Relation of Candidate Genes that Encode for Endothelial Function to Migraine and Stroke. The Stroke Prevention in Young Women Study

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Relation of Candidate Genes that Encode for Endothelial Function to Migraine and Stroke
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Background and Purpose—Migraine with aura is a risk factor for ischemic stroke, but the mechanism by which these disorders are associated remains unclear. Both disorders exhibit familial clustering, which may imply a genetic influence on migraine and stroke risk. Genes encoding for endothelial function are promising candidate genes for migraine and stroke susceptibility because of the importance of endothelial function in regulating vascular tone and cerebral blood flow.

Methods—Using data from the Stroke Prevention in Young Women study, a population-based case-control study including 297 women aged 15 to 49 years with ischemic stroke and 422 women without stroke, we evaluated whether polymorphisms in genes regulating endothelial function, including endothelin-1 (*EDN*), endothelin receptor type B (*EDNRB*), and nitric oxide synthase-3 (*NOS3*), confer susceptibility to migraine and stroke.

Results—*EDN* SNP rs1800542 and rs10478723 were associated with increased stroke susceptibility in whites (OR, 2.1; 95% CI, 1.1–4.2 and OR, 2.2; 95% CI, 1.1–4.4; $P=0.02$ and 0.02, respectively), as were *EDNRB* SNP rs4885493 and rs10507875, (OR, 1.7; 95% CI, 1.1–2.7 and OR, 2.4; 95% CI, 1.4–4.3; $P=0.01$ and 0.002, respectively). Only 1 of the tested SNP (*NOS3* rs3918166) was associated with both migraine and stroke.

Conclusions—In our study population, variants in *EDN* and *EDNRB* were associated with stroke susceptibility in white but not in black women. We found no evidence that these genes mediate the association between migraine and stroke. (*Stroke*. 2009;40:00-00.)

Key Words: endothelium ■ ischemia ■ migraine ■ stroke in young adults

Several observational studies have shown that migraine is a risk factor for ischemic stroke, specifically among young women.[1–4] At least 1 study[4] has reported that women with a family history of migraine are at increased risk of stroke, regardless of their personal history of migraine. The mechanism by which these disorders are associated remains unclear. However, there appears to be a genetic contribution to both disorders,[5–9] which may imply a genetic influence on the association between migraine and stroke in young adults.

Genes encoding for endothelial function are promising candidate genes for both migraine and stroke susceptibility because of the importance of endothelial function in regulating vasoconstriction and vasodilation of blood vessels and cerebral blood flow. The nitric oxide synthase-3 (*NOS3*) gene encodes for nitric oxide (NO) synthase, which is responsible for the conversion of L-arginine to NO, a primary vasodilator and regulator of blood flow and vascular tone.[10–12] The endothelin-1 (*EDN*) gene encodes for endothelin-1 (ET-1), a primary vasoconstrictor of vascular smooth muscle cells.

Depending on the potency and type of blood vessel, ET-1 may also cause vasodilation via *ETB* receptors on endothelial cells (encoded for by the endothelin receptor type B [*EDNRB*] gene), an action that is mediated by NO.[10–12] Therefore, NO and ET-1 released from endothelial cells act reciprocally to maintain vascular tone (Figure).

Previous studies have reported on the role of impaired NO-dependent vasodilation in the pathophysiology of migraine[13] and stroke,[10,14,15] ET-1 dose-dependent ischemia,[16] and associations between ET-1 plasma levels and migraine.[17,18] Consequently, variants in genes that encode for endothelial function have been studied in relation to migraine,[19–21] stroke,[22–24] and related conditions such as high blood pressure,[25,26] pulse pressure,[27] and myocardial infarction.[28] However, a systematic study has not been performed to assess the individual or combined contribution of these variants to stroke or to migraine as a risk factor for ischemic stroke. We have sought to evaluate whether polymorphisms in genes regulating endothelial function and vascular tone
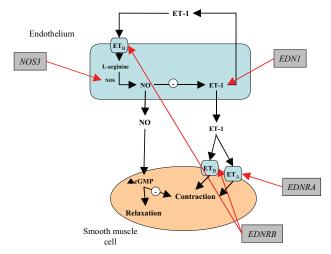
Figure. Candidate genes encoding for endothelial function.

