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ORIGINAL ARTICLE

Association of NOS3 Glu298Asp SNP with hypertension and possible effect modification of dietary fat intake in the ARIC study

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Endothelial nitric oxide synthase breaks down nitric oxide and has a key role in blood pressure regulation. Earlier studies have shown associations between single nucleotide polymorphisms (SNPs) in the NOS3 gene and hypertension. Studies also suggest that such associations may vary by dietary fat intake. We investigated associations between the NOS3 Glu298Asp SNP (rs1799983) and hypertension, as well as the interaction between NOS3 genotypes and dietary fat intake using data from baseline examination in white and African American participants in the Atherosclerosis Risk in Community (ARIC) study. Dietary fat intake was measured by a Food Frequency Questionnaire during the baseline examination in 15 792 individuals aged 45–64 years in ARIC study participants. Race-stratified unconditional logistic regression was performed to investigate the association between prevalent hypertension and NOS3 Glu298Asp genotypes. There was no significant interaction between dietary fat intake and NOS3 Glu298Asp genotype with regards to hypertension status in either African Americans or whites (*P* for interaction=0.3 and 0.4, respectively). We found a significant relationship between NOS3 Glu298Asp and triglycerides in African Americans. We did not find an association between the NOS3 Glu298Asp polymorphism and hypertension, and dietary fat intake did not interact with NOS3 genotypes to influence hypertension. We recommend further exploration of the relationship between NOS3 Glu298Asp and triglycerides in African Americans.
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INTRODUCTION

In the United States, one out of every three persons aged 20 and older has hypertension, with more than 100 million people having either pre-hypertension or definite hypertension.[1,2] Both human and animal studies have shown onset of hypertension because of loss of nitric oxide, which acts as the endothelium-derived relaxing factor.[3] Nitric oxide is catalyzed by endothelial nitric oxide synthase (eNOS), an enzyme with multiple genetic variants that might confer risk for hypertension.[4] Three single nucleotide polymorphisms (SNPs) in the NOS3 gene have been shown to be associated with hypertension, including Glu298Asp, T786C and a VNTR in intron 4.[5] However, inconsistent findings on the relationship between NOS3 polymorphisms and hypertension have been observed in earlier studies, with significant associations reported from Japan[6] and Singapore,[7] but no associations observed in China[8] and Australia.[9] In a recent meta-analysis by Pereira *et al*,[10] the association between the NOS3 Glu298Asp variant and hypertension was shown to be modified by specific dietary factors (for example, dietary saturated fat) and other conditions (for example, hypercholesterolemia). This meta-analysis included subjects from Europe, China and Japan, but did not include subjects from the United States. The most common limitation among these previously reported studies is low power to detect the strength of association because of small sample sizes.

The Atherosclerosis Risk in Community (ARIC) study offers an opportunity to investigate the association between NOS3 genetic variation and hypertension in a large population-based sample of whites and African Americans, with additional analyses of gene–diet interactions. Earlier studies have shown that the impact of n-3 fatty acids on endothelial function depend on NOS3 genotype, with the influence of n-3 fatty acid level being greater in Asp298 carriers.[11] Interactions between cholesterol and regulatory mechanisms of blood pressure (BP) are poorly understood and the role of individual fatty acids in BP regulation remains unclear.[12–14] We proposed to evaluate the association between the NOS3 Glu298Asp SNP (rs1799983) and hypertension, as well as the possible effect modification of dietary fat intake (that is saturated fat, monounsaturated fat, polyunsaturated fat) using a population-based sample of African Americans and whites from the ARIC study.

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METHODS

Study population

Study participants were selected from the ARIC study, a prospective investigation of atherosclerosis and its clinical sequelae involving 15 792 individuals aged 45–64 years at recruitment (1987–1989). Institutional review boards approved the ARIC study, and all participants provided written informed consent. Details on sampling frames and methods, as well as examination procedures, have been described elsewhere.[15] Briefly, elements of baseline examination included sitting BP, anthropometry, venipuncture, electrocardiogram, ultrasound-postural change, interview (medical history, physical activity, medical use, food frequency, and so on), pulmonary function, physical exam (brief exam including heart, lung and extremities, neurologic and breast exam) and medical data review.[16] The current cross-sectional investigation used data collected at the baseline examination.

