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# 한국인 통풍환자에서 *ABCG2* 와 *SLC2A9* 유전자 다형성 에 관한 연구

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Genetic analysis of *ABCG2* and *SLC2A9* gene polymorphism in Korean population with gout

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지도교수 정 종 훈

이 논문을 의학 박사학위신청 논문으로 제출함

2013년 4월

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# CONTENTS

ABSTRACT ····· iv

I. Introduction ····· 1

II. Patients and Methods ····· 2

III. Results ····· 4

IV. Discussion ····· 7

V. Conclusion ····· 10

Reference ····· 18

# LIST OF TABLES

Table 1. Demographic and clinical characteristics of the study population. ····· 11

Table 2. Single Nucleotide Polymorphisms of *ABGG2* and *SLC2A9* · ····· 12

Table 3. Genotype distribution and relative allele frequencies of c.421C>A (rs2231142) polymorphism of *ABCG2*. ····· 13

Table 4. Genotype distribution and relative allele frequencies of c.410+4190A>G (rs6449213) polymorphism of *SLC2A9*. ···· 14

Table 5. Genotype distribution and relative allel frequencies of c.844A>G (rs16890979) polymorphism of *SLC2A9*. ····· 15

Table 6. Genotype distribution and relative allel frequencies of c.881A>G polymorphism of *SLC2A9*. ····· 16

Table 7. Genotype distribution and relative allel frequencies of c.1002+78G>A polymorphism of *SLC2A9*. ····· 17

# 초 록

## 한국인 통풍환자에서 *ABCG2* 와 *SLC2A9* 유전자 다형성에 관한 연구

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통풍은 관절내 요산결정의 침착에 의한 비교적 흔한 관절염이다. 지난 수세기 동안 통풍이 유전과 관계가 있을것이라 생각해 왔지만 일부 특정 유전자가 가족력과 관련이 있다는 몇몇 보고들을 제외하면 정확히 알려지 사실은 없었다.

그러나 최근 몇 년 동안 genome-wide association studies (GWAS) 의 괄목할 만한 발전을 통해 다양한 질환의 발병 대한 유전적 연관성을 밝혀냈다. 그 중 통풍발병과 밀접한 관련이 있는 요산 대사에 관여하는 몇몇 유전자가 보고되었는데, 대표적인 유전자들로는 *SLC2A9*, *ABCG2*, *SLC13A3, SLC17A1*, *SLC17A3*, *SLC17A4*, *SLC22A11*, *SLC22A12* 등이 있다. 특히 이들 유전자의 단일뉴클레오티드다형태(single neucleotide polymorphism, SNP) 이 통풍환자들에서 더 많이 변이됨이 알려졌다.

본 연구는 한국인 통풍환자들에서 지금까지 밝혀진 요산대사와 관련된 유전자의 SNP 이 건강한 대조군보다 더 많이 존재하는지를 알아보고자 하였다. 저자들은 비교적 한국인과 유사한 인종적 배경을 가진 중국한족을 대상으로 한이전 보고들을 고려하여 *ABCG2* 유전자 의 rs2231142 SNP 과 *SLC2A9* 의 rs6449213, rs16890979 SNP 이 과연 한국인 통풍환자들에서도 존재하는지 알아보고자 하였다. 더울어 알려진 유전자 전좌(locus)를 중심으로 주위 부분에 아직까지 보고되

지 않은 새로운 SNP 가 존재하는지도 연구하고자 하였다.

연구 대상은 109명의 통풍 환자와 102명의 건강 대조군, 23명의 질환 대조군으로 구성되었다. 통풍환자군에서 2명을 제외하면 모두 남성이었다. 통풍은 미국류마티스학회(American College of Rhematology, ACR) 분류에 따라 진단하였고, 유전자 각 SNP 에 대해서 중합효소연쇄반응(polymerase chain reaction,PCR) 을 통한 직접 염기서열분석(full sequencing) 을 시행하였다. 통계는 SPSS version 17.0을 통해 chi-square test , *t*-test 와 multivariate logistic regression test 를 사용하였다.

