Effect of Genetic Polymorphisms on Development of Gout

Wako Urano, Atsuo Taniguchi, Eisuke Inoue, Chieko Sekita, Naomi Ichikawa, Yumi Koseki, Naoyuki Kamatani, and Hisashi Yamanaka

ABSTRACT. Objective. To validate the association between genetic polymorphisms and gout in Japanese patients, and to investigate the cumulative effects of multiple genetic factors on the development of gout.
Methods. Subjects were 153 Japanese male patients with gout and 532 male controls. The genotypes of 11 polymorphisms in the 10 genes that have been indicated to be associated with serum uric acid levels or gout were determined. The cumulative effects of the genetic polymorphisms were investigated using a weighted genotype risk score (wGRS) based on the number of risk alleles and the OR for gout. A model to discriminate between patients with gout and controls was constructed by incorporating the wGRS and clinical factors. C statistics method was applied to evaluate the capability of the model to discriminate gout patients from controls.
Results. Seven polymorphisms were shown to be associated with gout. The mean wGRS was significantly higher in patients with gout (15.2 ± 2.01) compared to controls (13.4 ± 2.10; $p < 0.0001$). The C statistic for the model using genetic information alone was 0.72, while the C statistic was 0.81 for the full model that incorporated all genetic and clinical factors.
Conclusion. Accumulation of multiple genetic factors is associated with the development of gout. A prediction model for gout that incorporates genetic and clinical factors may be useful for identifying individuals who are at risk of gout. (J Rheumatol First Release June 1 2013; doi:10.3899/jrheum.121244)

Key Indexing Terms:
GOUT   URIC ACID   SINGLE-NUCLEOTIDE POLYMORPHISMS

Epidemiological studies have shown an increasing prevalence of gout. For example, the prevalence of gout in the United States was reported to be 3.9% by the National Health and Nutrition Examination Survey 2007-2008[1]. In Japan, the prevalence of gout has been rising, and has been reported to be 1.1% in Japanese men[2]. Gout has been reported to be associated with a high risk of death from all causes and coronary heart disease[3]. Therefore, the identification of individuals with a high risk of developing gout is important.

Multiple genetic and environmental factors are associated with the development of gout. Environmental factors, particularly dietary factors, have been established through epidemiological studies[4]. Genome-wide association studies (GWAS) in white and African American populations have recently revealed polymorphisms on several loci that are associated with serum uric acid (SUA) levels[5,6,7,8,9]. Gout is a urate deposition disease, and epidemiological studies have demonstrated that hyperuricemia is the most important risk factor for developing gout[10]. Several genetic factors associated with SUA have been shown to be risk factors for gout[6,7]. Studies in Japanese populations have revealed susceptibility loci for SUA and gout[11,12,13,14,15]. These studies have suggested that the prediction of gout before onset is possible using genetic and environmental factors.

The aims of our study were to validate the associations between multiple genetic loci reported to be associated with SUA level and gout in Japanese; to examine the effects of multiple genetic factors on the development of gout; and to develop a model to discriminate between patients with gout and controls using genetic and clinical factors and to evaluate it using C statistics.

MATERIALS AND METHODS

Study participants. The subjects in this study were previously described[12]. From April to October 2004, patients with primary gout in the outpatient clinic at the Institute of Rheumatology at the Tokyo Women's Medical University were asked to participate. The diagnosis of acute gouty arthritis

was based on the preliminary classification criteria described by Wallace, *et al*[16]. Informed consent was obtained from 188 Japanese patients with gout. Seven samples were excluded because of ethnicity, sex, and availability of DNA; thus 181 male patients were studied. On the first visit, blood samples were collected from all patients and tested for SUA, creatinine, and triglycerides using a Hitachi H7700 autoanalyzer (Hitachi). Estimation of the glomerular filtration rate (eGFR) was based on the equation recommended by the Japanese Society of Nephrology[17]. In our previous study, genomic DNA from Japanese volunteers was collected in Tokyo[18]. A total of 1032 Japanese individuals were recruited. Of those, 595 males were selected randomly and served as controls in the present study. Informed consent for the use of their samples was obtained from each subject. Genomic DNA was prepared from peripheral blood using a genomic DNA isolation kit (Qiagen).

