Coffee, Caffeine-Related Genes, and Parkinson's Disease: A Case–Control Study

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Abstract

An inverse association between coffee and Parkinson's disease (PD) has been reported. However, it remains uncertain why some but not all coffee drinkers are less susceptible to PD. We considered the possibility of a pharmaco-genetic effect. In our study, we included 1,208 subjects (446 case-unaffected sibling pairs and 158 case-unrelated control pairs) recruited from an ongoing study of the molecular epidemiology of PD in the Upper Midwest (USA). We collected information on lifetime coffee drinking and we studied two genes: *ADORA2A*, which encodes the major receptor activity of caffeine in the brain (variants rs5751876 and rs3032740), and *CYP1A2*, which encodes the major rate-limiting step of caffeine metabolism (variants rs35694136 and rs762551). We did not observe significant associations of coffee drinking or of the genetic variants with PD susceptibility, either independently or jointly, in the sample overall and in most strata. Our study neither supports the hypothesis that coffee protects against PD nor provides evidence for a pharmacogenetic effect.

Keywords

Parkinson's disease; coffee; genes

An inverse association of coffee drinking with Parkinson's disease (PD) has been reported.[1–6] However, this association has not always been replicated,[7–10] and in some studies has been observed primarily in men[1] or to a lesser extent in women.[4] Avoidance of coffee may be a manifestation of premorbid PD personality, or caffeine in coffee may exert a neuroprotective effect.[11–14] Assuming that coffee has a protective effect, it is unclear why some coffee drinkers are protected against PD and others are not, and why men are more protected than woman.

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The individual variability and between-studies variability in the effect of coffee may be due to gene-environment interactions. Yet to date, there has been only one limited study of coffee, caffeine-related genes, and PD.[15] The identification of genetic modifiers of the putative neuroprotective effect could inform the design of clinical trials of coffee or caffeine in PD, and might provide additional insights on the pathophysiology of the disease.[16] Therefore, the aims of this study were to study the main and joint effects of coffee, caffeine-related genes, and PD.

PATIENTS AND METHODS

Study Sample

All subjects were recruited as part of an ongoing study of the molecular epidemiology of PD. Cases were patients with PD referred sequentially to the Department of Neurology of the Mayo Clinic in Rochester, MN, during the 10-year period from June 1, 1996 to May 31, 2006. Cases resided in Minnesota or in one of the surrounding four states (Wisconsin, Iowa, North Dakota, and South Dakota). Control subjects consisted primarily of unaffected siblings[17] of PD cases who screened negative for PD or parkinsonism via telephone interview,[18] or siblings who screened positive but were confirmed to be free of parkinsonism at clinical examination. We also recruited unrelated controls from the same geographic region. Controls of age 65 or older were randomly selected from the Centers for Medicare and Medicaid Services (CMS) lists. Controls younger than 65 years were selected using random digit dialing, according to standard techniques.[19,20] All unrelated controls screened negative for PD or parkinsonism via telephone interview; unrelated controls screening positive could not be examined and were excluded from the study. All examinations (cases and siblings screening positive) were performed in a standardized fashion by neurologists specialized in Movement Disorders, and employing a detailed protocol for clinical assessment.

Exposure Assessment

Exposures were obtained by direct interview (or by proxy for deceased or incapacitated subjects) using a structured questionnaire that was administered via telephone by trained research assistants. To reduce interview and recall biases, interviewers were kept unaware of the case or control status of subjects, and the subjects (or their proxies) were kept unaware of the study hypotheses. The information collected was for the time period spanning from birth to the age at onset of PD in cases or the same age in matched controls (see later). The lag time between recruitment of cases and controls and their interview was kept to a minimum of 2 to 3 weeks to avoid intercurrent deaths or loss of interest in the study. Cases and controls were asked to provide information about coffee drinking, including number of cups, (quantified as small cups or regular coffee cup of about 6 ounces, or one shot of espresso; or as medium or large cup or coffee mug of about 12 ounces, or two shots of espresso) that they drank per day, with details about periods of life with different amounts. Subjects were instructed to not consider decaffeinated coffee in their responses. Information on tea and sodas was also collected: subjects were asked to provide similarly detailed information on both hot and iced tea, but they were asked to not consider herbal tea in their answers. Similarly, only caffeinated sodas were investigated.

