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Interaction of folate intake and the paraoxonase Q192R polymorphism with risk of incident coronary heart disease and ischemic stroke: the Atherosclerosis Risk in Communities Study

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Abstract

Purpose—To investigate the potential interaction between folate intake and the PON1 Q192R polymorphism with the risk of incident CHD and ischemic stroke in the ARIC study – a population-based prospective cohort of cardiovascular disease in 15,792 whites and African Americans.

Methods—Race-stratified Cox proportional hazards models were performed to examine the interaction between folate intake and the PON1 Q192R polymorphism.

Results—A significant inverse association between folate intake and risk of incident CHD among whites was found (HRR=1.30, 95% CI: 1.09, 1.56; *P*=0.004; folate intake ≤155 μg vs. ≥279 μg- reference group). An interaction effect was observed between the dominant genetic model and folate intake with regards to incident ischemic stroke in whites (HRR=0.68, 0.91, 0.99, and 1.24 from 1st-4th quartile, respectively; *P*-trend=0.05).

Conclusions—There was an interaction between folate intake and PON1 Q192 polymorphism with regard to the risk of ischemic stroke in whites. Future studies should investigate the interaction between additional polymorphisms within the PON1 gene and genetic variants in other folate metabolizing genes with folate intake on the risk of incident CHD and stroke.

Keywords

Coronary Heart Disease; Folate; Incidence; Genetic Polymorphism; Stroke

INTRODUCTION

Coronary heart disease (CHD) and stroke are two major causes of death worldwide. Death cases from CHD and stroke account for 13% and 10%, respectively, of total deaths

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throughout the world (1). Conventional risk factors studied with regard to CHD and stroke risk include hypertension, smoking and diabetes, but increasing attention has been placed upon studies of nutrition (e.g., folate intake) and genetic variation as risk factors and independent predictors of CHD and stroke.

The role of folate, through its influence on homocysteine, with regards to the risk of CHD has been extensively studied. Homocysteine (Hcy) is a sulfur-containing amino acid formed during normal metabolism of the essential amino acid methionine. Defects in intracellular Hcy metabolism lead to an elevation of plasma Hcy and may have a genetic or nutritional background (2). In 1969 and 1975, McCully et al. (3, 4) developed the Hcy theory in which they formulated that due to an inborn metabolic error leading to homocystinuria, patients with elevated Hcy concentrations both in plasma and urine would be at risk for occlusive vascular disease in early adulthood or even childhood. In early 2000, Ueland et al. (5) reported that the estimated odds ratio for CHD for a 5-μmol/L increase in Hcy was 1.20 (95% CI: 1.14, 1.25). This observation was consistent with additional longitudinal studies (6–8). Hcy levels are influenced by genetic factors (9) and dietary factors, including folate, vitamin B_6 and B_{12} intake (10, 11). Previous studies in Europe and the United States showed folate intake inversely associated with CHD and stroke (8, 12–18). However, these associations have not been successfully replicated in other populations (19, 20). The potential mechanism by which folate may be involved in cardiovascular disease is through its Hcy-lowering effect as a substrate in the remethylation of Hcy to methionine (3, 4).

In human plasma, Hcy exists in different molecular forms, including toxic Hcy thiolactone, which spontaneously modifies protein lysine residues in a process called N-homocysteinylation (21). Human serum PON1 carried on high density lipoprotein (HDL) has the ability to detoxify Hcy thiolactone due to its intrinsic Hcy-thiolactonase activity (22), which prevents protein N-homocysteinylation in vitro (23) and in vivo (24). Recently, Jakubowski et al. (24, 25) showed that genetic or nutritional disorders in Hcy or folate metabolisim would elevate plasma Hcy-thiolactone and lead to vascular and/or brain pathologies. Specifically, they found these disorders to increase *N*-Hcy-protein levels and that there was an inverse relationship between plasma *N*-Hcy-protein and serum Hcy-thiolactonase activity (25). They also found a weak or no association between enzymatic activities of PON1 protein and plasma *N*-Hcy-protein (24). Therefore, they concluded that the Hcy-thiolactonase activity of PON1 is a determinant of plasma *N*-Hcy-protein levels and that Hcy-thiolactonase/PON1 protects proteins against N-homocysteinylation.