(*NOS3*, *EDN*, and *EDNRB*) may confer susceptibility to migraine and stroke in a large, biracial, population-based, case-control study of young onset-stroke performed in young women. In our analyses we considered risk factor status, migraine, as well as stroke subtype on an exploratory basis.

Materials and Methods

Study Population
The Stroke Prevention in Young Women Study (SPYW) is a population-based, case-control study initiated to examine risk factors for ischemic stroke in young women. Study recruitment and data collection occurred in 2 waves: recruitment for SPYW-1 was conducted between 1992 and 1996, and recruitment for SPYW-2 was conducted between 2001and 2003. In both waves, cases were women hospitalized with a first cerebral infarction identified by discharge surveillance from 1 of 59 hospitals in the greater Baltimore–Washington area and direct referral from regional neurologists. The methods for discharge surveillance, chart abstraction, case adjudication, and assignment of probable and possible underlying causes have been described elsewhere.[29–31] Control subjects were women with no history of stroke identified by random-digit dialing and were matched to cases by age (within 10 years) and geographic region of residence in both waves, and additionally matched for race in SPYW-2. SPYW-1 included cases aged 15 to 44 years recruited within 1 year of stroke and was designed with a 1:2 case-to-control ratio. SPYW-2 included cases aged 15 to 49 years recruited within 3 years of stroke and was designed with a 1:1 case-to-control ratio. For both study periods, additional cases were recruited after completion of control recruitment.

SPYW participants for whom DNA was available include 743 subjects (321 cases and 423 controls). Subjects were excluded from the current study if they had genetic or other known specific etiologies for their stroke that would impair detection of new genetic associations. These conditions were: sickle cell disease, sickle thalassemia disease, central nervous system vasculitis by angiogram and clinical criteria, endocarditis, neurosyphillis, mechanical heart valve, postradiation arteriopathy, and cocaine use within 48 hours of stroke. These criteria resulted in the exclusion of 24 cases and 1 control, leaving 297 cases and 422 controls in these analyses.

A pair of neurologists evaluated each case to establish ischemic stroke, with disagreements resolved by a third neurologist. Methods for stroke subtype categories have been previously described[29,31] and include: (1) large artery atherosclerosis; (2) cardioembolism; (3) lacunar stroke; (4) stroke of other determined etiology; and (5) stroke of undetermined etiology. Lifetime headache history was collected from case and control subjects by standardized questionnaire as described previously.[32] Briefly, subjects were classified as having migraine with visual aura if they: (1) reported ever seeing spots, lines, or flashing lights around the time of their probable migraine; or (2) if they reported ever experiencing loss of vision and also reported a frequency of probable migraine with visual aura of at least twice per year. Subjects were identified as having probable migraine without visual aura if they reported no history of visual aura and reported nausea, vomiting, or sensitivity to light during a probable migraine and probable migraine frequency of at least 5 times per year. Traditional stroke risk factors and other study variables, including age, ethnicity, and history of hypertension, diabetes, myocardial infarction, current smoking status, and current oral contraceptive use (both defined as use within 1 month of stroke event for cases and at time of interview for controls), were also collected during standardized interview and were included as covariates in our analyses.

This study was approved by Institutional Review Boards at the University of Maryland, the Centers for Disease Control and Prevention, and at all participating hospitals. Each patient gave written informed consent before enrollment.

