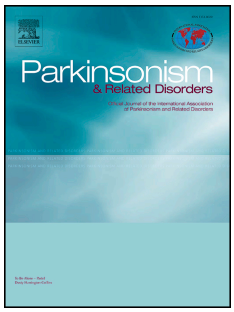
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Genetic variants related to urate and risk of Parkinson's disease

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Abstract

Introduction: Higher urate concentrations have been associated with a lower risk of developing Parkinson's disease (PD) and with slower rates of clinical decline in PD patients. Whether these associations reflect a neuroprotective effect of urate is unclear. Our objective was to assess whether genetic variants that modify circulating urate levels are also associated with altered PD risk.

Methods: Participants were from three large ongoing cohort studies: the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), and the Cancer Prevention Study II Nutrition Cohort (CPS-IIN). We examined associations between single nucleotide polymorphisms (SNPs) in *SLC2A9* and other genes involved in urate transport and PD risk using conditional logistic regression among 1,451 cases and 3,135 matched controls. We assessed associations between SNPs and plasma urate levels in a subset of 1,174 control participants with linear regression models.

Results: We found the expected associations between SNPs in *SLC2A9* and plasma urate levels among men and women; however, SNPs in other genes tended not to be associated with urate. Each SNP in *SLC2A9* explained less than 7% of the variance in plasma urate. We did not find significant associations between the SNPs in *SLC2A9* and PD risk among men or women.

Conclusion: Our results do not support an association between genetic variants associated with circulating urate levels and risk of PD, but larger investigations are needed to determine whether the modest genetic effects on blood urate contribute to predict PD risk.

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Urate is a powerful antioxidant that circulates at high concentrations in humans and is responsible for most of the antioxidant capacity in human plasma[1]. Because of the proposed role of oxidative stress in the pathology of Parkinson's disease (PD), it has been suggested that high plasma urate levels could be protective against PD[2]. Support for this hypothesis has come from a variety of sources. Laboratory models of PD have demonstrated that urate attenuates MPP+ toxicity in dopaminergic neurons[3] and 6-OHDA toxicity[4], and urate oxidase knock-out mice with increased concentrations of urate in the brain exhibit attenuated toxic effects of 6-OHDA on dopaminergic cells[5]. Several studies have reported that patients with PD have lower urate levels in serum[6, 7] and plasma[8] than individuals without PD. In addition, higher serum and cerebrospinal fluid concentrations of urate have been associated with slower rates of clinical decline in PD patients[9, 10]. Prospective studies have shown that people with lower urate levels have an increased risk of developing PD[11]. However, several of the above studies reported associations only among men[7, 9, 11].

Evidence of an association between urate and PD suggests that urate could be a suitable target for neuroprotective therapies, since plasma urate levels can be increased through pharmacologic or dietary interventions. However, despite consistent evidence supporting an association between urate and PD, it is difficult to establish causality due to the inherent limitations of observational studies, particularly confounding and reverse causation. One approach to address these concerns, known as Mendelian randomization, is to take advantage of genetic variants that affect urate levels to investigate causality. Since alleles are assigned randomly during meiosis, genotypes should be unrelated to confounding factors typical of epidemiologic studies. Several genetic loci have been associated with urate concentration in genome-wide association studies (GWAS), and one locus that has been consistently identified is solute carrier family 2, member 9 (*SLC2A9*)[12-18]. *SLC2A9* encodes glucose transporter 9 (*GLUT9*), which can reabsorb urate in renal tubules[19]. Polymorphisms in *SLC2A9* have been associated with age at onset of PD[20] as well as PD risk when combined with polymorphisms from other genes

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using a genetic score[21]. However, no association between 12 *SLC2A9* polymorphisms and PD risk was found in a separate study[22].

