

Coffee, *ADORA2A*, and *CYP1A2*: the caffeine connection in Parkinson's disease

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Background and purpose: In 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine animal models of Parkinson's disease (PD), caffeine protects neurons by blocking the adenosine receptor *A2A* (*ADORA2A*). Caffeine is primarily metabolized by cytochrome P450 1A2 (*CYP1A2*). Our objective was to examine whether *ADORA2A* and *CYP1A2* polymorphisms are associated with PD risk or modify the caffeine-PD association.

Methods: Parkinson's Epidemiology and Genetic Associations Studies in the United States (PEGASUS) included five population-based case-control studies. One laboratory genotyped four *ADORA2A* and three *CYP1A2* polymorphisms in 1325 PD cases and 1735 age- and sex-matched controls. Information regarding caffeine (coffee) consumption and other lifestyle factors came from structured in-person or telephone interviews. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using logistic regression.

Results: Two *ADORA2A* polymorphisms were inversely associated with PD risk – rs71651683, a 5' variant (adjusted allelic OR = 0.51, 95% CI 0.33–0.80, permutation-adjusted *P* = 0.015) and rs5996696, a promoter region variant (adjusted OR for AC and CC genotypes compared with the AA wild-type genotype were 0.76 (95% CI 0.57–1.02) and 0.37 (95% CI 0.13–1.01), respectively (permutation-adjusted *P* for trend = 0.04). *CYP1A2* polymorphisms were not associated with PD risk; however, the coffee-PD association was strongest among subjects homozygous for either variant allele rs762551 (*P*_{interaction} = 0.05) or rs2470890 (*P*_{interaction} = 0.04).

Conclusion: In this consortium study, two *ADORA2A* polymorphisms were inversely associated with PD risk, but there was weak evidence of interaction with coffee consumption. In contrast, the coffee-PD association was strongest among slow metabolizers of caffeine who were homozygous carriers of the *CYP1A2* polymorphisms.

Introduction

Coffee drinking has been associated with lower risk of Parkinson's disease (PD) in several case-control and

cohort studies. A recent meta-analysis showed that coffee drinkers had a 30% reduction in PD risk compared to non-drinkers [1]. The biological basis of the putative neuroprotective effect of caffeine is not completely understood; however, caffeine has been shown to protect neurons in the 1-methyl-4-phenyl 1,2,3,6-tetrahydropyridine (MPTP) neurotoxin model of PD by blocking the adenosine *A2A* receptor (*ADORA2A*) [2–5]. Hence, polymorphisms in *ADORA2A*, the gene that encodes the *ADORA2A* receptor, might mediate

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the caffeine–PD association. Caffeine is primarily metabolized in the body by cytochrome P450 1A2, an enzyme encoded by the gene *CYP1A2* [6,7]. Therefore, polymorphisms in *CYP1A2* may affect caffeine availability and, thereby, modify caffeine effects on PD risk.

Previous studies in ethnically homogeneous populations composed primarily of non-Hispanic Whites [8] or Asians [9,10] have evaluated the role of *ADORA2A* and *CYP1A2* variants on caffeine–PD association, but did not find any interaction.

We used information from five population-based studies to evaluate whether variations in *ADORA2A* or *CYP1A2* were associated with PD risk and whether the caffeine–PD association was modified by these genetic variants.

Methods

Study design and populations

This consortium study [Parkinson's Epidemiology and Genetics Association Studies in the United States (PEGASUS)] combined DNA and risk factor data from five population-based case–control studies, of which, two were nested within cohorts [11–16]. Characteristics of the study populations are presented in Table 1 and other details, including the research diagnostic criteria [17,18], are summarized in Table S1. The pooled data included 1325 PD cases and 1735 age- and sex-matched controls.

Data collection methods

Data were collected by structured in-person or telephone interviews. Data for each subject on the following variables were obtained from the lead investigators of the five component studies: date of diagnosis or reference date, sex, self-reported race/ethnicity, date of birth, family history of PD, smoking history, and caffeine consumption. Race/ethnicity was self-reported according to one of the following categories: Hispanic White, non-Hispanic White, Asian, or African-American. The Human Subjects Committees at the various institutions approved the study, and informed consent was obtained from all cases and controls.

