



# Lifestyle, Genetics, and Their Interactions in Determining Parkinson's Disease Risk

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LIFESTYLE, GENETICS, AND THEIR INTERACTIONS IN DETERMINING  
PARKINSON'S DISEASE RISK

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A Dissertation Submitted to the Faculty of  
The Harvard T.H. Chan School of Public Health  
in Partial Fulfillment of the Requirements  
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in the *Department of Epidemiology*

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Boston, Massachusetts

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## **Lifestyle, Genetics, and Their Interactions in Determining Parkinson's Disease Risk**

### **Abstract**

Parkinson's disease is the second most common neurodegenerative disease, afflicting 1-2% of the population over the age of 65, and its prevalence is expected to rise as the population of older adults increases<sup>1</sup>, highlighting the importance of prevention and treatment.

In this dissertation, we investigated the associations between dietary, lifestyle, and genetic factors and PD risk, using data from three large prospective cohorts: the Nurses' Health Study, the Health Professionals Follow-up Study, and the Cancer Prevention Study II Nutrition Cohort. For all analyses, we assessed diet using validated food frequency questionnaires; self-administered biennial questionnaires were used to identify PD cases, who were then confirmed by neurologists specializing in movement disorders via medical records.

Cox proportional hazards models and conditional logistic models were used to calculate the relative risks (RR) of PD and 95% confidence intervals (CI). In addition, we assessed multiplicative interactions by conducting likelihood ratio test and additive interaction using three indices: the relative risk due to interaction (RERI), the attributable proportion (AP), and the synergy index (SI). In Chapter 1, we found that total caffeine intake was protective against PD in women who have never used postmenopausal hormone therapy (PMH) and men; the pooled multivariable-adjusted RR comparing the highest to lowest quintile of caffeine intake in men and women with never PMH use was 0.62 (95% CI= 0.39, 0.98;  $p = 0.04$ ). In addition, as the pathogenesis of many complex diseases involve multiple components, we examined how genetic factors may influence the association between caffeine intake and PD risk in Chapter 3, but we

did not find sufficient evidence for the presence of an interaction between caffeine intake and GRIN2A and CYP1A2 polymorphisms. In Chapter 2, we found that risk scores composed of predetermined lifestyle risk factors and family history of PD was strongly associated with overall risk for men and women; we report that factors may combine to influence PD risk by interacting with each other.

Our findings show that in addition to individual risk factors, there may be a complex interplay between multiple factors to potentially contribute to the neurodegeneration in Parkinson's disease.

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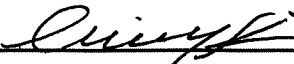
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Iris Y. Kim

**CHAPTER 1 DIFFERENCES IN PARKINSON’S DISEASE RISK WITH CAFFEINE  
INTAKE AND POSTMENOPAUSAL HORMONE USE**

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## **ABSTRACT**

**Background:** Caffeine intake has been associated with a lower risk of Parkinson's disease (PD).

This association is robust in men, but inconsistent in women due to a possible interaction with post-menopausal hormone (PMH) use. We sought to further elucidate this association in two large, prospective cohorts.

**Objective:** To (1) evaluate the association between caffeine intake and Parkinson's disease (PD) risk and (2) assess potential effect modification of the association by post-menopausal hormone (PMH) use among women.

**Methods:** We examined associations between caffeine intake and incident PD risk in the Nurses' Health Study (NHS) (N= 121,701 women; 32 years of follow-up) and the Health Professionals Follow-up Study (HPFS) (N= 51,530 men; 26 years of follow-up). We excluded participants with onset of PD at or before the study baseline (1980 for the NHS and 1986 for the HPFS), participants with extreme caloric intake, and participants who lacked data on caffeine intake, leaving 90,254 women and 47,474 men in our analyses. Dietary data on coffee and caffeine from other sources were collected every four years using a validated semi-quantitative food frequency questionnaire (FFQ) for both cohorts. Information on lifestyle and incident PD diagnosis was updated biennially and PD diagnoses were confirmed by medical record review. We estimated hazard ratios (HRs) and 95% confidence intervals (CIs) using Cox proportional hazards models, adjusting for age, smoking, physical activity, alcohol consumption, and other potential confounders.

**Results:** During the combined 58 years of follow-up, we documented a total of 1,219 PD cases (590 cases in HPFS, 629 cases in NHS). The multivariable-adjusted RRs comparing the highest

quintile of caffeine intake to the lowest quintile of caffeine intake were 0.50 (95% CI: 0.37, 0.68;  $p_{\text{trend}} < 0.0001$ ) in the HPFS and 0.90 (95% CI: 0.69, 1.16);  $p_{\text{trend}} = 0.30$  in the NHS. Among women, there was a suggestion of an interaction between coffee intake and PMH use ( $p = 0.08$ ). In the pooled analyses combining men and women who have never used PMH, the risk of PD was lower as coffee intake increased ( $p_{\text{trend}} < 0.001$ ).

**Conclusions:** Our results support previous findings that increased caffeine intake may reduce PD risk in women who have never used PMH and men.

## INTRODUCTION

A lower risk of Parkinson's disease (PD) among coffee drinkers has been observed in numerous longitudinal studies.<sup>1-10</sup> This inverse association was also present for caffeine from non-coffee sources, such as cola beverages, chocolate, and non-herbal tea. However it was not present for decaffeinated coffee, suggesting that the inverse association is largely due to caffeine, rather than niacin or other biologically active compounds found in coffee.<sup>3,6,11</sup> Caffeine serves as a non-selective adenosine receptor antagonist, and appears to have neuroprotective effects in animal models of PD by blocking the A<sub>2A</sub> subtype of adenosine receptor.<sup>12-15</sup>

While a robust inverse correlation between caffeine intake and incident PD risk exists for men, a more complex association exists for women. Whereas a 'beneficial' association of caffeine was observed among women who do not use postmenopausal hormones (PMH) in most studies, conflicting results were observed among PMH users. However tests of interaction showed conflicting results, possibly due to low power.<sup>1,2,5,6,16</sup> In the present study, we prospectively examined the associations between caffeine consumption and PD risk in the Nurses' Health Study (NHS) and the Health Professional Follow-Up Study (HPFS). Results from the early follow-up of these cohorts have been published previously,<sup>1,2,17</sup> but we present here updated results after an additional 14 years of follow-up for the NHS and 16 years for the HPFS. With 908 additional cases and increased power, we sought to elucidate the possible interaction between caffeine intake and PMH on PD risk.

## METHODS

### *Study population*

The Nurses' Health Study (NHS) enrolled 121,700 female registered nurses of ages 30-55 who returned mailed, self-administered questionnaires regarding medical histories, lifestyle and dietary factors in 1976. The Health Professionals Follow-up Study (HPFS) enrolled 51,529 male health professionals of ages 40-75 who returned similar questionnaires in 1986. The baseline for the analyses of this study for the NHS was 1980, while the baseline for the HPFS was 1986, as the first dietary assessments of caffeine consumption were collected then. For both cohorts, follow-up information on lifestyle factors was collected every two years and dietary information was updated every 4 years. Participants with onset of PD or participants who died before the study baseline, participants who reported extreme caloric intakes (<800 or >4,200 kcal for men; <600 or >3,500 for women), and participants who lacked data on baseline coffee intake were excluded. Therefore, our analytic cohort included 90,254 women from the NHS and 47,474 men from the HPFS. This study was approved by the Human Research Committees at the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

### ***Dietary assessment coffee consumption and other covariates***

Nutritional information was ascertained via validated food frequency questionnaires (FFQs). The FFQs were self-administered and asked to report participants' average intake pattern of a food or beverage item over the past 12 months. Nine possible multiple-choice responses were provided for intake frequency for each item, ranging from "never or less than once per month" to "6 or more times per day" for both cohorts. The 1980 FFQ included questions on servings of coffee with caffeine (cups), tea (cups), cola beverages (glasses), and chocolate (in 1-oz servings). From 1984 onwards, all FFQs additionally included items on decaffeinated coffee (cups), caffeinated and caffeine-free soda beverages (glasses). Information obtained from the U.S. Department of Agriculture food-composition sources was used to convert



participants' reported average intake of one serving of a caffeinated beverage or food over the preceding year into average daily intake of caffeine. The average caffeine content used for these calculations was estimated to be 137 mg caffeine per cup of coffee, 47 mg of caffeine per cup of non-herbal tea, 46 mg of caffeine per can or bottle of cola beverage, and 7 mg per serving of chocolate. The reproducibility and validity of the FFQs have been previously reported for the NHS<sup>18</sup> and the HPFS<sup>19</sup>. The validation studies on a subsample of the cohorts show a high correlation between self-reported intake of caffeinated beverages via the FFQ and four 1-week diet records. The Pearson correlation for coffee was 0.93 in the HPFS and 0.78 in the NHS, while the correlation for non-herbal tea was 0.77 in the HPFS and 0.93 in the NHS. Both cohorts had the same correlation for cola beverages ( $r=0.84$ ).<sup>20,21</sup> Information on other covariates regarding lifestyle characteristics was collected, including information on menopausal status and postmenopausal hormone therapy (PMH) use in the NHS.

### *Ascertainment of PD cases*

Biennial self-report questionnaires were administered to ascertain new illness diagnoses. Lifetime occurrence of PD was first asked in the 1994 (NHS) and 1988 (HPFS) questionnaires and incident PD diagnoses were documented every two years thereafter via subsequent questionnaires. We asked self-reported PD cases for permission to contact their neurologist to request copies of their medical records to confirm the diagnosis. After obtaining their permission, we contacted patients' neurologists and requested for them to either return a self-administered diagnostic questionnaire that asked to confirm the case or to send a copy of the patient's medical records. In previous years, PD cases were considered confirmed if they fulfilled at least one of the following conditions: the treating neurologist rated the certainty of diagnosis as definite or probable; the final diagnosis of PD by a neurologist was included in the

medical record; or the medical record indicated the presence of at least two out of three cardinal signs of PD (i.e. resting tremor, rigidity, bradykinesia) in the absence of features indicating other illness diagnoses. After 2003, a similar procedure was used to identify confirmed PD cases; however, medical records were requested from all PD cases, which were then reviewed by a neurologist specializing in movement disorders. If the diagnosis of the neurologist specializing in movement disorders conflicted with that of the original neurologist, the diagnosis of the movement disorder neurologist was used.

### ***Statistical analysis***

We conducted all analyses separately in each cohort and subsequently pooled cohort-specific estimates. Participants contributed person-years starting from age in months at baseline (1980 for NHS; 1986 for HPFS) until the age in months at the date of first PD symptoms, date of death, date of the latest completed questionnaire, or end of follow-up (June 2012 for NHS; January 2012 for HPFS), whichever occurred first. The analysis was stratified jointly by age in months at the start of follow-up and calendar year of the current questionnaire cycle in order to finely control for confounding by time.

For both cohorts, we compared incident PD risk in quintiles of coffee intake. Within each cohort, cumulative averages of time-updated covariates, such as alcohol intake and physical activity, were categorized into quintiles. Nutrient intake (e.g., flavonoids, fructose, dairy protein, vitamin C, and vitamin E) was adjusted for total energy intake using the residual method to account for its correlation with nutrient intake.<sup>22</sup> We calculated age-adjusted and multivariable-adjusted hazard ratios and corresponding 95% confidence intervals using Cox proportional hazards model. The multivariable regression adjusted for the following time-updated covariates:

pack years of smoking (never smoker, 1 to <5, 5 to <10, 10- <15, or  $\geq 15$ ), alcohol intake (in quintiles), and physical activity (in quintiles). Further analyses were conducted using baseline caffeine intake levels.

For tests of trend, the mid-category scores (median caffeine intake value within each quintile) was modeled as a continuous variable to allow for possible nonlinear associations. Secondary analyses included incorporating a lag period by excluding the first years of follow-up (2, 4, and 6 years), evaluating total caffeine from non-coffee sources, and using cumulative averages of coffee intake in categories: less than one cup of coffee per day (“<1”), 1-3 cups per day (“1-3”), 3-5 cups per day (“3-5”), 5 or more cups per day (“ $\geq 5$ ”), and the reference group of no coffee intake or less than 1 cup per month (“0”). We also conducted analyses additionally adjusting for BMI, dairy intake, dietary intake of antioxidants, and total energy intake. Similar analyses were performed among women in the NHS cohort, stratified by PMH use (ever/never). PMH status was updated over the study period. If a woman ever initiated PMH use, she would be considered an ever-user for all consequent follow-up questionnaires. To determine statistical significance of a possible interaction between coffee intake and PMH use, we conducted likelihood ratio tests (LRT) by comparing the log-likelihood of a model including interaction terms to that of a model without the interaction terms on the multiplicative scale in each cohort. Results from the HPFS and NHS cohorts were pooled using random-effects methods. All statistical analyses were conducted using SAS (SAS Institute, Cary, NC).

## **RESULTS**

There were a total of 1,219 cases (590 in HPFS, 629 in NHS) observed over the 3,485,803 person-years of follow-up. Women had a higher baseline mean coffee intake

compared to men (women: 2.22 cups/day, men: 1.34 cups/day). Participants with the highest caffeine intake tended to smoke more, were generally more likely to have higher alcohol intake, and be less physically active compared to participants with the lowest caffeine intake (**Table 1.1**).

**Table 1.1** Age-adjusted characteristics of the study population at baseline by caffeine intake

	Caffeine intake in quintiles (mg/day)				
	1	2	3	4	5
<b>Health Professionals Follow-Up Study, 1986-2012</b>					
	n=9,151	n=9,279	n=9,579	n=9,738	n=9,725
Caffeine intake, mg/day	8.9(7.5)	57.6(20.7)	150.6(24.3)	327.1(57.9)	589.2(165.0)
Age, years*	55.3(10.0)	54.6(9.9)	55.2(10.0)	54.7(9.6)	52.7(9.0)
Current smoker, %	5.2	6.1	8.5	11.2	17.9
Past smoker, %	36.0	39.0	44.7	47.8	49.1
Body Mass Index, kg/m <sup>2</sup>	24.5(4.8)	24.9(4.9)	25.0(5.0)	25.0(5.0)	25.3(4.9)
Exercise, met-h/week	20.3(26.2)	19.8(28.6)	18.9(26.3)	17.9(23.5)	17.2(25.2)
Alcohol, g/day	7.9(13.4)	9.3(13.3)	11.3(14.7)	14.1(16.9)	13.7(17.2)
Tea, servings/day	0.0(0.0)	0.3(0.4)	0.5(0.8)	0.6(1.1)	0.7(1.2)
Coca cola, servings/day	0.0(0.1)	0.2(0.3)	0.2(0.5)	0.2(0.5)	0.2(0.5)
Decaffeinated coffee, servings/day	0.7(1.2)	0.9(1.3)	0.7(1.1)	0.5(1.0)	0.4(0.9)
Total energy intake, kcal/day	1,862(586)	1,952(601)	1,977(612)	2,006(607)	2,142(657)
<b>Nurses' Health Study, 1980-2012</b>					
	n=17,677	n=18,013	n=18,683	n=17,552	n=18,329
Caffeine intake, mg/day	53.5(41.1)	208.0 (56.1)	365.0(19.6)	523.6(107.5)	805.5(96.5)
Age, years*	52.7(7.4)	52.5(7.4)	53.3(7.1)	53.0(7.1)	52.6(6.8)
Current smoker, %	19.5	18.7	28.0	31.1	46.3
Past smoker, %	26.5	27.4	30.3	29.1	25.5
Body Mass Index, kg/m <sup>2</sup>	25.4(5.0)	25.4(5.0)	24.8(4.4)	25.1(4.5)	25.2(4.5)
Exercise, met-h/week	14.8(21.1)	14.1(20.0)	14.2(20.2)	13.9(21.4)	13.0(18.6)
Alcohol, g/day	4.8(9.4)	5.3(9.5)	7.9(11.6)	6.9(10.7)	7.0(11.1)
Tea, servings/day	0.3(0.4)	1.5(1.3)	0.6(1.3)	1.3(1.4)	0.8(1.2)
Coca cola, servings/day	0.2(0.5)	0.3(0.7)	0.1(0.3)	0.2(0.6)	0.2(0.5)
Decaffeinated coffee, servings/day <sup>†</sup>	3.4(2.6)	2.9(2.4)	3.1(2.5)	3.1(2.6)	3.1(2.8)
Postmenopausal Hormone Therapy use, %	27.8	26.2	25.4	25.7	24.9
Total energy intake, kcal/day	1,533(494)	1,576(503)	1,531(479)	1,593(496)	1,621(511)

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

\*Value is not age adjusted

† Value from 1984 questionnaire

Consistent with previous studies, higher total caffeine intake was associated with a lower risk of PD among men in the HPFS (multivariable-adjusted  $p_{\text{trend}} < 0.0001$ ) (**Table 1.2**). The risk of PD decreased monotonically with increasing quintile of caffeine intake; the RR comparing the highest quintile to the lowest quintile of caffeine consumption 0.50 (95% CI= 0.37, 0.68;  $p < 0.0001$ ) in men. For women, neither caffeine nor coffee intake was associated with PD risk ( $p_{\text{trend}} = 0.30$ ,  $p_{\text{trend}} = 0.39$ , respectively).