Therefore, we examined genetic variants that have previously been associated with altered urate levels in relation to PD risk among cases and controls selected from three large prospective cohort studies—the Nurses' Health Study (NHS), Health Professionals Follow-up Study (HPFS), and the Cancer Prevention Study II Nutrition Cohort (CPS-IIN). In addition, as detailed exposure histories have been collected from all members of these cohorts as well as measured plasma urate for a subset of participants, we have a unique ability to assess relationships among genetic variants, plasma urate, and lifestyle factors.

Methods

Study population

The NHS was established in 1976 when 121,700 female registered nurses aged 30-55 years completed a mailed questionnaire regarding their medical histories and baseline health-related exposures. The HPFS was established in 1986 when 51,529 male health professionals aged 40-75 years responded to a similar questionnaire. The CPS-IIN, a sub-cohort of the larger CPS-II, includes 184,190 individuals (86,404 men and 97,786 women) aged 50-74 in 1992 who completed a questionnaire regarding nutrition and other risk factors. For all cohorts, follow-up questionnaires have been sent every two years to update exposure data and disease diagnoses. Follow-up in all cohorts has been approximately 90% or higher. The study was approved by the Human Research Committees at the Harvard T. H. Chan School of Public Health and the Brigham and Women's Hospital.

Case ascertainment and control selection

Procedures for case ascertainment have been described previously[11] . Briefly, cases were first identified through biennial self-report questionnaires. With consent of the participant, we asked the

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treating neurologist to complete a questionnaire confirming or refuting the PD diagnosis and to send a copy of the medical records, which were reviewed by a neurologist specializing in movement disorders. Cases were confirmed if a diagnosis was considered definite or probable by the treating neurologist, or if the medical record included either a final diagnosis of PD made by a neurologist, or evidence of at least 2 of the 3 cardinal signs (rest tremor, rigidity, bradykinesia) in the absence of features suggesting alternative diagnoses.

For each case, we randomly selected controls who were alive and had not reported PD at the time of the cases' diagnosis. We selected between 2-6 controls per case in the NHS and HPFS and one control per case in the CPS-IIN cohort. Controls were matched to the cases based on cohort, sex, birth year (+/-1 year), race (white vs. other), fasting status (>8 hours vs. less/unknown), and year, month, and time of blood draw (in two-hour intervals).

Plasma urate assessment

Blood samples were collected from 32,826 members of NHS between 1989-1990, 18,000 members of HPFS between 1993-1995, and 40,000 members of CPS-IIN between 1998-2001. For NHS and HPFS, participants used collection kits provided to them by the studies and returned blood samples via overnight delivery to our lab. More than 95% of samples were delivered within 26 hours of being drawn. Upon arrival, blood samples were centrifuged and blood components were aliquoted into cryotubes and stored in the vapor phase of liquid nitrogen freezers at -130 degrees C or colder until being sent to the laboratory for analysis. For CPS-IIN, participants went to participating hospitals in their communities for blood draws. Hospital staff centrifuged the samples to separate blood components, then shipped samples overnight to a central repository where the samples were aliquoted and frozen in the vapor phase of liquid nitrogen freezers for long-term storage[23]. Samples from cases and controls were handled identically. Concentrations of plasma urate were measured using a colorimetric enzyme assay

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(Hitachi 911; Roche Diagnostics, Indianapolis, Indiana). Coefficients of variation (CVs) were determined using blinded quality control samples included with the study samples. All reported CVs were <10%.