Methodology for ascertaining caffeine exposure differed slightly among component studies and is briefly summarized in Table S1. The Columbia University studies did not ascertain information regarding caffeine consumption; therefore, caffeine–genotype interactions analyses included 925 cases and 1249 controls. Questions pertaining to caffeine use from the other four studies allowed the construction of the following exposure measures: broad category of consumption (ever/never) and average number of 6-oz cups consumed daily. Because the average amount of caffeine per cup is highest in coffee, we evaluated genotype–caffeine interactions separately for caffeinated coffee, tea, and sodas, and only present results for genotype–coffee interactions in the study.

Table 1 Characteristics of Parkinson's disease (PD) cases and controls in the consortium study

Characteristic	Overall		PEAK		PEG		FAME		COLUMBIA		HAAS	
	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl	Cases	Ctrl
Total number	1325	1735	578	630	279	254	100	367	296	299	72	185
Male (%)	61.1	65.4	61.8	63.0	54.5	52.0	74.0	76.3	52.4	47.2	100.0	100.0
Age ^a												
≤60	37.2	24.2	43.3	42.1	22.6	29.5	49.0	10.9	41.9	6.0	9.7	11.4
>60	62.8	75.9	56.8	57.9	77.4	70.5	51.0	89.1	58.1	94.0	90.3	88.7
Mean age	64.9	68.4	64.3	64.6	67.6	65.8	62.0	69.6	61.9	72.9	75.2	75.5
Race/ethnicity												
African-American (%)	2.2	3.8	2.8	5.7	1.1	3.5	2.0	1.6	2.7	5.0	0.0	0.0
Asian (%)	9.1	13.0	7.4	5.1	1.8	3.5	0.0	0.0	0.0	0.0	100.0	100.0
White Hispanic	10.6	9.5	8.3	7.3	10.4	7.5	1.0	0.5	21.3	32.4	0.0	0.0
White, non-Hispanic (%)	78.1	73.7	81.5	81.9	86.7	85.4	97.0	97.8	76.0	62.5	0.0	0.0
Family history of PD ^b (%)	10.4	5.9	10.4	2.9	12.5	11.8	13.7	7.9	6.0	5.2	4.4	5.3
Smoking status												
Never (%)	54.3	46.8	52.1	45.8	52.9	40.6	74.5	61.8	56.1	43.6	43.1	34.4
Former (%)	41.2	41.3	44.3	41.2	40.3	51.2	24.5	31.5	39.5	41.8	45.8	45.9
Current (%)	4.7	12.0	3.6	13.0	6.8	8.3	1.0	6.7	4.4	14.5	11.1	19.7

The totals for the variables may not equal the number of cases and controls because of missing values; FAME, Farming and Movement Evaluation; HASS, Honolulu Asia Aging Study; PEAK, Parkinsonism Epidemiology at Kaiser; PEG, Parkinson's disease Epidemiology and Genetics; ^aAge is age of diagnosis for PD cases; ^bOne or more first-degree relatives with PD.

Laboratory methods

Component studies provided the consortium a DNA sample from each of their subjects. *ADORA2A* and *CYP1A2* were sequenced by the Stanford Human Genome Center in 24 patients with early-onset PD randomly selected from the Parkinsonism Epidemiology at Kaiser (PEAK) case-control study. Functional regions of both genes were resequenced, including the exons, intron-exon junctions, and regions within 500 bp of the 5' and 3' UTR regions [19,20]. Variants occurring at polymorphic frequencies (minor allele frequency >1%) were identified and polymorphisms were prioritized for genotyping based on function, location, and frequency, with emphasis given to variants affecting protein sequence and function (i.e., exonic variants producing nonsense and missense changes) and variants affecting gene expression or mRNA stability (i.e., variants located in the promoter region, 5'UTR, 3' UTR, splice-site, and intron-exon boundaries). In all samples, we genotyped four *ADORA2A* and three *CYP1A2* single nucleotide polymorphisms (SNPs) on PEGASUS samples (Table 2).