기존에 보고되었던 *ABCG2* 유전자 의 rs2231142 SNP 은 본 연구에서도 기존 연구들과 마찬가지로 통풍환자에게서 유의하게 높게 나왔으나 *SLC2A9* 유전자는 이전 연구들에서 의미있게 보고되었던 2가지 SNP 인 rs6449213, rs16890979 는 환자군과 대조군 모두 한가지 대립유전자(allele)로 일치해서 통계상 유의하지 않았다. 오히려 이번 연구에서 새롭게 시행했던 SNP 중 SLC2A9 gene 의 881A>G 와 1002+78G>A 가 통풍환자에서 유의하게 높게 나왔다.

본 연구는 처음으로 한국인의 통풍 환자들을 대상으로 *ABCG2* 와 SLC2A9 유전자의 SNP 이 존재하는지 여부를 알아봤다는데 의의가 있으며, 또한 이전에 보고되지 않은 새로운 SNP을 찾아냄으로서 향후 한국인 통풍 환자들에서 유전적 연관성을 연구하는 토대를 제공했다는 점은 주목할만하다. 단, 본 연구는 제한된 유전자를 대상으로 SNP 이 존재하는지의 여부를 조사했을 뿐이며 앞으로 더 많은 환자들을 대상으로 하여 다양한 SNP 가 임상양상에 미치는 영향과 연관성에 대한 연구도 진행되어야 할 것이다.

**핵심어 :** 통풍, 유전자, 단일뉴클레오티드다형태, *ABCG2* 유전자, SLC2A9 유전자, rs2231142, 881A>G, 1002+78G>A

# I. Introduction

Gout is a common inflammatory arthritis triggered by the crystallization of uric acid within the joints.[1] It is characterized by painful joint, inflammation, and obstructive tophi, and can result in joint destruction and disability if untreated. A strong genetic predisposition to gout has been recognized for centuries but our understanding of the roles of specific genes had been primarily limited to families with uncommon mutations. These rare familial forms of gout and hyperuricemia include mutational defects in purine metabolic pathway constituents [e.g. X-linked superactivity of PRPS1 and deficiency of hypoxanthine-guanine phosphoribosyl transferase (HGPRT)] and those associated with hereditary renal disorders (e.g. autosomal dominant familial juvenile hyperuricemic nephropathy, medullary cystic kidney disease type 1 and 2).[2] Over the last few years, there has been a remarkable expansion of our knowledge on regards to the genetics of hyperuricemia and gout primarily derived from genome-wide association studies (GWAS).[3] Recently, GWAS identified substantial associations between single nucleotide polymorphism (SNPs), rs2231142 of *ABCG2* and rs6449213, rs16890979 of *SLC2A9* and uric acid concentration and gout invarious ethnic.[4,5]

# II. Patients and Methods

A total of 109 gout patients and 102 gout-free controls were recruited in this study from Chosun University hospital and Daegu Catholic University medical center. The 109 gout patients were composed of 107 male and 2 female. Age of mean were 55 years. Control group consisted of 102 subjects. All of them were male sex and age of mean were 51 years. Controls did not have a self-reported history of arthritis. For both group, participants were interviewed using a structured questionnaire to collect personal history and demographic characteristics (age, sex, etc). Data were expressed as mean ± standard deviation (SD). All participants gave written imformed consent. Demographic and clinical characteristics (mean ± SD) of the study population are listed in Table 1. The diagnosis of gout was based on the preliminary criteria which was published by the American College of Rheumatology (ACR) in 1977 of the classification of gout for use in either clinical settings or population-based epidemiologic sudies.[6] All patients had a diagnosis of gout confirmed by rheumatologists that be educated.

For laboratory assays, serum was separated from peripheral venous blood samples obtained from each participant and stored at -70°C and then we performed polymerase chain reaction (PCR)-direct sequencing for detection of SNP. The rs2231142, rs6449213, rs16890979 and nearby regions in patients and controls were amplified by PCR and PCR product was sequenced by ABI 3730XL instrument (Applified Biosystems, Foster City, CA) to perform genetic mutational analysis. The genotype frequencies and allele frequencies were compared between case and

control samples. Allele frequency was defined as the percentage of the individuals carrying the allele among the total number of the individuals. Statistical analyses were performed using SPSS version 17.0. The chi-square test , $t$-test and multivariate logistic regression test were used to compare between groups. Significant differences between or among groups were indicated by a $p$ value $< 0.05$. The $\chi 2$ test used to estimate the Hardy-Weinberg equilibrium (HWE).

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of the National Research Institute for Family Planning.

The Nomenclature for SNPs used Human genome variant society (HGVS) recommendation and the SNP database from National Center for Biotechnology information (NCBI).