Genotyping

Participants who had not provided blood samples were invited to provide cheek cell samples. Participants were sent a package contained a small empty cup with a cap and a bottle of mouthwash, and were asked to swish the mouthwash in their mouths and then spit into the cup; samples were mailed back to our lab and processed within a week of receipt. Genotyping using either blood or cheek cells was conducted for 322 confirmed cases and 1337 controls in NHS, 310 confirmed cases and 979 controls in HPFS, and 819 cases and 819 controls in CPS-IIN (the 819 cases in CPS-IIN included 307 individuals whose medical records were incomplete or could not be obtained; these individuals were excluded in sensitivity analyses). Genotyping was carried out through the Harvard Partners Center for Genetics and Genomics at the Harvard Partners Genotyping Facility using the OpenAssay SNP Genotyping System (BioTrove, Woburn, Massachusetts, USA). Our primary gene of interest, *SLC2A9*, has been identified in several genome-wide association studies as the strongest genetic predictor of serum urate levels and gout[12-15, 24]. Although the causal variant has not been identified, we genotyped three SNPs due to their strong associations with urate in previous studies: rs6855911[12, 15, 18, 24], located within intron 7 with minor allele frequency (MAF) of 0.31 (G allele); rs7442295[12, 13, 15, 18], located within intron 6 with a MAF of 0.21 for G allele; and rs16890979[14, 17, 18], a missense mutation in exon 8 with a MAF of 0.22 for T allele (using HapMap data from Utah residents with ancestry from northern and western Europe, abbreviated CEU[25]). These three SNPs are in strong linkage disequilibrium (LD; pairwise r^2 range from 0.68-0.76 from Haploview[26] with HapMap CEU data) and each minor allele of these SNPs has been associated with a 0.30-0.43 mg/dL decrease in serum urate in individuals of European descent[12, 14]. In addition to *SLC2A9*, we selected for analysis other genes of

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interest due to their role in the transport of urate, including solute carrier family 22, member 12 (*SLC22A12/URAT1)*, ATP-binding cassette sub-family G member 2 *(ABCG2)*, and solute carrier family 17, member 3 *(SLC17A3/NPT4)*.

Covariate assessment

Data on covariates, including age, body mass index (BMI), smoking, and usual diet, including coffee and alcohol intake, were collected via self-report questionnaires every two years, as previously described[27].

Statistical analyses

Basic characteristics of the study population were assessed using means for continuous variables and percentages for discrete variables. Given previously reported sex differences for the association between urate and PD risk[11] and different ranges, we performed analyses separately in men and women. We used histograms and q-q plots to check for normality and then examined associations between individual SNPS and urate using linear regression under an additive genetic model for individuals with measured urate. We used R^2 as a measure of the proportion of the variation in plasma urate explained by each SNP. Because only SNPs located within *SLC2A9* demonstrated statistically significant associations with urate, we also created a genetic score by summing the number of *SLC2A9* alleles that have been associated with lowered urate in previous GWAS. Finally we explored possible modification of the association between the genetic score and urate by factors associated with altered urate levels, including BMI, alcohol, fructose, vitamin C in all three cohorts, and additionally dairy protein and the dietary urate index in the NHS and HPFS, by including cross-product terms between these variables (higher vs. lower, based on the median values) and the genetic score.[27, 28] We then conducted analyses of the genetic score and urate within levels of variables identified as effect modifiers through testing of the interaction terms.

We then assessed the association between each SNP and PD risk under an additive genetic model using conditional logistic regression. In a second model we additionally adjusted for smoking status, coffee intake, BMI, and alcohol consumption. Covariates were obtained from the questionnaire preceding blood draw. In addition to the individual SNPs, we also examined the association between the genetic score and PD using conditional logistic regression. We performed analyses first separately by study and gender, and then used a random meta-analysis approach to pool results and assess potential heterogeneity. Finally, we estimated the association between genetically determined plasma urate and PD risk using the two-stage regression described below. First we fit a linear regression model within the subset of controls with measured urate and SNPs, with plasma urate as the dependent variable and the three *SLC2A9* SNPs as independent variables, and obtained fitted urate levels. Fitted urate levels for cases and controls without measured urate were obtained by substituting their genotypes into the first-stage regression model. Then we used the fitted urate levels as a continuous independent variable in a conditional logistic regression model for PD. In sensitivity analyses, we repeated analyses after excluding cases without confirmation from medical records and those who were diagnosed with PD after age 80.