SNP Selection and Genotyping Methods
SNP selection and genotyping methods for *NOS3* have been previously described.[24,33] For *EDN* and *EDNRB* genes, we identified haplotype tag SNP (htSNP) from the International HapMap Project European Caucasian and Yoruban data.[34] Using the Tagger program, we specified the pair-wise tagging method,[35] an r^2 of ≥ 0.8, and a minor allele frequency of at least 5% from the 2 populations. The chromosome location, size, number of exons, known SNP and htSNP for each candidate gene are shown in Table 1. All alleles for *EDN* and *EDNRB* SNP were determined using the Taqman method developed by Applied Biosystems Inc.

Statistical and Genetic Analyses
We compared risk factor distributions between stroke cases and controls using t tests for continuous variables and χ^2 tests for categorical variables. All polymorphisms were biallelic; therefore, frequencies of minor alleles were compared between stroke cases and controls and between women with and without history of migraine and migraine with aura using χ^2. For our primary analyses, we used a race-stratified additive model adjusted for age and geographic region to test the effect of genotype on ischemic stroke. A fully adjusted risk factor model (age, geographic region, smoking, diabetes, hypertension, myocardial infarction, and oral contraceptive use) was also evaluated. Stroke subtype analyses were also performed; however, given the small samples sizes these were considered exploratory or hypothesis generating in nature.

To test whether identified variants mediated the association between migraine and stroke, we modeled the probability of stroke given migraine, with stroke as the response (dependent) variable and

Table 1. Size, Chromosome Location, SNP, and Tag SNP of Candidate Genes

Gene	Size	Chromosome	Exons	Known SNP With MAF >5%	N of Haplotype Bins	N of Bins Tested (Black/White)
NOS3	23 530 bp	7	26	15/5	13/7	5/2
EDN	6119 bp	6	5	10/5	6/4	6/4
EDNRB	24 288 bp	13	7	75/66	20/9	11/7

bp indicates basepairs; MAF, minor allele frequency.

Table 2. Demographic and Clinical Characteristics of Stroke Cases and Controls in the Stroke Prevention in Young Women Study

	Cases (n=297)	Controls (n=422)	P
Age	40.0±7.9	37.9±7.5	0.0003
Black	135 (45%)	169 (40%)	0.170
Hypertension	108 (36%)	58 (14%)	<0.0001
Diabetes	46 (15%)	21 (5%)	<0.0001
MI or angina	38 (13%)	15 (4%)	<0.0001
Smoking	138 (47%)	113 (27%)	<0.0001
Use oral contraceptives	39 (13%)	33 (8%)	0.03
Migraine			
No migraine	152 (51%)	255 (60%)	0.02
Migraine no aura	20 (7%)	49 (12%)	0.02
Migraine with aura	125 (42%)	118 (28%)	0.0001

history of migraine as the study (independent) variable, with and without the variant present in the model. We compared the outcomes to evaluate whether the association between migraine and stroke remained elevated when variants were controlled for. If the association between migraine and stroke weakened after adjustment for allelic variation, then this suggested that the identified htSNP at least partially explains the association between migraine and stroke in our study.

All statistical analyses were performed using SAS version 8.2 software.[37] Hardy-Weinberg equilibrium and linkage disequilibrium were calculated with Haploview version 3.2.[36]

Two-tailed $P<0.05$ was considered statistically significant. To test whether the overall difference in allele distribution between cases and controls was greater than might be expected by chance, taking into account multiple comparisons, we used Haploview[36] to randomly permute case and control status, simulating 10 000 race-stratified data sets for each candidate gene. A summary χ^2 distribution was obtained for the simulated data sets and compared to that of our observed data.

Results

Clinical characteristics of stroke cases (n=297) and non-stroke controls (n=422) are shown in Table 2. Cases were slightly older on average compared to controls and were more likely to be black. As expected, cases were also more likely than controls to report a history of hypertension, diabetes, and myocardial infarction, and were more likely to be current smokers and current oral contraceptive users. Cumulatively, cases were also more likely than controls to report a history of migraine, including migraine with visual aura.