Candidate Susceptibility Genes

We selected two genes for consideration in this study. Both genes encode proteins that are related to caffeine responsiveness or metabolism in humans. The *ADORA2A* gene encodes the adenosine receptor A2a.

This receptor is the major target of caffeine in the central nervous system and it is abundant in the basal ganglia, the motor control pathway perturbed in patients with PD.[21] The cytochrome P450 1A2 gene (*CYP1A2*) encodes an enzyme responsible for caffeine N3-demethylation. This enzyme is the major rate-limiting step of caffeine metabolism in humans, converting almost 90% of caffeine to paraxantine.[22]

Blood Sampling, DNA Extraction, and Genotyping

The study was approved by the Institutional Review Board of the Mayo Clinic in Rochester, MN. After written informed consent, a blood sample was obtained directly from each subject who underwent a clinical assessment or via mail-in kit for subjects who did not undergo a clinical assessment. Blood was processed for DNA extraction via the Puregene procedure (Gentra Systems®). The *ADORA2A* and *CYP1A2* SNPs were analyzed using a chip-based platform (Nanogen®, San Diego, CA), employing previously described methods.[23]

We genotyped two single nucleotide polymorphisms (SNPs) within the *ADORA2A* gene and two within the *CYP1A2* gene that occurred with greater than 1% frequency in Caucasian European subjects.[24–26] *ADORA2A* rs5751876 is a synonymous polymorphism (Tyr to Tyr substitution in exon 4 at amino acid position 361). *ADORA2A* rs3032740 is characterized by an insertion of a train of thymidines in intron 3, which seems to reduce protein expression.[27] *CYP1A2* rs35694136 is positioned in the 5′ promoter region and *CYP1A2* rs762551 is positioned in the first intron of the gene; both SNPs are haplotype tagging.[28]

Data Analyses

We matched cases to a single unaffected sibling of the same sex (when able) and then of closest age at study. For cases without an available sibling, we matched an unrelated control for sex, age (±2 years), and self-reported ethnicity. Hardy-Weinberg equilibrium was assessed for each SNP (in controls), and linkage disequilibrium (LD) maps were built for the genes of interest.[29] For analyses of PD susceptibility, we employed conditional logistic regression to study the main and joint effects of coffee and genes. We considered autosomal dominant, autosomal recessive, and log-additive coding schemes for each SNP and calculated odds ratios (ORs) and 95% confidence intervals (95% CIs). All analyses were done using SAS version 8.2 (SAS Institute, Cary, NC), and statistical tests were performed at the two-tailed alpha level of 0.05.

Quality Measures and Reliability Study

Recruitment of cases, matching of controls, telephone screening interviews of unaffected siblings and unrelated controls to rule out PD, physical examinations of cases or siblings screening positive for PD, and risk factors interviews of all subjects were performed in a standardized manner by trained personnel. Interviewers were kept masked to the clinical

status of the interviewed subjects. We also performed a reliability study of coffee exposure as measured via telephone interview. Specifically, we randomly selected 20 pro-bands, 20 unaffected siblings, and 20 unrelated controls from our sample and conducted a second risk factor assessment via telephone interview. The second interview was performed by an interviewer other than the one who performed the initial one (by design). The median lag time between the first and the second interview was 4.8 months (range 2.3–14.6). DNA extraction and genotyping were performed with standardized techniques and without knowing the clinical status of the subjects.

RESULTS

We included 604 case–control pairs (446 case-unaffected sibling pairs and 158 case-unrelated control pairs), for whom data were available for coffee and at least one SNP. Table 1 summarizes demographic characteristics and ancestry information of our sample. More than 85% of eligible PD cases participated in the study (all provided DNA and underwent risk factor interviews). The participation rates among siblings were 92% for the risk factor interview and 81% for the blood sample donation. The participation rate among unrelated controls for both the interview and the blood sample was 57%. Information was obtained by proxies in 9.1% of the cases and in 2.5% of controls. Spouses were the main source of proxy information (70%), followed by the offspring (14.3%) and the siblings of the subjects (7.1%). For two cases information was obtained by a brother- or sister-in-law (2.9%), and only in one case each (1.4%) by a parent, a niece or nephew, a son- or daughter-in-law, and a stepchild. The average time between PD diagnosis and the time of the risk factor interview was 5.6 years.