Human serum PON is an antioxidative enzyme present in HDL which eliminates oxidative radicals in the circulation and protects against coronary diseases (26). The association between PON1 polymorphism and the risks of CHD and/or stroke is found to be inconsistent. While the Q192R polymorphism has been found to be associated with CHD and stroke in some populations (i.e., European, North American, Japanese and South Asian) (27–35) these associations could not be replicated in other populations (i.e., Scandinavian) (36, 37). In fact, results from the British Women's Heart and Health cohort study and a meta-analysis from the same study (38) showed no association between PON1 Q192R polymorphism with CHD risk in Caucasian women or men. Recently, Dahabreh et al. (39) reported an association between PON1 polymorphism and ischemic stroke (pool OR=1.10, 95% CI: 1.04, 1.17).

While the independent association between folate intake and the risk of CHD and/or stroke have been established and there has been existed controversies in the independent relationship between PON1 Q192R polymorphism and the risk of CHD and/or stroke, their combination effect (i.e., interaction) on CHD and stroke has not been studied. The current study investigated whether folate intake and the PON1 Q192R polymorphism interacts to

modify the risk of CHD and ischemic stroke in the Atherosclerosis Risk in Communities Study (ARIC).

METHODS

Study population

ARIC is a prospective cohort study of atherosclerosis and its clinical sequelae involving 15,792 individuals aged 45 to 64 years at recruitment (1987–1989). Institutional review boards approved the ARIC study, and all participants provided their written informed consent. A detailed description of the ARIC study design and methods was published elsewhere (40–42). Briefly, subjects were selected by probability sampling from four communities: Forsyth County, North Carolina; Jackson, Mississippi; northwestern suburbs of Minneapolis, Minnesota; and Washington County, Maryland. Incidence of CHD and ischemic stroke were determined by contacting participants annually to identify hospitalizations during the previous year and by surveying discharge lists from local hospitals and death certificates from state vital statistics offices for potential cardiovascular and cerebrovascular events (40–42). Incident CHD cases were defined as a definite or probable myocardial infarction (MI), a silent MI between examinations by ECG, a definite CHD death, or a coronary revascularization. Ischemic stroke cases were defined as validated definite or probable hospitalized embolic or thrombotic brain infarctions.

Participants were excluded from analyses (n=2,272) if they 1) prohibited use of their DNA for research purposes, 2) had an ethnic background other than white or African-American, 3) had a positive or unknown history of prevalent CHD or stroke at baseline, 4) were missing dietary data information >10 items in the Food Frequency Questionnaire (FFQ), or 5) were missing genotype information for the PON1 Q192R polymorphism. Following exclusions, a total sample of 13,520 participants (including 1,469 incident cases of CHD and 594 incident cases of ischemic stroke) were available for analysis.

Baseline examination and laboratory measures

Body mass index (kg/m^2) was calculated from height and weight measurements. Plasma total cholesterol was measured by an enzymatic method (43). HDL cholesterol was measured after dextran-magnesium precipitation of non-HDL lipoproteins (44). Seated blood pressure was measured three times using a random-zero sphygmomanometer and the last two measurements were averaged. Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mmHg or diastolic blood pressure (DBP) ≥ 90 mmHg or self-reported use of antihypertensive medications 2 weeks before the baseline exam (45). Diabetes was defined as a fasting glucose level ≥ 126 mg/dL, a non-fasting glucose level ≥ 200 mg/dL and/or a history of or treatment for diabetes (46). For smoking status, current smokers were compared to former and/or never smokers.