Participants were excluded from the analyses (n=1636) if they had (1) prohibited use of their DNA for research purposes, (2) an ethnic background other than white or African American, (3) missing genotype information for the NOS3 Glu298Asp SNP, (4) missing information on hypertension status, (5) inadequate dietary data (missing >10 items in the Food Frequency Questionnaire (FFQ)) and (6) extreme energy intakes (intakes, kcal intake <600 or >4200 for men or <500 or >3600 for women; approximate lower and upper 1 percentiles of the energy-intake distribution).

Following exclusions, a total of 14 156 participants were available for analysis.

Baseline examination and laboratory measures

Seated BP was measured three times with a random-zero sphygmomanometer and the last two measurements were averaged. Hypertension was defined as systolic BP $\geqslant$140 mm Hg or diastolic BP $\geqslant$90 mm Hg or current use of antihypertensive medications. Questionnaires and in-person interviews were used to assess use of antihypertensive medications as participants brought to the examination all medications they had taken in the preceding 2 weeks.[16] Diabetes was defined by a fasting glucose level $\geqslant$126 mg per 100 ml (7.0 mmol l^{-1}), a non-fasting glucose level $\geqslant$200 mg per 100 ml (11.1 mmol l^{-1}) and/or history of or treatment for diabetes.[17] Cigarette-smoking status was analyzed by comparing current smokers to individuals who had formerly or never smoked. Body mass index (kg m^{-2}) was calculated from height and weight measurements. Plasma total cholesterol was measured by an enzymatic method.[18] Physical activity was assessed using a modified Baeke Physical Activity Questionnaire comprising of three summed components (Baeke score): the work index reflected physical activity at work, the sport index reflected physical activity during sports and the leisure time index reflected physical activity during leisure.

Dietary assessment

A 66-item semi-quantitative FFQ was modified[19] from the 61-item FFQ designed and validated by Willet *et al*.[20] The FFQ was used at the baseline (1987–1989) and third (1993–1995) exams. Briefly, participants provided information regarding frequency of specific foods and beverages they consumed in nine predefined frequency categories, ranging from never or <1 time per month to >6 times per day. Standard portion sizes were given as a reference for intake estimation. Additional information such as brand name of breakfast cereal most commonly consumed (open-ended response), the type of fat usually used in frying and in baking (butter, margarine, vegetable oil, vegetable shorting, lard) and use of salt in cooking and at the table (two questions) were also ascertained. A separate form was used to obtain information of intakes of wine, beer or liquor. The Harvard Nutrient Database was used to derive nutrient intakes from FFQ responses.

Genotype determination

Genotyping of the eNOS (NOS3) Glu298Asp variant was performed using the TaqMan assay (Applied Biosystems, Foster City, CA, USA). A 175-base pair product was amplified utilizing 0.9 μM each of the forward primer 5′-CCCCACAGCTCTGCATTCA-3′ and the reverse primer 5′-CACCCAGT CAATCCCTTTGG-3′, 30 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 62 °C. A total of 0.2 μM of each of the sequence-specific probes 5′-6FAM-CCCCAGATGATCCCCCAGAACTC-TAMRA-3′ and 5′-VIC-CCCCAG-ATGAGCCCCCAGAAC-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).[21] The ARIC study has a rigorous blind duplicate program. The percentage of agreement for blind duplicate data were 95% for the NOS3GLU298ASP polymorphism.

Statistical analysis

Statistical analyses were performed using STATA 10.0 software (College Station, TX, USA). Allele frequencies were estimated by gene counting. Hardy–Weinberg equilibrium expectations were tested using a χ^2 goodness-of-fit test. As allele frequencies for the NOS3 Glu298Asp SNP were different between whites and African Americans, all analyses were performed separately by race. Proportions, means and standard errors of the mean (s.e.m.) of covariates used in the analyses were calculated. Unconditional logistic regression was performed to estimate the odds ratio and 95% confidence intervals. Covariates included age, body mass index, smoking status, diabetes, physical activity (Baeke score), daily dietary sodium intake, total daily energy and total fats (% of total energy). Evidence for a dietary fat-specific effect of variation was assessed by including a genotype-by-dietary fat interaction term in the model, with statistical significance assessed by the standard t-test. Each dietary fat intake variable (that is percentage of energy from total, saturated, monounsaturated and polyunsaturated fat) was classified into two groups (low and high; separately by race) according to the race-specific mean value. Dietary fat variables were dichotomized to enable comparison between groups of high versus low fat dietary consumers. The likelihood ratio test was also used to evaluate the significance of interaction by comparing models with and without interaction terms.