Coffee drinking was not associated with PD susceptibility, either overall (ever/never) or by number of cups per day (Table 2). Adjusting our analyses for the level of education (years of school in quartiles) and cigarette smoking (ever/never) did not change the results (data not shown). On the other hand, cigarette smoking (ever/never) was inversely associated with PD susceptibility in our sample, overall (OR: 0.49, 95% CI: 0.37–0.64, $P < 0.0001$) and in several strata (data not shown). No significant differences in the pattern of coffee use in cases and controls at different periods of life were found; both groups showed a stable intake of coffee over time (data not shown). Agreement between the first and second telephone risk factor interview was 90% for ever versus never coffee drinking ($\kappa = 0.72$, 95% CI: 0.51, 0.93). For age-started drinking coffee and coffee-cup/years, the intraclass correlation coefficient was 0.87 for both variables.

All four SNPs of *ADORA2A* and *CYP1A2* were in Hardy-Weinberg equilibrium in controls. For a smaller group of subjects, we also had data available for additional SNPs in the two candidate genes, because those subjects were also included in a whole-genome association study of PD reported elsewhere.[30] Using information for the additional SNPs, we constructed LD maps under the Malecot model using LDMAP[29] for the region surrounding the *ADORA2A* and the *CYP1A2* loci. The two *ADORA2A* SNPs selected for this study (rs5751876 and rs3032740) were in LD (see Fig. 1). On the other hand, for the *CYP1A2* SNPs (rs35694136 and rs762551) there was less LD between the two SNPs and with other regions of the gene (see Fig. 1). For this reason, to study the association of *CYP1A2* and

ADORA2A loci with PD, we analyzed data for both *CYP1A2* SNPs but only for *ADORA2A* rs3032740, as the function of that SNP has been defined.[28]

None of the three SNPs were associated with PD susceptibility in the sample overall, either before or after adjusting for coffee and smoking (Table 3). However, findings were significant for the *ADORA2A* SNP rs3032740 in men (OR: 0.71, 95%; CI: 0.52–0.96; *P* = 0.03). No global differences in haplotype frequencies were revealed in two-locus haplotype analyses for the *CYP1A2* gene. No significant interactions were observed for coffee and any of the SNPs, after adjusting for age at study and gender (Table 4).

DISCUSSION

In this study we neither observed a significant inverse association of coffee use (ever/never) with PD nor a dose effect or an association in men only. To date, the inverse association of coffee drinking with PD has been assessed mainly in population-based case-unrelated control studies or in cohort studies.[1–5] By contrast, our study is different because it is primarily family-based. For studies testing genetic hypotheses, the use of unaffected siblings as controls is well established as a method to circumvent possible population stratification bias.[17] Our use of unaffected siblings as controls for this study of exposures to coffee (as well as caffeine-related genes) is also justified by published family-based and discordant twin-based studies of exposures to smoking and PD,[31,32] and more recently of coffee and PD.[33] Those family-based studies yielded results similar to the results of population-based case–control studies. However, consistent with our study, a Swedish twins study and some population-based studies also failed to observe a significant inverse association between caffeine intake and PD susceptibility[8–10] or disease-modifying effects.[34] Some of these negative studies were not included in the extensive meta-analysis of coffee and PD published by Hernan et al.,[7] because they were published after January 1, 2002.

Our choice of caffeine-related genes was well justified because *ADORA2A* encodes the receptor at which caffeine exerts its effect in the central nervous system, and *CYP1A2* encodes the rate-limiting enzyme in caffeine metabolism. However, SNPs were selected according to the literature accessible before 1999 and prior to the availability of the International Haplotype Map (www.hapmap.org). Our LD maps demonstrated adequate linkage disequilibrium mapping of the *ADORA2A* locus (except perhaps at the 3′-end), but limited mapping of the *CYP1A2* locus. Therefore, our negative findings for the SNPs that we genotyped need to be interpreted with caution. It is reassuring that one whole-genome association study of PD that included three SNPs for *CYP1A2* and three SNPs for *ADORA2A*,[30] and another whole-genome association study of PD that included one SNP for *ADORA2A*,[35] also revealed no associations with PD. In addition, Tan et al.[15] failed to report any main effects of *ADORA2A* 2592C>Tins (now coded as rs3032740) on PD susceptibility or any joint effects with coffee (consistent with our study).