Usual dietary intake was assessed with the use of a 66-item, interviewer-administered, semi-quantitative food-frequency questionnaire (FFQ) administered at the baseline examination. The questionnaire was a modified version of the 61-item FFQ designed and validated by Willett et al. (47). Participants were asked to report the frequency of consumption of each food/beverage on the basis of nine categories, ranging from never or <1 time/month to ≥ 6 times/day. Additional information such as brand name of breakfast cereal most commonly consumed (open-ended response), type of fat used in frying and baking (butter, margarine, vegetable oil, vegetable shortening, lard), and amount of salt used in cooking were also ascertained. Intake of nutrients (including folate) was calculated by multiplying the daily serving of each food item by its nutrient content. Nutrient content of food items were primarily taken from US Department of Agriculture (48). Total caloric intake was

determined by Willett's nutrient coding and adding calories from ethanol. Ethanol intake was usually measured as gram/week and then converted to kcal/day using a scale of 1 gram ethanol equals 7kcal and 1 week equals 7 days. Total caloric intake variable did not include non-ethanol calories from alcoholic beverages (49).

Genotype determination

Genotyping of the Paraoxanase 1 (PON1) Q192R polymorphism was performed using the TaqMan assay (Applied Biosystems). A 82-bp product was amplified utilizing 0.9 μM each of the forward primer 5′-GCACTTTTATGGCACAAATGATCA-3′ and the reverse primer 5′-CGACCACGCTAAACCCAAA-3′, 50 ng DNA, 5.0 mM MgCl2, and 1X TaqMan Universal PCR Master Mix containing AmpliTaq Gold DNA Polymerase in a 22 μl reaction volume. After an initial step of 2 min at 50°C and 10 min at 95°C to activate the AmpliTaq Gold, the products were amplified using 40 cycles of 15 s at 95°C and 1 min at 60°C. A total of 0.2 μM of each of the sequence-specific probes 5′-6FAM-TGACCCCTACTTACAATCCTGGGAGATGT- TAMRA-3′ and 5′-VIC-TGACCCCTACTTACGATCCTGGGAGAT-TAMRA-3′ was used in the allele discrimination assay, and allele detection and genotype calling were performed using the ABI 7700 and the Sequence Detection System software (Applied Biosystems).

Statistical analysis

All statistical analyses were conducted utilizing STATA 11.1 (College Station, TX). Allele frequencies were estimated by gene counting. Hardy-Weinberg equilibrium expectations were tested using a χ^2 goodness-of-fit test. In the analysis of baseline characteristics, folate intake was treated as a continuous variable. Then, in further analysis of Cox proportional hazard models folate intake was categorized using quartile scale (1^{st} quartile: 0-155μg; 2^{nd} quartile: 156-211μg; 3^{rd} quartile: 212-278μg; and 4^{th} quartile: ≥279μg). Proportions, means and standard errors of the mean (SEM) of baseline measurements were calculated, and the student t-test or log-rank test were used to assess differences between whites and African Americans. Because PON1 allele frequencies were different between whites and African Americans, all analyses were performed separately by race. The variant allele (192R) was identified as the low frequency allele in whites, and the homozygous non-variant genotype (192QQ) was designated as the referent group in the statistical analyses (i.e., dominant, recessive, and per allele model). Cox proportional hazards (PH) models were used to estimate the hazard rate ratios (HRRs) for incident CHD and stroke. For analyses of incident CHD and stroke, follow-up time intervals were defined as the time between the initial clinical visit and the date of the first event. For non-cases, follow-up continued until December 31, 2005, the date of death, or the date of last contact if lost to follow-up, whichever came first. For analyses of incident CHD the covariates included age, gender, BMI, HDL and total cholesterol, smoking, diabetes, total caloric intake, and hypertension status. For stroke, the covariates (identified by the National Institute of Neurological Disorders and Stroke at www.ninds.nih.gov) included age, gender, smoking, diabetes, total caloric intake, and hypertension status. Covariates were assessed for statistical significance in the models by the Wald χ^2 statistic.