# III. Results

The majority of individuals in this study were male-sex. Among all of our patients, only 1.8% was female. Mean levels of uric acid 5.8± 1.9 in patients with gout and 6.1 ± 1.2 in controls (Table 1). Levels of uric acid were not significant difference between two groups. Probably, the result was why majority of patient with gout have received uric acid lowering agent such as allopurinol, febuxostat, benzbromarone. General characteristics of study population including age, height, weight, BMI were not significant difference. Table 2 shows nine different kinds of SNPs. The SNPs rs2231142, rs6449213 and rs16890979 which have previously been shown to be associated with control of serum urate levels and gout, were genotyped first. The rs2231142, also known as Q141K and C421A, is a aSNP in the *ABCG2* gene, indicating a missense variant. A allele is widely known as the risk allele. The rs2231142 (c.421C>A) showed a higher A/A genotype and A allele frequency in the gout cases than controls (29.3% vs 4.9% by genotype; 52.8% vs 26.5% by allele). And the association to gout reached significance (chi-square=29.42, $p<0.001$, OR=3.32, 95% CI 2.11-5.20) (Table 3). It was almost similar to the results of previous studies. However, there were not significant differencein rs6449213 and rs16890979 genotype between gout cases and controls. The rs6449213 is basically a surrogate for rs7442295 which is in fairly tight linkage ($r^2$=0.88), and thus associated with higher serum urate levels. The rs6449213 was a highA/A genotype distribution in both gout patients and controls (98.2% vs 99.0%). A/G genotype was 2 case in patient group and only 1case in controls (Table 4). The rs16890979 has

been found to be associated with gout in several independent sudies. It may be a variation in the *SLC2A9* gene, which is more commonly known as *GLUT9*. The most of rs16890979 also appeared a high A/A genotype distribution(100% vs 98.1%) (Table 5). They mainly shared A/A of one major genotype. On the other hand, new polymorphism was founded in nearby regions of these SNPs. A c.881A>G and c.1002+78G>A, SNP polymorphism in *SLC2A9* were significant difference in genotype frequency between gout cases and controls respectively. The c.881A>G was a higher G/G genotype frequency and G allele frequency in the gout cases than controls (20.1% vs 5.8% by genotype; 36.2% vs 25.0% by allele, chi-square=9.36, $p < 0.001$, OR=1.54, 95% CI 1.03-2.28) (Table 6). The c.1002+78G>A was a higher A/A genotype distribution and A allele frequency in the gout than controls (10.1% vs 2.9% by genotype; 27.1% vs 17.2% by allele , chi-square=5.93, $p$=0.05 , OR=1.64, 95% CI 1.03-2.62)(Table7). In addition, the c.871+386A>G of *ABCG2* and c.410+4124C>T and c.410+4242G>A of *SLC2A9* were not significant difference in genotype frequency between gout cases and controls. The c.871+386A>G of *ABCG2* was a high A/A genotype distribution with 95.4%. The c.410+4124C>T of *SLC2A9* was a high CC genotype distribution with 98.2%. The c.410+4242G>A of *SLC2A9* a high GG genotype distribution with 99.1%. Finally, c.813-18A>G which is nearby rs16890979 of *SLC2A9* also a high AA genotype distribution with 99.1%. Notably, the minor allele was not observed at any of these four SNPs in the gout patients. Therefore, Odd ratios could not be calculated.

In summary, we demonstrate association of rs2231142 in *ABCG2* gene with gout in a Korean population. Also, we newly found the association

of c.881A>G, c.1002+78G>A in *SLC2A9* gene with gout in a Korean population. Therefore, we report the association of genotype and allele frequencies of three SNPs (rs2231142 in *ABCG2*, c.881A>G and c.1002+78G>A in *SLC2A9*) between gout patients and healthy control groups in a korean population.