Despite negative results, our study has a number of strengths. Our sample size (cases and matched controls) was large and of sufficient size to detect main and joint effects. Our controls were preferentially unaffected siblings, thus limiting possible confounding due to population stratification.[36] We extended the study of the joint effects of coffee and caffeine-

related genes to include *CYP1A2*, which encodes the major rate limit- ing step of caffeine metabolism, in addition to *ADORA2A* (which encodes the caffeine receptor in the brain). We were also able to compare findings from this study with the findings from two whole-genome association studies of PD, and we were able to use overlapping data from our study and from one of the whole-genome association studies[30] to construct detailed linkage disequilibrium maps.

Our study also has limitations. Our sample was not population-based. However, cases were recruited prospectively and resided in a geographically defined region. We have previously shown that the demographic and clinical characteristics were similar for PD patients residing in Olmsted County, MN, (a defined population) or within a 120 mile radius of the County (about one half of the cases). However, the cases residing in the broader five-state region tended to be younger (possibly biasing toward increased heritability and genetic effects).[37] Our controls were primarily unaffected siblings; our intention was to limit possible confounding due to population ethnic stratification. However, unaffected sibling controls can be over-matched for genetic and environmental factors, leading to false negative findings. For this reason, we performed sensitivity analyses, which showed that the ORs for the explanatory variables were similar when restricting the sample to case-unaffected sibling pairs or to case-unrelated control pairs only.

The exposure assessments were interview-based and the information collected was for the time period spanning from birth to the time of the study for cases and controls. Recall bias may have compromised the validity of the study, or PD may have changed coffee drinking habits in cases (personality changes or altered smell and taste early after disease onset). Therefore, we restricted our analyses to exposures preceding the age at onset of PD in cases or the same age in matched controls. Our study did not consider the possible effects of passive exposures to coffee during gestation. We limited our analyses to caffeinated coffee. It is possible that chemicals in coffee other than caffeine are associated with PD. Our subjects drank other beverages that contained caffeine, such as tea and sodas. However, we performed separate analyses for tea or soda drinking and found that they were not significantly associated with PD (data not shown). In addition, we did not assess possible hormonal influences that might confound studies of gene-environment interactions in PD.[38,39] For this reason, we also performed analyses stratified by or adjusted for gender.

Genotyping was performed only for two SNPs each within the two selected genes, and the LD mapping of the two gene loci was not complete. Nevertheless, our findings converged with those of two published whole-genome association studies of PD that considered additional SNPs in the genes.[31,35] One or more pieces of information regarding genetic or environmental factors were occasionally missing from either or both members of a case–control pair. This limited the number of pairs available for the matched pair analyses and could have limited the statistical power of the study, particularly for the case-control pairs.

In summary, our study neither supports the hypothesis that coffee protects against PD nor provides evidence for pharmacogenetic effects. Although our study had strengths, it also had weaknesses that warrant caution in the interpretation of our findings and warrant additional consideration of the hypotheses. Future studies of the association of coffee and PD might

consider genomic pathways-based analyses of additional caffeine responsiveness and metabolism genes, sources of caffeine in addition to coffee, and exposures during gestation as well as later in life.[40]