The main effect of PON1 polymorphism on CHD and stroke (independently), as well as the main effect of folate on CHD and stroke (independently) was analyzed first. The independent association between PON1 polymorphism and CHD as well as stroke was examined in genetic models of dominant, recessive, and per allele. To determine if there was an interaction between folate intake and the PON1 Q192R polymorphism with regard to risk of incident CHD and ischemic stroke, an interaction term was included in the model. If a significant interaction was detected, stratified analyses were performed by folate intake and

PON1 polymorphism. All tests were two-sided with significance defined by a *P*-value ≤ 0.05.

RESULTS

SNP genotype frequencies were in Hardy-Weinberg equilibrium. Mean length of follow-up was 16.5 years. Race-specific baseline characteristics of the study population are presented in Table 1. African Americans were significantly older, had a significantly higher BMI and significantly higher HDL cholesterol levels compared to whites. A significantly higher proportion of African Americans were hypertensive, diabetic and current smokers compared to whites. Whites were more likely to have incident CHD compared to African Americans (13.1% vs. 11.2%, respectively), while African Americans were more likely to have incident ischemic stroke (6.8% vs. 3.0%, respectively). The PON1 192Q allele was more commonly observed in whites (frequency (Q) = 0.71) than African Americans (frequency (Q)=0.34). There was no difference in PON1 Q192R polymorphism frequencies between incident CHD cases and non-cases or between incident ischemic stroke cases and non-cases, in whites or African Americans (Table 2).

Associations between incident CHD and ischemic stroke with folate intake and PON1 Q192R polymorphism by race are presented in Table 3. A significant inverse association between folate intake and risk of incident CHD was observed in whites (*P*-trend=0.003), such that persons with a folate intake < 155 μg were 30% more likely to be at risk for a CHD event compared to persons with a folate intake ≥ 279 μg (HRR=1.30, 95% CI: 1.09, *P*=0.004). A1.56; similar inverse relationship between folate intake and incident CHD risk was observed in African Americans (*P*-trend=0.02), but this finding was not statistically significant (HRR=1.36, 95% CI: 0.99, 1.88; *P*= 0.06). Even though there was a dose-response between folate intake and the risk of ischemic stroke in whites, no significant associations were observed in either whites or African Americans. No significant association was observed between the PON1 Q192R polymorphism and the risk of either incident CHD or stroke in whites or African Americans. However, there was a marginal association, when considering a recessive genetic model, such that the RR genotype was associated with risk of incident CHD in whites (HRR=1.18, 95% CI: 0.99, 1.40; *P*=0.06).

In stratified analysis (Table 4), a significant interaction was observed only when considering a dominant genetic model and folate intake with the risk of incident ischemic stroke in whites. The HRRs for this analysis, from 1st to 4th quartiles, were 0.68, 0.91, 0.99, and 1.24, respectively (*P*-trend = 0.05).

DISCUSSION

In this study, we found that ARIC white participants consuming lower levels of folate (<155μg) were at an increased risk of CHD, while PON1 genotype was not associated with either incident CHD or ischemic stroke in whites or African Americans. In a dominant genetic model, we found an interaction between folate intake and PON1 genotype with regard to incident ischemic stroke in whites.

The frequency of the PON1 RR genotype in African-Americans was 5 times higher than those in Caucasians (43.2% vs. 8.5%, respectively). This finding is consistent with results reported by Jakubowski et al. (39.4% vs. 2.9%) (23). We found an inverse dose-response relationship between folate intake and incident CHD in both whites and African Americans, as well as with incident ischemic stroke in whites only. However, we only found a significant relationship between whites consuming ≤155μg folate and incident CHD risk. The association between African Americans consuming ≤155μg folate and incident CHD

risk was marginally significant (HRR=1.36, 95% CI: 0.99, 1.88; *P*= 0.06). While the frequency of African Americans is one-third that of whites and the HRR is comparable (1.36 vs. 1.30, respectively), it would be interesting to examine this association in a larger sample size of African Americans. Also, there appears to be a marginal association between PON1 and CHD in whites when considering a recessive model (HRR=1.18, 95% CI: 0.99, 1.40; *P*=0.06).

