98727906 | 15 | A | | A | G | | | |
| | | | | | Case | 0.51 | 0.49 | 0.029 | 0.033 | 1.23 [1.05-1.44] |
| | | | | | Control | 0.47 | 0.53 | | | |
| rs7224610 | HLF | 55287427 | 17 | C | | A | C | | | |
| | | | | | Case | 0.85 | 0.15 | 0.926 | 0.990 | 1.03 [0.83-1.28] |
| | | | | | Control | 0.86 | 0.14 | | | |
| rs1967017 | PDZK1 | 145711421 | 1 | T | | T | C | | | |
| | | | | | Case | 0.89 | 0.11 | 0.009 | 0.009 | 1.43 [1.12-1.82] |
| | | | | | Control | 0.86 | 0.14 | | | |

| SNP | Detail | | | P | Dominant | | P | OR[95% CI] | Recessive | | P | OR[95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A/G | G/G | A/A | | AG/AA | GG | | | AA | AG/GG | | |
| rs6598541 | | | | | | | | | | | | |
| Case | 0.52 | 0.23 | 0.25 | 0.099 | 0.77 | 0.23 | 0.056 | 1.28 [1.04-1.57] | 0.25 | 0.75 | 0.361 | 1.17 [0.95-1.44] |
| Control | 0.51 | 0.28 | 0.22 | | 0.72 | 0.28 | | | 0.22 | 0.79 | | |
| | T/T | C/C | T/C | | CT/TT | CC | | | TT | CT/CC | | |
| rs1967017 | | | | | | | | | | | | |
| Case | 0.79 | 0.01 | 0.19 | 0.015 | 0.99 | 0.01 | 0.997 | 1.07 [0.52-2.19] | 0.79 | 0.21 | 0.024 | 1.33 [1.08-1.65] |
| Control | 0.73 | 0.02 | 0.25 | | 0.98 | 0.02 | | | 0.73 | 0.27 | | |

SNP: single nucleotide polymorphism;

IGF1R: insulin-like growth factor 1 receptor; HLF: hepatic leukemia factor; PDZK1: PDZ domain containing 1.

P*: before adjusting by uric acid level.

