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Caffeine, creatine, *GRIN2A* and Parkinson's disease progression

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Abstract

Caffeine is neuroprotective in animal models of PD and caffeine intake is inversely associated with the risk of Parkinson's disease (PD). This association may be influenced by the genotype of *GRIN2A*, which encodes an NMDA-glutamate-receptor subunit. In two placebo-controlled studies, we detected no association of caffeine intake with the rate of clinical progression of PD, except among subjects taking creatine, for whom higher caffeine intake was associated with more rapid progression. We now have analyzed data from 420 subjects for whom DNA samples and caffeine intake data were available from a placebo-controlled study of creatine in PD. The *GRIN2A* genotype was not associated with the rate of clinical progression of PD in the placebo group. However, there was a 4-way interaction between *GRIN2A* genotype, caffeine, creatine and the time since baseline. Among subjects in the creatine group with high levels of caffeine intake, but not among those with low caffeine intake, the *GRIN2A* T allele was associated with more rapid progression ($p = 0.03$). These data indicate that the deleterious interaction between caffeine and creatine with respect to rate of progression of PD is influenced by *GRIN2A* genotype. This example of a genetic factor interacting with environmental factors illustrates the complexity of gene-environment interactions in the progression of PD.

Keywords

Parkinson's disease; Caffeine; Coffee; GRIN2A; Creatine; Progression

1. INTRODUCTION

The pathogenesis of Parkinson's disease (PD) is complex, and in most patients likely involves interactions between multiple genetic and environmental factors. Here, we report a complex interaction between caffeine, creatine and *GRIN2A* genotype with the rate of progression of PD. Several studies demonstrate a dose-dependent inverse association between caffeine intake and the risk of developing PD[1, 2]. Experimental data *in vitro* and in animal models of PD supports a role for caffeine and other A2a receptor antagonists in protection against cell death[3–5]. We previously hypothesized that caffeine might slow disease progression in patients already diagnosed with PD. To test this hypothesis, we analyzed data on caffeine intake that was collected during a large phase 3 placebo-controlled study of creatine as a potential disease-modifying therapy[6]. Contrary to our hypothesis, among PD subjects randomized to placebo there was no association of caffeine with the rate of progression of PD. However, in the subgroup of PD subjects randomized to take creatine, high levels of caffeine intake were associated with significantly more rapid progression of PD, suggesting a deleterious interaction between caffeine and creatine[7]. This interaction between caffeine and creatine replicated a similar finding in a previous study[8]. The mechanism of this interaction is unknown, although prior reports have indicated that caffeine can counteract the effects of creatine on muscle contraction, possibly by reducing calcium uptake by the sarcoplasmic reticulum.[9, 10]

In the current study, we assessed whether the *GRIN2A* genotype is associated with rate of progression of PD as measured by the Unified Parkinson's Disease Rating Scale (UPDRS) and tested for an interaction between *GRIN2A* genotype, creatine and caffeine. The *GRIN2A* gene encodes an NMDA-glutamate-receptor subunit. Adenosine A2a receptors enhance NMDA-induced calcium influx, and caffeine is an A2a receptor antagonist, providing a potential mechanistic link between caffeine and *GRIN2A*. Two studies have found that the T-allele of the *GRIN2A* single nucleotide polymorphism (SNP) rs4998386 strongly influences the association of caffeine with the risk of PD[11, 12], although this association was not replicated in a third study[13]. In the initial paper by Hamza et al[11], among heavy coffee drinkers, carriers of a *GRIN2A* T allele had a 59% lower risk of PD compared to light coffee drinkers. In contrast, the reduction in risk associated with heavy coffee drinking was only 18% for subjects with the CC genotype. We hypothesized that *GRIN2A* genotype also might influence the rate of progression of PD, and that there would be interactions with caffeine and creatine.

2. MATERIALS AND METHODS

2.1 Subjects

A total of 1,741 subjects with early PD (within 5 years of diagnosis) participated in the Long-term Study 1 (LS1) study and were randomized 1:1 to creatine 5 grams twice daily or to placebo for 5 years, although the study was terminated early for futility based on an interim analysis when the median follow-up time was 4 years[6]. Of these subjects, 1,549 completed a caffeine intake questionnaire at the 18-month study visit that focused on caffeine intake during the prior week. DNA samples were available for 451 LS1 subjects. Baseline characteristics were compared among subjects with and without DNA genotype data to verify the homogeneity of the subgroup sample. *GRIN2A* genotype data were available from 420 of these 451 subjects. The final subgroup analysis used these 420 samples. Additional details of the LS1 study have been published previously[6].