RESULTS

Race-specific characteristics of the study sample are presented in Table 1. NOS3 Glu298Asp genotype frequencies were significantly different between whites and African Americans, and genotype distributions for each racial group were in accordance with Hardy–Weinberg equilibrium expectations. There were a significantly higher proportion of African American hypertensives (56%), diabetics (20%) and current smokers (30%) compared to whites (27, 9 and 25%, respectively). Whites were more physically active and had significantly higher intake of total fat, polyunsaturated fat and saturated fat as a percentage of total daily energy compared to African Americans. Mean systolic and diastolic BPs were significantly higher in African Americans compared to whites. The NOS3 TT genotype (Asp/Asp) was more commonly observed in whites (10%) compared with African Americans (1%).

NOS3 Glu298Asp genotype frequencies in hypertensives and normotensives are presented in Table 2 by racial group. Genotype frequencies did not differ between hypertensives and normotensives in whites or African Americans. Results from logistic regression models to estimate odds ratios of hypertension for individuals with the NOS3 Glu298Asp SNP are presented in Table 3, by racial group. After adjusting for hypertension risk factors and dietary factors, no significant findings were observed for analyses of hypertension for NOS3 in whites or African Americans. Other models adjusting for all the individual fat variables did not reveal any significant findings (data not shown).

Tests for interaction between NOS3 genotypes and dietary fat intake are shown for African Americans and whites in Tables 4 and 5, respectively. Tests for interaction did not reveal any significant interaction between total dietary fat intake and NOS3 genotypes in either whites or African Americans (P for interaction=0.4 and 0.3,

Table 1 Baseline characteristics of the Atherosclerosis Risk in Community (ARIC) cohort

Characteristic	*White (N=10453)* *Mean (s.e.m.)*	*African American (N=3703)* *Mean (s.e.m.)*	*P-value
Age (years)	54.4 (0.05)	53.6 (0.09)	<0.001
Body mass index (kg m^{-2})	27.0 (0.05)	29.6 (0.01)	<0.001
Total cholesterol (mg per 100 ml)	215.0 (0.4)	215.1 (0.72)	0.9
Total calories (kcal per day)	1639.0 (5.7)	1586.1 (9.8)	0.001
Total fat (%kcal)	33.1 (0.06)	32.2 (0.10)	<0.001
Monounsaturated fat (%kcal)	12.7 (0.04)	12.6 (0.03)	0.5
Polyunsaturated fat (%kcal)	5.1 (0.01)	4.8 (0.02)	<0.001
Saturated fat (%kcal)	12.2 (0.03)	11.5 (0.04)	<0.001
Physical activity (Baeke score)	7.1 (0.1)	6.5 (0.02)	<0.001
Systolic blood pressure (mm Hg)	118.6 (0.2)	129 (0.3)	<0.001
Diastolic blood pressure (mmHg)	71.5 (0.1)	79.8 (0.2)	<0.001
Sodium (mg per day)	1518.0 (5.9)	1364.0 (9.2)	<0.001
	N (%)	*N (%)*	
Hypertension	3116 (27.4)	2339 (56.0)	<0.001
Diabetes	1043 (9.1)	817 (20.0)	<0.001
Current smoker	2836 (24.8)	1254 (29.9)	<0.001
NOS3 genotype			
GG	4966 (46.6)	2986 (78.1)	<0.001
GT	4620 (43.3)	782 (20.5)	<0.001
TT	1076 (10.1)	54 (1.4)	<0.001

**P*-value comparing whites and African Americans.

Table 2 NOS3 Genotype frequencies for hypertensives and normotensives

NOS3 Genotype	*Hypertensive* n *(%)*	*Normotensive* n *(%)*	P[a]
White			
GG	1370 (47.0)	3575 (46.5)	0.8
GT	1248 (42.8)	3351 (43.5)	
TT	296 (10.2)	768 (10.0)	
African American			
GG	1668 (78.7)	1256 (77.3)	0.5
GT	420 (19.8)	347 (21.4)	
TT	31 (1.5)	21 (1.3)	

[a]*P*-value comparing genotype frequencies between hypertensives and normotensives, with the GG genotype serving as the referent group.