SNP analyzed from the *NOS3*, *EDN*, and *EDNRB* genes and their call rates are shown in Tables 3, 4, and 5 in order of physical location within the gene. In addition, race-stratified minor allele frequencies and Hardy-Weinberg χ^2 and P values are given. The SNP selection strategy used for *NOS3* resulted in

Table 3. SNP Analyzed From *NOS3* Gene Stratified by Race and Case/Control Status

			Allele Frequency*									
			Black					White				
Marker	Position	Call Rate	Cases	N	Controls	N	HW P	Cases	N	Controls	N	HW P
rs2373962 (C/**G**)	150118625	0.90	0.12	126	0.13	157	0.87	0.41	129	0.39	196	0.85
rs10277237 (**A**/G)	150120992	0.92	0.48	125	0.45	166	0.95	0.24	129	0.22	210	0.64
rs6946415 (**A**/G)	150122196	0.93	0.10	129	0.14	164	0.26	0.41	133	0.38	212	0.58
rs1800783 (**A**/T)	150127045	0.96	0.40	135	0.45	168	0.92	0.42	135	0.41	217	0.44
rs1800779 (A/**G**)	150127591	0.96	0.14	134	0.23	169	0.38	0.41	135	0.40	217	0.32
rs2070744 (**C**/T)	150127727	0.92	0.13	126	0.23	163	0.45	0.42	133	0.40	207	0.65
rs3918162 (A/**G**)	150127934	0.91	0.04	128	0.02	156	1.00	0.00	133	0.00	203	1.00
rs3918166 (**A**/G)	150131204	0.95	0.07	134	0.02	166	1.00	0.00	132	0.00	215	1.00
rs1799983 (G/**T**)	150133759	0.95	0.14	134	0.16	164	0.74	0.33	132	0.31	217	0.63
rs1800780 (**A**/G)	150136527	0.94	0.45	131	0.41	168	0.62	0.48	133	0.45	213	0.63
rs3918184 (C/**T**)	150139867	0.95	0.40	132	0.40	168	0.43	0.35	135	0.40	215	0.33
rs7792133 (**A**/G)	150141894	0.94	0.00	129	0.01	168	1.00	0.00	134	0.00	214	1.00
rs3730305 (**A**/C)	150142048	0.96	0.20	135	0.18	168	0.66	0.08	135	0.11	219	0.30
rs3918232 (**A**/G)	150144288	0.95	0.00	132	0.00	168	1.00	0.00	133	0.00	215	1.00
rs3918201 (G/**T**)	150144992	0.91	0.02	126	0.03	155	0.30	0.01	133	0.00	201	0.00
rs3918211 (A/**G**)	150148555	0.96	0.15	134	0.15	169	0.82	0.01	135	0.00	219	0.01
rs3918220 (**C**/G)	150151104	0.95	0.05	132	0.04	169	1.00	0.00	135	0.00	216	1.00
rs3800787 (C/**G**)	150151284	0.90	0.11	126	0.15	157	0.37	0.37	130	0.39	197	0.13
rs6464119 (**A**/G)	150154801	0.94	0.16	130	0.17	167	1.00	0.26	135	0.27	213	0.99
rs11769158 (C/**T**)	150159815	0.95	0.03	132	0.04	168	1.00	0.13	133	0.12	214	0.44
rs3763486 (A/**G**)	150160913	0.91	0.23	127	0.25	159	1.00	0.17	133	0.19	200	0.71
rs2303922 (**A**/C)	150163370	0.94	0.46	133	0.48	166	0.16	0.34	134	0.37	214	1.00

HW indicates Hardy-Weinberg.

*As listed by minor allele frequency (bolded in column 1).