2.2 Genotyping

A 433-bp fragment including rs4998386 in intron 3 (NM_000833) of *GRIN2A* was amplified by polymerase chain reaction (PCR) with the following primers: forward: GGAGGACAGGACACTAACTGG, reverse: CCTCGGAGGGATATATCTACAAG. PCR products were Sanger sequenced on an automated sequencing machine (ABI-3130 Genetic Analyzer, Applied Biosystems; Carlsbad, CA, USA) and genotype at the position of rs4998386 was determined using Mutation surveyor software (SoftGenetics; State College, PA, USA).

2.3 Statistical methods

In a case-only study, an association test between caffeine and a SNP is equivalent to testing for a caffeine-SNP interaction in a case-control design provided that caffeine and the SNP are independent in the general population. The *GRIN2A* rs4998386 SNP was not significantly associated with the baseline caffeine in any of 8 models tested.

For these analyses, “rate of progression of PD” is defined as the change over time in the total UPDRS score for Parts I – III, which is the same outcome measure used in our prior report of the interaction of caffeine and creatine with rate of progression of PD[7].

Our previous study[7] detected an interactive effect of caffeine consumption with creatine treatment on the rate of PD progression, as defined above. This gene-environment interaction analysis explores the interaction of *GRIN2A* genotypes with caffeine. Based on reports of an association of the GRIN2A T allele with a lower risk of PD among heavy coffee drinkers[11], we hypothesized that *GRIN2A* genotype would interact with caffeine or treatment to affect the rate of PD disease progression. For the current study, heavy caffeine drinkers are defined as in previous analyses by daily caffeine intake ≥300mg; and the low caffeine group as daily consumption <300mg[7]. Continuous baseline variables Levodopa Equivalent Dose, Beck Depression Index (BDI), uric acid, body mass index (BMI), age at enrollment, days since diagnosis and baseline UPDRS were tested by nonparametric Wilcoxon two-sample test which does not rely on the normal distribution assumption. The Chi-Square Test was used for binary variables of gender and treatment groups. Allele frequencies were calculated for subjects in high and low caffeine groups and a Fisher’s exact test was conducted to test the association between genotype distribution and caffeine consumption. The Fisher’s exact test was used here because of the low frequency of T alleles.

To assess the effect of genotype on disease progression, we used a mixed model by treating site as a random effect. The response variable is the annual total UPDRS score from years 1 to 5, with the coefficient estimate on years representing the rate of progression by this measure. The model includes all baseline covariates, baseline total UPDRS, caffeine group, treatment group, years of follow-up (continuous time from baseline), and added the genotype *GRIN2A* category main effect as well as all possible two-, three-, and four- way interaction with caffeine group, treatment, and years of follow-up. The mixed effect model used all available longitudinal annual measures of UPDRS, assuming the correlation structure of multiple measures within each individual to be heterogeneous first-order autoregressive. The autoregressive structure assumes the correlation of annual UPDRS is mainly related to the measurements in the neighboring years and the relationship decreases as time between measurements increases. The heterogeneous structure allows a different variance at each time point. The model was fit by SAS PROC MIXED using Restricted Maximum Likelihood method[14]. Fixed effect parameters were estimated for every model. Similarly, as before, missing data are considered missing at random[7]. The *GRIN2A* genotype was categorized into C allele group with genotype C/C and T allele group with genotype C/T + T/T because T/T type is rare (<2% in European HapMap samples (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4998386). The full model is hierarchical in the sense that lower order interaction terms are preserved if higher order interactions were significant. All interaction terms were considered significant at the 0.1 level and all main effects were considered significant at the 0.05 level. The model was broken up in to sub-models if the interaction terms were significant.

3. RESULTS

The minor allele frequency (MAF) of rs4998386 in *GRIN2A* was 0.0944 in the LS1 samples, compared with 0.0819 in the 1000 Genomes Project (MAF = 0.188 in Europeans, 0.087 in African Americans, and 0 in the Asian panel) [15]. This SNP achieved Hardy-Weinberg Equilibrium (HWE test p-value = 0.784). Race was not evenly distributed among the genotype groups (Tables 1 and 2). However, since there were only 12 African Americans, 1 American Indian, and 22 Asians, the inclusion of race in the analysis had a minimal effect on the reported results and so race was not included as a covariate in the reported analyses below.