Table 3 Odds ratios (ORs) relating NOS3 variant genotypes to hypertension

Genotype status[a]	*Model 1*[b] *OR (95% CI),* P	*Model 2*[c] *OR (95% CI),* P
White		
GG *vs.* GT	0.96 (0.87–1.06) 0.5	0.96 (0.87–1.06) 0.5
GG *vs.* TT	1.04 (0.89–1.23) 0.6	1.05 (0.89–1.23) 0.5
GG *vs.* GT+TT	0.98 (0.89–1.07) 0.7	0.98 (0.89–1.07) 0.7
African American		
GG *vs.* GT	0.99 (0.84–1.18) 0.9	0.99 (0.83–1.17) 0.9
GG *vs.* TT	1.12 (0.61–2.0) 0.7	1.10 (0.60–2.0) 0.8
GG *vs.* GT+TT	0.99 (0.85–1.18) 1.0	0.99 (0.84–1.17) 0.9

[a]GG genotype serves as the referent genotype.
[b]Adjusted for age, body mass index, current smoking status, diabetes, physical activity, daily dietary sodium intake and total daily energy (kcal per day).
[c]Adjusted for model 1 factors plus percentage of daily energy from total fat.

respectively). Additionally, no interaction effects were observed for saturated, polyunsaturated or monounsaturated fat intake in either whites or African Americans. Among lipid variables, we found a significant relationship between NOS3 Glu298Asp and triglycerides, which was encountered only in African Americans P=0.017 for Glu/Glu versus Glu/Asp+Asp/Asp and P=0.034 for Glu/Glu versus Glu/Asp versus Asp/Asp. We did not find any significant relationship between NOS3Glu298Asp and lipids in whites.

DISCUSSION

Earlier studies suggest that individuals with the NOS3Asp298 allele are more susceptible to develop essential hypertension,[6,22–24] with additional studies suggesting a possible interaction effect between the NOS3 Glu298Asp polymorphism and other factors that regulate hypertension (for example, dietary fats and lipids). In our study, we did not find an association between NOS3 Glu298Asp genotype and hypertension in either whites or African Americans in the ARIC study population. We did not observe an interaction effect between dietary fat intake and the NOS3 Glu298Asp variant to influence hypertension.

The relationship between dietary fat intake and hypertension has been well established, with evidence that dietary supplements containing omega-3 polyunsaturated fatty acids were associated with a mild favorable effect on BP in both hypertensive and normotensive subjects.[25] However, the mechanism underlying this process remains controversial. One possible mechanism, based on animal experimental data, relates to enhanced endothelial vasodilator function, which is due to deficient adenylyl cyclase activity on endothelial cells caused

Table 4 Interaction between percentage of daily energy intake from dietary fat and genotypes of endothelial nitric oxide synthase to influence hypertension (systolic blood pressure) in African Americans[a]

	Total fat		*Saturated fat*		*Monounsaturated fat*		*Polyunsaturated fat*	
Genotype	*Low <32.2*	*High ⩾32.2*	*Low <11.5*	*High ⩾11.5*	*Low <12.6*	*High ⩾12.6*	*Low <4.8*	*High ⩾4.8*
GG								
Mean	128.7	128.9	129	128.6	128.8	128.8	129.3	128.3
n	1400	1481	1439	1442	1398	1483	1488	1393
P[b,c]		0.9		0.6		1.0		0.2
GT/TT								
Mean	128.4	128.9	128.8	128.5	128.4	128.8	129.1	128.1
n	368	441	389	420	376	433	436	373
P[b,c]		0.8		0.8		0.8		0.5
P for interaction[c,d]		0.3		0.1		0.9		0.2

[a]Low and high refer to groups above and below the mean values of dietary fat expressed as a percentage of energy for each fat consumed.
[b]Test of means across dietary fat categories within each genotype category assessed from a multivariate model with individual consideration of genotypes.
[c]Adjusted for age, body mass index, smoking status, diabetes, physical activity, sodium intake and total daily energy.
[d]Interaction derived from interaction term included in the model.