This research was reviewed and approved by the Hospital Ethics Committee for Human Genome Research, and all subjects provided informed consent.

Genotype analysis. We selected 10 genes reported to be associated with SUA or gout based on the metaanalysis of GWAS by Kolz, *et al*[9] and a GWAS by Kamatani, *et al*[11]; the latter GWAS is the only one reported for SUA in a Japanese population (Table 1). Single-nucleotide polymorphisms (SNP) in *SLC2A9*, *SLC17A1*, and *SLC22A12* were selected based on previous studies because they have shown significant associations with the development of gout[12,14,15]. Because the variant rs12356193 in *SLC16A9*[9] was nearly monomorphic in the Japanese population, we selected rs2242206, which is estimated to be located in the same haplotype block as rs12356193 based on information obtained from HapMap JPT[19]. Two polymorphisms, rs2231142 and rs72552713, were selected for *ABCG2*. These variants have been shown to be genetic risk factors for gout in the Japanese population, and no linkage disequilibrium has been detected between them[13]. Genotyping was primarily performed using TaqMan SNP Genotyping Assays™ (Applied Biosystems). Detection of each genotype was performed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The data were processed and genotypes were determined using the Sequence Detection System (SDS) version 2.1 software (Applied Biosystems). The rs121907892 genotype in *SLC22A12* (c. G774A) was determined according to our previous study[12]. The rs72552713 genotype in *ABCG2* was determined by direct sequencing of PCR products using the forward primer (5'- AAA CCA TAG AAT TTC TTG ACC AG- 3') and the reverse primer (5' -CTG CCT TTT CAC ATA AGT GTC- 3'). Sequencing of PCR products was conducted using the Big Dye Terminator cycle sequencing kit and the ABI Prism 3100xl Genetic Analyzer (Applied Biosystems).

Weighted genotype risk score and statistical analysis. The background data

of the gout and control groups was compared using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Single genetic marker tests for gout and the controls were evaluated using Fisher's exact test. OR with 95% CI of each SNP for developing gout were calculated. Statistical analyses were performed using R software (The Institute of Statistical Mathematics; http://cran.ism.ac.jp).

Gene-gene interactions between pairwise combinations of SNP were tested by likelihood ratio tests based on the logistic model with and without interaction. Bonferroni correction for multiple testing was performed.

A weighted genotype risk score (wGRS) was constructed as follows. A weight of 0 was assigned to risk alleles without significant association with gout ($p > 0.05$). Risk alleles significantly associated with gout ($p < 0.05$) were assigned a weight of 1, 2, or 3 based on OR. Risk alleles with a $1 < OR \leq 2$ and $2 < OR \leq 3$ were assigned as weight 1 and 2, respectively. The OR of SNP rs121907892 (*SLC22A12*) was infinity. Because of the strong association between this SNP and gout, a weight of 3 was assigned to this allele. Table 1 lists the weight of each risk allele. The wGRS for an individual was the sum of the number of risk alleles for each SNP multiplied by its weight. The cumulative effect of the wGRS on gout was examined by a logistic regression model with adjustment for age, body mass index (BMI), triglyceride (TG) levels, and eGFR. The proportion of individuals with wGRS ≤ 12 was 26%; these patients served as a reference group[20]. The OR for individuals with each wGRS was calculated relative to the reference group.