Results

Baseline characteristics are presented in Table 1. A total of 1,451 cases and 3,135 controls were included. We examined associations between SNPs and urate in a subset of 1,174 controls with measured urate. As expected, urate levels were higher among men than women. In both men and women across all three cohorts, we found statistically significant associations between all three *SLC2A9* SNPs and urate (figure), with each explaining 3.93-5.79% of the overall variance in plasma urate among female controls and 1.45-6.43% among male controls. RS6855911 exhibited the strongest associations with plasma urate among women--a one-allele increment was associated with a 0.47 mg/dL decrease in urate levels (95% CI -0.62, -0.33). Among men, rs7442295 was most strongly associated with plasma

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urate, and a one-allele increment was associated with a 0.42 mg/dL decrease in urate levels (95% CI -0.60, -0.23). Associations were similar in men and women. The genetic score consisting of all three SLC2A9 SNPs explained 5.22% of the variation in urate levels in women and 3.97% in men. SNPs from other genes were not associated with urate levels (Supplementary Table) and did not contribute to explaining more of the variation—for example, an alternative genetic score that included all measured SNPs explained 3.06% of the variation in urate levels in women and 2.34% in men.

We then examined associations between each SNP and risk of PD. Only one SNP (rs1165202) within the *SLC17A3* gene was significantly associated with PD (pooled RR 0.87, 95% CI 0.79-0.96). Results from minimally and fully adjusted models did not show significant associations with PD risk for other SNPs in men or women (Table 2); pooled, multivariable-adjusted relative risks ranged from 0.92-1.08. The genetic score including the three SNPs within *SLC2A9* was not associated with PD risk: the pooled RR was 1.01 (95% CI: 0.97, 1.05). Results were similar after additional adjustment for diuretic and aspirin use (results not shown). Finally, we performed a two-stage regression to examine the association between genetically-predicted urate and PD risk. Consistent with the results above, we did not find significant associations for men (pooled RR for a 1mg/dL higher urate=1.00, 95% CI 0.69-1.45) or women (pooled RR for a 1mg/dL higher urate 1.09, 95% CI 0.76-1.57). Results did not change after excluding cases without confirmation from medical records or after excluding individuals whose PD was diagnosed after age 80.

In exploratory analyses, we investigated the association between SNPs and urate stratified by body mass index and the dietary urate index and its components. We found an interaction between BMI and *SLC2A9* variants (pooled *p* for interaction=0.0006), where the association between additional risk variants and lowered urate was stronger for men and women with BMI below the median. For those with BMI above the median level, each additional risk allele was associated with 0.12 mg/dL lowered

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urate (95% CI -0.21, -0.03), while for those with BMI below the median each additional risk allele was associated with 0.17 mg/DL lowered urate (95% CI -0.22, -0.12). No other significant interactions were found (p>0.05 for all). However, *SLC2A9* risk variants were not associated with PD risk after stratifying by BMI (RR for BMI below the median=1.02, 95% CI 0.95-1.10; RR for BMI above the median: 1.00, 95% CI 0.92-1.09).

Discussion

Previous research has demonstrated an association between urate and lowered PD risk; however, whether this association reflects a neuroprotective effect of urate is difficult to confirm in observational studies because of the possibility of unmeasured confounding. Since polymorphisms should be minimally confounded by other factors, studying the association between genetic determinants of urate levels and PD risk could provide important insights into the relationship between urate and PD. In this analysis, we found the expected associations between variants in *SLC2A9* and urate levels among both men and women in three cohorts; however, we did not find associations between these genetic variants and PD risk.