Among women who have never used PMH, there was a trend towards an inverse association with coffee intake, though the results were not statistically significant ( $p_{\text{trend}} = 0.16$ ) (**Table 1.3**). However, when specific types of hormonal therapies were examined (e.g., progesterone, estrogen, combination therapy), we observed a borderline significant interaction between coffee intake and hormonal therapy use ( $p = 0.08$ ). Furthermore, there was a significant interaction between coffee consumption and use of progestin only ( $p_{\text{interaction}} = 0.01$ ). However, this result should be interpreted cautiously due to the low number of cases ( $n = 8$ ). When we restricted our analyses to “definite” cases, an inverse association was observed specifically among women who have never used PMH (multivariable-adjusted  $p_{\text{trend}} = 0.01$ ).

Overall, higher caffeine intake was associated with a lower PD risk among men and women who have never used PMH (pooled multivariable-adjusted  $p_{\text{trend}} = 0.05$ ) (**Figure 1.1**); the pooled multivariable-adjusted RR comparing the highest to lowest quintile of caffeine intake in men and women with never PMH use was 0.62 (95% CI= 0.39, 0.98;  $p = 0.04$ ;  $p_{\text{heterogeneity}} = 0.09$ ). These findings were robust in additional analyses, including a lag analysis of 2, 4 and 6 years, and further adjusting for BMI, total energy intake, vitamin C, vitamin E, dietary urate index, dairy protein, total dairy, fructose, and flavonoid intake, and the Alternate Health Eating Index (derived from intake of fruit, vegetables, nuts, soy, and other dietary components).<sup>23</sup>

**Table 1.2** Hazard Ratio of PD by caffeine and coffee intake for the HPFS (men) and the NHS (women)

	Quintiles of caffeine intake					<i>P</i> <sub>trend</sub>
	1	2	3	4	5	
<b>HPFS</b>						
Median caffeine, mg	7	58	150	353	628	
Number of cases	155	141	117	109	68	
Person-years	178,199	191,609	191,895	204,090	194,726	
Hazard Ratio (95% CI)						
Age-adjusted <sup>a</sup>	1.0 (REF)	0.83 (0.66, 1.05)	0.69 (0.54, 0.88)	0.61 (0.48, 0.78)	0.46 (0.34, 0.61)	<0.0001
Multivariable <sup>b</sup>	1.0 (REF)	0.85 (0.67, 1.07)	0.72 (0.56, 0.92)	0.64 (0.50, 0.83)	0.50 (0.37, 0.68)	<0.0001
<b>NHS</b>						
Median caffeine, mg	51	191	363	501	816	
Number of cases	143	135	135	108	108	
Person-years	465,096	508,352	531,793	513,464	506,584	
Hazard Ratio (95% CI)						
Age-adjusted <sup>a</sup>	1.0 (REF)	0.83 (0.65, 1.05)	0.80 (0.63, 1.01)	0.69 (0.54, 0.88)	0.75 (0.58, 0.96)	0.01
Multivariable <sup>b</sup>	1.0 (REF)	0.84 (0.66, 1.07)	0.83 (0.65, 1.06)	0.74 (0.57, 0.96)	0.90 (0.69, 1.16)	0.30
Coffee intake in categories (servings/ day)						
	0	<1	1-3	3-5	≥5	
<b>HPFS</b>						
Median, cups/day*	0.0	0.4	2.5	4.5	6.0	
Number of cases	135	233	186	32	4	
Person-years	209,894	285,545	353,463	91,431	20,187	
Hazard Ratio (95% CI)						
Age-adjusted <sup>a</sup>	1.0 (REF)	1.06 (0.86, 1.32)	0.74 (0.59, 0.92)	0.53 (0.36, 0.78)	0.38 (0.14, 1.03)	<0.0001
Multivariable <sup>b</sup>	1.0 (REF)	1.11 (0.89, 1.38)	0.79 (0.63, 1.00)	0.59 (0.40, 0.88)	0.45 (0.16, 1.21)	<0.0001
<b>NHS</b>						
Median, cups/day*	0	0.4	2.5	4.5	6	
Number of cases	84	175	279	77	14	
Person-years	365,292	522,516	1,129,441	399,310	108,730	
Hazard Ratio (95% CI)						
Age-adjusted <sup>a</sup>	1.0 (REF)	0.96 (0.74, 1.25)	0.83 (0.65, 1.07)	0.76 (0.56, 1.04)	0.75 (0.43, 1.33)	0.02
Multivariable <sup>b</sup>	1.0 (REF)	0.97 (0.74, 1.26)	0.88 (0.68, 1.13)	0.90 (0.65, 1.23)	1.03 (0.58, 1.83)	0.39

Using cumulative average intake level

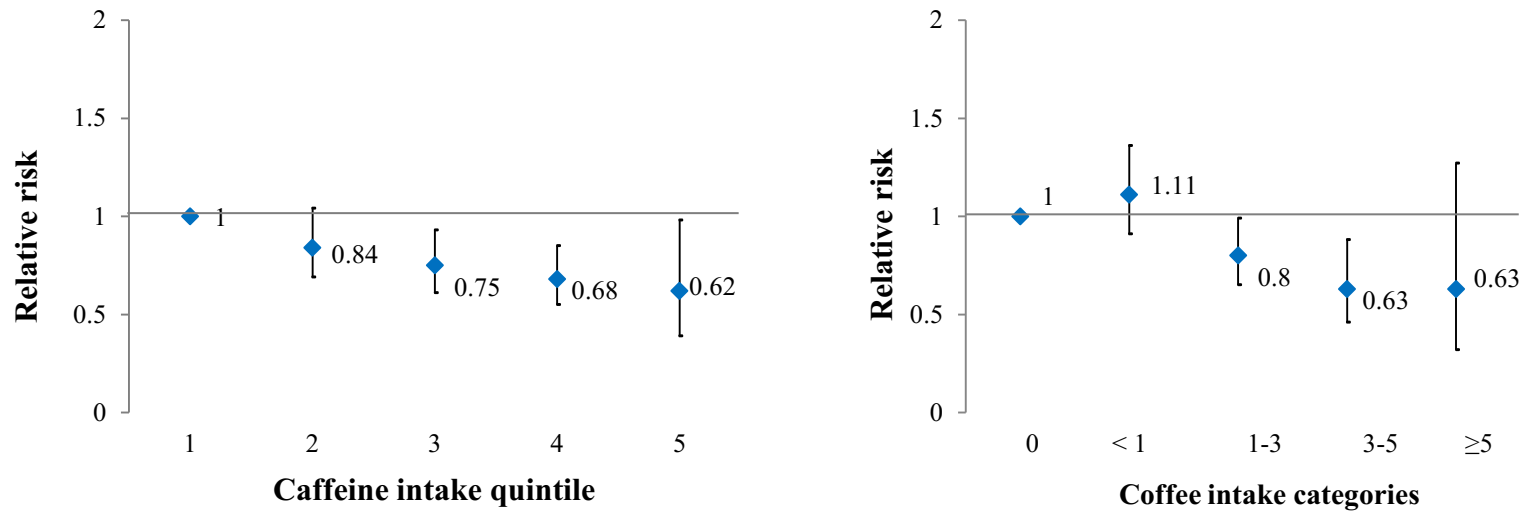
\* At cohort baseline

<sup>a</sup> Adjusted for age (years)<sup>b</sup> Adjusted for age, pack years of smoking, physical activity, and alcohol intake

**Table 1.3** Hazard ratio of Parkinson's disease by coffee intake and postmenopausal hormone use (PMH) in the NHS

	No. of cases	No. of person-years	Model 1 <sup>a</sup> (RR, 95%CI)	Model 2 <sup>b</sup> (RR, 95% CI)	<i>P</i> Trend <sup>b</sup>
<b>No PMH</b>					
Never or <1 month	29	197,015	1.0 (REF)	1.0 (REF)	
<1 cups/day	55	214,885	0.98 (0.62, 1.55)	1.01 (0.64, 1.61)	
1-3 cups/day	83	528,206	0.77 (0.50, 1.18)	0.85 (0.55, 1.32)	
3-5 cups/day	22	207,278	0.59 (0.34, 1.03)	0.73 (0.41, 1.29)	
5+ cups/day	5	69,178	0.60 (0.23, 1.56)	0.89 (0.34, 2.34)	0.16
Per cup	194	1,216,562	0.88 (0.79, 0.97)	0.93 (0.83, 1.04)	0.19
<b>PMH</b>					
Never or <1 month	55	163,746	1.0 (REF)	1.0 (REF)	
<1 cups/day	116	301,673	0.91 (0.66, 1.25)	0.90 (0.65, 1.25)	
1-3 cups/day	192	588,616	0.84 (0.62, 1.13)	0.87 (0.63, 1.18)	
3-5 cups/day	54	187,720	0.82 (0.56, 1.20)	0.94 (0.64, 1.39)	
5+ cups/day	9	38,196	0.89 (0.44, 1.80)	1.16 (0.56, 2.38)	0.99
Per cup	426	1,279,950	0.96 (0.89, 1.03)	1.00 (0.92, 1.08)	0.92
Interaction					0.08

<sup>a</sup>Adjusted for age (years)<sup>b</sup>Adjusted for age, pack years of smoking, physical activity, and alcohol intake

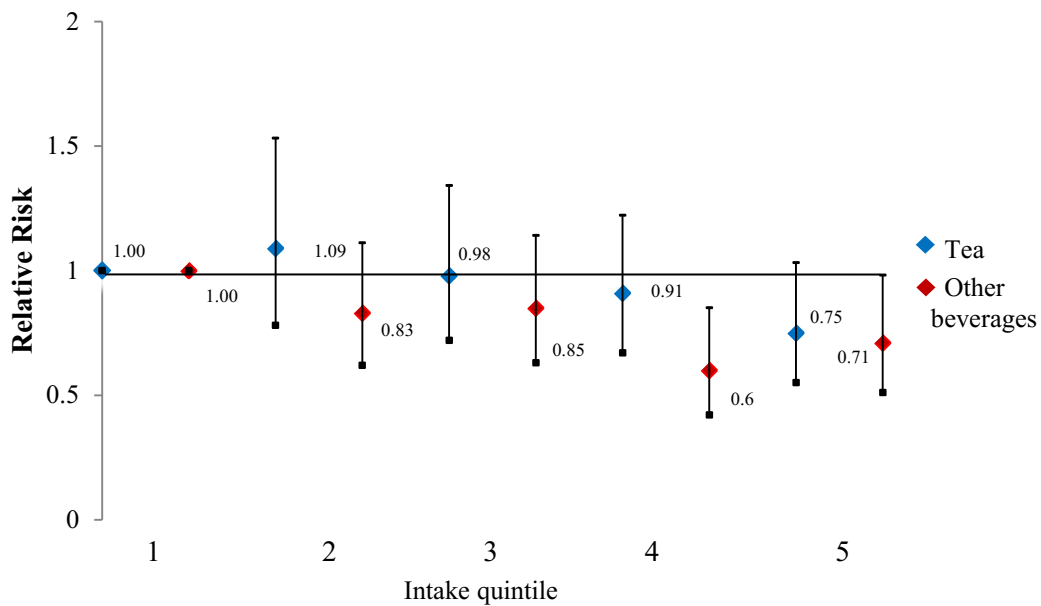


**Figure 1.1** Pooled Relative Risk of PD for men (HPFS) and women (NHS) who have never used PMH according to total caffeine intake quintiles ( $p_{\text{trend}}=0.05$ ) and coffee categories ( $p_{\text{trend}}<0.001$ ) using cumulative average intake levels and adjusted for pack years of smoking, alcohol intake, and physical activity.



Analyses considering servings of coffee linearly or total caffeine intake from non-caffeine sources did not change the results.

Furthermore, we investigated possible associations between decaffeinated coffee and caffeinated non-coffee beverages (e.g., soda) and PD risk among men and women who consumed less than 1 cup of coffee per day. Our results show that there was a significant inverse association between non-herbal tea consumption and PD risk among men across quintiles of cumulative averages (382 cases; multivariable-adjusted  $p$  trend = 0.03) (**Figure 1.2**).



**Figure 1.2** Associations of tea ( $p_{\text{trend}} = 0.03$ ) and other caffeinated beverages ( $p_{\text{trend}} = 0.08$ ) with PD using quintiles of cumulative average intake level, adjusting for pack years of smoking, physical activity, and alcohol intake among men who consume less than 1 cup of coffee per day

The association between other caffeinated beverage intake from non-coffee sources and PD risk was borderline significant (385 cases; multivariable-adjusted  $p$  trend= 0.08), while

evidence for a potentially harmful effect was unexpectedly detected among men who drank decaffeinated coffee; per each increasing cup of decaffeinated coffee, there was a 13% higher risk of PD (RR= 1.13, 95% CI: 1.01, 1.25;  $p = 0.03$ ). Finally, consistent with the direction of results for caffeine intake, marginally significant effects were found in the association between consumption of other caffeinated beverages from non-coffee sources and reduced risk of PD (72 cases; multivariable-adjusted  $p_{\text{trend}} = 0.05$ ) among women who have never used PMH. No statistically significant relation between tea and PD risk was found, possibly due to the low number of cases ( $n = 84$ ).

## **DISCUSSION**

In these analyses of two large prospective cohorts, greater intakes of coffee and total caffeine, but not intake of decaffeinated coffee, were associated with a lower risk of Parkinson's disease. These results were consistent in pooled analyses including men and women who have never used postmenopausal hormone therapy, confirming our previous findings.<sup>1,2,6,16</sup> In addition, the lack of protective association between consumption of decaffeinated coffee and PD risk suggests that caffeine, rather than other biological components of coffee, is the presumptive protective factor. The strengths of our study include a large number of cases for increased power, high follow-up rates in all cohorts, and repeated assessment of dietary intake and lifestyle factors using a prospective study design, which minimizes the potential for recall bias and reverse causation.

Our study has several limitations. First, during the early stages of disease onset, participants' dietary habits can change due to an altered sense of taste and smell, possibly influencing coffee-drinking behavior. Hence addressing this potential source of bias is

particularly important for insidious diseases, such as PD. However, bias from reverse causation appears to be minimal, as we consistently found a strong inverse association between coffee consumption and the onset of first PD symptoms even in lag-time analyses. In addition, the association remained robust in analyses using baseline caffeine levels for both men and women who have never used PMH. Second, there may have been measurement error of the exposure because the FFQ, as with any other method of measuring dietary data, may not have captured the exact coffee intake. However, the FFQ has been validated in all of our cohorts; in addition, any measurement error is expected to be non-differential with respect to PD, which would likely bias our results towards the null. It should be noted that due to the observational nature of the study, the possibility of residual confounding and/or unmeasured confounding should be considered. However, despite several sensitivity analyses performed to adjust for potential confounders, estimates remained largely unchanged. Therefore, any bias from unmeasured confounding is likely to be modest.

While there is conflicting evidence regarding the statistical presence of a caffeine-PMH interaction, most studies have reported that a significant protective effect of caffeine on PD is limited to women who have never used PMH.<sup>1,2,6,16</sup> Our pooled results indicated that an inverse association between coffee intake and PD risk was present in men and women who never used PMH. Because post-menopausal hormone therapies frequently include a combination of estrogens and progestin, we further explored possible effect modification by different types of hormonal therapy. Progesterone administration has been shown to confer neuroprotection in animal studies.<sup>24</sup> Interestingly, our findings suggest a significant interaction between coffee intake and PD risk among women who were only treated with progestin compared to women who were not treated with any PMH, regardless of type. However, the number of cases in this

subtype was low, partially because progestin-only therapy was rare; therefore, confirmation of these results are warranted. While we did not observe protective effects that were statistically significant of coffee intake among never users of PMH, we observed a marginally significant interaction between coffee intake and PMH use in the NHS when we considered the different types of PMH therapies. It is also important to note that potential effects of PMH may have been attenuated over time, as the prevalence of PMH use peaked in the 1980's and early 1990's and declined over the recent years.<sup>25</sup>

Caffeine is believed to be protective against excitotoxicity and dopaminergic neuron injury by antagonizing brain adenosine A<sub>2A</sub> receptors.<sup>12,26</sup> In animal studies, rodents that were administered caffeine were protected against loss of nigrostriatal dopaminergic neurons, induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or its active toxin metabolite.<sup>12,14,26–28</sup> Furthermore, studies have demonstrated that caffeine down-regulates neuroinflammatory responses and nitric oxide (NO) production, which may delay neuronal cell death.<sup>29</sup>

Our epidemiological results support experimental evidence for effect modification by PMH for the association between caffeine and PD risk in animal models. For instance, in ovariectomized aged (retired breeder) female mice the neurotoxic effect of MPTP, as reflected by striatal dopamine depletion, was reduced by caffeine.<sup>30</sup> Similarly, in young male mice caffeine treatment attenuated MPTP-induced striatal dopamine loss; however, in male mice that were pretreated with estrogen caffeine did not have a neuroprotective effect.

In summary, the results of this study with two large longitudinal cohorts support our previous reports that total caffeine intake is protective against PD in men and in women who have never used post-menopausal hormones.