# IV. Discussion

Gout is the most frequent inflammatory joint disease that can result in joint destruction and disability if untreated.[7] Emerging evidence suggest that gout and hyperuricemia are strongly associated with the metabolic syndrome and may lead to myocardial infarction, diabetes, and premature death.[8-12,38,39] It was estimated that up to 2% of men over the age of 30 and women over the age of 50 could develop gout at sometime.[13] Uric acid is the endproduct of purine metabolism in humans, and the rate of excretion and reabsorption of uric acid is determined by kidney and intestine.[14] Elevated uric acid levels are associated with a variety of adverse health risks including gout. GWAS have emerged as a comprehensive and powerful approach to identify genetic variants related to complex disease.[3] These genetic data add considerably to our understanding to the pathogenesis of hyperuricemia and gout. Since 2007, systematic, well-powered, GWAS have been performed throughout the world to explore the relationships between common sequence variation and disease predisposition.[39]

This approach has revealed over 50 disease-susceptibility loci and has provided insights into the allelic architecture of multifactorial traits. Recently, several GWAS indicated a substantial association between urate concentration and SNPs in ten genetic loci including transporter-coding genes such as *SLC2A9* (GLUT), *ABCG2* (BCRP), *SLC13A3, SLC17A1* (NPT1), *SLC17A3* (NPT4), *SLC17A4* (named as NPT5 provisionally), *SLC22A11* (OAT4), *SLC22A12* (URAT1), and *SLC16A9* (MCT9) as well as urate transport-related scaffolding protein PDXK1.[5,15-23,40]

Especially, considering their activities as urate transporters, as well as the strong associations between genetic variants of *SLC2A9* and *ABCG2* and serum uric acid levels, both genes appear to be important modulators of uric acid levels and potential determinants in the risk of gout. Several recent studies reported that rs6449213, rs16890979, rs7442295, rs6855911 and rs734553 SNPs within *SLC2A9* show highly significant associations with gout.[24-29,32-37] More recently, a GWAS in African-Americans reported significant genome-wide associations of four SNPs with uric acid levels : rs6449213, rs3775948, rs7663032 and rs68563969[25]. Also rs2231142, the missense SNP in *ABCG2* (Q141K) has been associated with hyperuricemia and gout in Caucasian, Han Chinese, Japanese and African-Americansamples[4-5,21,30-31]. It was especially worthy of notice in the way that research of *ABCG2* vaiants (rs2231142) was performed in Asian population such as Han Chinese and Japanese.

In this background, we carried out a replication study of rs6449213 and rs16890979 SNPs within *SLC2A9* and rs2231142 within *ABCG2*. The result of rs2231142 in *ABCG2* was similar to previous study. However, rs6449213 and rs16890979 genetic variant in *SLC2A9* were different comparing the earlier researchs. Maybe, the reason was that genetic variant were very different in different ethnity.

In our study, there are several limitations that have to be considered. Firstly, this study sample size is not sufficient in detecting the potential correlation effect of the genetic associations of these SNPs with gout in a Korean population. Secondly, Among all of our patients, female sex was only 2%, not sufficient to perform the statistical analysis. Therefore, this study was limited to male gout population. Thirdly, levels of uric acid

were not significant difference between two groups. Probably, the result was why majority of patient with gout have received uric acid lowering agent such as allopurinol, febuxostat, benzbromarone. Despite these limitations, this is the first report to indicate an association between gout and polymorphism (rs2231142, rs6449213 and rs16890979) in Korean population. Although studies on *ABCG2 and SLC2A9* variants have been conducted indifferent population, the presence and effects of these in a Korean population have not been reported.

We investigate the differences of allele and genotype distributions of several SNPs in the *ABCG2* and *SLC2A9* between 109 Korean patients with gout and 102 gout-free controls. A rs2231142 polymorphism in *ABCG2* gene and c.881A>G, c.1002+78G>A in *SLC2A9* gene is a potential candidate for the pathogenesis of gout in a male Korean population. We further have to analysis the differences of the haplotype distributions by these SNPs between the healthy controls and the patients with gout. In addition, follow up studies involving deep investigation of the these SNPs and functional experiments will be needed to unravel the exact mechanism by which this region is pathologically involved.

# V. Conclusion

We demonstrate association of rs2231142 in *ABCG2* gene with gout in a Korean population. Also, we newly found the association of c.881A>G, c.1002+78G>A in *SLC2A9* gene with gout in a Korean population. These study demonstrate the differences of allele and genotype distributions of several SNPs in the *ABCG2* and *SLC2A9* in Korean population.