PCR primers and TaqMan probes were designed based on the NCBI DNA sequence and purchased from ABI (Applied Biosystems, Foster City, CA, USA). PCR assays were run in TaqMan Universal Master Mix (Applied Biosystems). Fluorescence data files from each plate were analyzed by automated allele calling software (ABI Prism 7900 HT Sequence Detection System 2.1; Life Technologies Corporation, Carlsbad, CA, USA) and reviewed by a skilled operator. Laboratory

personnel were blinded to the identity and case-control status of the samples. For quality control purposes, a 15% repeat set of redundant genotypes was tested along with a small number of samples with known genotypes. The 'no call' rate was very low (<1% of samples), and thus, we are confident that we analyzed only high-quality genotyping data.

Statistical analyses

Each component study sent interview data and data documentation to Stanford University. For statistical analyses, we used SAS[®] statistical software (SAS Institute, Cary, NC, USA) [21]. We evaluated whether genotype distributions for control subjects were in Hardy-Weinberg equilibrium (HWE) among each racial/ethnic group separately with chi-square or Fisher's exact tests. We designated the minor allele based on white, non-Hispanic subjects and used it for all ethnicities, even when the designated minor allele was the more frequent allele in these other ethnic groups.

We used unconditional logistic regression analyses to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for allelic and genotypic associations with PD risk. To evaluate the risk associated with an increasing number of copies of the variant allele for a given polymorphism, we conducted a test of trend. All estimates were adjusted for sex, age, study site, and race/ethnicity.

We excluded subjects who identified their race/ethnicity as other ($n = 18$) and subjects whose genotyping assay results could not be called ($n = 58$), leaving 1325

Table 2 Polymorphic variants in the adenosine receptor A2A (*ADORA2A*) and cytochrome P450 1A2 (*CYP1A2*) genes genotyped in PEGASUS subjects

Gene and SNP	Type	Change	Minor/major allele	Minor allele frequency in controls (%)			
				White, non-Hispanic $n = 1279$	African-American $n = 66$	Asian $n = 226$	White, Hispanic $n = 164$
<i>ADORA2A</i>							
rs5751876 ^a	Exon	Synonymous (Tyr-Tyr) (also known as 1976 T>C)	T/C	41	67	56 ^b	48
rs71651683	5'	239 5' transcription start	T/C	2	9	0	3
rs3032740 ^a	Intron 3	3' UTR: reduces protein expression (also referred to as 2592C>Tins)	-/Tins	41	66	56 ^b	48
rs5996696	Promoter	5' transcription start	C/A	4	32	8 ^b	8
<i>CYP1A2</i>							
rs762551	Intron	151 5' exon 2 (also known as -163C>A) ^c	C/A	29	35	36	28
rs2472304 ^d	Intron	43 3' exon 4	G/A	36	82	77	56
rs2470890 ^d	Exon	Synonymous (Asn-Asn)	C/T	36	83	78	56

PEGASUS, Parkinson's Epidemiology and Genetics Association Studies in the United States; ^ars5751876 and rs3032740 in strong linkage disequilibrium ($D' = 0.997$ and $r^2 = 0.98$); ^b $P < 0.01$ for Hardy-Weinberg Equilibrium chi-square (HAAS study); ^cAA = fast caffeine metabolizers, CA or CC = 0 slow caffeine metabolizers; ^drs2470890 and rs2472304 were in strong LD ($D' = 0.993$, $r^2 = 0.986$).

cases and 1735 controls for analysis. For analyses of the newly discovered SNPs, we also excluded the 24 early-onset cases in the discovery sample. Information regarding variants in monogenic genes was only available for the PEAK case-control study (578 cases, 630 controls; 39.6% of all subjects in the PEGASUS consortium). In a sub-analysis, the risk estimates were unchanged when we excluded PEAK subjects who carried any of the known pathogenic variants in monogenic genes (*PARKIN*, α -synuclein, *DJI*, *PINK1*, and *LRRK2*). Because we did not have the information to exclude possible monogenic cases of Parkinsonism in the majority of subjects, we conducted our primary analyses using all subjects.

We evaluated whether polymorphisms in *ADORA2A* and *CYP1A2* were effect modifiers of the caffeine-PD associations (ever/never and average cups consumed among ever-drinkers, separately for caffeinated coffee, tea, and sodas). We evaluated effect measure modification on a multiplicative scale by testing the significance of the interaction terms in the logistic regression model using the likelihood ratio chi-square test, which compares the model with the interaction term to the model without it.