The inverse association between folate intake and risk of incident CHD in whites observed in our analysis is consistent with findings from other studies (12, 14–18). The National Health Examination Survey I Epidemiologic Follow-up Study (16) found an inverse relationship between folate intake and subsequent risk of stroke and cardiovascular disease (RR=0.79, 95% CI: 0.63, 0.99; *P*=0.03 and 0.86, 95% CI: 0.78, 0.95; *P*<0.001, respectively).

To our knowledge, our findings are the first to show an interaction between folate intake and PON1 Q192 polymorphism with regard to the risk of incident ischemic stroke. In our white population, there was a statistically significant linear trend for the risk of incident ischemic stroke as folate intake increased (HRR = 0.68, 0.91, 0.99 and 1.24 from 1st to 4th quartile, respectively; *P*-trend=0.05). This finding is important because the inconsistent findings from previous studies (27–37) might be due to a lack of consideration for interaction between genetic factors (e.g., PON1 Q192 polymorphism) and environmental factors (i.e., folate intake). If the power of this study is large enough, we would expect to see an interaction effect between the recessive model and folate intake in the association with incident ischemic stroke.

Recently, Agrawal et al. (33) found the PON1 RR genotype to be significantly associated with coronary artery disease (CAD) in a north Indian population, with the risk more pronounced among smokers (smokers: OR = 2.84, 95% CI: 1.40, 5.78; non-smokers: OR=1.31; 95% CI: 0.66, 2.63). The authors suggested that an interaction between PON1 genotype and smoking may impact the risk of CAD in this population. They postulated that the PON1 192R allele may have a more exaggerated effect for those living in a high risk environment of enhanced oxidative stress, such as smokers. *In vivo* studies have provided evidence that folate deficiency increases lipid peroxidation and decreases cellular antioxidant defense (50, 51). Vehaar et al. (2) hypothesized that folate intake might interact with endothelial nitric oxide synthase to affect cardiovascular risk. Also, elevated Hcy levels were found to be associated with cardiovascular disease (52–54). If folate intake and Hcy are independently associated with the risk of cardiovascular disease, one might investigate the interaction between Hcy levels and PON1 genotype with regards to cardiovascular disease risk.

Strengths of our study include its large sample size that comprises both whites and African Americans, as well as its careful assessment of cardiovascular events and risk factors. One limitation to our study is that we only examined a single polymorphism in the PON1 gene. Additionally, the nature of the ARIC food frequency questionnaire may have limited our ability to measure folate intake accurately. This might lead to an attenuation of a true association due to random measurement error. One important note is that the timeframe in our analysis was from baseline (1987–1989) until December 31, 2005, therefore spanning times both before and after the 1996 mandatory implementation of the folic acid fortification program in the US (55). For that reason we considered no lead time bias in our analysis.

In summary, we observed a significant inverse association between folate intake and risk of incident CHD such that whites consuming lower levels of folate (≤155μg) were at an increased risk of incident CHD compared to whites consuming higher levels of folate (≥279

μg). We also found an interaction between the dominant genetic model and folate intake with regard to the risk of ischemic stroke in whites. Further studies are warranted to investigate additional polymorphisms within the PON1 gene and genetic variation in other folate metabolizing genes that might interact with folate intake to modify the risk of incident CHD and stroke.

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Selected Abbreviations and Acronyms

ARIC	Atherosclerosis Risk in Communities
CHD	Coronary heart disease
PON1	Paraoxonase 1
Hcy	Homocysteine
HDL	high density lipoprotein
MI	myocardial infarction
FFQ	Food Frequency Questionnaire
ECG	electrocardiography
SBP	systolic blood pressure
DBP	diastolic blood pressure
CI	confidence interval
HR	hazard ratio
HRR	hazard rate ratio

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Table 1

Baseline Characteristics of the ARIC Study Population[a]