## **ACKNOWLEDGEMENTS**

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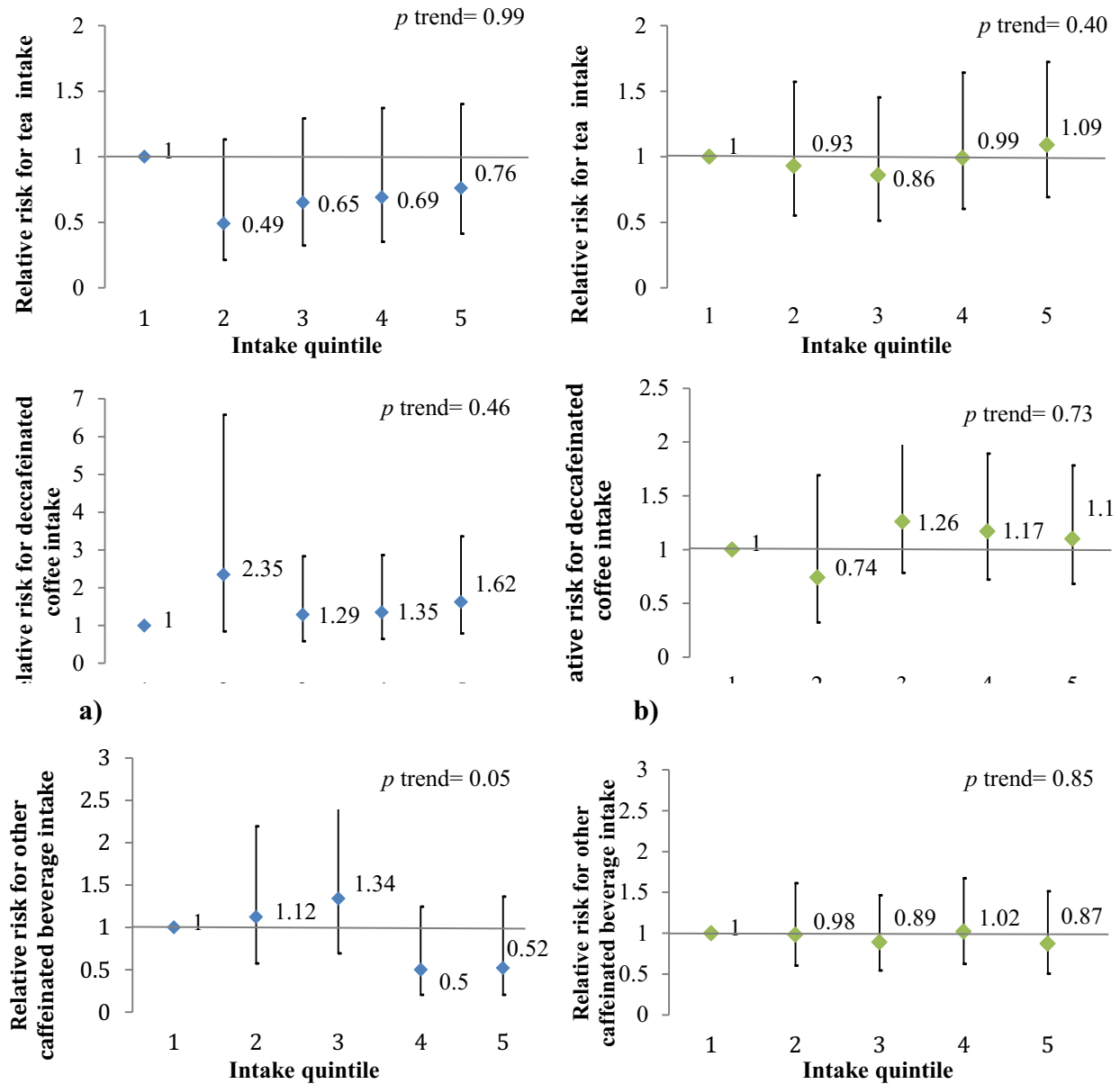
## **APPENDIX**

**Table 1.4** Hazard ratio of Parkinson’s disease by coffee intake and postmenopausal hormone use (PMH) among definite cases in the NHS

	No. of cases	No. of person-years	Model 1 <sup>a</sup> (RR, 95%CI)	Model 2 <sup>b</sup> (RR, 95% CI)	<i>P</i> Trend <sup>b</sup>
<b>No PMH</b>					
Never or <1 month	18	197,126	1.0 (REF)	1.0 (REF)	
<1 cups/day	26	215,092	0.86 (0.47, 1.59)	0.86 (0.46, 1.60)	
1-3 cups/day	33	528,500	0.54 (0.30, 0.96)	0.56 (0.31, 1.01)	
3-5 cups/day	9	207,320	0.38 (0.17, 0.85)	0.44 (0.19, 1.01)	
5+ cups/day	2	69,211	0.32 (0.07, 1.37)	0.43 (0.10, 1.90)	0.01
<b>PMH</b>					
Never or <1 month	22	163,975	1.0 (REF)	1.0 (REF)	
<1 cups/day	36	302,334	0.79 (0.46, 1.36)	0.81 (0.47, 1.39)	
1-3 cups/day	48	589,552	0.57 (0.34, 0.95)	0.60 (0.36, 1.02)	
3-5 cups/day	16	187,937	0.56 (0.29, 1.08)	0.65 (0.33, 1.27)	
5+ cups/day	9	38,196	1.65 (0.75, 3.65)	2.14 (0.95, 4.80)	0.88

<sup>a</sup>Adjusted for age (years)

<sup>b</sup>Adjusted for age, pack years of smoking, physical activity, and alcohol intake



**Figure 1.3** Associations of different types of caffeinated and decaffeinated beverages with PD using quintiles of cumulative average intake level, adjusting for pack years of smoking, physical activity, and alcohol intake among women who consume less than 1 cup of coffee per day: a) never PMH users and b) ever PMH users.

## Chapter 2 INTEGRATION OF RISK FACTORS FOR PARKINSON'S DISEASE IN TWO LONGITUDINAL COHORTS

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## ABSTRACT

**Objective:** To examine how selected lifestyle risk factors and family history of Parkinson's disease (PD) combine to determine overall PD risk.

**Background:** Numerous lifestyle factors have been related to risk of PD, but little is known on how these factors interact with each other. We sought to determine the overall combined effect of several known predictors of PD and to assess their interactions on the additive and multiplicative scale in two large prospective cohorts.

**Method:** We derived risk scores among 69,968 women in the Nurses' Health Study (NHS) (1984-2012) and 45,830 men in the Health Professionals Follow-up Study (HPFS) (1986-2012), free of PD at baseline. Risk scores were computed for each individual based on the following factors previously associated with PD risk: total caffeine intake, smoking, physical activity, and family history of PD for the NHS, and additionally total flavonoid intake and dietary urate index for the HPFS. We assigned one point per increase in quintile for each factor, with the exception of family history, for which we assigned a score of 5 for absence and 0 for presence of family history. The scores were summed to compute the overall score (NHS: 3-20; HPFS: 5-30). Hazard ratios (HRs) were estimated using Cox proportional hazards models. In addition, we performed tests of interactions on both the multiplicative and additive scale between pairs of risk factors.

**Results:** We documented 534 PD cases in NHS and 583 PD cases in the HPFS during follow up. The adjusted HRs comparing individuals in the highest category of the risk score to those in the lowest category of the risk score was 0.33 (95% CI: 0.21, 0.49;  $p_{\text{trend}} < 0.0001$ ) in the NHS and 0.18 (95% CI: 0.10, 0.32;  $p_{\text{trend}} < 0.0001$ ) in the HPFS. Results were similar when applying the risk scores computed by summing the predictors weighted by the log of their individual effect

sizes on PD risk in these cohorts. Additive interaction, possibly suggesting a synergic protective effect, was present between no family history of PD and caffeine intake in the HPFS and between caffeine and physical activity in NHS.

**Conclusions:** Our results suggest that known protective factors for PD have additive or super-additive effects, so that PD risk is very low in individuals with multiple protective risk factors.

## INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, and its prevalence is expected to rise as the population of older adults increases<sup>1</sup>. While several genes modulate PD risk<sup>2-6</sup>, 90% of PD cases have no discernable genetic cause<sup>7,8</sup>, and there is strong evidence for a role of lifestyle factors<sup>9</sup>.

In particular, caffeine intake, smoking, and physical activity have been consistently associated with a reduced PD risk in both men and women. Further, although data are more sparse, there is evidence that high flavonoid intake and dietary urate index are related to a reduced PD risk among men. It remains uncertain, however, how these factors interact with each other and with family history of PD. Both negative and positive synergisms are possible. Negative synergisms could arise if these factors act on similar mechanisms that can be saturated, whereas positive synergism could arise from activation of complementary or sequential pathways. We therefore generated risk scores and evaluated the association between these scores and long-term PD risk in two large prospective cohorts, the Nurses' Health Study (NHS) and the Health Professional Follow-up Study (HPFS). In addition, we assessed the presence of multiplicative and additive interactions between pairs of factors. Assuming no bias, multiplicative interaction corresponds to effect-measure modification in the relative risk or hazard scale and its presence is commonly determined by testing the statistical significance of the interaction term in a logistic or a Cox proportional hazards model to assess the departure from multiplicativity of effects. In contrast, additive interaction measures the deviation from additivity of absolute risks of two factors, which may be a better indicator of biological synergism<sup>10</sup>.



## **METHODS**

### ***Study population***

The participants of the current study were comprised of US women from the NHS and men from the HPFS. The NHS began in 1976 when 121,701 female registered nurses who were 30 to 55 years of age, returned detailed mailed questionnaires regarding health-related factors and medical histories. The HPFS enrolled 51,530 male health professionals aged 40 to 75 years who returned similar questionnaires in 1986. Every two years, follow-up information on lifestyle practices, health-related factors, and incident diseases was collected for members of both cohorts. The present study was restricted to 69,968 women from the NHS and 45,830 men from the HPFS with no history of Parkinson's disease and complete and reliable dietary data in 1984 (NHS) or 1986 (HPFS). This study was approved by the Human Research Committees at the Brigham and Women's Hospital and the Harvard T.H. Chan School of Public Health.

### ***Assessment of dietary and components of the risk score***

Nutritional information was ascertained every 4 years via validated food frequency questionnaires (FFQ). The study baseline was determined to be 1984 for the NHS and 1986 for the HPFS because dietary information was first comprehensively assessed in FFQs requested in those years. Participants were asked to self-report their average intake of approximately 130 foods or beverages over the past year using nine possible multiple-choice responses provided for intake frequency for each item, ranging from "never or less than once per month" to "6 or more times per day". Nutrient intake was then calculated using the quantity of nutrient in each item times the frequency of consumption. To account for correlation between an individual's daily

nutrient intake with overall caloric intake, we adjusted nutrients for total energy intake using the residual method<sup>11</sup>.

Updated information on other factors regarding lifestyle characteristics were assessed biennially. The risk score was composed of factors that have been previously found to be associated with PD risk in each of these two cohorts. For NHS, these predictors included total caffeine intake, smoking, physical activity, and family history of PD (i.e., mother, father, sibling). Additional predictors for the HPFS included dietary urate index (comprised of dairy protein, fructose, alcohol, and vitamin C intake) and total flavonoid intake.

### ***Computation of the risk score***

For each factor shown to be protective of PD, we ranked participants' levels into cohort-specific quintiles of cumulative averages up to the last questionnaire before the date of onset of PD symptoms, with the exception of smoking in pack-years for which we used the following categories: 0-9, 10-19, 20-49,  $\geq 50$ . We then assigned scores between 1 to 5— one point per increase in rank, with the lowest quintile or category being the reference. If the score could not be derived from a specific questionnaire due to missing values, we used the risk score derived from the previous questionnaire. All predictors are associated with a reduced risk of PD except for family history of PD, for which we assigned a score of 5 for absence of family history, and a score of 0 for presence of family history. The scores were summed to compute the overall score, which ranged from 3-20 for the NHS and 5-30 for the HPFS (the range is higher as expected because there are two additional factors assigned values of 1 to 5). Higher scores represent a lifestyle associated with lower PD risk.

### *Ascertainment of PD cases*

PD cases were identified via self-administered questionnaires and incident diagnoses were biennially documented thereafter. We then requested the patients' neurologists either to return a self-administered questionnaire confirming the PD diagnosis or to send a copy of the patient's medical records. Prior to 2003, PD cases were considered to be confirmed if the treating neurologist reported the diagnosis as definite or probable, or if the medical record either indicated a final diagnosis of PD made by a neurologist or the medical record indicated presence of at least two out of three cardinal signs (i.e., rest tremor, rigidity, bradykinesia) in the absence of features suggesting other illness diagnoses. After 2003, PD cases were confirmed using a similar procedure with the exception that medical records were requested from all PD cases, which were then reviewed by a movement disorder specialist.

### *Statistical analysis*

We conducted all analyses separately in each cohort using cohort-specific risk scores. Participants contributed person-time starting from the return date of the baseline questionnaire (June 1984 for NHS; January 1986 for HPFS) until the date of first PD symptoms, date of death, date of the latest completed questionnaire, or end of follow-up (June 2012 for NHS; January 2012 for HPFS), whichever occurred first. Our analyses were stratified jointly by age in months at the start of follow-up and calendar year of the current questionnaire cycle.

### *Risk score analysis*

We compared incident PD risk in quintiles of risk score in each cohort. We calculated hazard ratios (HR) and corresponding 95% confidence intervals (CI) using time-dependent Cox

proportional hazards model. Indicator variables were used to adjust for the number of missing FFQs.

To conduct tests of trend, mid-category scores (median lifestyle risk score value within each quintile) were modeled as a continuous variable to allow for possible nonlinear associations. Secondary analyses included using baseline risk scores while incorporating a lag period by excluding the first years of follow up (2, 4, 6, and 8 years). In addition to ranking the predictors into quintiles, we compared PD risk in five categories of the risk score: 5-11 (reference), 12-16, 17-20, 21-25, 25-30) for the HPFS; 3-9 (reference), 10-12, 13-14, 15-16, and 17-20 for NHS. We also computed individuals' weighted risk score by summing the predictors weighted by the log-transformed effect size of each predictor and its association with PD risk in each cohort; thus predictors with stronger hazard ratios contributed more to the weighted risk score compared to a predictor with weaker hazard ratios. The weighted risk scores were then analyzed similarly, comparing the risk of PD between quintiles of weighted risk scores. Additional analyses included comparing the PD risk in deciles, removing smoking as a risk factor, removing total flavonoid intake and dietary urate index as risk factors of the HPFS risk score, and replacing total flavonoid intake with total anthocyanin intake for the HPFS and including total anthocyanin intake for the NHS.

#### *Interaction analysis*

We used a 2x2 factorial design composed of two dichotomous risk factors with four corresponding possible exposure categories, among which the category with low/no exposure to either factor was the reference. In each cohort, predictors of PD were dichotomized at their respective median levels as the following: caffeine (high/low), physical activity (high/low), and

smoking (ever/never). We dichotomized having no family history of PD (mother, father, or sibling) vs. having any family history of PD. For men, dietary urate and total flavonoid intake were each dichotomized as high/low, and for women, PMH was categorized as never/ever use. Because the interpretations of the additive interaction indices are only appropriate for factors with harmful effects, we reversed the coding of all preventative factors before conducting tests of additive interaction<sup>12</sup>.

We compared the individual effects of exposures and their joint effect, each against the subgroup that is unexposed to either exposure to estimate three primary measures of additive interaction: the relative excess risk due to interaction (RERI), the attributable proportion due to interaction (AP), and the synergy index (SI), where an RERI and AP of 0 and S of 1 indicate exact additivity and therefore no additive interaction. This allowed us to determine whether the joint effect of both exposures is super-additive ( $RERI > 0$ ;  $AP > 0$ ;  $S > 1$ ) or sub-additive ( $RERI < 0$ ;  $AP < 0$ ;  $S < 1$ ) compared to the combined effect of each of the individual effects. To obtain the RERI and its 95% CI for the proportional hazards model, we followed the methods outlined by Li and Chambless (2007)<sup>13</sup>. For analyses of additive interaction where caffeine is categorized into tertiles, we again computed the additive interaction measures for each tertile of caffeine intake compared to lowest tertile (reference) using methods described by Andersson<sup>14</sup>.

In addition, we performed tests of statistical interaction between predictors of the risk score on the association of PD risk in on the multiplicative scale. We also conducted likelihood ratio tests comparing the model with interaction terms between caffeine intake (100 mg/day) and predictors (quintiles) to the model without the interaction terms. All statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Cary, NC).

## RESULTS

A total of 1,117 incident PD cases (534 in NHS, 583 in HPFS) were documented over 2,652,246 person-years of follow-up. The distribution of baseline characteristics across quintiles of the risk score for the NHS and HPFS are shown in **Table 2.1**. Women generally had a higher caffeine intake, were less physically active, and tended to smoke less compared to men.