We used a permutation-based approach to adjust *P*-values for multiple testing [22]. We randomly permuted the case-control status of subjects within strata defined by sex, race/ethnicity, and site. For each of 10 000 permuted data sets, we used logistic regression to compute an age-, sex-, race-, and site-adjusted per allele effect estimate for each polymorphism. The resulting empirical *P*-value distribution of 10 000 minimum *P*-values was used to estimate multiple comparison adjusted *P*-values.

Results

The five case-control studies were similar in some demographic characteristics but differed in others (Table 1). Mean age was fairly similar across the studies; however, Honolulu Asia Aging Study (HAAS) subjects were older. Subjects from the PEAK, Farming and Movement Evaluation (FAME), and PEG studies were primarily White, HAAS subjects were all Asians, and the Columbia University study was comprised of 28% Hispanics. History of caffeinated coffee consumption was associated with a 28% reduced risk of PD (adjusted OR = 0.72, 95% CI 0.58–0.88); and, among coffee drinkers, the risk decreased 12% with each one cup increase in daily average consumption (adjusted OR = 0.88, 95% CI 0.83–0.94; data not shown). We did not observe effect modification by sex for caffeine-PD associations; hence, all our genotype-caffeine interaction-related analyses combined men and women and

adjusted for sex as a covariate. Inverse associations were also observed with caffeinated tea (adjusted OR = 0.81, 95% CI 0.67–0.96). Among tea drinkers, PD risk decreased by 7% per cup of average daily consumption; however, this estimate was not statistically significant (adjusted OR = 0.93, 95% CI 0.83–1.03). Consumption of caffeinated soda was not associated with PD risk (adjusted OR = 1.00, 95% CI 0.82–1.22).

ADORA2A polymorphisms

The four *ADORA2A* SNPs we selected were in HWE among non-Hispanic White, African-American, and Hispanic controls (Table 2). The Asian subgroup from HAAS was not in HWE at $P < 0.01$ (rs5751876, rs3032740, and rs5996696); however, no substantial differences in the *ADORA2A*-PD associations were observed after excluding these samples. Therefore, genotypic associations for *ADORA2A* SNPs include subjects from all five studies (Table 3).

SNPs rs5751876 and rs3032740 were in strong linkage disequilibrium ($D' = 0.997$ and $r^2 = 0.98$) in all racial/ethnic groups; hence, further discussion will be limited to rs3032740, which has functional relevance as it shown to reduce protein expression [23]. The deletion for rs3032740, identified as the variant among White controls (non-Hispanic and Hispanic), was more frequent than the *Tins* among African-Americans and Asians. After adjustment for age, sex, race/ethnicity, and site, we did not find an overall association of rs3032740 genotypes with PD risk (Table 3), and associations were similar across racial/ethnic groups (Table S2).

The frequency of variant allele for rs71651683 was 1.1% in cases and 2.1% in controls (adjusted allelic OR 0.51, 95% CI 0.33–0.80, permutation-adjusted $P = 0.015$). The variant allele was only present in Whites (non-Hispanic and Hispanic) and a few African-American control subjects (9.1%). Because no cases carried two copies of the variant allele, only genotypic associations involving heterozygotes were estimable, and genotype-coffee interactions could not be evaluated.

The *ADORA2A* promoter variant, rs5996696, was inversely associated with PD risk (3.7% cases, 5.6% controls; adjusted allelic OR 0.70, 95% CI 0.54–0.91). Compared to subjects homozygous for the wild-type allele (AA), the adjusted OR for PD risk among subjects with one (AC) or two copies of the variant allele (CC) were 0.76 (95% CI 0.57–1.02) and 0.37 (95% CI 0.13–1.01), respectively (permutation-adjusted P -value for trend = 0.04, Table 3).

