A Genome Wide Association Study of Plasma Uric Acid Levels in Obese Cases and Never-Overweight Controls

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Introduction

Uric acid is the end product of purine metabolism. The prevalence of hyperuricemia (uric acid $\geq$ 420 $\mu$mol/L in males, $\geq$360 $\mu$mol/L in females) has increased rapidly over the past two decades (1,2). The connection between hyperuricemia and gout has long been known; however, hyperuricemia is much more common than gout. Increasing evidence shows that hyperuricemia is a risk factor for metabolic syndrome (3) and cardiovascular diseases (4).

Although obesity and hyperuricemia are correlated, the genetic background of this association is not well understood. Several candidate genes, including SLC2A9 and ABCG2 (5,6), have been identified in genome-wide association studies (GWASs) and follow-up replications. To investigate the possible role of these genes in obese individuals, we performed a GWAS for plasma uric acid in 1,060 obesity cases/controls using our previous genotyping data for body weight traits (7).

Methods and Procedures

Subjects

All subjects gave informed consent, and the protocol was approved by the Committee on Studies Involving Human Beings at the University of Pennsylvania. Five hundred and twenty (520) European-American obesity cases (BMI $>35$ kg/m$^2$) and 540 normal-weight controls (BMI $<25$ kg/m$^2$) were selected for analysis from ongoing studies (8). Clinical characteristics have been described previously (9). In 961 samples with uric acid data, 924 were females.
Genotyping

DNA was extracted from whole blood or lymphoblastoid cell lines using a high-salt method. All samples were genotyped on Illumina HumanHap550 SNP arrays (Illumina, San Diego, CA) with approximately 550,000 SNP markers, at the Center for Applied Genomics, Children’s Hospital of Philadelphia.

Data analyses

Uric acid outliers (>3SD) were deleted from the dataset. Quantitative association studies were performed using PLINK 1.07 based on the Wald test (10). To investigate the plausible influence of obesity status on uric acid levels, we also performed GWAS separately in obesity cases (BMI > 35 kg/m²) and normal weight controls (BMI < 25 kg/m²).

### TABLE 1 Traits distributions of plasma uric acid in obese individuals (BMI > 35 kg/m²), normal weight (BMI < 25 kg/m²), and combined samples

|          | N  | Minimum | Maximum | Mean   | SD    | Skewness | Kurtosis |
|----------|----|---------|---------|--------|-------|----------|----------|
| All      |    |         |         |        |       |          |          |
| Uric acid| 962| 1.500   | 8.800   | 4.667  | 1.4037| 0.441    | −0.219   |
| Female   |    |         |         |        |       |          |          |
| Uric acid| 926| 1.500   | 8.800   | 4.602  | 1.3628| 0.441    | −0.219   |
| BMI > 35 |    |         |         |        |       |          |          |
| Uric acid| 487| 2.200   | 9.100   | 5.523  | 1.258 | 0.127    | −0.095   |
| BMI < 25 |    |         |         |        |       |          |          |
| Uric acid| 472| 1.500   | 6.300   | 3.768  | 0.881 | 0.147    | −0.344   |

**FIGURE 1** Q-Q plots of plasma uric acid levels in (A) all subjects, (B) obesity cases (BMI > 35 kg/m²), and (C) normal-weight controls (BMI < 25 kg/m²).
TABLE 2 Significant associations between SLC2A9 gene SNPs and plasma uric acid