**Table 2.1** Age-standardized characteristics of the study population at baseline by quintiles of risk score

	Risk score quintiles				
	1	2	3	4	5
<b>Health Professionals Follow-Up Study, 1986-2010</b>	n=10,528	n=8,645	n=9,841	n=8,552	n=8,264
Median risk score	14.4(1.7)	17.5(0.5)	19.5(0.5)	21.5(0.5)	24.3(1.3)
Age at study baseline*	53.8(9.9)	54.4(9.9)	54.5(9.9)	54.9(9.7)	55.4(9.4)
Caffeine intake, mg/day	85.8(137.4)	170.8(195.2)	233.8(218.1)	296.0(226.4)	390.8(229.6)
Pack-years smoked	4.3(11.3)	9.1(16.1)	13.0(18.1)	17.8(20.3)	25.0(20.8)
Body Mass Index, kg/m <sup>2</sup>	24.9(5.2)	24.9(5.1)	25.0(4.9)	25.0(4.7)	24.8(4.9)
Exercise, met-h/week	9.8(16.5)	15.3(22.8)	18.2(24.8)	22.6(29.0)	29.6(30.8)
Alcohol, g/day	4.6(8.2)	7.7(11.1)	11.1(14.4)	14.8(17.0)	20.4(19.8)
Total flavonoid intake, mg/day	192.9(120.9)	267.1(208.8)	320.1(257.7)	387.3(316.8)	492.8(364.8)
Dietary urate index	-0.1(0.2)	0.0(0.2)	0.1(0.2)	0.1(0.2)	0.2(0.3)
Family history of PD, % <sup>a</sup>	0.1(0.3)	0.0(0.1)	0.0(0.1)	0.0(0.1)	0.0(0.0)
Total energy intake, kcal/day	1,891(599)	1,929(602)	1,995(621)	2,041(621)	2,122(632)
<b>Nurses' Health Study, 1984-2012</b>	n=12,335	n=17,720	n=9,720	n=17,174	n=13,019
Median risk score	9.0(1.3)	11.6(0.5)	13.0(0.0)	14.5(0.5)	16.9(1.0)
Age at study baseline*	52.6(7.4)	52.9(7.3)	53.1(7.2)	53.2(7.0)	53.7(6.8)
Caffeine intake, mg/day	174.1(143.3)	250.6(173.8)	329.5(185.8)	423.2(199.1)	571.2(193.5)
Pack-years smoked	1.9(6.0)	4.7(10.5)	8.8(14.1)	16.1(17.6)	29.6(19.5)
Body Mass Index, kg/m <sup>2</sup>	26.6(5.5)	26.0(5.0)	25.8(4.9)	25.5(4.6)	25.1(4.3)
Exercise, met-h/week	4.7(8.7)	10.3(15.0)	14.6(19.0)	17.3(24.0)	23.7(26.9)
Alcohol, g/day	3.8(7.8)	5.2(8.7)	6.5(9.7)	8.2(11.3)	9.3(12.0)
Family history of PD, % <sup>a</sup>	0.2(0.4)	0.0(0.1)	0.0(0.1)	0.0(0.0)	0.0(0.0)
Postmenopausal Hormone Therapy use, %	28.0	26.8	27.4	27.5	28.1
Total energy intake, kcal/day	1,721(528)	1,736(525)	1,749(526)	1,752(527)	1,762(534)

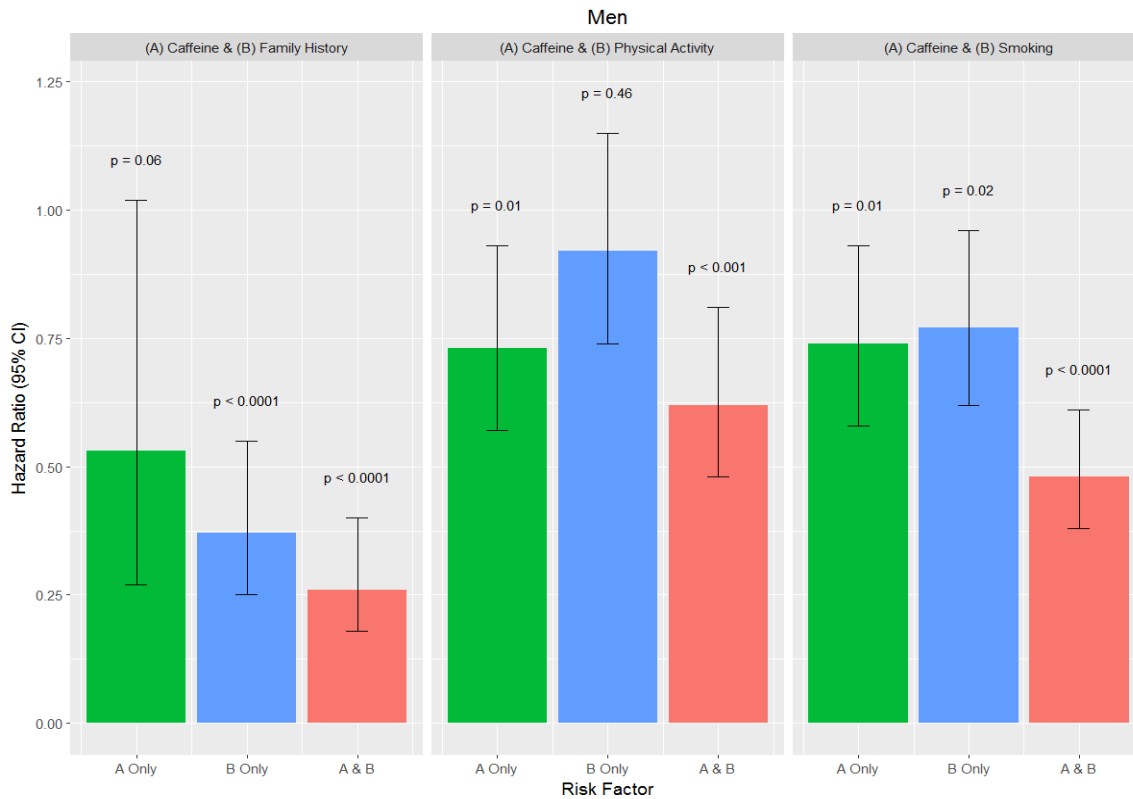
Values are means(SD) or percentages and are standardized to the age distribution of the study population.

\* Value is not age adjusted

<sup>a</sup> Family history of PD in 2008

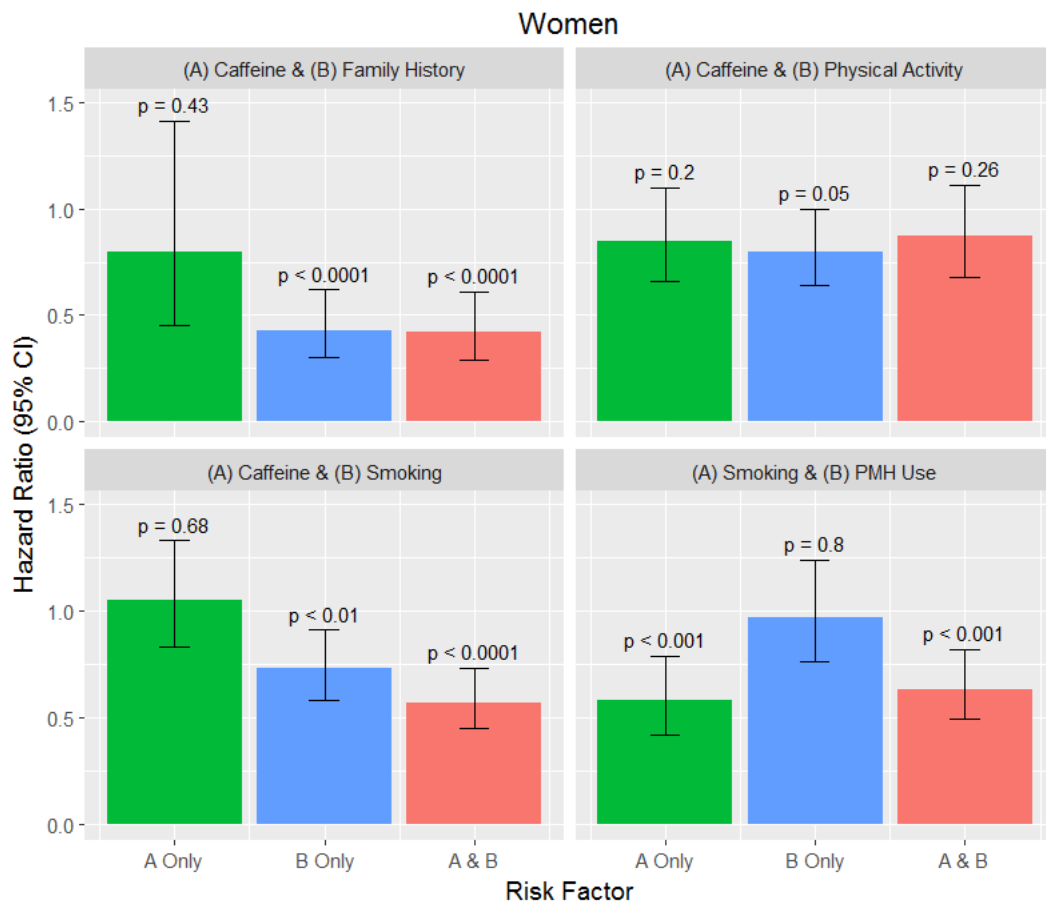
*Interaction analyses*

**Figures 2.1 and 2.2** show the results of subgroup analyses between two dichotomized predictors of PD in the HPFS and NHS, respectively. Overall, among the four exposure categories, men and women who were in the high category for both predictors had the lowest PD risk compared to participants who were in the low category for both predictors. For the HPFS, ever smokers with high caffeine intake had a 52% decreased PD risk compared to the referent group of never smokers with low caffeine intake (HR = 0.48, 95% CI: 0.38, 0.61;  $p < 0.0001$ ) (**Figure 2.1**). Men who were ever smokers with low caffeine intake and men who were never smokers with high caffeine intake also had a reduced risk compared to the referent group, though the strongest protective effect was observed among ever smokers with high caffeine intake.



**Figure 2.1** Associations of individual and combined risk factors and PD risk among men (HPFS).

However, there was neither evidence of additive interaction nor effect modification on the multiplicative scale ( $p_{\text{RERI}} = 0.35$ ;  $p_{\text{multi}} = 0.18$ ). For the NHS, women who had ever smoked with high caffeine consumption had 0.57 times the PD risk compared to women who had never smoked with low caffeine intake (95% CI: 0.45, 0.73;  $p < 0.0001$ ) (**Figure 2.2**). In addition, ever smokers who had ever used PMH had significantly decreased risk of PD compared to never smokers who have never used PMH (HR= 0.63, 95% CI: 0.49, 0.82;  $p < 0.001$ ).



**Figure 2.2** Associations of individual and combined risk factors and PD risk among women (NHS).

There was no evidence for multiplicative or additive interaction between caffeine and physical activity in the HPFS, but evidence for additive interaction between caffeine and having no family history was present;  $AP = 0.38$  95% CI = 0.04, 0.72;  $p_{\text{AP}} = 0.03$ ). Furthermore, when



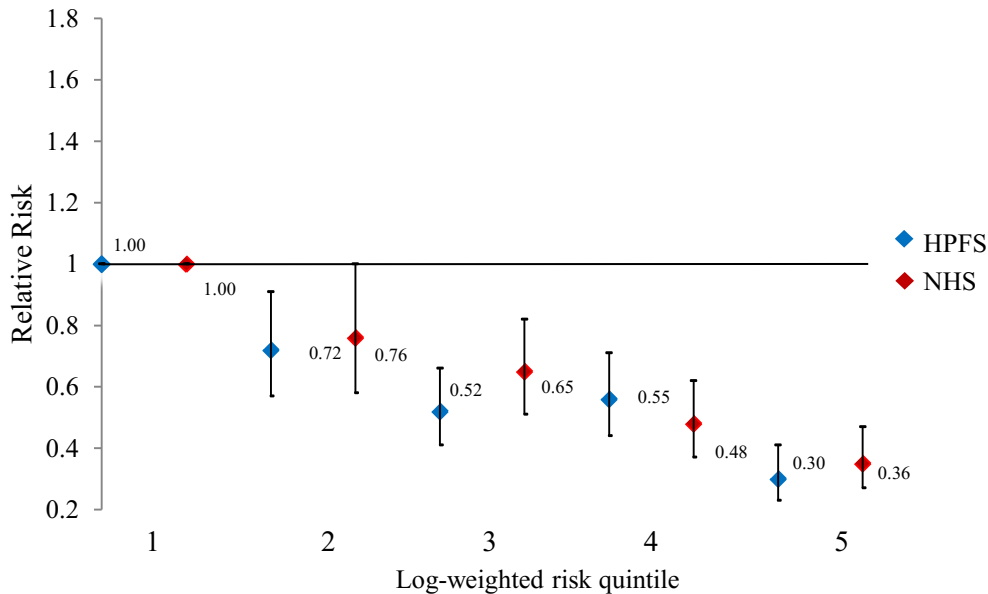
caffeine intake was categorized into tertiles, additive interaction between men who were in the highest tertile of caffeine intake and family history was present ( $RERI = 2.0$ ;  $p_{RERI} < 0.05$ ;  $AP = 0.48$   $p_{AP} < 0.01$ ), but not for men who were in the middle tertile of caffeine intake. In women, additive interaction between caffeine and physical activity was significant ( $AP = 0.21$ , 95% CI = 0.04, 0.39;  $p_{AP} = 0.02$ ).

### *Risk score analyses*

In both cohorts, a higher category of risk score was associated with a decreased risk of PD as expected ( $p_{trend} < 0.0001$ ) (**Table 2.2**). Participants who were in the highest category compared to the lowest category of the score had an 82% decreased risk (HR = 0.18, 95% CI: 0.10, 0.32;  $p < 0.0001$ ) in the HPFS and a 67% decreased risk in the NHS (HR = 0.33, 95% CI: 0.21, 0.49;  $p < 0.0001$ ). A 1-point increase in the risk score was associated with a 9% decrease in risk in men (HR = 0.91, 95% CI: 0.89, 0.93;  $p < 0.0001$ ) and a 10% decrease in risk in women (HR = 0.90, 95% CI: 0.87, 0.93;  $p < 0.0001$ ).

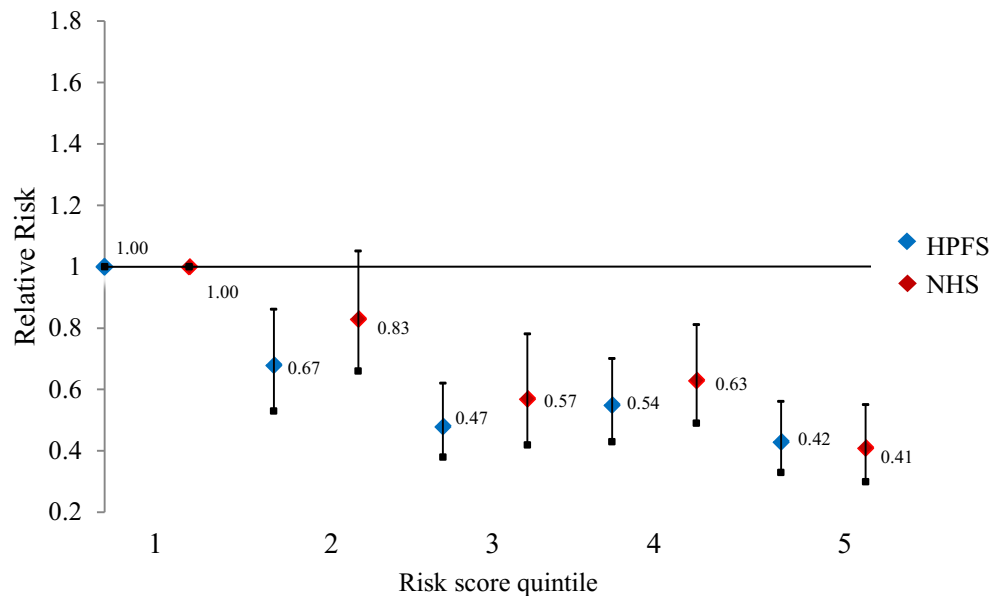
<b>Table 2.2</b> Hazard Ratio of PD by categories of risk score for HPFS (men) and NHS (women)						
	Categories of “risk” score					<i>P</i> <sub>trend</sub>
	1	2	3	4	5	
<b>HPFS*</b>						
Median risk score	11	15	19	22	25	
Number of cases	24	165	216	154	24	
Person-years	14,588	188,489	373,742	288,822	59,409	
Hazard Ratio Risk score <sup>a</sup> (95% CI)	1.0 (REF)	0.45 (0.29, 0.70)	0.27 (0.18, 0.42)	0.24 (0.16, 0.38)	0.18 (0.10, 0.32)	<0.0001
Linear/per point					0.91 (0.88, 0.93)	<0.0001
<b>NHS*</b>						
Median risk score	9	11	13	15	17	
Number of cases	82	206	128	87	31	
Person-years	163,583	565,854	461,566	354,145	182,046	
Hazard Ratio Risk score <sup>b</sup> (95% CI)	1.0 (REF)	0.71 (0.55, 0.92)	0.53 (0.40, 0.70)	0.46 (0.34, 0.63)	0.33 (0.21, 0.49)	<0.0001
Linear/per point					0.90 (0.87, 0.93)	<0.0001
* At baseline: 1986 for HPFS, 1984 for NHS						
<sup>a</sup> Caffeine, smoking, physical activity, dietary urate index, flavonoid intake, and family history of PD						
<sup>b</sup> Caffeine, smoking, physical activity, and family history of PD						

Similarly, a strong inverse association was observed when comparing the PD risk in the highest quintile to the lowest quintile of the log-weighted risk score for both men (HR = 0.30, 95% CI: 0.23, 0.41;  $p < 0.0001$ ) and women (HR = 0.36, 95% CI: 0.27, 0.47;  $p < 0.0001$ ) (**Figure 2.3**).