The coffee (ever/never)-PD association was similar among rs3032740 genotypes (Table 4). However, among ever-drinkers, the inverse association with daily

Table 3 Genotype frequency (%), adjusted odds ratios (OR), and 95% confidence intervals (CI) for the association between adenosine receptor A2A (*ADORA2A*) and cytochrome P450 1A2 (*CYP1A2*) polymorphisms and Parkinson's disease in PEGASUS

Variant	Overall				White, non-Hispanic			
	Cases (n = 1325)	Controls (n = 1735)	OR ^a	95% CI	Cases (n = 1035)	Controls (n = 1279)	OR ^b	95% CI
<i>ADORA2A</i>								
rs5751876 ^c								
CC	35.7	32.3	1.00	–	37.4	35.0	1.00	–
CT	45.7	46.3	0.93	0.78–1.1	45.6	48.1	0.91	0.75–1.09
TT	18.7	21.4	0.85	0.69–1.05	17.0	16.8	0.93	0.73–1.2
rs3032740 ^e								
TT	36.5	32.6	1.00	–	38.1	35.2	1.00	–
Td	45.4	46.3	0.91	0.77–1.07	45.4	48.1	0.89	0.74–1.08
dd	18.2	21.1	0.83	0.67–1.03	16.5	16.7	0.91	0.71–1.18
rs71651683								
CC	97.7	95.8	1.00	–	97.6	96.1	1.00	–
CT	2.3	4.2	0.53	0.34–0.83	2.4	3.8	0.61	0.36–1.02
TT	0.0	0.1	0.00	NE	0.0	0.1	0.00	NE
rs5996696								
AA	93.0	90.0	1.00	–	95.1	93.2	1.00	–
AC	6.6	8.8	0.76 ^d	0.57–1.02	4.8	6.5	0.70 ^e	0.48–1.02
CC	0.4	1.2	0.37 ^d	0.13–1.01	0.1	0.2	0.30 ^e	0.03–2.93
<i>CYP1A2</i>								
rs762551								
AA	49.7	49.5	1.00	–	51.4	51.1	1.00	–
AC	40.1	41.4	0.99	0.85–1.16	39.6	40.4	0.99	0.83–1.19
CC	10.1	9.1	1.16	0.9–1.51	9.0	8.6	1.04	0.76–1.43
rs2472304 ^f								
AA	36.0	33.1	1.00	–	43.1	40.9	1.00	–
AG	42.3	43.8	0.93	0.78–1.1	44.3	46.3	0.91	0.76–1.1
GG	21.7	23.1	1.01	0.81–1.27	12.6	12.8	0.89	0.67–1.17
rs2470890 ^f								
TT	35.9	33.5	1.00	–	43.4	41.1	1.00	–
TC	42.3	43.5	0.95	0.8–1.12	44.2	46.1	0.92	0.76–1.1
CC	21.8	23.1	1.02	0.81–1.28	12.4	12.8	0.88	0.67–1.16

NE = not estimable; PEGASUS, Parkinson's Epidemiology and Genetics Association Studies in the United States; ^aAdjusted for age, sex, site, and race/ethnicity; ^bAdjusted for age, sex and site; ^crs5751876 and rs3032740 were in strong linkage disequilibrium ($D' = 0.997$ and $r^2 = 0.98$); ^d P for trend = 0.01, permutation-adjusted P for trend = 0.04; ^e P for trend = 0.03, permutation-adjusted P for trend = 0.1; ^frs2470890 and rs2472304 were in strong linkage disequilibrium ($D' = 0.993$, $r^2 = 0.986$).

number of cups of coffee was strongest among those homozygous for the deletion (adjusted OR = 0.70, 95% CI 0.55–0.86, $P_{interaction} = 0.08$, Table 4). Results were similar when coffee–genotype interactions were restricted to non-Hispanic Whites only (Table S3). No interactions of *ADORA2A* genotypes were observed with caffeinated tea or soda (data not shown).

CYP1A2

All three *CYP1A2* SNPs were in HWE within every ethnic group. For rs762551, homozygous wild-type carriers (AA) are rapid caffeine metabolizers, and heterozygotes (AC) and homozygotes (CC) are slow caffeine metabolizers [24,25]. We did not find an overall association of rs762551 genotypes with risk of PD (Table 3).

SNPs rs2470890 and rs2472304 were in strong linkage disequilibrium ($D' = 0.993$, $r^2 = 0.986$); hence, further discussion will be limited to rs2470890, the exonic variant. The allele 'C' for rs2470890, identified as the variant based on non-Hispanic Whites controls, was the more frequent allele among the other race/ethnic groups (Table 2). We did not find an overall association of rs2470890 genotypes with risk of PD among non-Hispanic Whites, African-Americans, and Asians (Table 3 and Table S2). However, among Hispanic subjects with one (TC) or two copies (CC) of the variant allele, the adjusted OR for PD risk were 1.67 (95% CI 0.8–3.4) and 2.1 (95% CI 1.0–4.3), respectively (P for trend = 0.05, permutation-adjusted P -value for trend = 0.2).