| CHR | SNP    | bp       | P(all subjects) | P(cases)   | P(controls) | Gene |
|-----|--------|----------|----------------|------------|-------------|------|
| 4   | rs6449213 | 9603313  | 3.15 x 10^-12  | 1.61 x 10^-7 | 1.01 x 10^-12 | SLC2A9 |
| 4   | rs1014290 | 9610959  | 1.13 x 10^-9   | 3.59 x 10^-6 | 4.26 x 10^-12 | SLC2A9 |
| 4   | rs7660895 | 9594543  | 1.47 x 10^-9   | 8.48 x 10^-7 | 1.91 x 10^-9  | SLC2A9 |
| 4   | rs6832439 | 9533417  | 5.64 x 10^-11  | 6.09 x 10^-6 | 3.19 x 10^-12 | SLC2A9 |
| 4   | rs13129697 | 9536065  | 1.12 x 10^-10  | 2.53 x 10^-6 | 3.48 x 10^-10 | SLC2A9 |
| 4   | rs13131257 | 9590987  | 9.17 x 10^-11  | 1.15 x 10^-5 | 1.44 x 10^-11 | SLC2A9 |
| 4   | rs737267  | 9543842  | 2.73 x 10^-11  | 7.34 x 10^-6 | 1.79 x 10^-12 | SLC2A9 |
| 4   | rs10805364 | 9884161  | 1.67 x 10^-9   | 0.00019    | 1.41 x 10^-12 | SLC2A9 |
| 4   | rs4698014 | 9898399  | 1.67 x 10^-9   | 0.00069    | 7.55 x 10^-13 | SLC2A9 |
| 4   | rs4698036 | 9940392  | 2.89 x 10^-9   | 0.0013     | 7.78 x 10^-12 | SLC2A9 |
| 4   | rs714436  | 9923765  | 2.48 x 10^-8   | 0.00072    | 6.46 x 10^-11 | SLC2A9 |
| 4   | rs10022911 | 9749649  | 3.29 x 10^-6   | 0.0057     | 5.42 x 10^-13 | SLC2A9 |
| 4   | rs17420080 | 9954646  | 3.53 x 10^-8   | 0.00051    | 1.55 x 10^-10 | SLC2A9 |
| 4   | rs4698050 | 10018946 | 2.05 x 10^-8   | 0.0022     | 1.22 x 10^-10 | SLC2A9 |
| 4   | rs4643800 | 10016670 | 1.08 x 10^-7   | 0.0030     | 8.53 x 10^-11 | SLC2A9 |
| 4   | rs12498956 | 9559803  | 3.14 x 10^-7   | 0.00026    | 8.71 x 10^-7  | SLC2A9 |
| 4   | rs4447863 | 9548067  | 5.06 x 10^-6   | 0.00074    | 2.26 x 10^-6  | SLC2A9 |
| 4   | rs3733585 | 9645437  | 2.36 x 10^-5   | 0.0015     | 7.19 x 10^-7  | SLC2A9 |
| 4   | rs6845554 | 9622271  | 1.30 x 10^-5   | 0.0019     | 3.18 x 10^-7  | SLC2A9 |
| 4   | rs6827754 | 9627251  | 1.22 x 10^-5   | 0.0020     | 3.05 x 10^-7  | SLC2A9 |
| 4   | rs1860910 | 9884568  | 1.35 x 10^-7   | 5.25 x 10^-5 | 3.28 x 10^-5  | SLC2A9 |

RESULTS

Female-only analyses were also carried out after quantitative associations were conducted in all samples.

Of the 1,060 obese cases and normal controls, 961 had plasma uric acid data. Thirty-seven (37) of those 961 individuals were male; 924 were female. Average age of the 961 subjects was 41.9 ± 9.1 years (range, 16-65 years). Distributions of uric acid levels in all samples, cases, and controls are shown separately in Table 1. Q-Q plots showed normal distributions of uric acid levels in those three groups (Figure 1).

Significant associations were found between SLC2A9 gene SNPs and plasma uric acid. The most significant result was for the SNP