Table 4. SNP Analyzed From *EDN* Gene Stratified by Race and Case/Control Status

			Allele Frequency*									
			Black					White				
Marker	Position†	Call Rate	Cases	N	Controls	N	HW *P*	Cases	N	Controls	N	HW *P*
rs1800542 (**A**/G)	12400514	0.92	0.25	126	0.26	147	0.45	0.08	135	0.04	202	0.52
rs2070699 (G/**T**)	12400758	0.91	0.12	113	0.11	136	1.00	0.46	127	0.47	195	0.01
rs9296343 (C/**G**)	12401519	0.92	0.20	128	0.20	147	0.37	0.07	134	0.04	204	0.12
rs1800543 (**C**/T)	12402123	0.99	0.22	128	0.22	147	0.18	0.23	135	0.22	205	0.03
rs10478723 (**A**/G)	12403447	0.86	0.38	119	0.41	138	0.28	0.08	131	0.04	196	0.50
rs1626492 (**A**/G)	12403489	0.91	0.37	125	0.38	145	0.51	0.28	134	0.28	202	0.88
rs6912834 (A/**G**)	12403521	0.99	0.09	126	0.09	146	0.38	0.12	136	0.12	208	0.59
rs2071943 (**A**/G)	12403800	0.90	0.19	123	0.16	145	1.00	0.21	132	0.19	203	0.10
rs1629862 (**A**/G)	12403862	0.92	0.13	128	0.11	147	0.47	0.09	135	0.12	201	0.62
rs5370 (G/**T**)	12404241	0.87	0.19	120	0.17	144	0.05	0.19	120	0.21	190	0.00

HW indicates Hardy-Weinberg.

*As listed by minor allele frequency (bolded in column 1).

†Position refers to NCBI Build 36.2.

the selection of 22 SNP with an average intra-SNP distance of 2.1 kb and a maximum intra-SNP distance of 5 kb.[33] As mentioned, we used HapMap data to identify htSNP for *EDN* and *EDNRB*. The number of haplotype bins or htSNP that were identified and the number that we tested from each gene are shown in Table 1. For *EDN*, we identified 6 haplotype bins from the Yoruban data and 4 from the white data. HtSNP from each of the identified bins in *EDN* were tested in our analyses.

For *EDNRB*, 20 haplotype bins were identified in Yorubans and 9 were identified in white. Of these, htSNP from 11 of the Yoruban haplotype bins and 7 of the white haplotype bins were tested, resulting in moderate coverage.

Associations between *NOS3* polymorphisms and stroke risk in our study population have been previously published.[24,33] However, in the current analyses, we stratified these associations by stroke subtype and found that

Table 5. SNP Analyzed From *EDNRB* Gene Stratified by Race and Case/Control Status

			Allele Frequency*									
			Black					White				
Marker	Position†	Call Rate	Cases	N	Controls	N	HW *P*	Cases	N	Controls	N	HW *P*
RS4885491 (**A**/G)	77368351	0.62	0.08	91	0.10	99	1.00	0.16	88	0.12	138	0.78
RS12585038 (**A**/G)	77378576	0.96	0.15	127	0.14	140	0.12	0.11	133	0.18	200	0.40
RS3027111 (C/**T**)	77379869	0.97	0.23	123	0.23	145	0.16	0.11	134	0.20	203	0.58
RS4885493 (C/**G**)	77381064	0.84	0.45	122	0.45	131	1.00	0.19	122	0.31	185	0.96
RS7982910 (**C**/T)	77382132	0.85	0.10	120	0.13	138	1.00	0.47	122	0.42	188	0.61
RS3027129 (C/**T**)	77384402	0.95	0.01	118	0.01	142	1.00	0.10	129	0.08	200	0.48
RS9544636 (C/**T**)	77393935	0.98	0.13	127	0.12	146	1.00	0.09	133	0.11	208	0.05
RS9544634 (**A**/G)	77397570	0.99	0.04	127	0.02	147	0.40	0.08	136	0.10	204	0.11
RS17068573 (**A**/G)	77399614	0.98	0.28	126	0.26	146	0.88	0.10	134	0.18	205	0.07
RS12720154 (**C**/T)	77401188	0.98	0.16	126	0.16	145	0.22	0.10	135	0.18	204	0.17
RS11618814 (C/**T**)	77402775	0.96	0.42	124	0.36	144	0.00	0.46	131	0.34	202	0.00
RS9574124 (C/**G**)	77403587	0.97	0.47	115	0.47	122	0.00	0.31	120	0.37	164	0.05
RS10507875 (A/**G**)	77415255	0.85	0.15	122	0.16	137	0.78	0.09	123	0.17	186	0.21
RS1924914 (A/**G**)	77420315	0.87	0.42	121	0.45	138	0.72	0.26	130	0.29	197	0.01
RS7319342 (A/**G**)	77421485	0.37	0.44	42	0.39	46	0.00	0.36	59	0.42	114	0.00
RS1537063 (C/**T**)	77426235	0.90	0.03	126	0.01	139	0.17	0.08	129	0.10	193	1.00
RS11838546 (**A**/G)	77429615	0.91	0.18	128	0.18	147	0.65	0.11	134	0.12	201	0.67
RS4884075 (A/**C**)	77439622	0.96	0.37	125	0.38	143	0.25	0.11	130	0.18	198	0.21