**Figure 2.3** Relative Risk of PD for the HPFS and NHS according to quintiles of their respective log-weighted risk scores.  $P_{\text{trend}} < 0.0001$  for both.

Conducting analyses using quintiles of the risk score did not change results (**Figure 2.4**). These results were robust in lag analyses (2, 4, 6, and 8 years), comparing deciles of the risk scores and using baseline risk scores (data not shown). Further analyses adjusting for reported ibuprofen use, dairy intake, antioxidants (e.g., vitamin C and E) and head trauma injury (data only available in men) yielded consistent results. Finally, results remained unchanged when the components of dietary urate index and total flavonoid intake were excluded in the HPFS risk score to match that of NHS and when total anthocyanin intake was included in the NHS risk score while it replaced total flavonoid intake in the HPFS.



**Figure 2.4** Relative Risk of PD for the HPFS and NHS according to quintiles of their respective risk scores.  $P_{\text{trend}} < 0.0001$  for both.

Using the population attributable risk proportion (PAR%) we estimated the proportion of the new PD cases that hypothetically could have been prevented if all participants in the highest quintile of the risk score had instead been in the lowest quintile of the risk score, assuming a causal relationship between the risk score and PD. For men, the PAR% was 80% for the original risk score, 50% for the risk score excluding family history of PD as a component, and 44% for the risk score excluding both family history of PD and smoking behavior. For women, the PAR% was 63%, 40%, and 15%, for the original risk score, risk score without family history of PD, and risk score without family history of PD and smoking behavior, respectively.

## DISCUSSION

In our two large, prospective cohorts, a risk score that included caffeine intake, smoking, physical activity, family history of PD for the NHS and additionally, total flavonoid intake and dietary urate index for the HPFS, was associated with a decreased risk of PD. We also found evidence for additive interaction between total caffeine intake and family history of PD on incident PD risk in men; presence of additive interaction between total caffeine intake and physical activity on PD risk was detected in women.

Our risk score was based on independent predictors supported in the literature that have previously been found to be associated with PD risk in our two health professional cohorts. Evidence for an inverse association between tobacco smoking and PD is robust and has been widely studied in many longitudinal studies<sup>9,15-17</sup>. Similarly, caffeine is a well-established neuroprotective factor; the effect is stronger among men compared to women, most likely due to effect modification by postmenopausal hormone use<sup>18,19</sup>. Physical activity is another factor that is suggested to be associated with a reduced PD risk in several longitudinal studies across different cohorts<sup>20-24</sup>. In our health professional cohorts, total physical activity, as well as vigorous activity, was associated with a lower risk of PD in men even after lag analyses, suggesting evidence against reverse causation<sup>25</sup>. Among women, physical activity was not associated with a reduced risk of PD, though women who reported strenuous exercise during early adulthood had a lower risk. In addition, because plasma urate<sup>26-28</sup> and total flavonoid<sup>29</sup> have been found to be protective against PD in men but not in women, they were both included as additional factors contributing to the risk score only for men. Alternatively, we performed sensitivity analyses using risk scores including anthocyanin, a subclass of flavonoids, because it was associated with reduced PD risk in both men and women. Finally, although family

history of PD is not a modifiable risk factor, it was included as a hereditary component of the risk score due to its moderate genetic association with PD risk. In an alternate risk score, we removed smoking as a factor, as it would be immaterial from a public health perspective to recommend smoking due to its adverse effects on respiratory, cardiovascular, and other health outcomes. However, if nicotine or other biological agents explained the protective effects of smoking, potential therapeutic interventions would be possible.<sup>9</sup> For this reason, we calculated population attributable risk also for a score including smoking.

In addition to applying risk scores to our cohorts, we also assessed effect modification by each predictor on the multiplicative and additive scale. Among the predictors of PD for the HPFS, there was evidence for additive interaction between total caffeine intake and family history of PD on incident PD risk, i.e. the increased PD risk associated with a positive family history plus caffeine abstinence is higher than the sum of the risks associated with each factor alone. Furthermore, additive interaction between total caffeine intake and physical activity was evident in women; the adverse “effect” of being both physical inactive and having a low caffeine intake was greater than the sum of the effects combined (AP: 0.21, 95% CI: 0.04, 0.39;  $p = 0.02$ ).

The main strengths of our study include large number of cases for greater power, a high active follow-up rate in both cohorts (~94% in both cohorts), and minimized potential for recall bias and reverse causation due to a prospective collection of repeated detailed data on dietary intake and lifestyle factors. We also report three indices of additive interaction. While it is generally agreed that measuring interaction on the additive scale is particularly important for public health implications, many studies only report effect modification on the multiplicative scale<sup>30–32</sup>. Knol et al. (2009) report that among a random sample of 138 studies assessing interaction, only three studies mentioned the use of additive interaction and none reported the

RERI or AP<sup>33</sup>. Assessing additive interaction would provide insight into possible biological mechanisms through which two factors can interact to have a greater effect than the combined effect of each individual factor alone.

We recognize that there is potential for measurement error of lifestyle data from the FFQ. However, they have been validated in both cohorts<sup>34-36</sup>, and any measurement error would be expected to bias our results towards the null since it is likely to be non-differential with respect to PD due to our prospective design. In addition, although we cannot disregard the possibility of unmeasured confounding, our results remained robust after conducting several sensitivity analyses adjusting for other potential confounders. Finally, because we dichotomized predictors to assess additive interaction for simplicity, we may have failed to detect potential additive interactions if the associations between predictors are not best captured by dichotomizing the predictors. However, in sensitivity analyses, categorizing caffeine into tertiles did not change results.

Future research should focus on the validation of risk scores, such as those presented here, in other populations. Since our risk score included nutrient data that is not easily measured, other modified risk scores could be developed for rapid use in a clinical setting.

In conclusion, our results show that the risk score was associated with decreased risk of PD, and that the combination of family history and known lifestyle factors can explain 80% of cases of PD in men and 63% in women. Further analyses on additive interactions support evidence for additive interaction between caffeine intake and family history of PD for both men and women, as well as interaction between caffeine intake and physical activity for women.

## **ACKNOWLEDGEMENTS**

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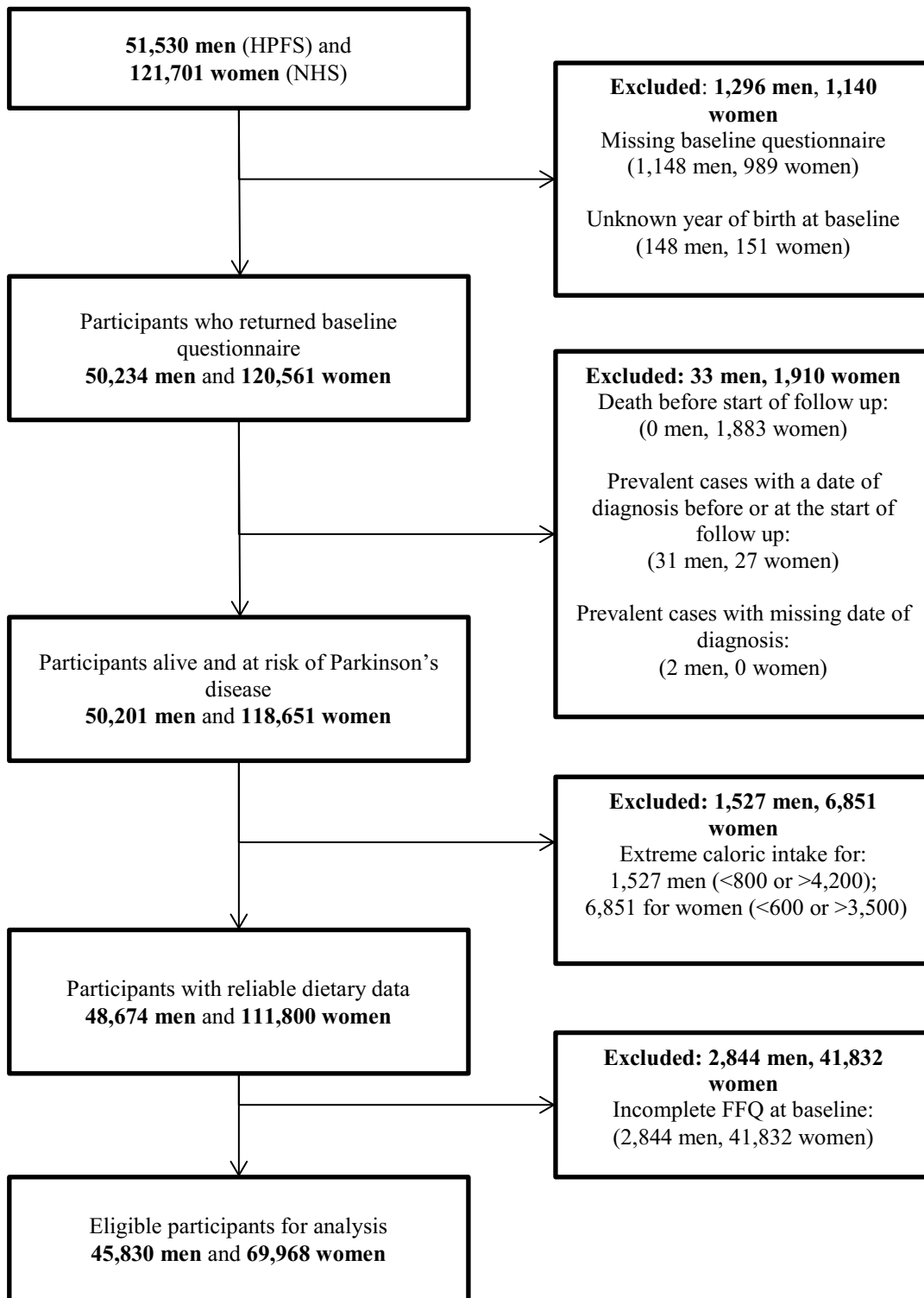
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## APPENDIX



**Figure 2.5** Flowchart of study inclusion and exclusion criteria for the HPFS and NHS

<b>Table 2.3</b> Associations between individual risk factors and PD risk		
	HR (95% CI)	p-value
<b>HPFS</b>		
Caffeine		
1	1.00 (reference)	
2	0.90 (0.71, 1.14)	0.37
3	0.79 (0.61, 1.01)	0.06
4	0.70 (0.53, 0.90)	<0.01
5	0.54 (0.39, 0.73)	<0.0001
Smoking		
1	1.00 (reference)	
2	0.82 (0.61, 1.11)	0.20
3	0.82 (0.63, 1.07)	0.14
4	0.70 (0.56, 0.88)	<0.01
5	0.47 (0.30, 0.73)	<0.01
Physical activity		
1	1.00 (reference)	
2	0.78 (0.60, 1.02)	0.07
3	0.72 (0.55, 0.94)	0.02
4	0.80 (0.61, 1.03)	0.09
5	0.64 (0.48, 0.84)	<0.01
Family history		
Present	1.00 (reference)	
Absent	0.44 (0.32, 0.61)	<0.0001
Flavonoid		
1	1.00 (reference)	
2	0.84 (0.64, 1.11)	0.22
3	0.85 (0.66, 1.12)	0.25
4	0.99 (0.76, 1.29)	0.94
5	0.82 (0.62, 1.09)	0.18
Dietary urate index		
1	1.00 (reference)	
2	0.99 (0.78, 1.27)	0.95
3	0.91 (0.71, 1.17)	0.47
4	0.91 (0.70, 1.17)	0.45
5	0.75 (0.57, 0.99)	0.05



**Table 2.3 (Continued)**

<b>NHS</b>		
<b>Caffeine</b>		
1	1.00 (reference)	
2	0.90 (0.70, 1.15)	0.40
3	0.84 (0.65, 1.09)	0.20
4	0.76 (0.58, 1.00)	0.05
5	1.04 (0.79, 1.38)	0.76
<b>Smoking</b>		
1	1.00 (reference)	
2	0.82 (0.64, 1.04)	0.10
3	0.65 (0.47, 0.89)	<0.01
4	0.62 (0.48, 0.79)	<0.001
5	0.27 (0.16, 0.46)	<0.0001
<b>Physical activity</b>		
1	1.00 (reference)	
2	1.13 (0.84, 1.50)	0.42
3	1.02 (0.76, 1.36)	0.92
4	1.00 (0.75, 1.34)	0.98
5	0.89 (0.66, 1.20)	0.44
<b>Family history</b>		
Present	1.00 (reference)	
Absent	0.48 (0.36, 0.64)	<0.0001

**Table 2.4** Tests of interactions on the multiplicative and additive scale for the HPFS

<b>Predictor 1</b>	<b>Predictor 2</b>	<b>HR (95% CI)</b>	<b>p-value</b>
Caffeine	Smoking		
Low	Never	1.00 (reference)	
High	Never	0.74 (0.58, 0.93)	0.01
Low	Ever	0.77 (0.62, 0.96)	0.02
High	Ever	0.48 (0.38, 0.61)	<0.0001
		Additive interaction:	
		RERI	-0.25 (95% CI:-0.78, 0.28)
		$P_{RERI}$ for interaction	0.35
		AP (95% CI)	-0.13 (-0.41, 0.14)
		$P_{AP}$ for interaction	0.35
		Synergy Index	0.79 (0.50, 1.24)
		Multiplicative interaction:	
		Caffeine (high/low) * smoking (never/ever)	0.84 (0.64, 1.09) $P_{multi} = 0.18$
		Caffeine (100 mg /day) * smoking categories	$P_{multi} = 0.68$
Caffeine	Physical Activity		
Low	Low	1.00 (reference)	
High	Low	0.73 (0.57, 0.93)	0.01
Low	High	0.92 (0.74, 1.15)	0.46
High	High	0.62 (0.48, 0.81)	<0.001
		Additive interaction:	
		RERI	-0.03 (-0.52, 0.46)
		$P_{RERI}$ for interaction	0.91
		AP (95% CI)	-0.02 (-0.27, 0.23)
		$P_{AP}$ for interaction	0.90
		Synergy Index	0.97 (0.55, 1.69)
		Multiplicative interaction:	
		Caffeine (high/low) * physical activity (high/low)	0.93 (0.66, 1.32) $P_{multi} = 0.69$
		Caffeine (100 mg/day) * physical activity (quintiles)	$P_{multi} = 0.77$
Caffeine	Family History		
Low	Yes	1.00 (reference)	
High	Yes	0.53 (0.27, 1.02)	0.06
Low	No	0.37 (0.25, 0.55)	<0.0001
High	No	0.26 (0.18, 0.40)	<0.0001
		Additive interaction:	
		RERI	1.46 (-0.27, 3.18)
		$P_{RERI}$ for interaction	0.10
		AP (95% CI)	0.38 (0.04, 0.72)
		$P_{AP}$ for interaction	0.03
		Synergy Index	2.07 (0.83, 5.02)
		Multiplicative interaction:	
		Caffeine (high/low) *family history (yes/no)	1.34 (0.68, 2.64); $P_{multi} = 0.40$
		Caffeine (100mg/day) * family history (yes/no)	1.11 (0.92, 1.33); $P_{multi} = 0.26$

<b>Table 2.5</b> Tests of interactions on the multiplicative and additive scale for the NHS			
<b>Predictor 1</b>	<b>Predictor 2</b>	<b>HR (95% CI)</b>	<b>p-value</b>
Caffeine	Smoking		
Low	Never	1.00 (reference)	
High	Never	1.05 (0.83, 1.33)	0.68
Low	Ever	0.73 (0.58, 0.91)	<0.01
High	Ever	0.57 (0.45, 0.73)	<0.0001
		Additive interaction:	
		RERI	-0.44 (-1.01, 0.12)
		$P_{RERI}$ for interaction	0.12
		AP (95% CI)	-0.25 (-0.56, -0.07)
		$P_{AP}$ for interaction	0.12
		Synergy Index	0.64 (0.40, 1.03)
		Multiplicative interaction:	
		Caffeine (high/low) * smoking (never/ever)	0.75 (0.53, 1.07); $P_{multi} = 0.11$
		Caffeine (100 mg /day) * smoking categories	$P_{multi} = 0.55$
Caffeine	Physical Activity		
Low	Low	1.00 (reference)	
High	Low	0.85 (0.66, 1.10)	0.20
Low	High	0.80 (0.64, 1.00)	0.05
High	High	0.87 (0.68, 1.11)	0.26
		Additive interaction:	
		RERI	0.24 (-0.08, 0.57)
		$P_{RERI}$ for interaction	0.15
		AP (95% CI)	0.21 (0.04, 0.39)
		$P_{AP}$ for interaction	0.02
		Synergy Index	No estimate
		Multiplicative interaction:	
		Caffeine(high/low) * physical activity (high/low)	1.28 (0.90, 1.81); $P_{multi} = 0.17$
		Caffeine (100 mg/day) * physical activity (quintiles)	$P_{multi} = 0.33$
Caffeine	Family History		
Low	Yes	1.00 (reference)	
High	Yes	0.80 (0.45, 1.41)	0.43
Low	No	0.43 (0.30, 0.62)	<0.0001
High	No	0.42 (0.29, 0.61)	<0.0001
		Additive interaction:	
		RERI	0.55 (-0.65, 1.74)
		$P_{RERI}$ for interaction	0.37
		AP (95% CI)	0.22 (-0.17, 0.61)
		$P_{AP}$ for interaction	0.27
		Synergy Index	1.59 (0.53, 4.77)