One explanation of our results is that the relatively small proportion of the variation in urate levels explained by the SNPs in our participants mean that the association between urate and PD could not be detected using these variants. Since the effects of individual variants on phenotypes are often small, this is a common drawback to Mendelian randomization studies.[29] Since the *SLC2A9* score only accounted for 5.22% of the variation in urate concentrations in women and 3.97% in men, genetic factors other than the common variants assessed in this analysis may contribute more to between-person differences in circulating urate levels. In a recent analysis in these cohorts, we found approximately a 40% reduced risk of PD among men in the highest quartile of plasma urate (6.3-9.0 mg/dL) compared to men in the lowest quartile (<4.9 mg/dL)[11]. Based on data from our cohorts, we estimated that the approximately

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0.80mg/dL decrease in plasma urate associated with having two copies of the risk allele compared to having none for one of the SNPs in *SLC2A9* would predict a 10.5% increase in PD risk, assuming a linear association. Power of our study may thus have been insufficient to detect this relatively modest difference in genetically determined PD risk.

Another possible explanation of our results is that plasma urate does not have a causal effect on PD. While possible, these findings should be weighed against the evidence from a variety of sources supporting the hypothesis that urate is neuroprotective. Higher urate levels have been associated with decreased risk of PD in prospective studies[11] and with slower rates of clinical decline in PD patients[9, 10]. Dietary determinants of urate have also been associated with altered PD risk[27]. Of note, a recent analysis using the same *SLC2A9* variants found a hazard ratio for disease progression of 1.27 for a 0.5mg/dL genetically conferred decrease in serum urate[30], which may suggest that urate is a stronger predictor of PD progression than risk. Further, it is possible that genetic determinants of plasma urate differ from genetic determinants of CNS urate. While cerebrospinal fluid, brain, or neuronal urate may be neuroprotective, as plasma urate does not directly determine urate levels in the immediate environment of the degenerating neurons, it may be unrelated or only weakly related to PD risk. This explanation could be supported by our finding of a significant association between rs1165202 and PD risk. While the T allele at this locus was associated with lowered serum urate and with gout in white participants[14] and was not associated with plasma urate in our study, it has been associated with significantly higher cerebrospinal fluid urate levels.[31]

The strengths of our study include the availability of genetic data as well as plasma urate measurements and data on diet and lifestyle factors, as well as the relatively large sample size. A previous study examining associations between variants in *SLC2A9* and risk of PD also found no association, yet had roughly half the number of cases compared to our study[22]; taken together, the agreement between

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these studies lends more confidence to the results. One weakness is that the participants are almost exclusively of European descent, limiting the generalizability of our findings. However, restricting our analysis to individuals of European descent also minimizes the potential for confounding by population stratification. In addition, for *SLC2A9* in particular, associations with urate levels have been found in many different populations[12-18].

In conclusion, our results do not support an association between genetic variants associated with circulating urate levels and risk of PD, but larger investigations are needed to determine whether the modest genetic effects on blood urate contribute to predict PD risk.

Author Contributions

1. Research project. Conception: AA, XG, MAS. Organization: XG, MAS, and AA. Execution: XG, MAS, and AA.

2. Statistical Analysis. Design: KCH, AA. Execution: KCH. Review and Critique: KCH, XG, EJO, IYK, MW, MGW, MAS, and AA.

3. Manuscript Preparation. Writing of the first draft: KCH. Review and Critique: KCH, XG, EJO, IYK, MW, MGW, MAS, and AA.

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Figure caption

Figure 1. Plasma urate level by number of *SLC2A9* minor alleles in PD cases and controls

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Table 1 Age-adjusted characteristics of the study population

	Women		Men	
	Controls (n=1631)	Cases (n=616)	Controls (n=1504)	Cases (n=835)
Age, years*	61.2(7.0)	63.9(7.7)	68.0(7.4)	69.0(6.7)
UA	4.8(1.1)	4.7(1.2)	5.6(1.1)	5.5(1.0)
Body mass index, kg/m	25.8(4.1)	25.5(3.9)	26.1(3.3)	26.2(3.4)
Current smoker, %	8.2	3.1	4.9	1.8
Past smoker, %	38.9	34.9	51.6	49.5
Caucasian, %	98.8	98.5	99.5	95.8
Alcohol, g/day	4.8(8.0)	4.1(7.6)	8.7(13.2)	7.4(10.1)
Coffee, servings/day	1.6(1.5)	1.7(1.6)	1.8(1.7)	1.9(1.7)
Fructose intake, gm	21.0(10.4)	20.3(10.4)	22.6(12.6)	22.1(10.3)
Vitamin C intake, mg	271.8(281.1)	217.3(229.9)	215.8(256.4)	170.2(163.7)
High blood pressure, %	29.3	30.8	28.9	33.4
Use of diuretics, %	16.5	15.1	7.3	10.7

Values are means(SD) or percentages and are standardized to the age distribution of the study population.