**Table 3.Associations between genotypes and clinical characteristics of rs1967017 in gout patient.**

| dbSNP ID (C/T) | TT | CT | CC | TT Vs CT Vs CT P | TT Vs CT +CC P | TT Vs CT +CC OR [95% CI] | CC Vs CT+ TT P | CC Vs CT+ TT OR [95% CI] |
|---|---|---|---|---|---|---|---|---|
| BMI (kg/m$^2$) | 27.10 ± 3.75 | 26.83 ± 3.56 | 27.67 ± 4.15 | 0.421 | 0.765 | 0.99[0.92-1.07] | 0.127 | 0.91[0.79-1.04] |
| WHR(cm/cm) | 0.93 ± 0.06 | 0.93 ± 0.04 | 0.90 ± 0.03 | 0.140 | 0.261 | 0.25[0.21-0.32] | **0.025** | 76.6[40.98-143.91] |
| Systolic pressure (mmHg) | 134.92 ± 18.82 | 135.64 ± 17.02 | 140.83 ± 18.17 | 0.304 | 0.193 | 1.00[0.98-1.02] | 0.442 | 0.94[0.87-1.01] |
| Diastolic pressure (mmHg) | 88.35 ± 11.9 | 90.42 ± 13.5 | 88.00 ± 15.1 | 0.139 | 0.129 | 1.02[0.99-1.05] | 0.966 | 1.05[0.97-1.14] |
| Blood glucose (mmol/L) | 6.18 ± 1.45 | 6.05 ± 1.21 | 7.04 ± 3.32 | 0.635 | 0.973 | 0.99[0.78-1.24] | 0.471 | 1.96[1.80-2.14] |
| Triglycerides (mmol/L) | 2.40 ± 1.74 | 2.38 ± 1.79 | 3.34 ± 3.02 | 0.534 | 0.702 | 0.88[0.71-1.09] | 0.656 | 0.94[0.57-.55] |
| Total cholesterol (mmol/L) | 5.15 ± 1.13 | 5.20 ± 1.13 | 5.10 ± 1.28 | 0.931 | 0.872 | 1.27[9.97-1.67] | 0.211 | 1.19[0.54-2.59] |
| Urea nitrogen (mmol/L) | 5.30 ± 2.86 | 5.66 ± 2.71 | 5.33 ± 2.63 | 0.098 | 0.131 | 1.07[0.89-1.29] | 0.248 | 0.60[0.39-1.22] |
| Urica cid (μmo1/L) | 464.59 ± 116.72 | 448.02 ± 129.67 | 400.38 ± 101.53 | 0.078 | 0.104 | 0.99[0.98-1.01] | 0.174 | 1.01[1.00-1.02] |
| Creatinine (μmo1/L) | 89.84 ± 45.90 | 97.47 ± 71.75 | 88.41 ± 22.58 | 0.405 | 0.213 | 1.01[0.99-1.02] | 0.984 | 1.03[0.98-1.09] |
| ALT | 33.43 ± 25.18 | 31.76 ± 33.76 | 27.81 ± 8.49 | 0.777 | 0.236 | 1.01[0.99-1.02] | 0.629 | 1.02[0.96-1.09] |
| AST | 24.72 ± 12.68 | 23.83 ± 13.66 | 25.29 ± 6.57 | 0.365 | 0.277 | 0.99[0.97-1.02] | 0.598 | 1.00[0.98-1.02] |
| Age (years) | 49.67 ± 13.26 | 50.16 ± 13.65 | 50.00 ± 13.48 | 0.959 | 0.942 | 0.97[0.95-0.98] | 0.994 | 1.23[1.19-1.27] |
| Disease duration (years) | 6.48 ± 7.49 | 6.14 ± 6.66 | 7.83 ± 8.41 | 0.766 | 0.639 | 0.99[0.95-1.03] | 0.938 | 1.04[0.96-1.12] |
| Age at diagnosis (years) | 43.21 ± 12.78 | 44.10 ± 12.97 | 40.17 ± 11.34 | 0.427 | 0.901 | 1.00[0.98-1.02] | 0.218 | 1.11[1.09-1.13] |
| <29 | 41/679 | 9/164 | 1/13 | | 0.846 | 1.07[0.53-2.19] | 0.553 | 1.32[0.17-10.37] |
| 30-49 | 313/679 | 74/164 | 5/13 | | 0.728 | 1.06[0.76-1.48] | 0.593 | 0.74 [0.24-2.27] |
| 50-69 | 265/679 | 66/164 | 5/13 | | 0.792 | 0.96[0.63-1.34] | 0.953 | 0.97[0.31-2.98] |
| 70-89 | 60/679 | 15/164 | 2/13 | | 0.750 | 0.91[0.52-1.61] | 0.329 | 1.86 [0.41-8.56] |
| Hypertension | 320/679 | 96/164 | 9/13 | | **0.004** | 0.61[0.44-0.86] | 0.155 | 2.31 [0.71-7.56] |
| Diabetes | 135/679 | 29/164 | 3/13 | | 0.590 | 1.12[0.73-1.72] | 0.726 | 1.24[0.34-4.56] |
| Obesity | 102/679 | 18/164 | 4/13 | | 0.383 | 1.25[0.76-2.04] | 0.105 | 2.68[0.81-8.83] |

BMI: body mass index. WHR: waistline/hipline.

ALT:alanine aminotransferase. AST:aspartate aminotransferase.

Hypertension: systolic blood pressure ≥140mmHg or diastolic blood pressure ≥90 mmHg or receiving anti- hypertensive medication with a previous history of hypertension.

Diabetes: fasting blood glucose ≥7.0 mmol/l (126 mg/dl) or non-fasting blood ≥11.1 mmol/l (200 mg/dl) or under treatment of diabetes.

Obesity: BMI ≥30 kg/m$^2$.