Characteristic	White (*n*=9,930) Mean (SE)	African American (*n*=3,590) Mean (SE)	*P* value
Age	54.2 (0.06)	53.4 (0.1)	<0.001
Body mass index (kg/m^2)	27.0 (0.05)	29.6 (0.1)	<0.001
Total cholesterol (mg/dl)	214.2 (0.4)	214.5 (0.8)	0.7
HDL (mg/dl)	51.1 (0.2)	55.3 (0.3)	<0.001
Folate intake (μg)	227.3 (1.0)	228.7 (1.8)	0.5
Caloric intake (kcal/day)	1,624.2 (6.0)	1,579.3 (10.3)	<0.001
	n (%)	*n* (%)	
Hypertension	2,530 (25.6)	1,959 (54.8)	<0.001
Diabetes	809 (8.2)	647 (18.3)	<0.001
Current smoker	2,421 (24.4)	1,045 (29.1)	<0.001
Coronary heart disease (CHD)	1,429 (13.1)	440 (11.2)	0.01
Ischemic stroke	327 (3.0)	267 (6.8)	<0.001
PON1 Q192R Polymorphism			
QQ	4,955 (49.9)	419 (11.7)	Reference
QR	4,133 (41.6)	1,618 (45.1)	<0.001
RR	842 (8.5)	1,553 (43.2)	<0.001

Abbreviations: HDL, high density lipoprotein; PON1, Paraoxonase 1; SE, standard error.

[a] *P* values were t-test for continuous variables and log-rank test for categorical variables.

Table 2

PON1 Q192R Polymorphism Frequencies for Incident CHD Cases, Incident Ischemic Stroke Cases and Non-cases

	CHD		
PON1 Q192R genotype	**Incident Cases (*n*, %)**	**Non-cases (*n*, %)**	***P* value***
White			
QQ	719 (50.3)	4236 (49.8)	Ref.
QR	569 (39.8)	3564 (41.9)	0.1
RR	141 (900.9)	701 (8.3)	0.1
African American			
QQ	48 (10.9)	371 (11.8)	Ref.
QR	186 (42.3)	1432 (45.5)	0.3
RR	206 (46.8)	1347 (42.7)	0.3
	Ischemic stroke		
	Incident Cases (*n*, %)	Non-cases (*n*, %)	*P* value*
White			
QQ	168 (51.4)	4787 (49.9)	Ref.
QR	135 (41.3)	3998 (41.6)	0.7
RR	24 (7.3)	818 (8.5)	0.7
African American			
QQ	39(14.6)	380 (11.4)	Ref.
QR	119 (44.6)	1499 (45.1)	0.3
RR	109 (40.8)	1444 (43.5)	0.3

Abbreviations: CHD, Coronary heart disease; PON1, Paraoxonase 1.

**P*-value comparing genotype frequencies between cases and non-cases, with the QQ genotype serving as the referent group.

Table 3

Relationship Between Folate Intake and PON1Q192R Polymorphism with Incident CHD and Incident Ischemic Stroke Case Status, by Racial Group

	Incident CHD[a]		Incident Ischemic Stroke[b]	
	n	**HR (95% CI), *P* value**	***n***	**HR (95% CI), *P* value**
Folate intake (μg)				
White	9,858		9,875	
4th quartile (≥279)		1		1
3rd quartile (212–278)		1.02 (0.88,1.19), 0.76		0.83 (0.59, 1.15), 0.26
2nd quartile (156–211)		1.14 (0.97, 1.34), 0.11		1.31 (0.94, 1.82), 0.10
1st quartile (0–155)		1.30 (1.09, 1.56), 0.004*		1.34 (0.93, 1.95), 0.12
p-trend		0.003		0.004
African American	3,442		3,510	
4th quartile (≥279)		1		1
3rd quartile (212–278)		1.02 (0.77, 1.37), 0.87		1.04 (0.73, 1.48), 0.82
2nd quartile (156–211)		1.27 (0.94, 1.71), 0.12		1.01 (0.69, 1.48), 0.96
1st quartile (0–155)		1.36 (0.99, 1.88), 0.06		1.03 (0.69, 1.57), 0.86
p-trend		0.022		0.98
PON1Q192R Polymorphism				
White	9,858		9,875	
Dominant model				
QQ		1		1
QR or RR		0.96 (0.86, 1.06), 0.43		0.96 (0.77, 1.19), 0.69
Recessive model				
QQ or QR		1		1
RR		1.18 (0.99, 1.40), 0.06		0.82 (0.54, 1.24), 0.35
Genotypic model				
QQ		1		1
QR		0.92 (0.83, 1.03), 0.16		0.99 (0.79, 1.24), 0.91
RR		1.14 (0.95, 1.36), 0.16		0.81 (0.53, 1.25), 0.35
African American	3,442		3,510	
Dominant model				
QQ		1		1
QR or RR		1.08 (0.79, 1.46), 0.63		0.77 (0.55, 1.09), 0.16
Recessive model				
QQ or QR		1		1
RR		1.13 (0.94, 1.37), 0.20		0.90 (0.70, 1.15), 0.40
Genotypic model				
QQ		1		1
QR		1.01 (0.73, 1.40), 0.95		0.80 (0.55, 1.16), 0.24
RR		1.14 (0.83, 1.57), 0.41		0.76 (0.52, 1.10), 0.14