Table 2.5 (Continued)			
		Multiplicative interaction:	
		Caffeine (high/low) *family history (yes/no)	1.23 (0.68, 2.22); $P_{\text{multi}} = 0.50$
		Caffeine (100mg/day) * family history (yes/no)	1.04 (0.91, 1.18); $P_{\text{multi}} = 0.62$
Smoking	PMH		
Never	Never	1.00 (reference)	
Ever	Never	0.58 (0.42, 0.79)	<0.001
Never	Ever	0.97 (0.76, 1.24)	0.80
Ever	Ever	0.63 (0.49, 0.82)	<0.001
		Additive interaction:	
		RERI	-0.05 (-0.42, 0.32)
		$P_{\text{RERI}}$ for interaction	0.80
		AP (95% CI)	-0.04 (-0.32, 0.23)
		$P_{\text{AP}}$ for interaction	0.75
		Synergy Index	No estimate
		Multiplicative interaction:	
		Smoking (never/ever) * PMH use (ever/never)	1.13 (0.77, 1.64); $P_{\text{multi}} = 0.54$
		Smoking (packyears) * PMH use *ever/never)	1.00 (1.00, 1.00); $P_{\text{multi}} = 0.55$

<b>Table 2.6</b> Tests of interactions on the additive scale in tertiles of caffeine intake for the HPFS			
<b>Predictor 1</b>	<b>Predictor 2</b>	<b>HR (95% CI)</b>	<b>p-value</b>
Caffeine	Smoking		
Tertile 1	Never	1.00 (reference)	
Tertile 2	Never	0.69 (0.53, 0.90)	<0.01
Tertile 3	Never	0.73 (0.55, 0.96)	0.02
Tertile 1	Ever	0.91 (0.70, 1.17)	0.44
Tertile 2	Ever	0.67 (0.52, 0.88)	<0.01
Tertile 3	Ever	0.46 (0.35, 0.61)	<0.0001
		Additive interaction:	
		RERI <sub>1</sub>	-0.55 (-1.22, 1.12)
		AP <sub>1</sub> (95% CI)	-0.36 (-0.81, 0.09)
		<i>P</i> <sub>AP1</sub> for interaction	0.11
		RERI <sub>2</sub>	-0.38 (-0.91, 0.15)
		AP <sub>2</sub> (95% CI)	-0.17 (-0.50, 0.15)
		<i>P</i> <sub>AP2</sub> for interaction	0.30
Caffeine	Physical activity		
Tertile 1	Low	1.00 (reference)	
Tertile 2	Low	0.90 (0.68, 1.20)	0.48
Tertile 3	Low	0.66 (0.49, 0.90)	<0.01
Tertile 1	High	1.02 (0.79, 1.31)	0.89
Tertile 2	High	0.59 (0.43, 0.79)	<0.001
Tertile 3	High	0.61 (0.45, 0.83)	<0.01
		Additive interaction:	
		RERI <sub>1</sub>	0.44 (-0.02, 0.90)
		AP <sub>1</sub> (95% CI)	0.30 (-0.01, 0.61)
		<i>P</i> <sub>AP1</sub> for interaction	0.06
		RERI <sub>2</sub>	-0.11 (-0.52, 0.31)
		AP <sub>2</sub> (95% CI)	-0.07 (-0.40, 0.27)
		<i>P</i> <sub>AP2</sub> for interaction	0.70
Caffeine	Family history		
Tertile 1	Yes	1.00 (reference)	
Tertile 2	Yes	0.57 (0.28, 1.15)	0.11
Tertile 3	Yes	0.38 (0.17, 0.85)	0.02
Tertile 1	No	0.38 (0.25, 0.59)	<0.0001
Tertile 2	No	0.28 (0.18, 0.44)	<0.0001
Tertile 3	No	0.24 (0.15, 0.38)	<0.0001
		Additive interaction:	
		RERI <sub>1</sub>	0.62 (-1.12, 2.36)
		AP <sub>1</sub> (95% CI)	0.26 (-0.37, 0.89)
		<i>P</i> <sub>AP1</sub> for interaction	0.42
		RERI <sub>2</sub>	2.0 (0.19, 3.83)
		<i>P</i> <sub>RERI2</sub> for interaction	<0.05
		AP <sub>2</sub> (95% CI)	0.48 (0.14, 0.83)
		<i>P</i> <sub>AP2</sub> for interaction	<0.01

<b>Table 2.7</b> Tests of interactions on the additive scale in tertiles of caffeine intake for the NHS			
<b>Predictor 1</b>	<b>Predictor 2</b>	<b>HR (95% CI)</b>	<b>p-value</b>
Caffeine	Smoking		
Tertile 1	Never	1.00 (reference)	
Tertile 2	Never	1.08 (0.83, 1.40)	0.58
Tertile 3	Never	1.21 (0.91, 1.60)	0.19
Tertile 1	Ever	0.73 (0.54, 0.99)	<0.05
Tertile 2	Ever	0.76 (0.58, 1.01)	0.06
Tertile 3	Ever	0.58 (0.43, 0.77)	0.0001
		Additive interaction:	
		RERI <sub>1</sub>	-0.51 (-1.23, 0.21)
		AP <sub>1</sub> (95% CI)	- 0.27 (-0.67, 0.12)
		P <sub>AP1</sub> for interaction	0.17
		RERI <sub>2</sub>	-0.60 (-1.05, -0.15)
		AP <sub>2</sub> (95% CI)	-0.35 (-0.76, 0.07)
		P <sub>AP2</sub> for interaction	0.10
Caffeine	Physical activity		
Tertile 1	Low	1.00 (reference)	
Tertile 2	Low	1.05 (0.79, 1.39)	0.76
Tertile 3	Low	0.91 (0.67, 1.23)	0.52
Tertile 1	High	0.84 (0.63, 1.12)	0.23
Tertile 2	High	0.95 (0.71, 1.26)	0.71
Tertile 3	High	0.98 (0.72, 1.32)	0.87
		Additive interaction:	
		RERI <sub>1</sub>	0.13 (-0.28, 0.54)
		AP <sub>1</sub> (95% CI)	0.12 (-0.26, 0.50)
		P <sub>AP1</sub> for interaction	0.53
		RERI <sub>2</sub>	0.24 (0.04, 0.52)
		AP <sub>2</sub> (95% CI)	0.23 (-0.14, 0.60)
		P <sub>AP2</sub> for interaction	0.22
Caffeine	Family history		
Tertile 1	Yes	1.00 (reference)	
Tertile 2	Yes	0.96 (0.50, 1.83)	0.89
Tertile 3	Yes	0.94 (0.49, 1.82)	0.86
Tertile 1	No	0.43 (0.27, 0.68)	<0.001
Tertile 2	No	0.47 (0.30, 0.75)	<0.01
Tertile 3	No	0.45 (0.28, 0.71)	<0.001
		Additive interaction:	
		RERI <sub>1</sub>	-0.01 (-1.48, 1.46)
		AP <sub>1</sub> (95% CI)	0.00 (-0.69, 0.68)
		P <sub>AP1</sub> for interaction	0.99
		RERI <sub>2</sub>	0.15 (-0.85, 1.15)
		AP <sub>2</sub> (95% CI)	0.07 (-0.56, 0.69)
		P <sub>AP2</sub> for interaction	0.84

<b>Table 2.8</b> Hazard ratios by quintiles for the HPFS using the same predictors as NHS						
	Quintiles of risk score					<i>P</i> <sub>trend</sub>
	1	2	3	4	5	
<b>HPFS</b>						
Median risk score*	9	12	13	14	17	
Number of cases	157	146	76	125	79	
Person-years	141,009	227,704	130,443	238,171	187,723	
Hazard Ratio (95% CI)						
Risk score <sup>a</sup>	1.0 (REF)	0.54 (0.43, 0.67)	0.49 (0.37, 0.65)	0.43 (0.34, 0.54)	0.33 (0.25, 0.43)	<0.0001
Linear/per point			0.87 (0.84, 0.90)			<0.0001

\* In 1986 (at baseline)

<sup>a</sup> Caffeine, smoking, physical activity, and family history of PD

<b>Table 2.9</b> Hazard Ratio of PD by quintiles of risk score without smoking as a risk factor for HPFS (men) and NHS (women)						
	Quintiles of risk score					<i>P</i> <sub>trend</sub>
	1	2	3	4	5	
<b>HPFS</b>						
Median risk score*	13	16	17	18	21	
Number of cases	178	129	74	103	99	
Person-years	195,218	192,447	114,678	215,627	207,078	
Hazard Ratio (95% CI)						
Risk score <sup>a</sup>	1.0 (REF)	0.67 (0.53, 0.84)	0.67 (0.51, 0.88)	0.49 (0.38, 0.62)	0.50 (0.39, 0.65)	<0.0001
<b>NHS</b>						
Median risk score*	8	10	11	12	13	
Number of cases	107	146	102	74	105	
Person-years	239,458	467,386	345,312	283,633	391,405	
Hazard Ratio (95% CI)						
Risk score <sup>b</sup>	1.0 (REF)	0.68 (0.53, 0.88)	0.65 (0.49, 0.85)	0.56 (0.42, 0.75)	0.60 (0.46, 0.78)	<0.0001

\* At baseline: 1986 for HPFS, 1984 for NHS

<sup>a</sup> Caffeine, physical activity, dietary urate index, flavonoid intake, and family history of PD

<sup>b</sup> Caffeine, physical activity, and family history of PD

<b>Table 2.10</b> Hazard Ratio of PD by quintiles of risk score with anthocyanin as a risk factor for HPFS (men) and NHS (women)						
	Quintiles of risk score					<i>P</i> <sub>trend</sub>
	1	2	3	4	5	
<b>HPFS</b>						
Median risk score*	15	18	20	21	24	
Number of cases	195	97	122	95	74	
Person-years	201,958	173,341	200,617	176,187	172,947	
Hazard Ratio (95% CI)						
Risk score <sup>a</sup>	1.0 (REF)	0.56 (0.44, 0.72)	0.58 (0.46, 0.73)	0.51 (0.40, 0.66)	0.39 (0.30, 0.51)	<0.0001
<b>NHS</b>						
Median risk score*	12	15	16	17	20	
Number of cases	131	142	113	48	100	
Person-years	326,006	356,670	422,475	213,224	408,819	
Hazard Ratio (95% CI)						
Risk score <sup>b</sup>	1.0 (REF)	0.94 (0.74, 1.19)	0.61 (0.47, 0.79)	0.56 (0.40, 0.78)	0.55 (0.42, 0.71)	<0.0001

\* At baseline: 1986 for HPFS, 1984 for NHS

<sup>a</sup> Caffeine, physical activity, dietary urate index, anthocyanin intake, and family history of PD

<sup>b</sup> Caffeine, physical activity, anthocyanin intake, and family history of PD



## **Chapter 3 POLYMORPHISMS IN GLUTAMATE RECEPTOR GENE GRIN2A AND CAFFEINE INTERACTION ON THE RISK OF PARKINSON'S DISEASE**

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## ABSTRACT

**Background:** Caffeine intake has been inversely associated with Parkinson's disease (PD) risk. This relationship may be modified by polymorphisms of GRIN2A, a gene encoding an NMDA-glutamate receptor subunit, and CYP1A2, which is involved in caffeine metabolism, but the results of previous studies have been inconsistent.

**Method:** We examined the interaction of caffeine intake with GRIN2A (rs4998386) and CYP1A2 (rs762551) polymorphisms in influencing PD risk among 829 incident cases of PD and 2,754 matched controls selected among participants in three large prospective ongoing cohorts: The Nurses' Health Study (women aged 30-55 recruited in 1976), the Health Professionals' Follow-up Study (men aged 40 to 75 recruited in 1986), and the Cancer Prevention Study II Nutrition Cohort (men and women aged 50-74 recruited in 1992). Matching factors included cohort, birth year, source of DNA (blood or buccal sample), date of DNA collection, and race. Relative risks (RRs) and corresponding 95% confidence intervals (CI) were estimated using conditional logistic models. Interactions were tested both on the multiplicative scale and on the additive scale; the magnitude of additive interactions was assessed by calculating the relative excess risk due to interaction (RERI) and the attributable proportion due to interaction (AP).

**Results:** Overall, caffeine intake was associated with a lower PD risk (adjusted RR for highest vs lowest tertile = 0.70; 95% CI: 0.57, 0.86;  $p < 0.001$ ). In analyses stratified by GRIN2A-rs4998386 genotype, the inverse association between caffeine and PD appeared to be stronger among individuals homozygous for the C allele (RR comparing the highest to the lowest tertile: 0.66; 95% CI: 0.52 to 0.83;  $p < 0.001$ ), than among carrier the T allele (RR: 0.82; 95% CI: 0.53 to 1.26; ns), but there were no significant interactions between caffeine and GRIN2A in either

the multiplicative or additive scale. We also did not observe significant interactions for CYP1A2-rs762551 and incident PD risk.

**Conclusion:** Our findings do not support the hypothesis of an interaction between the GRIN2A-rs4998386 or CYP1A2-rs762551 polymorphism and caffeine intake in determining PD risk.

## INTRODUCTION

Numerous cohort and case-control studies have suggested a large environmental component in predicting Parkinson's disease (PD) risk.<sup>1,2</sup> Among these determinants, caffeine intake is one of the most well-established protective factors of PD.<sup>3-8</sup> In 2011, Hamza et al. conducted a genome-wide association and interaction study (GWAIS) in Parkinson's disease (PD) with the joint test for each SNP's main effect and its interaction with coffee.<sup>9</sup> The investigators identified rs4998386 (C->T) and 11 neighboring SNPs in the GRIN2A gene to have significant interactions with caffeine on PD risk. This interaction is intriguing, because GRIN2A encodes a subunit of the N-methyl-D-aspartate (NMDA) glutamate receptor that regulates excitatory neurotransmissions in the brain and could thus plausibly influence the course of neurodegeneration leading to PD.<sup>10-12</sup> The results of subsequent studies, however, have failed to confirm this interaction – in an investigation in Sweden a significant interaction was observed in the opposite direction,<sup>13</sup> whereas in a third investigation in a pooled, diverse population from Rochester, Seattle, France, and Denmark, no interactions were found.<sup>14</sup>

Similarly conflicting results were reported on the caffeine-gene interaction with CYP1A2,<sup>15-18</sup> which encodes the cytochrome P450 CYP1A2 enzyme that is responsible for metabolizing over 90% of caffeine into paraxanthine.<sup>19,20</sup> Individuals with the rs762551 A->C SNP have lower CYP1A2 inducibility, rendering slower caffeine metabolism.

Important limitations of previous studies include the uncertain representativeness of the control groups, the inclusion of prevalent cases of PD, insufficient matching and adjustment of confounders, and that caffeine consumption was largely assessed retrospectively.

Therefore, we evaluated the presence of additive and multiplicative interaction between caffeine intake and GRIN2A-rs4998386 and CYP1A2-rs762551 polymorphisms on PD risk in a case-control study nested within three longitudinal cohorts.

## **METHODS**

### ***Study population***

The current study was comprised of participants who provided blood or buccal cell samples from three longitudinal cohorts: the Nurses' Health Study (NHS), the Health Professionals Follow-up Study (HPFS), and the Cancer Prevention Study II Nutrition Survey cohort (CPS-IIN).

In 1976, the NHS enrolled 121,700 female registered nurses of ages 30-55 who returned mailed, self-administered questionnaires regarding lifestyle factors and disease occurrence. In 1986, the HPFS enrolled 51,529 male health professionals of ages 40-75 who returned similar questionnaires as in the NHS; these questionnaires were collected every two years for both cohorts. Nutritional information, including coffee consumption and other caffeinated beverages, was ascertained via validated semi-quantitative food frequency questionnaire (SFFQ) generally every four years for the NHS and HPFS with an approximately 94% overall response rate.<sup>21</sup> Between 1992 and 1993, the CPS-IIN was established as a subgroup of the American Cancer Society CPS-II Cohort, which included 1.2 million American men and women. The CPS-IIN included 184,190 participants (86,404 men and 97,786 women) of ages 50-74 years. Questionnaires were administered in 1992, 1997, and every 2 years thereafter, with the response rate of 90%.<sup>22</sup>

We collected fasting blood samples from 1989-1990 in the NHS (n=32,825), 1993-1995 in the HPFS (n=18,159), and 1998-2001 in the CPS-IIN (n=39,371). Participants who had not provided blood samples were invited to provide buccal cell samples. The invitation package for the HPFS included two cytobrushes (one for each cheek), instructions for use, and a consent form for permission to examine genetic markers of diseases. The invitation package for the NHS contained a small empty cup with a cap, a bottle of Scope mouthwash, instructions for use, and a similar consent form. Since the latter procedure produces a higher DNA yield and greater genotyping success, it was chosen over the cytobrush collection procedure used in the HPFS. The buccal sample collection procedure via mouthwash for the CPS-IIN was very similar to that of NHS. In total, buccal samples were collected from an additional 33,744 women from 2002-2004 in the NHS, 13,979 men from 1993-1995 in the HPFS, and approximately 67,000 individuals from 1998-2001 in the CPS-IIN.