* Value is not age adjusted

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Table 2. Associations between urate transporter-related SNPs and risk of Parkinson's disease

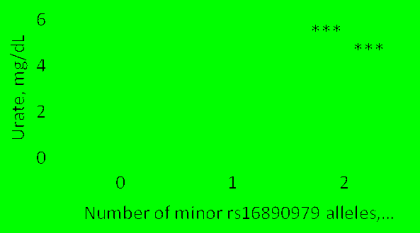
		Women		Men		Pooled
N cases, controls		616, 1631		835, 1504		
	EA/NEA[1]	RR (95% CI)[2]	RR (95% CI), fully adjusted[3]	RR (95% CI)	RR (95% CI), fully adjusted[3]	RR (95% CI), fully adjusted[3]
SLC2A9						
rs16890979	(C,T)	0.97 (0.82, 1.14)	1.00 (0.85, 1.19)	1.03 (0.90, 1.19)	1.04 (0.89, 1.20)	1.02 (0.91, 1.14)
rs7442295	(A,G)	0.99 (0.83, 1.17)	1.01 (0.85, 1.21)	0.99 (0.85, 1.14)	0.98 (0.85, 1.14)	1.00 (0.89, 1.12)
rs6855911	(A,G)	0.97 (0.83, 1.14)	1.00 (0.84, 1.18)	1.04 (0.91, 1.19)	1.04 (0.91, 1.20)	1.02 (0.92, 1.14)
SNP score[4]		0.99 (0.93, 1.05)	1.00 (0.94, 1.06)	1.01 (0.96, 1.06)	1.01 (0.96, 1.06)	1.01 (0.97, 1.05)
URAT1						
rs11231825	(T,C)	1.04 (0.90, 1.21)	1.07 (0.91, 1.24)	1.05 (0.72, 1.54)	1.07 (0.76, 1.51)	1.06 (0.91, 1.25)
rs11602903	(A,T)	0.98 (0.85, 1.14)	1.00 (0.86, 1.18)	1.08 (0.79, 1.46)	1.09 (0.83, 1.44)	1.05 (0.92, 1.20)
rs3825016	(C,T)	1.01 (0.87, 1.17)	1.03 (0.88, 1.20)	1.06 (0.77, 1.46)	1.08 (0.81, 1.45)	1.06 (0.92, 1.21)
rs3825018	(A,G)	0.96 (0.82, 1.12)	0.94 (0.78, 1.13)	0.95 (0.73, 1.25)	0.94 (0.73, 1.20)	0.94 (0.83, 1.07)
rs475688	(C,T)	0.97 (0.80, 1.18)	0.94 (0.75, 1.18)	0.93 (0.72, 1.21)	0.92 (0.74, 1.14)	0.92 (0.81, 1.05)
rs476037	(A,G)	1.01 (0.76, 1.34)	1.05 (0.76, 1.45)	1.09 (0.70, 1.69)	1.09 (0.73, 1.64)	1.08 (0.87, 1.33)
rs7932775	(C,T)	1.08 (0.91, 1.29)	1.06 (0.82, 1.36)	1.02 (0.87, 1.20)	1.02 (0.86, 1.21)	1.05 (0.93, 1.18)
rs893006	(A,C)	0.98 (0.84, 1.14)	0.96 (0.82, 1.13)	0.94 (0.75, 1.19)	0.93 (0.77, 1.13)	0.94 (0.85, 1.05)
ABCG2						
rs2231142	(G,T)	0.81 (0.64, 1.02)	0.76 (0.60, 0.97)	1.23 (0.98, 1.56)	1.21 (0.96, 1.53)	0.97 (0.73, 1.28)
SLC17A3						
rs1165205	(A,T)	0.90 (0.76, 1.06)	0.86 (0.67, 1.12)	0.87 (0.76, 0.99)	0.86 (0.75, 0.98)	0.87 (0.79, 0.96)