Models to discriminate patients with gout from controls were constructed using genetic and/or clinical factors. The wGRS was incorporated as a genetic factor. Clinical factors were constructed from age, BMI, TG, and eGFR. The latter 3 variables were included as clinical factors because their distributions were significantly different between patients with gout and controls (Table 2). We developed 5 models: model 1 was constructed with a genetic factor (wGRS) alone; model 2 was constructed with age and BMI; model 3 included age, BMI, and TG; model 4 included all clinical factors; and model 5 was constructed with all clinical and genetic factors. The discriminative capability of the models was evaluated using logistic regression to generate C statistics[21]. A C statistic < 0.6 has a low clinical value, while a C statistic > 0.8 has clinical utility[22].

RESULTS

Genotyping was completed in 153 patients in the gout group and 532 controls. The demographic data of the subjects are shown in Table 2.

Association of individual SNP with gout. The distribution of allelic frequencies is shown in Table 1. The allele frequency

Table 1. Genotype and allele distribution of single-nucleotide polymorphism (SNP) in patients with gout and controls.

SNP	Genes	Risk Allele	Risk Allele Frequency, %		OR of Gout for Risk Allele (95% CI)	p	Weights for Genotype Risk Scores	Ref*
			Gout	Controls				
rs12129861	*PDZKI*	G	85.9	85.1	1.10 (0.77–1.59)	0.66	0	9
rs780094	*GCKR*	A	63.4	55.6	1.48 (1.15–1.91)	1.74×10^{-3}	1	9
rs2231142	*ABCG2*	A	45.4	28.1	2.16 (1.68–2.78)	7.40×10^{-10}	2	9
rs72552713	*ABCG2*	T	5.2	2.0	2.50 (1.22–5.00)	9.49×10^{-3}	2	13
rs742132	*LRRC16A*	T	74.5	69.2	1.37 (1.03–1.82)	0.028	1	9
rs2544390	*LRP2*	T	57.5	49.9	1.32 (1.03–1.69)	0.025	1	11
rs1165196	*SLC17A1*	T	89.5	82.8	1.85 (1.27–2.78)	1.03×10^{-3}	1	14
rs1014290	*SLC2A9*	T	71.9	59.0	1.67 (1.27–2.17)	1.18×10^{-4}	1	15
rs2242206	*SLC16A9*	T	59.2	58.4	1.11 (0.86–1.43)	0.426	0	NA
rs17300741	*SLC22A11*	G	4.2	3.3	1.18 (0.58–2.22)	0.622	0	9
rs121907892	*SLC22A12*	G	100.0	98.4	Inf (1.19 – inf)	0.02	3	12

* Each SNP was selected based on the corresponding reference. Inf: infinity; NA: not available.

Table 2. Characteristics of patients with gout and controls in this study. Data are expressed as mean (SD) except for family history of gout (%). Age in the gout group represents age at onset of gout. Age in controls indicates age at entry to the study as described[17].

Characteristic	Gout, n = 153	Controls, n = 532	p
Age, yrs	39.5 (11.3)	40.5 (11.5)	0.687
Height, cm	168.9 (5.3)	170.5 (6.2)	0.0069
Weight, kg	70.8 (8.8)	67.4 (9.8)	< 0.0001
Body mass index	24.8 (2.7)	23.2 (2.9)	< 0.0001
Uric acid, mg/dl	9.5 (1.3)	5.7 (1.2)	< 0.0001
Triglycerides, mg/dl	220.7 (162.1)	146.4 (95.1)	< 0.0001
Creatinine, mg/dl	0.88 (0.16)	0.83 (0.14)	0.0011
eGFR, ml/min/1.73 m^2	79.2 (18.3)	86.8 (19.4)	< 0.0001
Family history of gout, %	30.7	NA	

eGFR: estimated glomerular filtration rate; NA: not available.

for each polymorphism was in Hardy-Weinberg equilibrium in the controls. The polymorphisms in *GCKR*, *ABCG2*, *LRRC16A*, *LRP2*, *SLC17A1*, *SLC2A9*, and *SLC22A12* were significantly associated with gout (p < 0.05).