Participants were followed from the return date of the baseline questionnaire until the date of first PD symptoms, date of death, date of the latest completed questionnaire or end of follow-up (NHS: June 1980 - 2012; HPFS: January 1986 - 2012; CPS-IIN: October 1999 - 2009), whichever occurred first.

### ***PD case ascertainment and control selection***

PD cases identified via biennial self-report questionnaires were asked for permission to contact their neurologist to confirm their diagnoses. We then contacted patients' neurologists and requested for them to either return a self-administered diagnostic questionnaire that asked to confirm the case or to send a copy of the patient's medical records. In years prior to 2003, PD cases were considered confirmed if the treating neurologist considered the diagnosis as definite

or probable, the final diagnosis of PD by a neurologist was included in the medical record, or the medical record indicated the presence of at least two out of four cardinal signs of PD (among which, one being resting tremor or bradykinesia) in the absence of features indicating other diagnoses. After 2003, a similar procedure was used to identify confirmed PD case with the exception that the medical records requested from all PD cases were reviewed by a neurologist specializing in movement disorders. We used the diagnosis of the neurologist specializing in movement disorders to determine cases if it differed from that of the original neurologist. Only confirmed cases were included in the analyses.

In NHS and HPFS between 2 and 6 controls who were alive on the date of the case's diagnosis and had never reported a diagnosis of PD were randomly selected and matched to the case; for the CPS-IIN cohort, one control was selected per case. Within each cohort, we matched the controls to cases based on sex, birth year ( $\pm 1$  year), race (white vs. other), source of DNA (blood or buccal smear), fasting status ( $>8$  hours vs. less or unknown) and time of blood draw (in 2 hour intervals) for participants with blood samples.

### ***Caffeine and other covariate assessment***

Information on lifestyle practices, such as smoking status and physical activity was collected biennially by self-report questionnaires for all cohorts. Dietary data, including coffee and caffeine intake, was collected via self-administered SFFQs every four years for the NHS and HPFS. The SFFQs captured average intake pattern of a food and beverages over the past 12 months using nine possible multiple-choice responses for each item's intake frequency, ranging from "never or less than once per month" to "6 or more times per day". Information on coffee



and caffeine was comprehensively assessed on the 1999 FFQ for the CPS-IIN; therefore, we considered 1999 as the study baseline for the CPS-IIN.

We used the U.S. Department of Agriculture food-composition sources to estimate participants' reported average intake of one serving of a caffeinated beverage or food into total daily average intake of caffeine using the following caffeine content values: 137 mg caffeine per cup of coffee, 47 mg of caffeine per cup of non-herbal tea, 46 mg of caffeine per can or bottle of cola beverage, and 7 mg per serving of chocolate.<sup>23</sup> The reproducibility and validity of the FFQs have been previously reported for the NHS,<sup>23</sup> HPFS,<sup>24,25</sup> and the CPS-IIN<sup>26</sup>.

### ***Laboratory analyses***

Genomic DNA was extracted from buffy coat with QIAamp (Qiagen Inc., Chatsworth, CA), which was then genotyped using the TaqMan assay on the ABI PRISM 7900HT Sequence Detection System, a high-throughput real-time PCR system (Applied Biosystems, Foster City, CA).

### ***Statistical analyses***

We tested the Hardy-Weinberg equilibrium (HWE) assumption for both the rs4998386 and rs762551 SNP using a  $\chi^2$  test, comparing the observed to expected genotype frequencies in all of the cohorts. Conditional logistic regression models were used to estimate relative risks (RRs) and corresponding 95% confidence intervals (CIs) to assess the association between each of the exposure categories and PD risk. We adjusted for the matching factors (e.g., year of birth, race, source of DNA) to account for the matched design of our study.

Using cohort-specific distribution of covariates, we dichotomized total caffeine intake (time-updated for the NHS and HPFS; at baseline for the CPS-IIN) as high caffeine intake compared to low caffeine intake at the median intake level among controls (366.0 mg/day for NHS; 156.5 mg/day for HPFS, 80.8 mg/day for CPS II-N). In addition, we used cohort-specific tertiles of caffeine intake, and, in selected analyses, caffeine as a continuous variable. Physical activity was dichotomized at each cohort's respective median levels and smoking status was categorized as never/ever. Because the homozygous TT genotype of the rs4998396 SNP was very rare in all of our cohorts (<1%), the TT and CT genotypes were combined together for the dominant model of inheritance (i.e., CC vs. TC/TT). The exposure categories were composed of the four possible combinations of the GRIN2A SNP genotypes and dichotomized caffeine levels, among which the reference was the category with low caffeine intake and having a TT or CT genotype (category with lowest PD risk). This 2x2 factorial design allowed us to calculate two indices of additive interaction: relative excess risk due to interaction (RERI) and the attributable proportion due to interaction (AP), where an RERI and AP of 0 suggests exact additivity (i.e., no additive interaction), an RERI or AP >0 indicates presence of super-additive interaction, and sub-additive interaction if the RERI or AP <0. We followed the methods outlined by Andersson to calculate the RERI and AP and their 95% CIs in the conditional logistic regression models.<sup>27</sup> To conduct tests of additive interactions, we reversed the coding of both the GRIN2A SNP (i.e., TC/TT vs. CC(ref)) and caffeine categorization (i.e., low vs. high(ref)), as the interpretations of the additive interaction indices are only meaningful for factors with harmful effects.<sup>28</sup> In addition, we performed tests of multiplicative interaction between dichotomized caffeine intake and the GRIN2A SNP genotype on the risk of PD by testing the significance of the product term between the SNP and caffeine intake. Multiplicative interaction was also assessed using

continuous caffeine intake (per additional 100 mg/day) due to the large variation in caffeine intake across cohorts.

We conducted analyses separately for the NHS and HPFS, in addition to combining them to increase power. Because the CPS-IIN lacked sufficient genotyped data, we could not perform cohort-specific analyses for the GRIN2A SNP. Rather, we performed analyses including the CPS-IIN cohort with the health professional cohorts. Since genotyping information for the rs762551 SNP was not available in the CPS-IIN, we performed similar interaction analyses only in the NHS and HPFS using an additive model of inheritance (i.e., per increasing minor allele). We conducted similar analyses with coffee, since it is the main source of caffeine in the cohorts.

## **RESULTS**

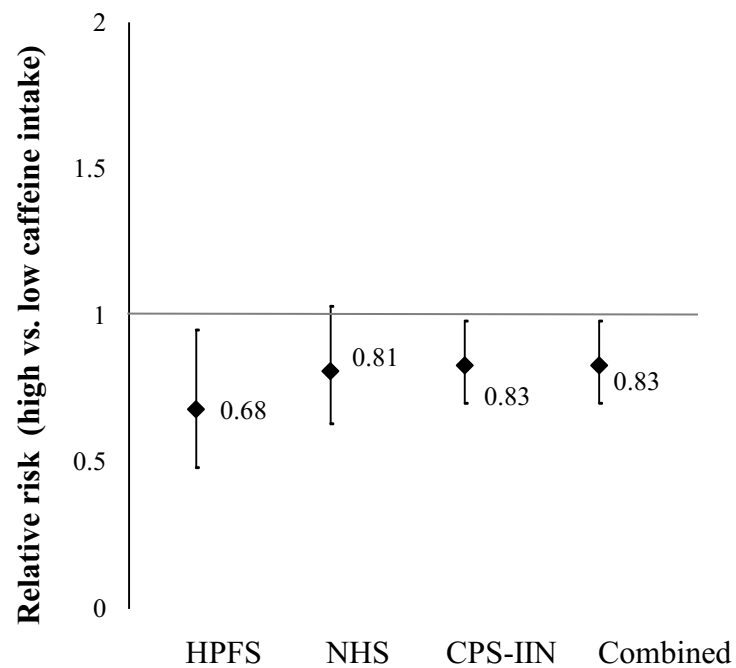
A total of 829 incident PD cases (286 from HPFS; 393 from NHS; 150 from CPS-IIN) were documented and matched with 2,754 controls. The genotype and allele frequencies of the GRIN2A SNP rs4998386 were comparable between cases and controls and the Hardy-Weinberg equilibrium assumption was confirmed in all cohorts ( $p > 0.05$ ) (**Table 3.1**).

**Table 3.1** Genotype and allele frequencies for GRIN2A rs4998386 in Parkinson's disease

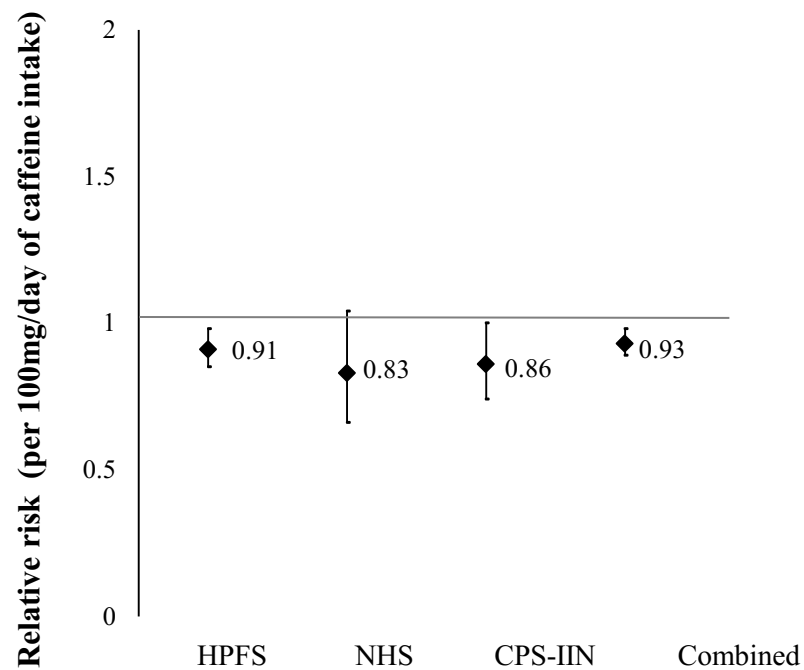
	N	Allele frequency (%)		HWE P-value	Genotype counts (%)			RR (95% CI)	p-value
		C	T		CC	TC	TT		
<b>HPFS</b>									
PD	286	527 (92.1)	45 (7.9)		242 (84.6)	43 (15.0)	1 (0.4)	0.98 (0.68, 1.42)*	0.92
Control	964	1,764 (91.5)	164 (8.5)	0.40	809 (83.9)	146 (15.2)	9 (0.9)	0.95 (0.65, 1.40)**	0.81
<b>NHS</b>									
PD	393	692 (88.0)	94 (12.0)		301 (76.6)	90 (22.9)	2 (0.5)	0.82 (0.63, 1.07)*	0.15
Control	1,625	2,902 (89.3)	348 (10.7)	0.23	1,291 (79.5)	320 (19.7)	14 (0.9)	0.80 (0.61, 1.05)**	0.11
<b>CPS-IIN<sup>†</sup></b>									
PD	150	264 (88.0)	36 (12.0)		114 (76.0)	36 (24.0)	0 (0.0)	0.88 (0.50, 1.56)*	0.66
Control	165	295 (89.4)	35 (10.6)	0.13	130 (78.8)	35 (21.2)	0 (0.0)		
<b>HPFS, NHS, CPS-IIN</b>									
PD	829	1,483 (89.5)	175 (10.6)		657 (79.3)	169 (20.4)	3 (0.4)	0.88 (0.72, 1.07)*	0.20
Control	2,754	4,961 (90.1)	547 (9.9)	0.38	2,230 (81.0)	501 (18.2)	23 (0.8)	0.85 (0.70, 1.05)**	0.13
<p>* Dominant model (CC vs. TC/TT)  ** Model excluding the rs4998386_TT genotype  <sup>†</sup> There were no participants with the TT genotype for CPS-IIN</p>									

Although there was a trend towards a protective effect among homozygous CC carriers, there was no significant association between the genotype frequencies and PD risk under the dominant model in any of the cohorts. In the combined cohorts (NHS, HPFS, and CPS-IIN), participants with high caffeine intake had a 17% reduced risk of PD compared to participants with a low caffeine intake, adjusting for the matching factors (RR= 0.83, 95% CI: 0.70, 0.98;  $p=0.03$ ) (**Figures 3.1a, 3.1b**).

Results of the joint effects of GRIN2A SNP genotype and total caffeine intake in the combined cohorts are presented in **Table 3.2**. Participants with the GRIN2A-rs4998386\_CC genotype and high caffeine intake was associated with a 26% reduced risk compared to referent group with a TC/TT genotype and low caffeine intake (RR= 0.74, 95% CI: 0.56, 0.99;  $p=0.04$ ). In addition, participants with high caffeine intake had 19% reduced risk of PD compared to those with low caffeine intake among CC carriers (RR=0.81, 95% CI: 0.67, 0.97;  $p=0.02$ ). However, these results were attenuated after additional adjustment for smoking status and physical activity. There was no sufficient evidence for additive (RERI= -0.11; 95% CI:-0.60, 0.39;  $p_{\text{RERI}}=0.68$ ; AP= -0.08; 95%CI: -0.48, 0.31;  $p_{\text{AP}}= 0.69$ ) or multiplicative interaction ( $p_{\text{mult}}= 0.61$ ). Similarly, no significant multiplicative interactions were found between rs4998386 genotype and caffeine intake as a continuous variable (per 100mg/day) (HR=0.92;  $p_{\text{mult}}= 0.15$ ).



**Figure 3.1a** Relative risk of PD comparing high vs. low caffeine intake, adjusting for matching factors (i.e., year of birth, race/ethnicity, month and year of DNA collection (blood draw or buccal cell collection)).



**Figure 3.2b** Relative risk of PD per 100mg/day of caffeine intake, adjusting for matching factors (i.e., year of birth, race/ethnicity, month and year of DNA collection (blood draw or buccal cell collection)).

**Table 3.2** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake on the risk of PD in the NHS, HPFS, and CPS-IIN

		GRIN2A SNP genotype				ORs (95%CI) for genotype within strata of caffeine
		CT/TT		CC		
		N cases/controls	OR (95%CI)	N cases/controls	OR (95%CI)	
Caffeine level	Low	94/258	1.0 (REF)	385/1,117	0.92 (0.70, 1.21)	0.92 (0.70, 1.21)
	High	78/266	0.91 (0.63, 1.30)	272/1,113	0.74 (0.56, 0.99)	0.82 (0.61, 1.10)
ORs (95%CI) for caffeine within strata			0.91 (0.63, 1.30)		0.81 (0.67, 0.97)	
of genotype			P=0.60		P=0.02	
Measure of interaction on additive scale:						
RERI (95%CI)					-0.13 (-0.63, 0.37)	P=0.61
AP (95% CI)					-0.10 (-0.48, 0.29)	P=0.62
Measure of interaction on multiplicative scale: Ratio of ORs (95%CI)						
High/low caffeine*GRIN2A					0.89 (0.59, 1.33)	P= 0.57
Per 100 mg/day*GRIN2A					0.92 (0.82, 1.03)	P= 0.13
RRs are adjusted for matching factors only (i.e., year of birth, race/ethnicity, month and year of DNA collection (blood draw or buccal cell collection)).						

When assessing caffeine intake in tertiles, we found that CC carriers with the highest tertile of caffeine intake had 0.60 times the PD risk compared to TC/TT carriers with the lowest tertile of caffeine intake (95% CI: 0.42, 0.86;  $p < 0.01$ ) (**Table 3.3**). Furthermore, among CC carriers, participants with a higher tertiles of caffeine consumption had a significantly decreased risk of PD compared to participants who had the lowest tertile of caffeine consumption ( $RR_{2vs1} = 0.77$ ; 95% CI: 0.62, 0.96;  $p = 0.02$ ;  $RR_{3vs1} = 0.66$ ; 95%CI: 0.52, 0.83;  $p < 0.001$ ). However, neither additive interaction nor multiplicative interaction was detected. Further adjustment for smoking status and physical activity did not change results.

In addition to assessing potential interaction of GRIN2A rs4998386 polymorphism and caffeine intake, we tested the caffeine interaction with the CYP1A2 rs762551 polymorphism on PD risk. No significant additive or multiplicative interactions were found (**Table 3.4**).