HW indicates Hardy-Weinberg.

*As listed by minor allele frequency (bolded in column 1).

†Position refers to NCBI Build 36.2.

Table 6. Effect of *EDN* and *EDNRB* Polymorphisms on Risk of Stroke

| Marker | Cases/Controls | Ischemic Stroke | | | |
| | | Minimally Adjusted Model* | | Full Model† | |
		OR (95% CI)	P	OR (95% CI)	P
EDN					
rs1800542					
Black	122/147	1.0 (0.7–1.5)	0.970	1.0 (0.7–1.5)	0.991
White	134/202	2.1 (1.1–4.2)	0.030	2.3 (1.1–4.8)	0.024
EDNRB					
rs4885493					
Black	118/131	1.0 (0.7–1.4)	0.972	1.1 (0.7–1.7)	0.619
White	121/185	0.5 (0.3–0.8)	0.002	0.6 (0.4–0.9)	0.010
rs10507875					
Black	118/137	0.9 (0.6–1.5)	0.746	1.0 (0.6–1.7)	0.872
White	123/186	0.5 (0.3–0.8)	0.004	0.4 (0.2–0.7)	0.002

*Adjusted for study period, age, and geographic region.

†Adjusted for study period, age, geographic region, smoking, diabetes, hypertension, myocardial infarction, and oral contraceptive use.

rs2070744 and rs3918166 were both associated with stroke of undetermined etiology in blacks (N=286; case/control=123/163; OR, 0.5; 95% CI, 0.2–0.9; P=0.01) and (N=296; case/control=130/166; OR, 2.9; 95% CI, 1.0–8.0; P=0.02), respectively, but not among whites.

We also found an association between *EDN* htSNP rs1800542 and increased stroke risk among white women; those with the minor allele (allele A) had a 2.1-fold increase in the odds of stroke (95% CI, 1.1–4.2; P=0.03) compared to those with GG homozygotes. This association remained statistically significant in our full model after adjustment for risk factors (Table 6). In exploratory stroke subtype analyses, rs1800542 was significantly associated with cardioembolic stroke in whites (N=211; case/control=9/202; OR, 6.2; 95% CI, 1.8–21.7; P=0.005).

Two *EDNRB* htSNP (rs4885493 and rs10507875) were significantly associated with stroke in white women (Table 6). Both associations remained statistically significant after adjustment for risk factors in our full model, and both were associated with stroke of undetermined etiology in the exploratory stroke subtype analyses after adjustment for risk factors (Table 7).

Because of the ligand/receptor relationship between ET-1 and ETB, we considered a possible interaction between *EDN* and *EDNRB*. We tested 2 potential interactions in whites, 1 between rs1800542 from *EDN* and rs10507875 from *EDNRB*, and 1 between rs1800542 and rs4885493 from *EDNRB*. We did not find an association in either instance (Breslow-Day test for homogeneity of the odds ratios; χ^2=0.07; P=0.78; and χ^2=1.92; P=0.17, respectively).