Gene-gene interactions. The allele frequencies of rs72552713 in *ABCG2* and rs121907892 in *SLC22A12* were too low to examine the gene-gene interactions. The gene-gene interactions between some combinations were not evaluated because the logistic regression was not convergent. Although the combination of *SLC16A9* (rs2242206) and *ABCG2* (rs2231142) showed p values of 0.018, the Bonferroni correction removed significance (p < 0.0014). Therefore, no combinations with statistical significance were detected.

Distribution of weighted genotype score and the risk of gout. The distribution of patients with gout and controls according to the wGRS is shown in Figure 1. The means (SD) of wGRS of the patients with gout and the controls were 15.2 (2.01) and 13.4 (2.10), respectively (p < 0.0001). Figure 2 shows OR for gout in individuals with each wGRS relative to individuals with wGRS ≤ 12 by logistic regression
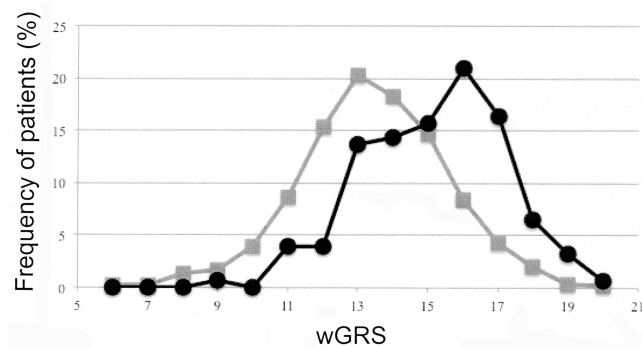

Figure 1. Distribution of weighted genotype risk score (wGRS) in patients with gout (black circles) and controls (gray squares). Mean (SD) wGRS of patients = 15.2 (2.01); controls = 13.4 (2.10; p < 0.0001).

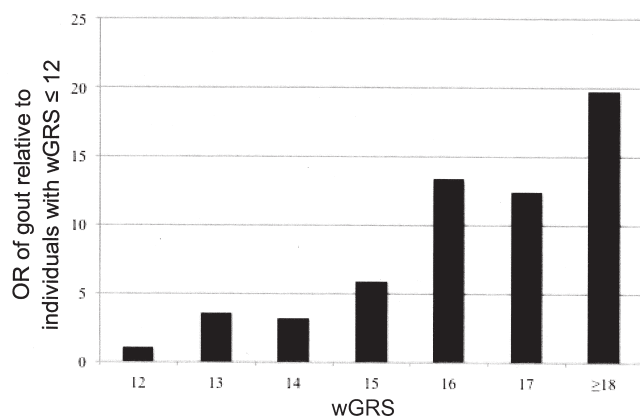*Figure 2.* OR of gout for individuals with each weighted genotype risk score (wGRS) relative to those of individuals with wGRS ≤ 12. p < 0.005 for each comparison with the reference group.

analysis. OR increased with the increasing wGRS. The OR of developing gout in individuals with wGRS ≥ 18 relative to individuals with wGRS ≤ 12 was 19.7 (95% CI 7.1–55.0, p = 1.2×10^{-8}). The logistic regression analysis showed that the increase of one wGRS resulted in an increase of 1.5 OR (95% CI 1.4–1.70, p = 2.4×10^{-14}).

The discriminative capability of a prediction model that includes genetic and clinical factors. We constructed a model to discriminate between patients with gout and the controls using wGRS or clinical factors (Table 3). In model 1, which incorporated wGRS alone, the C statistic was 0.72. Models 2, 3, and 4 were constructed only with clinical factors, and an improvement in the C statistic ranging from 0.69 to 0.73 was observed as the number of clinical factors increased. The highest C statistic was obtained when both genetic and clinical factors were incorporated into the model (model 5, with a C statistic = 0.81).