**Table 3.3** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake in tertiles on the risk of PD in the NHS, HPFS, and CPS-IIN

		Caffeine tertiles						ORs (95%CI) for	ORs (95%CI) for
		1		2		3		2 <sup>nd</sup> tertile of	3 <sup>rd</sup> tertile of
		N cases/ controls	OR (95% CI)	N cases/ controls	OR (95%CI)	N cases/ controls	OR (95%CI)	caffeine intake within strata of genotype	caffeine intake within strata of genotype
GRIN2A SNP genotype	CT/TT	70/159	1.0 (REF)	48/185	0.75 (0.48, 1.17)	54/180	0.82 (0.53, 1.26)	0.75 (0.48, 1.17)	0.82 (0.53, 1.26)
					P= 0.20		P=0.36	P= 0.20	P=0.36
	CC	292/747	0.91 (0.65, 1.27)	198/731	0.70 (0.50, 0.99)	167/752	0.60 (0.42, 0.86)	0.77 (0.62, 0.96)	0.66 (0.52, 0.83)
			P=0.58		P=0.05		P <0.01	P=0.02	P <0.001
ORs (95%CI) for genotype within strata caffeine		0.91 (0.65, 1.27) P=0.58		0.94 (0.65, 1.36) P=0.75		0.74 (0.52, 1.06) P=0.10			
Measure of interaction on additive scale:									
RERI (95%CI)				2 vs. 1: -0.26 (-1.16, 0.64); p=0.57		3 vs. 1: -0.21 (-1.02, 0.60); p=0.61			
AP (95% CI)				2 vs. 1: -0.21 (-0.76, 0.34); p=0.46		3 vs. 1: -0.12 (-0.57, 0.32); p=0.59			
Measure of interaction on multiplicative scale (95%CI)				2 vs. 1: 1.04 (0.63, 1.70); p= 0.89		3 vs. 1: 0.81 (0.50, 1.33); p=0.40			
RRs are adjusted for matching factors only (i.e., year of birth, race/ethnicity, month and year of DNA collection (blood draw or buccal cell collection)).									

**Table 3.4** Interaction between CYP1A2 SNP RS762551 genotype and total caffeine intake on the risk of PD in the NHS and HPFS

		CYP1A2 SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CA/CC		AA		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	84/303	1.0 (REF)	71/285	0.91 (0.63, 1.30)	0.91 (0.63, 1.30)
	High	64/276	0.90 (0.61, 1.32)	47/284	0.65 (0.44, 0.97)	0.72 (0.48, 1.10)
RRs (95%CI) for caffeine within strata of genotype		0.90 (0.61, 1.32)		0.72 (0.47, 1.09)		
		P=0.59		P=0.12		
Measure of interaction on additive scale:						
RERI (95%CI)				-0.20 (-0.95, 0.56)		P= 0.61
AP (95% CI)				-0.12 (-0.60, 0.35)		P= 0.61
Measure of interaction on multiplicative scale (95%CI)						
High/low caffeine*CYP1A2				0.80 (0.46, 1.39)		P= 0.43
Per 100 mg/day*CYP1A2				1.01 (0.86, 1.20)		P= 0.90
RRs are adjusted for matching factors and smoking and physical activity						

## DISCUSSION

A previous GWAIS study has identified a GRIN2A SNP to have a significant interaction with coffee intake. In our present study of three large, prospective cohorts, we found no evidence of an interaction between caffeine intake and GRIN2A SNP rs4998386 in PD risk.

Consistent with the previous studies, the main effect of high caffeine intake compared to low caffeine intake was protective in the HPFS, CPS-IIN, and in the combined cohorts including the HPFS, NHS, and CPS-IIN. High caffeine intake was marginally significant in the NHS cohort; the protective association was most likely attenuated due to effect modification by postmenopausal hormone (PMH) use.<sup>3,6,29</sup> In contrast, we found a potentially protective main effect of the rs4998386\_CC genotype on PD risk in our combined cohorts, particularly in the model excluding the rare TT genotype (CC vs. TC) (**Table 3.1**). Although, these results were not significant, they deviate from those of the original study that reported a trend toward a protective effect of the T allele. In addition, the median caffeine intake among our three cohorts varied widely. Therefore, one possible explanation of our results is that the cohorts were too heterogeneous to be pooled together, which may have masked interaction effects. However, the main effects of the rs4998386\_T allele and caffeine on PD risk were in the same direction in all of our cohorts. In addition, cohort-specific estimates of the main effects of both the presence of T minor allele and caffeine intake have met the acceptable standards for pooling ( $p_{\text{heterogeneity}} > 0.05$ ). Finally, results from sensitivity analyses only including the health professional cohorts (i.e., NHS and HPFS) also support our lack of interaction on the additive or multiplicative scale.

Another explanation for our results is that we may not have had enough power to detect a caffeine-GRIN2A SNP interaction on PD risk. We calculated the power to be 37% for the

combined cohorts, and 28% for the health professional cohorts. Interaction studies generally require substantially larger sample sizes compared to those required to detect a main effect of the same magnitude; this may explain our lack of association in our cohorts, as the Hamza et al. (2011) performed pooled analyses among 2,474 cases and 2,848 controls.<sup>9</sup> Therefore, we additionally modeled caffeine intake linearly to maximize power. The results suggested that there was a trend towards significance of an interaction in the combined cohorts ( $p_{\text{mult}} = 0.15$ ) and in the NHS ( $p_{\text{mult}} = 0.10$ ). Given the potential limitations of our study, our null results should be interpreted cautiously.

Although we were not able to replicate the interaction results, we found that among GRIN2A-rs4998386\_CC carriers, high caffeine consumption was associated with a reduced risk of PD compared to light caffeine consumption, which is in concordance to results reported by both Yamada-Fowler et al and Hamza et al. However, our findings showed this protective effect of caffeine intake was greater among CC carriers, not carriers of GRIN2A\_rs4998386-T allele, corroborating the direction of the interaction effect found by Yamada-Fowler et al., but not Hamza et al. Nonetheless, we did not find statistically significant interaction effects.

Finally, we found that the CYP1A2 rs762551 SNP was associated with an increased risk in the NHS, but not in the HPFS. No interaction was detected between the SNP and caffeine intake on PD risk, which is consistent with studies across multiple cohorts.<sup>15,16,18</sup>

The main strengths of our study include our nested case-control study design with prospectively collected data. Furthermore, we performed interaction analyses on the multiplicative and additive scale, which could provide more insight and lead to a better understanding of the biological mechanism through which multiple factors are involved in

disease pathogenesis. A limitation of our study is the restricted generalizability of our results to other populations, as the participants of our cohorts are predominantly European descent. However, our homogenous population is instrumental in reducing the risk of confounding by population stratification bias.

In conclusion, our results do not suggest that caffeine consumption and GRIN2A-rs4998386 SNP interact to affect PD risk in our three longitudinal cohorts. In addition, there was no evidence for an interaction between caffeine intake and the CYP1A2-726551 polymorphism. Further investigations are warranted in different populations to determine the presence of an interaction between caffeine and GRIN2A-4998386 SNP on PD risk.

## **ACKNOWLEDGEMENTS**

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## APPENDIX

**Table 3.5** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake in tertiles on the risk of PD in the NHS, HPFS, and CPS-IIN

		Caffeine tertiles						RRs (95%CI) for 2 <sup>nd</sup> tertile of caffeine intake within strata of genotype	RRs (95%CI) for 3 <sup>rd</sup> tertile of caffeine intake within strata of genotype
		1		2		3			
		N cases/ controls	OR (95%CI)	N cases/ controls	OR (95%CI)	N cases/ controls	OR (95%CI)		
GRIN2A	CT/TT	70/159	1.0 (REF)	48/185	0.78 (0.50, 1.22)	54/180	0.85 (0.55, 1.32)	0.78 (0.50, 1.22)	0.85 (0.55, 1.32)
SNP					P=0.27		P=0.47	P=0.27	P=0.47
genotype	CC	292/747	0.92 (0.66, 1.28)	198/731	0.73 (0.52, 1.03)	167/752	0.64 (0.45, 0.91)	0.79 (0.64, 0.99)	0.69 (0.55, 0.88)
			P=0.62		P=0.07		P=0.01	P=0.04	P <0.01
RRs (95%CI) for		0.92 (0.66, 1.28)		0.94 (0.65, 1.35)		0.75 (0.52, 1.08)			
genotype within		P=0.62		P=0.72		P=0.12			
strata caffeine									
Measure of interaction on additive scale:									
	RERI (95%CI)			2 vs. 1: -0.26 (-0.91, 0.40); p=0.44		3 vs. 1: -0.21 (-0.73, 0.31); p=0.43			
	AP (95% CI)			2 vs. 1: -0.21 (-0.78, 0.36); p=0.47		3 vs. 1: -0.13 (-0.60, 0.34); p=0.58			
	Measure of interaction on multiplicative scale			2 vs. 1: 1.02 (0.62, 1.67); p=0.94		3 vs. 1: 0.82 (0.50, 1.34); p=0.42			
	(95%CI)								
RRs are adjusted for matching factors, smoking, and physical activity									

**Table 3.6** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake on the risk of PD in the NHS

		GRIN2A SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CT/TT		CC		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	42/144	1.0 (REF)	152/541	0.87 (0.60, 1.26)	0.87 (0.60, 1.26)
					P=0.46	P= 0.46
	High	37/139	0.92 (0.57, 1.47)	114/555	0.70 (0.48, 1.02)	0.76 (0.51, 1.13)
			P=0.72		P=0.06	P=0.17
RRs (95%CI) for caffeine within strata			0.92 (0.57, 1.47)		0.80 (0.62, 1.04)	
of genotype			P=0.72		P=0.09	
Measure of interaction on additive scale:						
RERI (95%CI)					-0.11 (-0.83, 0.60)	P = 0.76
AP (95% CI)					-0.08 (-0.54, 0.38)	P = 0.76
Measure of interaction on multiplicative scale (95% CI)						
High/low caffeine*GRIN2A					0.87 (0.51, 1.49)	P = 0.62
Per 100 mg/day*GRIN2A					0.92 (0.82, 1.05)	P= 0.22
RRs are adjusted for matching factors only (i.e., year of birth, race/ethnicity, month and year of DNA collection (blood draw or cheek collection)).						

**Table 3.7** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake on the risk of PD in the NHS

		GRIN2A SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CT/TT		CC		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	42/144	1.0 (REF)	152/541	0.99 (0.66, 1.48)	0.99 (0.66, 1.48)
					P= 0.95	P=0.95
	High	37/139	1.10 (0.65, 1.87)	114/555	0.77 (0.51, 1.18)	0.70 (0.45, 1.09)
			P=0.72		P=0.23	P=0.11
RRs (95%CI) for caffeine within strata			1.10 (0.65, 1.87)		0.78 (0.60, 1.03)	
of genotype			P=0.72		P=0.08	
Measure of interaction on additive scale:						
RERI (95%CI)				-0.38 (-1.19, 0.43)		P = 0.36
AP (95% CI)				-0.29 (-0.96, 0.38)		P = 0.40
Measure of interaction on multiplicative scale (95%CI)						
High/low caffeine*GRIN2A				0.71 (0.39, 1.29)		P=0.26
Per 100 mg/day*GRIN2A				0.89 (0.77, 1.02)		P= 0.10
RRs are adjusted for matching factors, smoking, and physical activity						

**Table 3.8:** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake on the risk of PD in the HPFS

		GRIN2A SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CT/TT		CC		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	27/77	1.0 (REF)	144/417	0.94 (0.59, 1.50)	0.94 (0.59, 1.50)
	High	16/59	0.61 (0.30, 1.26)	80/303	0.64 (0.38, 1.07)	1.04 (0.56, 1.91)
					P=0.80	P=0.80
			P=0.18		P=0.09	P=0.90
RRs (95%CI) for caffeine within strata			0.61 (0.30, 1.25)		0.68 (0.48, 0.96)	
of genotype			P=0.18		P=0.03	
Measure of interaction on additive scale:						
RERI (95%CI)				0.28 (-0.62, 1.19)		P= 0.54
AP (95% CI)				0.18 (-0.34, 0.69)		P= 0.50
Measure of interaction on multiplicative scale (95% CI)						
High/low caffeine*GRIN2A				1.10 (0.51, 2.37)		P=0.80
Per 100 mg/day*GRIN2A				1.07 (0.89, 1.28)		P= 0.49
RRs are adjusted for matching factors only (i.e., year of birth, race/ethnicity, month and year of DNA collection (blood draw or cheek collection)).						

**Table 3.9** Interaction between GRIN2A SNP RS4998386 genotype and total caffeine intake on the risk of PD in the HPFS

		GRIN2A SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CT/TT		CC		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	27/77	1.0 (REF)	144/417	0.96 (0.59, 1.57)	0.96 (0.59, 1.57)
	High	16/59	0.76 (0.36, 1.59)	80/303	0.67 (0.39, 1.17)	0.89 (0.47, 1.68)
RRs (95%CI) for caffeine within strata of genotype			0.76 (0.36, 1.59)		0.70 (0.47, 1.02)	
			P=0.46		P=0.16	P=0.88
						P=0.88
Measure of interaction on additive scale:						
	RERI (95%CI)				0.07 (-0.85, 1.00)	P=0.88
	AP (95% CI)				0.05 (-0.60, 0.70)	P=0.87
Measure of interaction on multiplicative scale (95%CI)						
	High/low caffeine*GRIN2A				0.92 (0.41, 2.06)	P=0.84
	Per 100 mg/day*GRIN2A				1.04 (0.86, 1.26)	P= 0.68
RRs are adjusted for matching factors, smoking, and physical activity						



<b>Table 3.10</b> Genotype and allele frequencies for CYP1A2-rs762551 in Parkinson's disease									
	N	Allele frequency (%)		HWE P-value	Genotype counts (%)			RR (95% CI)*	p-value
		A	C		AA	AC	CC		
<b>HPFS</b>									
PD	124	183 (73.8)	65 (26.2)		66 (53.2)	51 (41.1)	7 (5.7)	0.89 (0.65, 1.22)	0.47
Control	496	713 (71.9)	279 (28.1)	0.47	253 (51.0)	207 (41.7)	36 (7.3)		
<b>NHS</b>									
PD	145	185 (63.8)	105 (36.2)		55 (37.9)	75 (51.7)	15 (10.3)	1.42 (1.07, 1.89)	0.02
Control	663	939 (70.8)	387 (29.2)	0.05	322 (48.6)	295 (44.5)	46 (6.9)		
<b>HPFS and NHS</b>									
PD	269	368 (68.4)	170 (31.6)		121 (45.0)	126 (46.8)	22 (8.2)	1.15 (0.93, 1.41)	0.21
Control	1159	1652 (71.3)	666 (28.7)	0.05	575 (49.6)	502 (43.3)	82 (7.1)		
* With each increasing minor allele									

**Table 3.11** Interaction between CYP1A2 SNP RS762551 genotype and total caffeine intake on the risk of PD in the NHS

		CYP1A2 SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CA/CC		AA		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	52/177	1.0 (REF)	35/155	0.75 (0.44, 1.28)	0.75 (0.43, 1.28)
					P=0.29	P=0.29
	High	39/165	1.03 (0.62, 1.72)	20/163	0.44 (0.24, 0.81)	0.43 (0.23, 0.80)
			P=0.92		P<0.01	P<0.01
RRs (95%CI) for caffeine within strata			1.03 (0.62, 1.72)		0.59 (0.31, 1.13)	
of genotype			P=0.92		P=0.11	
Measure of interaction on additive scale:						
RERI (95%CI)				-0.80 (-2.46, 0.87)		P= 0.35
AP (95% CI)				-0.35 (-1.07, 0.37)		P = 0.34
Measure of interaction on multiplicative scale (95%CI)						
High/low caffeine* CYP1A2				0.57 (0.25, 1.32)		p = 0.19
Per 100 mg/day* CYP1A2				0.96 (0.82, 1.13)		P= 0.63
RRs are adjusted for matching factors and smoking and physical activity						

**Table 3.12** Interaction between CYP1A2 SNP RS762551 genotype and total caffeine intake on the risk of PD in the HPFS

		CYP1A2 SNP genotype				RRs (95%CI) for genotype within strata of caffeine
		CA/CC		AA		
		N cases/controls	RRs (95%CI)	N cases/controls	RRs (95%CI)	
Caffeine level	Low	40/155	1.0 (REF)	43/151	1.01 (0.62, 1.65)	1.01 (0.62, 1.65)
	High	13/63	0.75 (0.36, 1.57)	18/74	0.79 (0.40, 1.57)	1.06 (0.58, 2.38)
		RRs (95%CI) for caffeine within strata of genotype		RRs (95%CI) for caffeine within strata of genotype		
		0.75 (0.35, 1.57)		0.78 (0.62, 1.65)		
		P=0.44		P=0.50		
Measure of interaction on additive scale:						
		RERI (95%CI)		-0.26 (-1.24, 0.72)		P=0.60
		AP (95% CI)		-0.24 (-1.15, 0.66)		P=0.60
Measure of interaction on multiplicative scale (95%CI)						
		High/low caffeine* CYP1A2		1.05 (0.41, 2.70)		p= 0.92
		Per 100 mg/day* CYP1A2		1.03 (0.83, 1.28)		P= 0.79
RRs are adjusted for matching factors and smoking and physical activity						