**TABLE 3 Quantitative association studies (PLINK) for uric acid levels in obese cases and controls (P < 1 x 10^-4)**

| CHR | SNP    | Position (bp) | P(all subjects) | P(cases)   | P(controls) | Gene |
|-----|--------|---------------|----------------|------------|-------------|------|
| 1   | rs6030 | 167171782     | 3.05 x 10^-6   | 0.0013     | 7 x 10^-5   | F5   |
| 4   | rs4656687 | 17558037     | 3.81 x 10^-5   | 0.0028     | 0.041       | LCORL |
| 4   | rs2251890 | 79240461     | 1.69 x 10^-5   | 0.0012     | 0.183       | FRA51 |
| 4   | rs453783 | 79243548     | 1.21 x 10^-5   | 0.00095    | 0.21        | FRA51 |
| 4   | rs10033428 | 79259915    | 2.28 x 10^-5   | 0.0012     | 0.20        | FRA51 |
| 4   | rs9995229 | 79261976     | 2.02 x 10^-5   | 0.0011     | 0.26        | FRA51 |
| 4   | rs6845871 | 79267514     | 3.88 x 10^-5   | 0.00024    | 0.45        | FRA51 |
| 4   | rs17002988 | 79298781    | 6.45 x 10^-6   | 0.00079    | 0.098       | FRA51 |
| 8   | rs2979126 | 52590614     | 1.42 x 10^-5   | 0.0050     | 0.063       | PXDLN |
| 10  | rs7092652 | 746109       | 8.72 x 10^-7   | 0.006    | 0.0075      | DIP2C |
| 10  | rs11599917 | 752288      | 1.08 x 10^-6   | 0.028     | 0.011       | DIP2C |
| 10  | rs877282  | 767532       | 4.56 x 10^-8   | 0.0073     | 0.011       | DIP2C |
| 10  | rs1769242 | 777896       | 2.73 x 10^-6   | 0.019     | 0.018       | DIP2C |
| 10  | rs2256711 | 792272       | 3.91 x 10^-6   | 0.019     | 0.0085      | DIP2C |
| 11  | rs1385850 | 12191171     | 2.74 x 10^-5   | 3.22 x 10^-5 | 0.7647      | MICAL2 |
rs6449213 (all samples, \( P = 3.15 \times 10^{-12} \); female-only samples, \( P = 2.29 \times 10^{-12} \)) (Table 2).

DIP2C gene SNP rs877282 also reached genome-wide significance (\( P = 4.56 \times 10^{-5} \)). Many SNPs in the DIP2C gene also showed associations (\( P < 1 \times 10^{-5} \)) (Table 3).

Weaker associations (\( P < 1 \times 10^{-5} \)) were found in F5, PXDNL, FRAS1, LCORL, and MICAL2 gene SNPs. All five genes had multiple SNPs that were associated with uric acid levels (\( 3.05 \times 10^{-6} < P < 1 \times 10^{-5} \)) (Table 3). Three coding region nonsynonymous SNPs in the coagulation factor V (F5) gene, rs6030(Met 1764 Val), rs4525 (His 865 Arg), and rs4524 (Lys 858 Arg), were associated with plasma uric acid, \( P \)-values of those three SNPs for BMI adjusted uric acid were \( 3.05 \times 10^{-6}, 0.00018, \) and 0.00017, respectively.

Besides SLC2A9, three previous found uric acid-related genes ABCG2 (rs2622605, \( P = 0.0026 \), SLC17A1(rs3799344, \( P = 0.0017 \)), and RREB1 (rs1615495, \( P = 0.00055 \)) received marginal support in our study (Table 4).

**Discussion**

Hyperuricemia has been considered as an independent risk factor of cardiovascular diseases and type 2 diabetes. Single gene mutations, including deficiency of hypoxanthine guanine phosphoribosyltransferase, led to hyperuricemia; however, the risk attributable to these genes in the general population is minor (11).

Large (>10,000 individuals) GWASs and meta-analyses have shown that many genes are associated with plasma uric acid levels, including eight genes/regions [SLC2A9 (5,12,13), ABCG2 (6), SLC22A11, SLC17A1, GCKR, R3HDM2-INHBC gene region, RREB1, and PDZK1] that exceeded the genome-wide association level (\( P < 10^{-5} \)) (14). SLC2A9 has the most significant association with uric acid so far, which could explain 3.5% of uric acid variation in the general population (5).

SLC2A9 (GLUT-9) is a major transporter of uric acid. It controls uric acid influx in the basolateral and apical surface of the kidney proximal convoluted tubule (PCT). SLC2A9 is highly expressed in kidney and liver. Interestingly, ABCG2 is an efflux uric acid transporter that is expressed in the apical surface of the PCT. The SLC2A9 and ABCG2 associations are among the strongest of all uric acid associations so far (14).