DISCUSSION

We validated the association between 6 out of 11 SNP and gout in a Japanese population and demonstrated the cumulative effects of genetic variants on the development of gout using wGRS. A model that includes genetic and clinical factors was developed to predict gout. The C statistic of the model constructed by all clinical factors (model 4) was 0.73, which was comparable to that of the model that included the genetic factor alone (model 1). However, when the genetic factor was combined with all clinical factors (model 5), the C statistic was increased to 0.81, indicating that both genetic and clinical factors are important in the development of gout. The result of the case-control discrimination may suggest the usefulness of this approach to predict gout.

Previous studies, including GWAS, have revealed genetic variants that are associated with SUA level. Based on these data, additive genetic risk scores have been shown

Table 3. C statistic of each model to discriminate gout.

Model	C Statistic (95% CI)
Model 1: wGRS without adjustment	0.72 (0.68–0.76)
Model 2: age + BMI	0.69 (0.65–0.74)
Model 3: age + BMI + TG	0.73 (0.68–0.77)
Model 4: age + BMI + TG + eGFR	0.73 (0.69–0.78)
Model 5: age + BMI + TG + eGFR + wGRS	0.81 (0.78–0.85)

wGRS: weighted genotype risk score; BMI: body mass index; TG: trigly-ceride level; eGFR: estimated glomerular filtration rate.

to be associated with SUA[23,24,25,26]. However, associations between genetic risk scores and gout have not been studied extensively. Dehghan, *et al* reported that the OR for gout was increased 6 to 41 times across the GRS in white or African American cohorts[23]. Our study validated these results in Japanese males and demonstrated the polygenic character of gout. Further, the study suggested the capability of our model for predicting gout using genetic and clinical factors. The predictive power of this model should be evaluated in cohort studies.

The contribution of genetic variants in case discrimination or prediction has been investigated in several diseases other than gout. In type 2 diabetes, a disease commonly associated with gout, the C statistics of the prediction models using genetic information alone have been reported to range from 0.54 to 0.63[20,27,28,29]. The contribution of genetic variants in the prediction of type 2 diabetes is modest. Minimal effects of genetic variants for prediction have also been reported for obesity and premature coronary disease[30,31]. However, De Jager, *et al* indicated that the C statistic of the genetic model for multiple sclerosis was 0.7[32], comparable to the C statistic observed in our present study. Although the predictive or discriminatory capabilities of genetic factors are expected to vary between diseases, differences in methodologies may influence the divergent results. The genetic aspects of uric acid have been indicated to be a good example of the polygenic trait theory[33], and all of the genetic variants in this study have been shown to be associated with SUA level. The predictive and discriminatory powers of genetic variants in gout may be greater than in type 2 diabetes because gout develops as a result of hyperuricemia.

There are several limitations of our study. First, this was a case-control study with a small sample size and the predictive model was developed based on the study population data. We did not validate the models in separate subjects. Therefore, the discriminative power of our model needs to be interpreted with caution. The predictive ability of the model should be validated using a cohort with independent subjects. Second, a limited number of clinical factors were evaluated. A model including more susceptibility loci and clinical factors may be necessary for better prediction. Third, we could not evaluate gene-gene inter-

actions in some of the pairwise combinations of the common SNP. Gene-gene interaction should be evaluated in a larger sample size in a future study. Finally, β-coefficients were not used to construct the GRS in this study. In some studies, GRS calculation is based on a simple count[27], while in others, the GRS is weighted based on the β-coefficient. Although β-coefficients will represent the best estimates of risk available[34], no obvious differences have been reported between the simple count and wGRS methods[29,34]. The β-coefficient for rs121907892 could not be calculated in our study. However, it may be prudent to construct GRS using discrete weights. When the GRS was constructed by counting the number of risk alleles with $p < 0.05$, the mean (SD) GRS in patients with gout and controls were 10.1 (1.57) and 8.9 (1.70), respectively ($p < 0.0001$), indicating the association between gout and accumulation of genetic risks constructed by a nonweighted GRS. In our study, we used wGRS because some of the variants are rare alleles with considerably higher OR for the development of gout than others (rs2231142 and rs72552713 in *ABCG2* and rs121907892 in *SLC22A12*; Table 1).