For the rs762551 polymorphism, the effect of coffee consumption (ever versus never) was strongest among

Table 4 Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between caffeinated coffee consumption and Parkinson's disease in PEGASUS, by *ADORA2A* and *CYP1A2* genotypes

Caffeinated coffee	Wildtype			Heterozygote			Homozygote			Interaction				
	Cases	Controls	OR ^a	95% CI	Cases	Controls	OR ^a	95% CI	Cases	Controls	OR ^a	95% CI	P-value	
<i>ADORA2A</i>														
rs5751876														
Never	109	103	1.00	–	130	123	1.0	–	58	61	1.0	–	–	
Ever ^b	221	324	0.67	0.47–0.93	308	452	0.67	0.50–0.91	118	213	0.66	0.42–1.04	0.98	
Cups/day ^c , mean ± SD	1.8 ± 1.7	2.1 ± 2.5	0.93	0.84–1.03	1.6 ± 1.4	1.9 ± 1.6	0.90	0.80–1.00	1.5 ± 1.1	2.1 ± 1.8	0.69	0.55–0.86	0.05	
rs3032740														
Never	113	103	1.00	–	125	123	1.0	–	57	59	1.0	–	–	
Ever ^b	225	322	0.66	0.47–0.93	305	448	0.70	0.51–0.95	111	206	0.63	0.40–1.01	0.91	
Cups/day ^c , mean ± SD	1.7 ± 1.7	2.1 ± 2.5	0.92	0.83–1.03	1.6 ± 1.4	1.9 ± 1.6	0.91	0.81–1.02	1.5 ± 1.2	2.2 ± 1.9	0.70	0.55–0.86	0.08	
rs5996696														
Never	272	255	1.0	–	23	28	1.0	–	1	2	1.0	–	–	
Ever ^b	609	913	0.66	0.53–0.81	38	74	0.73	0.34–1.56	1	9	NE ^d	–	NE	
Cups/day ^c , mean ± SD	1.6 ± 1.4	2.1 ± 2.1	0.86	0.80–0.94	1.8 ± 2.5	1.8 ± 1.4	1.02	0.79–1.31	0.8 ± –	0.9 ± 0.2	NE	–	NE	
<i>CYP1A2</i>														
rs762551														
Never	145	128	1.00	–	113	139	1.0	–	37	21	1.0	–	–	
Ever ^b	337	523	0.61	0.45–0.81	254	391	0.9	0.66–1.23	62	84	0.33	0.16–0.68	0.05	
Cups/day ^c , mean ± SD	1.6 ± 1.4	2.0 ± 1.6	0.87	0.78–0.96	1.7 ± 1.6	2.1 ± 2.5	0.90	0.80–1.00	1.7 ± 1.4	1.8 ± 1.4	0.85	0.64–1.14	0.85	
rs2472304														
Never	106	104	1.0	–	119	131	1.0	–	71	48	1.0	–	–	
Ever ^b	255	337	0.73	0.52–1.01	257	418	0.80	0.58–1.09	132	228	0.46	0.28–0.73	0.07	
Cups/day ^c , mean ± SD	1.8 ± 1.8	1.9 ± 2.3	0.95	0.86–1.04	1.5 ± 1.2	2.1 ± 1.9	0.83	0.73–0.95	1.4 ± 1.3	2.0 ± 1.8	0.79	0.65–0.96	0.018	
rs2470890														
Never	106	107	1.0	–	120	132	1.0	–	71	46	1.0	–	–	
Ever ^b	254	341	0.75	0.54–1.03	258	411	0.80	0.59–1.10	132	227	0.43	0.27–0.69	0.04	
Cups/day ^c , mean ± SD	1.8 ± 1.8	1.9 ± 2.3	0.95	0.87–1.04	1.5 ± 1.2	2.1 ± 1.9	0.82	0.72–0.94	1.4 ± 1.3	2.0 ± 1.9	0.81	0.67–0.97	0.015	