Acknowledgments

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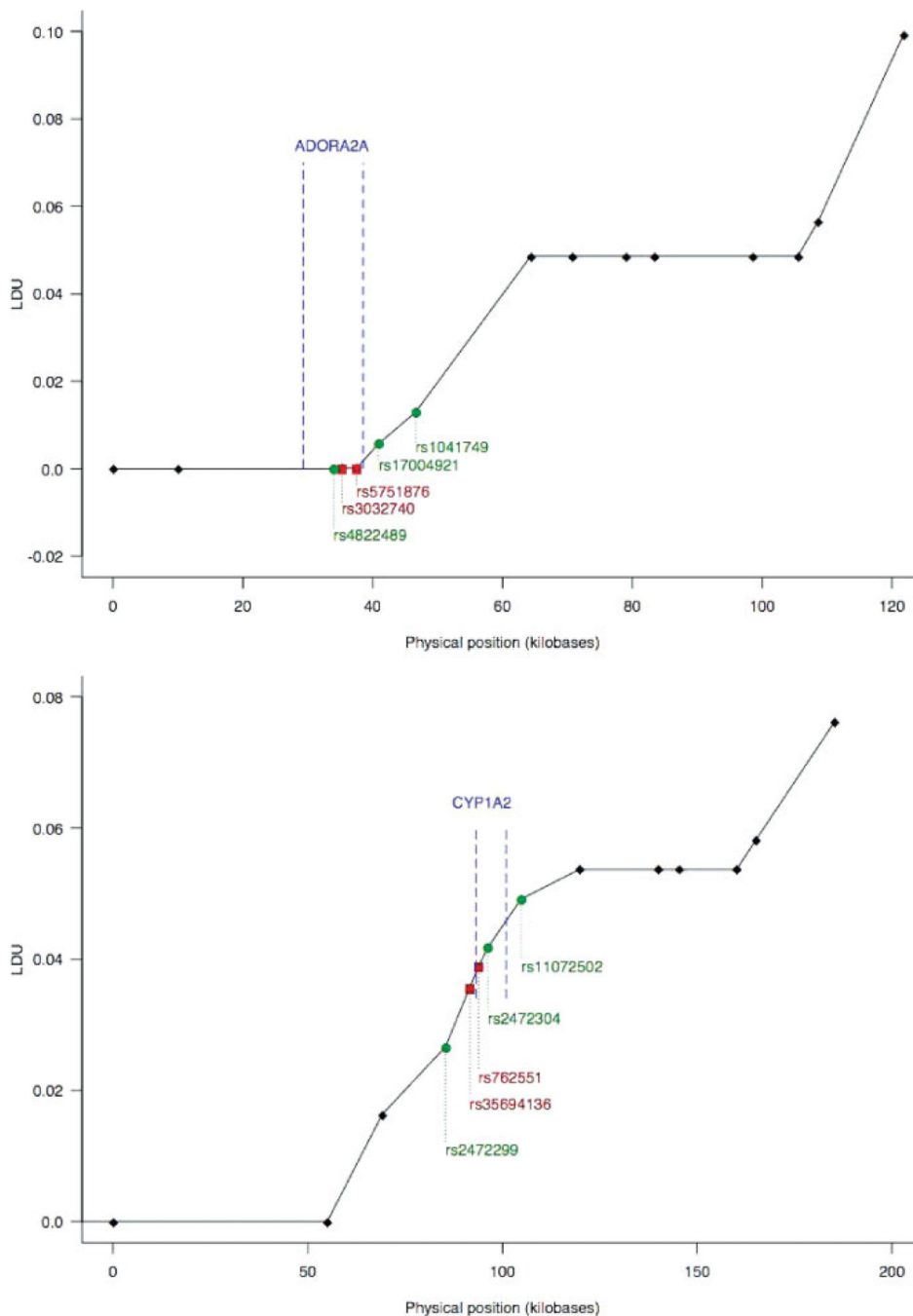
FIG. 1.
Linkage disequilibrium (LD) maps for *ADORA2A* (top panel) and *CYP1A2* (bottom panel)
genes. A "flat" line between two markers indicates a region of LD. When a gene lies
completely within a flat line segment, it is adequately mapped by any SNP marker within
that segment. SNPs designated by boxes and red color were genotyped as part of this study,
and SNPs designated by circles and green color were genotyped as part of a whole-genome

association study of PD.[30] SNPs are displayed from left to right in 5′ to 3′ direction. LDU, linkage disequilibrium units.