Our study revealed the multifactorial character of gout. Identifying individuals at high risk of developing gout in the general population is suggested to be feasible using a prediction model consisting of genetic and clinical factors. Because gout is a risk factor for cardiovascular disease and metabolic syndrome[3,35,36], prediction of gout can be beneficial in preventing these diseases. Additional studies that include more genetic and clinical factors in a larger number of subjects independent of the present study population will be necessary to refine the model proposed here.

REFERENCES

1. Zhu Y, Pandya BJ, Choi HK. Prevalence of gout and hyperuricemia in the US general population: The National Health and Nutrition Examination Survey; 2007-2008. Arthritis Rheum 2011;63:3136-41.
2. Smith EU, Díaz-Torné C, Perez-Ruiz F, March LM. Epidemiology of gout: an update. Best Pract Res Clin Rheumatol 2010;24:811-27.
3. Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. Circulation 2007;116:894-900.
4. Hak AE, Choi HK. Lifestyle and gout. Curr Opin Rheumatol 2008;20:179-86.
5. Li S, Sanna S, Maschio A, Busonero F, Usala G, Mulas A, et al. The GLUT9 gene is associated with serum uric acid levels in Sardinia and Chianti cohorts. PLoS Genet 2007;3:e194.
6. Vitart V, Rudan I, Hayward C, Gray NK, Floyd J, Palmer CN, et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat Genet 2008;40:437-42.
7. Döring A, Gieger C, Mehta D, Gohlke H, Prokisch H, Coassin S, et al. SLC2A9 influences uric acid concentrations with pronounced sex-specific effects. Nat Genet 2008;40:430-6.
8. Wallace C, Newhouse S, Braund P, Zhang F, Tobin M, Falchi M, et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum urate and dyslipidemia. Am J Hum Genet 2008;82:139-49.
9. Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, 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.

10. Campion EW, Glynn RJ, DeLabry LO. Asymptomatic hyperuricemia. Risks and consequences in the Normative Aging Study. Am J Med 1987;82:421-6.

11. Kamatani Y, Matsuda K, Okada Y, Kubo M, Hosono N, Daigo Y, et al. Genome-wide association study of hematological and biochemical traits in a Japanese population. Nat Genet 2010;42:210-5.

12. Taniguchi A, Urano W, Yamanaka M, Yamanaka H, Hosoyamada M, Endou H, et al. A common mutation in an organic anion transporter gene, SLC22A12, is a suppressing factor for the development of gout. Arthritis Rheum 2005;52:2576-7.

13. 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;1:5ra11.

14. Urano W, Taniguchi A, Anzai N, Inoue E, Kanai Y, Yamanaka M, et al. Sodium-dependent phosphate cotransporter type 1 sequence polymorphisms in male patients with gout. Ann Rheum Dis 2010;69:1232-4.

15. Urano W, Taniguchi A, Anzai N, Inoue E, Sekita C, Endou H, et al. Association between GLUT9 and gout in Japanese men. Ann Rheum Dis 2010;69:932-3.

16. Wallace S, Robinson H, Masi A, Decker J, McCarty D, Yü T. Preliminary criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977;20:895-900.

17. Imai E, Horio M, Nitta K, Yamagata K, Iseki K, Hara S, et al. Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease. Clin Exp Nephrol 2007;11:41-50.

18. Kamatani N, Sekine A, Kitamoto T, Iida A, Saito S, Kogame A, et al. Large-scale single-nucleotide polymorphism (SNP) and haplotype analyses, using dense SNP maps, of 199 drug-related genes in 752 subjects: the analysis of the association between uncommon SNPs within haplotype blocks and the haplotypes constructed with haplotype-tagging SNPs. Am J Hum Genet 2004;75:190-203.