Uric acid and glucose transport are often coupled, but SLC2A9 is not a major glucose/fructose transporter. In our study, uric acid levels correlated with fasting glucose. It is possible that SLC2A9 polymorphisms account for the uric acid–glucose connection. However, the SLC2A9 gene alone likely does not explain the 20% rate for hyperuricemia and almost the same rate for insulin resistance in general populations. Other genes with relatively minor genetic relative risk and/or gene–gene interactions may account for the rest of the genetic background for hyperuricemia.

The strength of the associations of SLC2A9 gene SNPs and uric acid was well beyond the threshold for genome-wide significance. This is particularly notable given the moderate sample size (961 individuals). The SLC2A9 associations have been replicated in several GWASs and follow-up association studies (5,6,13,14), including European, African-American (15), and Japanese populations. Although this is not the first study to examine a European American population, we are interested in the SLC2A9 association in extremely obese individuals. It is said that SLC2A9 is not the major glucose transporter, although it is the main uric acid transporter in proximal convoluted tubule (16). In our subjects, uric acid was correlated with almost all body weight, lipid (except LDL), and insulin resistance phenotypes (\( P < 0.001, \) data not shown). However, no direct association was found between SLC2A9 gene-region SNPs and these other phenotypes (7). These results suggest that the phenotypic associations between uric acid levels and metabolic syndrome phenotypes are through pathways independent of SLC2A9.

All uric acid-associated genes found in our GWAS, including SLC2A9, DIP2C (Homo sapiens DIP2 disco-interacting protein 2 homolog C [Drosophila]), F5 (coagulation factor V), FRAS1 ( Fraser syndrome 1), PXDNL (Homo sapiens peroxidin homolog [Drosophila]-like), LCORL (ligand-dependent nuclear receptor corepressor-like), and MICAL2 (microtubule-associated monooxygenase, calponin, and LIM domain containing 2), are expressed in kidney and/or liver. It is hard to predict functional connections among those genes and plasma uric acid levels, although we have already known that some genes have functions in transcription regulations (DIP2C and LCORL) and mesenchymal/epithelial transition (FRAS1).

Venous thromboembolism, insulin resistance, and hyperuricemia are correlated in general populations. Many studies have shown that Factor V (F5) mutations are associated with factor V Leiden thrombophilia characterized by deep vein thrombosis (17), however, no established connection between factor V and uric acid has been reported.

The SLC2A9 associations remained significant in both obese cases and controls. Several associations, including MICAL2, FRAS1, and LCORL, were more significant in obese individuals, while F5 was more significant in normal weight controls (Table 4). Although some of these associations varied in obese cases and controls, however, none of these genes were among the top BMI associations that were found in our GWAS (7).

We failed to replicate associations on SLC22A11, GCKR, and PDZK1 genes that were reported by previous large sample sized GWASs (18,19). We could not explain whether those lack of association were because of a smaller sample size, but no marginal significant association (\( P < 0.05 \)) was found in either original or BMI-adjusted uric acid levels.

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**TABLE 4 Previous uric acid associated genes were replicated in our GWAS**

| CHR | SNP   | BP        | All | Cases | Controls | Gene              |
|-----|-------|-----------|-----|-------|----------|-------------------|
| 4   | rs2622605 | 89298410  | 0.0026 | 0.037 | 0.00017 | ABCG2            |
| 4   | rs1481017 | 89316501  | 0.0044 | 0.11  | 0.0011  | ABCG2            |
| 6   | rs1615495 | 6979458   | 0.00055| 0.011 | 0.011   | RREB             |
| 6   | rs473437  | 6982476   | 0.00052| 0.0068| 0.068   | RREB             |
| 6   | rs3799344 | 25884972  | 0.0017 | 0.37  | 0.011   | SLC17A1          |
| 6   | rs2070642 | 25939191  | 0.0084 | 0.35  | 0.10    | SLC17A1          |
In summary, two genes/chromosome regions reached genome-wide association significance ($P < 1 \times 10^{-7}$, 550K SNPs) in our GWAS: SLC2A9, the chromosome 2 60.1 Mb region (rs6723995), and the DIP2C gene region. Five other genes (F5, PXDNL, FRAS1, LCORL, and MICAL2) yielded $P < 1 \times 10^{-5}$. Four previous reported associations were replicated in our study, including SLC2A9, ABCG2, RREB, and SLC17A1.

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