Both *GRIN2A* genotype and caffeine data were available from 420 out of the 1,741 total LS1 subjects. Table 1 compares baseline covariates among high and low caffeine groups. Differences between high and low caffeine groups used in the analysis included a higher body mass index (BMI) in the high caffeine group (28.2 versus 26.8; p=0.001) and a lower percentage of females in the high caffeine group (22.9% versus 39.1%; p<0.01). Similar differences were also seen in our prior report involving all subjects with caffeine data[7].

Next, the allele frequency of *GRIN2A* was explored for the 420 subjects with *GRIN2A* genotype data. A total of 2 out of these 420 subjects (less than 1%) had the T/T genotype, and so subjects harboring a T allele (C/T and T/T) were combined for these analyses. A two-way frequency table for caffeine group and genotype is shown in Table 2. There was no significant association between *GRIN2A* genotype and caffeine category (high versus low) detected. However, in the model of progression in the UPDRS there was a 4-way interaction between treatment category (creatine vs. placebo), caffeine (high vs. low), *GRIN2A* genotype and years since baseline (p=0.08).

In the creatine subgroup, there remained a 3-way interaction between caffeine, *GRIN2A* genotype and year (Table 3a; p=0.04).

This interaction was not detected in the placebo group (Table 3b; p = 0.62). Note that total subjects in tables 3a and 3b add up to 416 rather than 420 due to missing data on covariates in 4 subjects.

Among subjects in the creatine subgroup with a *GRIN2A* T allele (n=39), high caffeine intake was associated with a 4.98-unit higher rate of progression (Table 4a; p=0.03), whereas there was no significant association of caffeine with rate of progression among the 174 subjects in the creatine subgroup with a C allele (Table 4b; estimate 1.19 units; p=0.13).

Among the subset of subjects taking creatine who were in the high caffeine group (Table 5a; n = 34), the subgroup with a *GRIN2A* T allele had a progression rate of 5.45 units faster than that of the C/C genotype subgroup (p=0.03).

In contrast, among the subset of subjects taking creatine who were in the low caffeine group (Table 5b; n = 180), there was no significant interaction between rate of progression and *GRIN2A* genotype (p = 0.75).

Thus, the *GRIN2A* T-allele is associated with a significantly more rapid rate of progression among subjects taking creatine who also were in the high caffeine group. However, this interaction with genotype is not detected among low caffeine consumers who received creatine.

4. DISCUSSION

Caffeine intake has been associated with a lower risk of developing PD[1, 2], and an interaction of this association with *GRIN2A* genotype has been reported in two prior studies[11, 12], although this was not replicated in a third study[13]. Two prior studies indicated a deleterious interaction between caffeine and creatine, with higher rates of progression of PD among subjects taking creatine who also had high caffeine intake[7, 8]. Based on these studies, we hypothesized that *GRIN2A* genotype might interact with creatine, caffeine, and the rate of progression of PD. Consistent with this, among subjects taking creatine who had the *GRIN2A* T allele, high levels of caffeine intake were associated with a faster rate of progression compared to subjects with low caffeine intake. No interaction of *GRIN2A* genotype with caffeine and rate of progression was found among subjects in the placebo group. This suggests that our previous finding of a deleterious interaction between caffeine and creatine with respect to rate of progression of PD is influenced by *GRIN2A* genotype, and is mainly due to the T allele subgroup while the *GRIN2A* C allele subgroup is not sensitive to the caffeine and creatine interaction. Alternatively stated, the impact of *GRIN2A* on rate of progression of PD is dependent on both caffeine and creatine exposures. These data provide a rare example of a gene-environment-environment interaction, with *GRIN2A* genotype interacting with level of caffeine intake, creatine, and rate of progression of PD, and highlight the complexity of the factors that contribute to PD progression.

Caffeine is an antagonist of adenosine A2a receptors, and both caffeine and other A2a receptor antagonists are protective in mouse models of PD[3]. Mice lacking A2a receptors are likewise protected[5]. A strong interaction has been demonstrated between *GRIN2A* genotypes , caffeine intake, and a reduced risk of PD[11]. GRIN2A encodes a subunit of the NMDA-glutamate receptor and is involved in excitatory neurotransmission. Adenosine A2a receptors enhance NMDA-induced calcium influx, thus providing a plausible mechanistic link between *GRIN2A* and caffeine. The functional impact of the rs4998386 in *GRIN2A* is not known, and it’s possible that this SNP is in linkage disequilibrium with a different variant that is driving these results. Furthermore, it is unclear why the T allele reported in some prior studies to be associated with a lower risk of PD among heavy coffee drinkers would be associated with faster progression among heavy coffee drinkers who also take creatine. One possibility is that heavy coffee drinkers who develop PD despite a *GRIN2A* T allele do so because they have other genetic or environmental PD risk factors that are associated with more rapid progression.