TABLE 1 Demographic characteristics of Parkinson's disease cases, siblings, and unrelated controls

General characteristics	Case–sibling pairs		Case-unrelated control pairs		All case–control pairs	
	PD cases	Sibling controls	PD cases	Unrelated controls	PD cases	All controls
Total sample, n (%)[a]	446	446	158	158	604	604
Men	273 (61.2)	219 (49.1)	93 (58.9)	93 (58.9)	336 (60.6)	312 (51.7)
Women	173 (38.8)	227 (50.9)	65 (41.1)	65 (41.1)	238 (39.4)	292 (48.3)
Age at onset, median (range)	59.4 (30.6–86.9)	–	58.3 (36.7–69.7)	–	59.1 (30.6–86.9)	–
Age at study, median (range)	66.0 (32.8–91.4)	64.6 (32.0–90.4)	63.1 (44.3–70.8)	62.4 (44.9–66.8)	64.7 (32.8–91.4)	63.9 (32.0–90.4)
Region of origin of parents, n (%)[b]						
Both parents of European origin	383 (85.9)	369 (82.7)	121 (76.6)	137 (86.7)	504 (83.4)	506 (83.8)
Both parents Northern European	147 (38.4)	97 (26.3)	39 (32.2)	38 (27.7)	186 (36.9)	135 (26.7)
Both parents Central European	106 (27.7)	140 (37.9)	37 (30.6)	47 (34.3)	143 (28.4)	187 (37.0)
Both parents Southern European	3 (0.8)	3 (0.8)	1 (0.8)	1 (0.7)	4 (0.8)	4 (0.8)
Both parents European, mixed region	127 (33.2)	129 (35.0)	44 (36.4)	51 (37.2)	171 (28.3)	180 (35.6)
Only one parent of European origin	38 (8.5)	46 (10.3)	29 (18.4)	11 (7.0)	67 (11.1)	57 (9.4)
One parent declared "American"	17 (3.8)	16 (3.6)	10 (6.3)	7 (4.4)	27 (4.5)	23 (3.8)
Both parents declared "American"	17 (3.8)	18 (4.0)	5 (3.2)	5 (3.2)	22 (3.6)	23 (3.8)
Both parents Asian	1 (0.2)	2 (0.4)	1 (0.6)	0 (0.0)	2 (0.3)	2 (0.3)
Both parents Mexican	1 (0.2)	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.2)
Both parents African	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	4 (0.9)	9 (2.0)	1 (0.6)	2 (1.3)	5 (0.8)	11 (1.8)

[a] 446 case–sibling pairs and 158 case-unrelated control pairs used in conditional logistic regression analyses for *ADORA2A* rs3032740, *CYP1A2* (rs35694136 and rs762551), and coffee. Each member of the pair contained complete coffee data and at least 1 SNP genotyped.

[b] Region of origin of parents was self-reported by subjects. Note that Parkinson's disease cases and their siblings were not always in agreement. "Northern European" includes Scandinavian, Swedish, Norwegian, Finnish, Danish, Irish, or British origin. "Central European" includes French, Belgian, Dutch, Swiss, Luxemburgian, German, Austrian, Hungarian, Polish, Czechoslovakian, or Russian origins. "Southern European" includes Italian, Spanish, Portuguese, Greek, or Yugoslavian origins.

TABLE 2

Risk of Parkinson's disease: Conditional logistic regression analysis for coffee, adjusted for smoking