19. International HapMap project. [Internet. Accessed May 3, 2013.] Available from: http://hapmap.ncbi.nlm.nih.gov/

20. Miyake K, Yang W, Hara K, Yasuda K, Horikawa Y, Osawa H, et al. Construction of a prediction model for type 2 diabetes mellitus in the Japanese population based on 11 genes with strong evidence of the association. J Hum Genet 2009;54:236-41.

21. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44:837-45.

22. Ohman EM, Granger CB, Harrington RA, Lee KL. Risk stratification and therapeutic decision making in acute coronary syndromes. JAMA 2000;284:876-8.

23. Dehghan A, Köttgen A, Yang Q, Hwang S-J, Kao WL, Rivadeneira F, 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.

24. van der Harst P, Bakker SJ, de Boer RA, Wolffenbuttel BH, Johnson T, Caulfield MJ, et al. Replication of the five novel loci for uric acid concentrations and potential mediating mechanisms. Hum Mol Genet 2010;19:387-95.

25. Tabara Y, Kohara K, Kawamoto R, Hiura Y, Nishimura K, Morisaki T, et al. Association of four genetic loci with uric acid levels and reduced renal function: The J-SHIPP Suita Study. Am J Nephrol 2010;32:279-86.

26. Yang Q, Kottgen A, Dehghan A, Smith AV, Glazer NL, Chen MH, et al. Multiple genetic loci influence serum urate levels and their relationship with gout and cardiovascular disease risk factors. Circ Cardiovasc Genet 2010;3:523-30.

27. Meigs JB, Shrader P, Sullivan LM, McAteer JB, Fox CS, Dupuis J, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med 2008;359:2208-19.

28. Talmud PJ, Hingorani AD, Cooper JA, Marmot MG, Brunner EJ, Kumari M, et al. Utility of genetic and non-genetic risk factors in prediction of type 2 diabetes: Whitehall II prospective cohort study. BMJ 2010;340:b4838.

29. Qi Q, Li H, Wu Y, Liu C, Wu H, Yu Z, et al. Combined effects of 17 common genetic variants on type 2 diabetes risk in a Han Chinese population. Diabetologia 2010;53:2163-6.

30. Li S, Zhao JH, Luan J, Luben RN, Rodwell SA, Khaw KT, et al. Cumulative effects and predictive value of common obesity-susceptibility variants identified by genome-wide association studies. Am J Clin Nutr 2010;91:184-90.

31. Anderson JL, Horne BD, Camp NJ, Muhlestein JB, Hopkins PN, Cannon-Albright LA, et al. Joint effects of common genetic variants from multiple genes and pathways on the risk of premature coronary artery disease. Am Heart J 2010;160:250-6.

32. De Jager PL, Chibnik LB, Cui J, Reischl J, Lehr S, Simon KC, et al. Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score. Lancet Neurol 2009;8:1111-9.

33. Gunjaca G, Boban M, Pehli M, Zemunik T, Budimir D, Kolci I, et al. Predictive value of 8 genetic loci for serum uric acid concentration. Croat Med J 2010;51:23-31.

34. Cornelis MC, Qi L, Zhang C, Kraft P, Manson JA, Cai T, et al. Joint effects of common genetic variants on the risk for type 2 diabetes in US men and women of European ancestry. Ann Intern Med 2009;150:541-50.

35. Choi H, Ford E, Li C, Curhan G. Prevalence of the metabolic syndrome in patients with gout: The Third National Health and Nutrition Examination Survey. Arthritis Rheum 2007;57:109-15.

36. Kuo CF, See LC, Luo SF, Ko YS, Lin YS, Hwang JS, et al. Gout: an independent risk factor for all-cause and cardiovascular mortality. Rheumatology 2009;49:141-6.