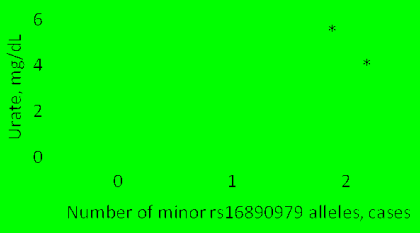
[1]Effect allele/noneffect allele

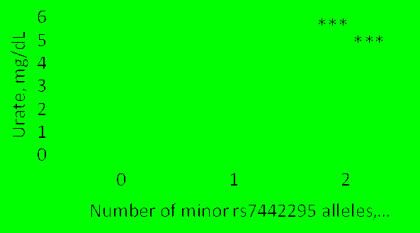
[2]Results from conditional logistic regression model

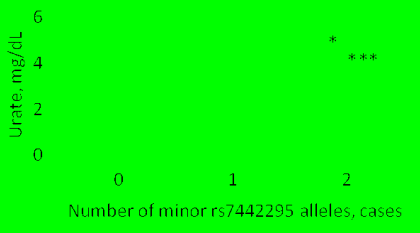
[3]Results from conditional logistic regression model with additional adjustment for BMI (<25, 25 to <30, ≥30), alcohol intake in grams per day (none, <5, 5 to <10, 10 to <15, ≥15 for women and none, <10, 10 to <20, 20 to <30, and ≥30 for men), coffee intake in servings per day (none, <1, 1, 2, ≥3), and smoking status (never, past, current)

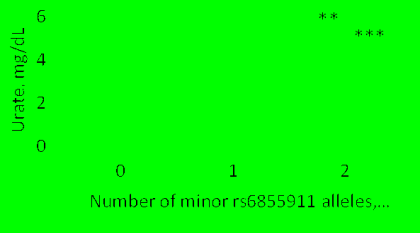
[4]Sum of *SLC2A9* effect alleles (0-6)

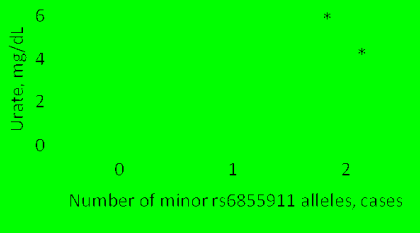

Urate, mg/dL
6
4
2
0
***
***
0
1
2
Number of minor rs16890979 alleles,...


Urate, mg/dL
6
4
2
0
*
*
0
1
2
Number of minor rs16890979 alleles, cases


Urate, mg/dL
6
5
4
3
2
1
0
***
***
0
1
2
Number of minor rs7442295 alleles,...


Urate, mg/dL
6
4
2
0
*
***
0
1
2
Number of minor rs7442295 alleles, cases


Urate, mg/dL
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4
2
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***
0
1
2
Number of minor rs6855911 alleles,...


Urate, mg/dL
6
4
2
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*
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0
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2
Number of minor rs6855911 alleles, cases


Urate, mg/dL
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0
1
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3
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5
6
SLC2A9 genetic score, controls


Urate, mg/dL
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6
SLC2A9 genetic score, cases

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Genetic variants related to urate and risk of Parkinson's disease—Highlights

SNPs in *SLC2A9* were associated with plasma urate levels in both men and women.

SNPs in other genes involved in urate transport were not associated with plasma urate in our cohorts.

SLC2A9 SNPs were not associated with risk of Parkinson's disease.

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