Abbreviations: CHD, Coronary heart disease; CI, confidence interval; HR, hazard ratio, PON1, Paraoxonose 1.

[a] Adjusted for age, gender, BMI, HDL, total cholesterol, current smoking status, diabetes, caloric intake, and hypertension.

[b] Adjusted for age, gender, current smoking status, diabetes, caloric intake, and hypertension.

* Significance at $P<0.05$.

Table 4

Relationship Between Folate Intake and Incident Ischemic Stroke Case Status, by PON1 Q192R Polymorphism among White Participants

Folate intake group (μg)	*n*	PON1 Polymorphism	Incident Stroke[a] HRR (95% CI), *P* value
1st quartile (0–155)	2,420		
		Dominant model	
		QQ	1
		QR or RR	0.68 (0.43, 1.07), 0.10*
		Recessive model	
		QQ or QR	1
		RR	0.81 (0.35, 1.87), 0.62†
		Genotypic model	
		QQ	1
		QR	0.68 (0.42, 1.10), 0.11‡
		RR	0.69 (0.29, 1.62), 0.40‡
2nd quartile (156–211)	2,514		
		Dominant model	
		QQ	1
		QR or RR	0.91 (0.60, 1.39), 0.66*
		Recessive model	
		QQ or QR	1
		RR	1.50 (0.82, 2.76), 0.19†
		Genotypic model	
		QQ	1
		QR	0.81 (0.52, 1.27), 0.37‡
		RR	1.37 (0.73, 2.59), 0.33‡
3rd quartile (212–278)	2,503		
		Dominant model	
		QQ	1
		QR or RR	0.99 (0.62, 1.62), 0.99*
		Recessive model	
		QQ or QR	1
		RR	0.30 (0.07, 1.20), 0.09†
		Genotypic model	
		QQ	1
		QR	1.16 (0.71, 1.90), 0.55‡
		RR	0.31 (0.08, 1.31), 0.11‡
4th quartile (≥279)	2,438		
		Dominant model	

Folate intake group (μg)	*n*	PON1 Polymorphism	Incident Stroke[a] HRR (95% CI), *P* value
		QQ	1
		QR or RR	1.24 (0.82, 1.88), 0.30[*]
		Recessive model	
		QQ or QR	1
		RR	0.55 (0.20, 1.51), 0.25[†]
		Genotypic model	
		QQ	1
		QR	1.36 (0.89, 2.07), 0.15[‡]
		RR	0.64 (0.23, 1.78), 0.39[‡]

Abbreviations: CI, confidence interval; HRR, hazard rate ratio; PON1, Paraoxonase 1.

[a] Adjusted for age, gender, BMI, HDL, total cholesterol, current smoking status, diabetes, caloric intake, and hypertension.

[*] *P*-trend (dominant model): 0.05;

[†] *P*-trend (recessive model): 0.49;

[‡] *P*-trend (genotypic model): 0.41