Table 5 Interaction between percentage of daily energy intake from dietary fat and genotypes of endothelial nitric oxide synthase to influence hypertension in whites [a]

	Total fat		*Saturated fat*		*Monounsaturated fat*		*Polyunsaturated fat*	
Genotype	*Low <33.1*	*High ⩾33.1*	*Low <12.2*	*High ⩾12.2*	*Low <12.7*	*High ⩾12.7*	*Low <5.1*	*High ⩾5.1*
GG								
Mean	118.8	118	118.6	118.2	118.6	118.2	118.7	118.1
n	2295	2563	2413	2445	2326	2532	2640	2218
P[b,c]		0.1		0.4		0.4		0.2
GT/TT								
Mean	118.5	118.8	118.5	118.8	118.3	119	118.8	118.5
n	2733	2810	2819	2724	2751	2792	3108	2435
P[b,c]		0.5		0.6		0.08		0.5
P for interaction[c,d]		0.4		0.4		0.4		0.7

[a]Low and high refer to groups above and below the mean values of dietary fat expressed as a percentage of energy for each fat consumed.
[b]Test of means across dietary fat categories within each genotype category assessed from a multivariate model with individual consideration of genotypes.
[c]Adjusted for age, body mass index, smoking status, diabetes, physical activity, sodium intake and total daily energy.
[d]Interaction derived from interaction term included in the model.

by altered G protein levels and function when exposed to omega-3 fatty acids. This overall cascade ends up with a decrease in cyclic adenosine monophosphate, a major second messenger in the development of hypertension.[26] Another major pathway involved in BP regulation is the endothelial nitric oxide pathway. Nitric oxide-related vascular relaxation tone is decreased in hypertensive subjects.[27] eNOS, which catalyzes nitric oxide, is a potent vasodilator, and some studies have reported a possible association between the NOS3 Glu298Asp variant and hypertension; however, a recent meta-analysis revealed no association with a pooled odds ratio of 1.[28] Another recent meta-analysis reported a possible gene–environment interaction between the NOS3 Glu298Asp variant and diets rich in saturated fats to influence BP.[10] To our knowledge, this study is the first to address the potential effect modification of the NOS3 Glu298Asp variant and dietary fat intake on hypertension status in a large US population of whites and African Americans. These results contradict earlier studies in other ethnic populations with smaller sample sizes. Significant findings from smaller studies are often not replicated in larger studies because of spurious results, which may be why our larger study of over 14 000 subjects did not corroborate results of earlier smaller studies. Of course, we cannot rule out the possibility that the different ethnicities studied may have contributed to the divergent results.

Very few studies have addressed a possible interaction between the NOS3 Glu298Asp polymorphism and lipids to regulate hypertension. Although our study emphasized on the interaction between dietary fats and NOS3 Glu298Asp to influence BP, understanding how lipids interact with NOS3 Glu298Asp could explain some of our findings. Earlier studies have reported a decrease in nitric oxide bioavailability to be a major mechanism through which high blood cholesterol levels may stimulate an elevation in BP.[29] Recent studies have shown significant reductions in NOS3 in caveolar membrane fractions for Glu/Asp and Asp/Asp variations compared with Glu/Glu in both basal and shear induced NOS3 activity hence advocating for altered caveolar localization of eNOS as a more explicit mechanism in the relationship between NOS3 and lipids.[30] As shown by Pereira *et al*,[31] eNOS genetic variation influenced the relationship between serum cholesterol levels and BP in a Brazilian population. They reported significant interactions between cholesterol levels and the NOS3 Glu298Asp SNP

(P=0.02), determining that the risk of hypertension was 2.8 times greater in subjects with the Asp298 homozygous genotype and a total cholesterol level above the median. In our study, we found a significant relationship between NOS3 Glu298Asp and triglycerides in African Americans, which will require further exploration as this might reveal possible explanations for the relationship between NOS3 and hypertension. A limitation to our study is that we only investigated a single SNP in the NOS3 gene. It is possible that this single variant might not be a reliable marker of hypertension risk, but rather a combination of polymorphisms may be involved in the development of hypertension. The abbreviated nature of our FFQ may have limited our ability to accurately measure dietary fat intake (greater potential for random measurement error, which would attenuate associations). Perhaps a much broader FFQ with more specific items will reveal much detail and reduce random error.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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