PEGASUS, Parkinson's Epidemiology and Genetics Association Studies in the United States; ^aAdjusted for age, sex, race/ethnicity, and site; ^bP for interaction [coffee (ever/never) × genotype] based on chi-square test with df = 02; ^cP for interaction among coffee drinkers (average coffee consumption × genotype) based on chi-square with df = 01; ^dNE = not estimable when the model included age, sex, race/ethnicity, and site; crude OR = 0.22 (95% CI 0.01–5.3).

subjects homozygous for the variant allele (adjusted OR = 0.33, 95% CI 0.16–0.68, $P_{interaction} = 0.05$; Table 4). Similarly, for the exonic variant rs2470890, the coffee–PD association was strongest among carriers of two copies of the variant allele (adjusted OR = 0.43, 95% CI 0.27–0.69; $P_{interaction} = 0.04$). Among ever coffee drinkers, a one 6-oz cup increase in coffee consumption was associated with an approximately 18% reduction in PD risk among heterozygotes (TC) and homozygous variants (CC) for rs2470890 compared to only a 5% reduction in PD risk among homozygous wildtypes ($P_{interaction} = 0.015$, Table 4). When analysis was restricted to non-Hispanic whites only the results were similar (Table S3); however, the power for genotype–coffee (ever/never) interactions was reduced.

No interactions of *CYP1A2* polymorphisms were observed with caffeinated tea or soda (data not shown).

Discussion

We report two interesting and novel findings in this consortium study that comprised five US case–control studies of PD. First, a polymorphism in the promoter region of *ADORA2A* (rs5996696) was associated with a 30% decreased risk of PD. Second, a newly identified polymorphism (rs71651683) in the 5' transcription start region of *ADORA2A* was associated with a 49% decreased risk of PD. The associations of the 5' and promoter *ADORA2A* variants with PD risk have not been previously reported. Because these associations remain after adjusting the *P*-values for multiple comparisons, they are less likely to represent false-positive findings.

In advance of the study, we hypothesized that any *ADORA2A* polymorphism resulting in reduced expression or function of the receptor would be protective. This hypothesis was based on findings from animal models of PD: knockout mice with non-functioning *ADORA2A* receptor showed protection against MPTP toxicity, and the effect was similar to those related to receptor blockade by caffeine or a pharmacologic agent (e.g., KW-600) [2–4]. While the functional importance of rs5996696 and rs71651683 *ADORA2A* SNPs is not currently known, they are likely to reduce protein expression by affecting transcription [26]. Therefore, our finding that these two *ADORA2A* SNPs are inversely associated with PD is consistent with the role of the *ADORA2A* receptor in caffeine-associated neuroprotection.

A previous study showed that rs3032740 reduces protein expression [23]; therefore, we expected the presence of this variant to be protective for PD. However, we did not find any suggestion of a protective effect of this polymorphism in any of the race/ethnicity

groups, a finding that is consistent with two other reports that did not find associations of rs3032740 (or rs5751876, a SNP in strong linkage disequilibrium (LD) with rs3032740) with PD risk [8,9].

Metabolism by *CYP1A2* is the primary pathway for the conversion of caffeine to paraxanthine. For the most frequently studied intronic variant, rs762551, we expected the risk of PD to be lower among slow metabolizers (AC or CC) compared to fast metabolizers (AA) as the former would have higher caffeine levels [24,25,27] resulting in greater neuroprotection. However, consistent with other reports [8,10], in our study, slow metabolizer status did not by itself render any protection against risk of PD. The other *CYP1A2* SNPs genotyped, rs2470890 (exon) and rs2472304 (intron), were in strong LD; their associations with PD risk have not been previously reported. Interestingly, the 'C' allele for rs2470890, the minor allele among non-Hispanic whites, was the more common allele among African-Americans, Asians, and Hispanics. We observed an increased PD risk associated with the 'C' allele among Hispanics, but the permutation-adjusted per allele effect was not statistically significant at $\alpha = 0.05$; hence, this finding should be interpreted with caution, especially as the functional impact of this exonic variant is not known.