Sample or stratum	N*	Coffee ever/never N (%)		Model**				Model**			
		Yes	No	OR (95% CI) coffee ever/never	P value	OR (95% CI) smoke ever/never	P value	OR (95% CI) coffee cups per day	P value	OR (95% CI) smoke ever/never	P value
Cases, all	604	490 (81.1)	114 (18.9)	1.13 (0.81–1.57)	0.49	0.48 (0.36–0.63)	<0.0001	1.00 (0.76–1.31)	0.99	0.49 (0.37–0.65)	<0.0001
Controls, all	604	501 (82.9)	103 (17.1)								
Cases, female	189	154 (81.5)	35 (18.5)	1.00 (0.57–1.74)	0.99	0.55 (0.32–0.93)	0.03	1.26 (0.78–2.05)	0.35	0.51 (0.30–0.88)	0.02
Controls, female	189	157 (83.1)	32 (16.9)								
Cases, male	263	218 (82.9)	45 (17.1)	1.18 (0.82–1.86)	0.52	0.54 (0.35–0.81)	0.003	0.76 (0.51–1.13)	0.17	0.57 (0.38–0.85)	0.006
Controls, male	263	214 (81.4)	49 (18.6)								
Cases	446	364 (81.6)	82 (18.4)	1.24 (0.82–1.86)	0.30	0.33 (0.23–0.48)	<0.0001	1.05 (0.76–1.46)	0.78	0.34 (0.24–0.50)	<0.0001
Controls, siblings	446	373 (83.6)	73 (16.4)								
Cases	158	126 (79.7)	32 (20.3)	1.08 (0.58–2.01)	0.81	0.85 (0.52–1.39)	0.52	0.97 (0.58–1.64)	0.92	0.87 (0.53–1.42)	0.57
Controls, unrelated	158	128 (81.0)	30 (19.0)								

*
N, number of pairs.

**
All models were adjusted for age at study (continuous variable) and gender, as appropriate. Coffee cups per day were coded continuously. Models adjusted for education were similar (data not shown).

TABLE 3

Risk of Parkinson's disease: Conditional logistic regression analyses for informative SNPs in caffeine-related genes

Gene, SNP	N*	Genotype, n (%)**			Model***	
		1/1	1/2	2/2	OR (95% CI)	*P*-value***
ADORA2A, rs3032740						
Cases, all	565	223 (39.5)	263 (46.5)	79 (14.0)	0.84 (0.62–1.14)	0.26
Controls, all	565	203 (35.9)	268 (47.4)	94 (16.6)		
CYP1A2, rs35694136						
Cases, all	455	397 (87.3)	55 (12.1)	3 (0.7)	0.69 (0.41–1.18)	0.17
Controls, all	455	389 (85.5)	65 (14.3)	1 (0.2)		
CYP1A2, rs762551						
Cases, all	391	207 (52.9)	150 (38.4)	34 (8.7)	0.73 (0.49–1.10)	0.13
Controls, all	391	194 (49.6)	166 (42.5)	31 (7.9)		

*
N = number of pairs.

**
1/1 = homozygous for the more common ("wild type") allele, 2/2 = homozygous for the less common (minor) allele; 1/2 = heterozygous for the minor allele.

***
Model = conditional logistic regression models adjusted for age at study (continuous variable), gender, and smoking. Models assume autosomal dominant genetic effect (2/2 or 1/2 vs. 1/1); autosomal recessive and log additive effects were also consistent (data not shown).

TABLE 4

Susceptibility to Parkinson's disease: Joint effect models for coffee drinking and caffeine-related genes

	Subjects, n [*]	Model [**]	
		HR (95% CI)	P-value
Coffee and *ADORA2A rs3032740*			
No coffee, no variant	90	1.00 (Reference)	–
No coffee, variant	116	0.90 (0.50–1.65)	0.74
Coffee, no variant	336	0.97 (0.58–1.61)	0.89
Coffee, variant	558	0.81 (0.49–1.33)	0.40
Coffee and *CYP1A2 rs35694136*			
No coffee, no variant	130	1.00 (Reference)	–
No coffee, variant	15	1.93 (0.54–6.94)	0.31
Coffee, no variant	656	1.06 (0.70–1.59)	0.79
Coffee, variant	109	0.74 (0.38–1.42)	0.37
Coffee and *CYP1A2 rs762551*			
No coffee, no variant	61	1.00 (Reference)	–
No coffee, variant	60	0.77 (0.36–1.65)	0.50
Coffee, no variant	340	0.93 (0.52–1.66)	0.81
Coffee, variant	321	0.70 (0.37–1.32)	0.28

[*] Subjects were cases, unaffected siblings, and unrelated controls (matched case–control pairs) for which complete data on coffee drinking and genotypes were available.

[**] All models were adjusted for gender and age at study (continuous variable), as appropriate. Coffee was coded dichotomously as ever or never. Each genetic variant was coded assuming an autosomal dominant genetic effect. Autosomal recessive and log-additive effects were also consistent (data not shown).