Table 1. Demographic and clinical characteristics of the study population.

| Characteristics | Patients with gout | Controls | *p*-value |
|---|---|---|---|
| | N = 109 (mean±SD) | N = 102 (mean±SD) | |
| Age, yrs | 55.0 ± 12.1 | 51.8 ± 6.7 | 0.018 * |
| Height, cm | 168.3 ± 6.4 | 169.4 ± 5.4 | 0.18 |
| Weight, Kg | 69.9 ± 8.6 | 69.1 ± 8.7 | 0.54 |
| BMI, $Kg/m^2$ | 24.6 ± 2.7 | 24.0 ± 2.7 | 0.12 |
| Serum uric acid, mg/dl | 5.8 ± 1.9 | 6.1 ± 1.2 | 0.17 |

Abbreviations: N, number of patients; SD, standard deviation; BMI, body mass index

* $p$-value $< 0.05$

Table 2. Single Nucleotide Polymorphisms (SNPs) of *ABCG2* and *SLC2A9*

| Gene | Polymorphism (dbSNP No) | Polymorphism (HGVS nomenclature) | Genotype frequency In gout patients(%) | OR (95% CI) |
|---|---|---|---|---|
| ABCG2 | rs2231142 | c.421C>A | AC(46.7) | 3.32 * |
| ABCG2 | - | c.871+386A>G | AA(95.4) | |
| SLC2A9 | rs6449213 | c.410+4190A>G | AA(98.2) | |
| SLC2A9 | - | c.410+4124C>T | CC(98.2) | |
| SLC2A9 | - | c.410+4242G>A | GG(99.1) | |
| SLC2A9 | rs16890979 | c.844A>G | AA(100) | |
| SLC2A9 | - | c.881A>G | AA(47.7) | 1.54 * |
| SLC2A9 | - | c.1002+78G>A | GG(55.9) | 1.64 * |
| SLC2A9 | - | c.813-18A>G | AA(99.1) | |

Abbreviations: dbSNP, The Single Nucleotide polymorphism Database; HGVS, Human Genome Variation Society; OR, odd ratio; 95% CI, 95% confidence interval.

* $p$ value $< 0.05$

Table 3. Genotype distribution and relative allele frequencies of c.421C>A(rs2231142) polymorphism of *ABCG2*.

| Group | Number | Genotype frequency(n,%) | | | Allele frequency(n,%) | |
|---|---|---|---|---|---|---|
| | | C/C | C/A | A/A | C | A |
| Patients | 109 | 26 (23.8) | 51 (46.7) | 32 (29.3) | 103 (47.2) | 115 (52.8) |
| Controls | 102 | 53 (51.9) | 44 (43.1) | 5 (4.9) | 150 (73.5) | 54 (26.5) |
| | | Chi-square 29.42, df = 2, $p < 0.001$* OR 3.32, 95% CI 2.11-5.20 | | | Chi-square 30.32, df =1 $p < 0.001$*, OR 3.10, 95% CI 2.04-4.76 | |

n, number ;
OR, odd ratio; 95% CI, 95% confidence interval

* $p$ value < 0.05

Table 4. Genotype distribution and relative allele frequencies of c.410+4190A>G (rs6449213) polymorphism of *SLC2A9*. There were not significant difference in genotype between gout cases and controls.

| Group | Number | Genotype frequency(n,%) | | | Allele frequency(n,%) | |
|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | A | G |
| Patients | 109 | 107 (98.2) | 2 (1.8) | 0 (0.0) | 216 (99.0) | 2 (1.0) |
| Controls | 102 | 101 (99.0) | 1 (1.0) | 0 (0.0) | 203 (99.5) | 1 (0.5) |
| | | | $p > 0.05$ | | $p > 0.05$ | |

n, number ;

* $p$ value $< 0.05$

Table 5. Genotype distribution and relative allele frequencies of c.844A>G (rs16890979) polymorphism of *SLC2A9*. There were not significant difference in genotype between gout cases and controls.

| Group | Number | Genotype frequency(n,%) | | | Allele frequency(n,%) | |
|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | A | G |
| Patients | 109 | 109 (100) | 0 (0.0) | 0 (0.0) | 218 (100) | 0 (0.0) |
| Controls | 102 | 100 (98.1) | 2 (1.9) | 0 (0.0) | 202 (99.0) | 2 (1.0) |
| | | | $p$ = 0.232 | | $p$ = 0.232 | |

n, number ;

* $p$ value < 0.05

Table 6. Genotype distribution and relative allele frequencies of c.881A>G polymorphism of *SLC2A9*.