Pooled analysis from the five case–control studies supported the inverse association of caffeinated coffee consumption with PD risk. A primary objective of this study was to evaluate whether the coffee–PD association was modified by *ADORA2A* or *CYP1A2* polymorphisms. Because variants that would result in a non-functioning *ADORA2A* receptor would probably not be influenced by caffeine, we hypothesized that caffeine would be more protective among homozygous carriers of the wild-type allele. However, our findings do not support this hypothesis. For the two *ADORA2A* polymorphisms in strong LD, rs3030274 and rs5751876, although the coffee–genotype interaction was stronger with cups consumed than with ever/never consumption, neither provided convincing evidence of interaction. These results are consistent with two other reports that did not find any effect modification of caffeine–PD association with these SNPs [8,9]. We were unable to adequately evaluate interactions of rs5996696 and rs71651683 *ADORA2A* polymorphisms with coffee consumption because the variant allele frequencies for these SNPs were relatively small (< 6%).

For the *CYP1A2* rs76551 variant, we hypothesized that the inverse coffee–PD association would be stronger among slow metabolizers compared to rapid metabolizers who carry two copies of the wild-type allele. We did observe that the coffee–PD association was strongest among subjects homozygous for the variant

allele; however, it was somewhat weaker for heterozygotes, who are also considered physiologically to be slow metabolizers. Furthermore, although the interaction was statistically significant at the $\alpha = 0.05$ level, interaction *P*-values were not adjusted for multiple comparisons and hence must be interpreted with caution. Similar to our results, Tan *et al.* [10] found that among Asian subjects, the caffeine–PD association was also stronger in slow compared to fast metabolizers (OR 0.19 vs. 0.40); however, the caffeine–genotype interaction was not statistically significant in multivariable analysis [10]. Fascheris *et al.* [8] did not find any effect of rs762551 variant on caffeine–PD association; however, in their study, caffeinated coffee consumption was not associated with PD risk.

For the *CYP1A2* exonic variant rs2470890, subjects homozygous for the variant allele also showed the strongest coffee–PD inverse association. The functional significance of this synonymous variant is not known, and it is possible that it has no effect on protein structure or function. A possible explanation for the minimal modification of the coffee–PD association by *CYP1A2* polymorphisms might be that paraxanthine, the primary metabolite produced from caffeine breakdown, also non-selectively inhibits *ADORA2A* receptor *in vitro*, and preliminary studies in mice show that, like caffeine, paraxanthine can also reduce MPTP toxicity [28].

Our consortium study had several strengths. The five constituent case–control studies in the consortium were methodologically rigorous and included careful selection of well-characterized cases, a majority of whom were newly diagnosed with PD, as well as population- or community-based controls. For genotype–PD associations, we used a permutation approach to adjust *P*-values for multiple comparisons, thereby minimizing type I error. Our study had some limitations as well. Although we included subjects from diverse racial/ethnic groups, we did not have sufficient numbers in all subgroups (e.g., African-Americans, $n = 95$) to estimate genotypic effects with precision or to have sufficient power to evaluate caffeine–genotype interactions. Methodology for ascertaining caffeine exposure information varied between studies; however, the methods were comparable enough to allow construction of relevant caffeine-related variables for our analyses.

This consortium study characterized *ADORA2A* and *CYP1A2* SNPs in Whites (non-Hispanic and Hispanic), Asians, and African-Americans. Two *ADORA2A* SNPs, which have not been previously studied, were inversely associated with PD risk. While the results of our study do not support the hypothesis that the inverse coffee–PD association was modified by putative functional polymorphisms in *ADORA2A*, two *CYP1A2*

variants appeared to modify the protective effects of coffee on PD risk.

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Disclosure of conflict of interest

The authors declare no financial or other conflict of interests.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Description of study populations in the Parkinson's Epidemiology and Genetics Association Studies in the United States (PEGASUS).

Table S2. Genotype frequency (%), adjusted odds ratios (OR), and 95% confidence intervals (CI) for the associations between adenosine receptor *A2A* (*ADORA2A*) and cytochrome P450 1A2 (*CYP1A2*) polymorphisms and Parkinson's disease in PEGASUS in other racial/ethnic groups..

Table S3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between caffeinated coffee consumption and Parkinson's disease in PEGASUS, by *ADORA2A* and *CYP1A2* genotypes among non-Hispanic Whites only.

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Appendix

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