Interestingly, the association of *GRIN2A* genotype with the risk of PD identified by Hamza et al[11] was not replicated in a subsequent study in a distinct study population[13]. The authors raised the possibility that the apparent association reported by Hamza et al may have resulted from the association of *GRIN2A* genotype with coffee drinking behavior in controls but not in PD patients[13]. However, our analyses were restricted to PD patients, and in any

case an impact of the *GRIN2A* genotype on coffee drinking behavior would not account for the 4-way interaction that we have identified for *GRIN2A* genotype, caffeine, creatine and rate of clinical progression.

This study has several strengths, including a large and clinically well-characterized group of PD patients examined longitudinally over several years by movement disorders specialists, allowing assessment of the rate of clinical progression. The availability of caffeine intake data as well as DNA samples from a substantial number of these subjects also has been an invaluable resource. On the other hand, this study also has several limitations. Although subjects were randomized to creatine or placebo, subjects were not randomized with respect to caffeine intake. Thus, these data demonstrate a correlation, but do not prove that high caffeine intake is causally related to more rapid progression among subjects with the *GRIN2A* T allele who also take creatine. An additional limitation is that no data on smoking were collected in this clinical trial. Caffeine and tobacco use are reported to be correlated[16], raising the possibility that tobacco use rather than high caffeine use might have contributed to our results. However, the rate of smoking among PD patients is quite low[16], and, as previously outlined[7], smoking status is unlikely to have had a major impact on these analyses. Another limitation is that we collected caffeine intake at only a single time point. Changes in caffeine intake over time could have influenced these results, although levels of caffeine intake among PD patients are reported to be relatively stable over time in most patients[8]. Caffeine also may have symptomatic effects[17, 18], and if these symptomatic benefits change over time as the disease progresses, then this could lead to an apparent association of caffeine intake with the rate of disease progression as measured by changes in UDPRS scores. An additional consideration is that coffee and other sources of caffeine have other constituents that may have contributed to the interactions that we detected[19]. Our study also focused on only a single genetic variant and did not consider other genetic factors that also may influence the association of caffeine with PD risk or progression[20].

In summary, we have identified a complex genotype-environment interaction. Among subjects taking creatine who also have high levels of caffeine intake, a *GRIN2A* T allele is associated with a significantly faster rate of progression of PD. Although our results are statistically significant, indicating that they are less likely to have occurred by chance, these results need to be replicated in an independent study population. However, such a study would require another large population of PD patients with extensive longitudinal clinical data as well as data on caffeine intake and availability of DNA samples. Given the potential importance of complex gene-environment interactions in PD risk and progression, it should be a priority to generate additional clinical and biospecimen resources to allow these types of studies in the future.

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Table 1

Baseline Characteristics for subjects in the low (<=300mg) and high (>300mg) caffeine groups

		Low Caffeine N=350	High Caffeine N=70	p-value
Levodopa Equiv. Dose at Baseline	Median	300.0	341.25	0.54
	SD	236.44	211.21	
	Q range	272.5	230	
Beck Depression Index	Median	6.0	5.5	0.82
	SD	5.99	5.53	
	Q range	6.0	7.0	
Uric Acid	Median	5.0	5.2	0.22
	SD	1.40	1.44	
	Q range	2.0	2.2	
Body Mass Index	Median	26.8	28.2	0.001**
	SD	4.93	5.25	
	Q range	5.86	6.9	
Age at Enrollment	Median	63.0	60.0	0.49
	SD	9.61	9.55	
	Q range	12.0	15.0	
Days since Diagnosis	Median	494.0	506.5	0.53
	SD	382.82	349.45	
	Q range	465	563	
UPDRS baseline	Median	25.0	23.0	0.53
	SD	11.47	10.31	
	Q range	17.0	14.0	
Female	Freq	137	16	<0.01**
	%	39.14	22.86	
Treatment(Creatine)	Freq	180	36	1.00
	%	51.43	51.43	

SD: standard deviation

Q range: 25%–75% inter-quartile range.