| Group | Number | Genotype frequency(n,%) | | | Allele frequency(n,%) | |
|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | A | G |
| Patients | 109 | 52 (47.7) | 35 (32.1) | 22 (20.1) | 139 (63.7) | 79 (36.2) |
| Controls | 102 | 57 (55.8) | 39 (38.2) | 6 (5.8) | 153 (75.0) | 51 (25.0) |
| | | Chi-square 9.36, df = 2, $p < 0.001$* OR 1.54, 95% CI 1.03-2.28 | | | Chi-square 6.24,df =1 $p = 0.015$*, OR 1.70, 95% CI 1.12-2.60 | |

n, number ;
OR, odd ratio; 95% CI, 95% confidence interval

* $p$ value < 0.05

Table 7. Genotype distribution and relative allele frequencies of c.1002+78G>A polymorphism of *SLC2A9*.

| Group | Number | Genotype frequency(n,%) | | | Allele frequency(n,%) | |
|---|---|---|---|---|---|---|
| | | A/A | A/G | G/G | A | G |
| Patients | 109 | 11 (10.1) | 37 (33.9) | 61 (55.9) | 59 (27.1) | 159 (72.9) |
| Controls | 102 | 3 (2.9) | 29 (28.4) | 70 (68.6) | 35 (17.2) | 169 (82.8) |
| | | Chi-square 5.93, df = 2, $P$ = 0.05* OR 1.64, 95% CI 1.03-2.26 | | | Chi-square 5.96,df =1 $P$ = 0.015*, OR 1.78, 95% CI 1.11-2.86 | |

n, number ;
OR, odd ratio; 95% CI, 95% confidence interval

* $p$ value < 0.05

# Reference


1. Choi HK, Mount DB, Reginato AM. Pathogenesis of gout. Ann InternMed 2005; 143:499-516.
2. Zaka R, Williams CJ. New developments in the epidemiology and genetics of gout. Curr Opinion Rheumatol Rep 2006; 8:215-23.
3. Choi HK, Yanyan Zhu, Mount DB. Genetics of gout. Curr Opinion Rheumaol 2010; 22:144-51.
4. Wang B, Miao Z, Liu S, Wang J, Zhou S, Han L et al. Genetics analysis of *ABCG2* gene C421A polymorphism with gout disease in Chinese Han male population. Hum Genet 2010;127:245-6.
5. Dehahan A, Kottgen A, Yang O, et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-association study. Lancet 2008; 372:1953-61.
6. Wallace SL, Robinson H, Masi AT, Decker JL, McCarty DJ, Yü Fu. Preliminary Criteria for the classification of the acute arthritis of primary gout. Arthritis Rheum 1977;20:895-900.
7. Ringel J, Kreutz R, Distler A, Sharma AM. The Trp64Arg polymorphism of the beta 3 adrenergic receptor gene is associated with hypertension in men with type 2 diabetes mellitus. Am J Hypertens 2000;13:1027-31.
8. Choi HK, Ford ES, Li C, Curhan G. Prevalence of the metabolic syndrome in patients with gout; the Third National Health and Nutrition Examination Survey. Arthritis Rheum 2007;57:109-115.

9. Abbott RD, rand FN, Kannel WB, Castelli WP. Gout and coronary heart disease; the Framingham Study. J Clin Epidemiol 1988;41:237-242.
10. Krishnan E, Baker JF, Furst DE, Schumacher HR. Gout and the risk of acute myocardial infarction. Arthritis Rheum 2006;54:2688-96.
11. Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. Circulation 2007;116:894-900.
12. Choi HK, De Vera MA, Krishnan E. Gout and the risk of type 2 diabetes among men with a high cardiovascular risk profile. Rheumatology (Oxford) 2008;47:1567-70.
13. Kramer HM, Curhan G. The association between gout and nephrolithiasis: the national Health and Nutrition Examination Survey III. Am J Kidney Dis 2002;40:37-42.
14. Garcia-Rubi E, Starling RD, Tchernof A, Matthews DE, Walston JD, Shuldiner AR, et al. Trp64Arg variant of the beta 3-adrenoceptor and insulin resistance in obese postmenopausal women. J Clin Endocrinol Metab 1998;83:4002-5.
15. 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 concentration and potential mediating mechanism. Hum Mol Genet 2010;19:387-95.
16. 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.
17. Wallace C, Newhouse SJ, Braund P et al. Genome-wide association study identifies genes for biomarkers of cardiovascular disease: serum