Table 2

Two-way Frequency Table of Caffeine Group and Genotype

Table of caffeine group by GRIN2A genotype N=420				
Caffeine group	**GRIN2A(rs4998386 (GRIN2A))**			
	C/C	**C/T**	**T/T**	**Total**
Low	285	64	1	350
High	60	9	1	70
Total	345	73	2	420

* Fisher's Exact test p=0.19

Table 3a

Model of GRIN2A for Creatine group (N=212).

Effect	GRIN2A	Estimate	Standard Error	DF	t Value	p value
Intercept		5.60	5.10	29	1.10	0.28
Baseline UPDRS		0.77	0.05	890	14.25	<.0001***
Years						0.15
Caffeine Category						0.12
Age						0.60
Years*Age		0.08	0.03	890	2.90	0.004**
Years*Caffeine Category[1]		1.11	0.80	890	1.39	0.17
GRIN2A	C/T+T/T					0.99
Caffeine Category*GRIN2A	C/T+T/T	−2.98	6.82	890	−0.44	0.66
Years*Caffeine Category[1]*GRIN2A	C/T+T/T	5.48	2.66	890	2.06	0.04**
Years*GRIN2A	C/T+T/T	0.31	0.74	890	0.42	0.68
Beck Depression Index (BDI)		0.33	0.12	890	2.82	0.005**

[1]Caffeine intake ≥ 300 coded as 1; caffeine intake <300 = 0

DF: degrees of freedom

Table 3b

Model of GRIN2A for placebo group (N=204).

Effect	GRIN2A	Estimate	Standard Error	DF	t Value	p value
Intercept		−1.50	4.94	28	−0.30	0.76
Baseline UPDRS		0.76	0.06	850	12.65	<.0001***
Years						0.18
Caffeine Category						0.38
Age						0.48
Years*Age		0.09	0.03	850	3.27	0.001**
Years*Caffeine Category		−0.96	0.76	850	−1.27	0.20
GRIN2A	C/T+T/T					0.18
Caffeine Category*GRIN2A	C/T+T/T	3.85	5.01	850	0.77	0.44
Years*GRIN2A	C/T+T/T	0.78	0.77	850	1.01	0.31
Years*Caffeine Category*GRIN2A	C/T+T/T	−0.88	1.76	850	−0.50	0.62
Beck Depression Index (BDI)		0.25	0.09	850	2.81	0.005**

DF: degrees of freedom

Table 4a

Model of GRIN2A by Creatine and T allele group (N=39).

Effect	Estimate	Standard Error	DF	t Value	p value
Intercept	−9.10	14.1	18	−0.64	0.53
Baseline UPDRS	0.85	0.16	149	5.21	<.0001***
Years					0.75
Caffeine Category					0.53
Age					0.68
Years*Age	0.07	0.07	149	0.93	0.36
Years*Caffeine Category	4.98	2.27	149	2.20	0.03**
Beck Depression Index (BDI)	1.15	0.35	149	3.24	0.002**

DF: degrees of freedom

Table 4b

Model of GRIN2A by Creatine and C allele group (N=174).

Effect	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept	8.29	5.35	26	1.55	0.13
Baseline UPDRS	0.76	0.06	727	13.76	<.0001***
Years					0.21
Caffeine Category	−3.30	2.05	727	−1.61	0.11
Age					0.40
Years*Age	0.08	0.03	727	2.58	0.01**
Years*Caffeine Category	1.19	0.78	727	1.53	0.13
Beck Depression Index (BDI)	0.23	0.12	727	1.94	0.05**

Table 5a

Model of GRIN2A by Creatine and high caffeine group (N=34).

Effect	GRIN2A	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		−7.73	11.6	16	−0.67	0.52
Baseline UPDRS		0.87	0.14	123	6.22	<.0001***
Years						0.55
Age						0.51
Years*Age		0.10	0.07	123	1.47	0.14
GRIN2A	C/T+T/T					0.81
Years*GRIN2A	C/T+T/T	5.45	2.54	123	2.14	0.03**

Table 5b

Model of GRIN2A by Creatine and low caffeine group (N=180).

Effect	GRIN2A	Estimate	Standard Error	DF	t Value	Pr > |t|
Intercept		10.68	5.47	29	1.95	0.06
Baseline UPDRS		0.81	0.05	753	14.77	<.0001***
Years						0.32
Age						0.22
Years*Age		0.07	0.03	753	2.35	0.02**
GRIN2A	C/T+T/T					0.89
Years*GRIN2A	C/T+T/T	0.23	0.73	753	0.31	0.75