urate and dyslipidemia. Am J Hum Genet 2008;82:139-149.
18. Caulfield MJ, Munroe PB, O'Neill D et al. SLC2A9 is a high-capacity urate transporter in humans. PLoS Med 2008;5:e197.
19. Vitart V, Rudan I, Hayward C, et al. SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat Genet 2008;40:437-42.
20. Doring A, Gieger C, Mehta D, et al. SLC2A9 influences uric acid concentrations with pronounced sex-specific effect. Nat Genet 2008;40:430-6.
21. Woodward OM, Kottgen A, Coresh J et al. Identification of a urate transporter, ABCG2, with a common functional polymorphism causing gout. Proc Nat Acad Sci USA. 2009;106:10338-42.
22. Iwai N, Mino Y, Hosoyamada M, et al. A high prevalence of renal hypouricemia caused by inactive SLC22A12 in Japanese. Kidney Int 2004;66:935-44.
23. Shima Y, Teruya K, Ohta H. Association betweenintronic SNP in urate-anion exchanger gene, SLC22A12, and serum uric acid levels in Japaneses. Life Sci 2006;79:2234-7.
24. Brandstatter A, Kiechi S, Kollerits B, et al. Sex-specific association of the putative fructose transporter SLC2A9 variants with uric acid levels is modified by BMI. Diabetes Care 2008;31:1662-7.
25. Charles BA, ShrinerD, Doumatey A, Chen G, Zhou J, Huang H et al. A genome-wide association study of serum uric acid in African Americans. BMC Med Genomics 2011;4(4):17.
26. Brandstatter A, Lamina C, Kiechl S, Hunt SC, Coassin S,

Paulweber B et al. Sex and age interaction with genetic association of atherogenic uric acid concentrations. Atherosclerosis 2010;210:474-8.

27. Matsuo H, Chiba T, Nagamori S, Nakayama A, Domoto H, Phetdee K et al. Mutations in glucose transporter 9 gene SLC2A9 cause renal hypouricemia. Am J Hum Genet 2008;744-51.
28. Preitner F, Bonny O, Laverrier A, Rotman S, Firsov D, Da Costa A et al. Glut9 is a major regulator of urate homeostasis and its genetic inactivation induces hyperuricosuria and urate nephropathy. Proc Natl Acad Sci USA 2009;106:15501-6.
29. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S et al. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease?. Hypertension 2003;41:1183-90.
30. Matsuo H, Takada T, Ichida K, Nakamura T, Nakayama A, Ikebuchi Y et al. Common defects of ABCG2, a high-capacity urate exporter, cuase gout a functional -based genetic analysis in a Japanese population. Sci Transl Med 2010;5.
31. Yamagishi K, Tanigawa T, Kitamura A, Ko¨ttgen A, Folsom AR, Iso H et al. The rs2231142 variant of the ABCG2 gene is associated with uric acid levels and gout among Japanese people. Rheumatology 2010;49:1461 - 1465.
32. Shima Y, Teruya K, Ohta H. Association between intronic SNP in urate-anion exchanger gene, SLC22A12, and serum uric acid levels in Japanese. Life Sci 2006;79:2234-7.
33. Johnson RJ, Rideout BA. Uric acid and diet: insights into the epidemic of cardiovascular disease. N Engl J Med 2004;350:1071-3.

34. Cassim B, Mody G<, Deenadayalu VK, Hammond MG. Gout in black South Africans: a clinical and genetic study. Ann Rheum Dis 1994;53:759-62.
35. Arromdee E, Michet CJ, Crowson CS et al. Epidemiology of gout: is the incidence rising? J Rheumatol 2002;29:2403-6.
36. Klemp P, Stansfield SA, Castle B, Robertson MC. Gout is on the increase in New Zealand. Ann Rheum Dis 1997;56:22-6.
37. Choi HK, Atkinson K, Karlson EW et al. Alcohol intake and risk of incident gout in men: a prospective study. Lancet 2004;363:1277-81.
38. Choi HK, Curhan G. Adiposity, hypertension, diuretic use and risk of incident gout in women: the Nurses health study. Arthritis Rheum 2005.
39. McCarthy MI, Abecasis GR, Cardon LR, Coldstein DB, Little J, Ioannidis JP et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. Nat Rev Genet 2008;9:356-69.
40. Naohiko A, Promsuk J, Sirirat A-T, Hiroyuki S. Recent advances in renal urate transport: characterization of candidate transporters indicated by genome-wide association studies.