With regard to migraine risk, 1 tested *NOS3* SNP showed an association with migraine: the minor allele A in rs3918166 was more common among blacks with migraine with visual aura (13%) than without (6%; χ^2=4.8; P=0.03). Because rs3918166 was also associated with stroke among blacks,[33] we considered this SNP as a potential mediator of the association between migraine and stroke in a regression model that included migraine with visual aura as the study variable and stroke as the outcome. However, controlling for rs3918166 had no impact on the association between migraine with visual aura and stroke in our model.

Two EDN htSNP, rs2070699 and rs1626492, were associated with migraine with visual aura among white women (χ^2=7.3; P=0.03; and χ^2=5.1; P=0.02, respectively), and *EDNRB* htSNP rs9544636 was significantly associated with the presence of migraine (with and without visual aura) among whites (χ^2=5.5; P=0.02). However, none of these htSNP were also associated with stroke; therefore, they were not considered potential mediators of the association between migraine with aura and stroke.

Results from Haploview permutations of our stroke data indicated that the best observed χ^2 for *EDN* rs1800542 was 4.7 in whites. A measure this extreme was observed in 20% of the 10 000 randomly permuted data sets; therefore, the corrected P was 0.20 and does not provide strong evidence in

Table 7. Effect of *EDNRB* Polymorphisms on Risk of Stroke of Undetermined Etiology

| Marker | Cases/Controls | Stroke of Undetermined Etiology | |
| | | Full Model* | |
		OR (95% CI)	P
rs4885493			
Black	58/131	1.2 (0.7–1.9)	0.478
White	72/185	0.5 (0.3–0.9)	0.013
rs10507875			
Black	59/137	0.8 (0.4–1.5)	0.448
White	76/186	0.5 (0.2–0.9)	0.018

*Adjusted for study period, age, geographic region, smoking, diabetes, hypertension, myocardial infarction, and oral contraceptive use.

favor of rejecting the global null hypothesis that there is no association between allele frequencies in *EDN* and case or control status. With regard to *EDNRB* htSNP, the best observed χ^2 was 10.2 (corrected $P=0.014$) for rs4885493, and the best observed χ^2 for rs10507875 was 7.6 (corrected $P=0.05$). Both of these measures provide evidence in favor of rejecting the null hypothesis that there was no association between allele frequencies in *EDNRB* and case or control status, and therefore support our findings. Further results from permutation analyses of our stroke data, stratified by migraine status, indicated no evidence in favor of rejection of the null hypothesis that there is no association between allele frequencies in *NOS3*, *EDN*, or *ENDRB* and case control status among blacks classified as having migraine with visual aura.

Discussion

The endothelium regulates vascular tone and blood flow by secreting vasoconstrictors such as ET-1 and vasodilators such as NO.[11,12] Thus, impaired dilation or exaggerated constriction of blood vessels may reflect reduced production of NO, which can be regarded as an important marker of endothelial cell dysfunction.[38] The *NOS3* gene encodes for NO synthase; therefore, any polymorphisms in the *NOS3* gene leading to decreased function in this gene could lead to NO deficiency. Evidence from experimental animal studies support a role for impaired NO regulation and vasodilation in the pathophysiology of stroke[14,15] and with migraine because of its role in vasodilation and with the central processing of migraine-related pain stimuli.[39,40] A few studies have reported associations between known single polymorphisms in *NOS3* and ischemic stroke in humans,[22–24] although these associations have not been well-replicated.[41]

As previously reported by members of our research team, there is increased prevalence of the A allele for rs1800779 and increased prevalence of the T allele for rs2070744 in cases compared to controls in our study population.[24] Although we do not know of any other studies that have reported similar associations with stroke, a recent meta-analysis reported an association between SNP rs2070744 and coronary heart disease in whites (OR, 1.12; 95% CI, 1.01–1.24) for T allele.[41] Exploratory stroke subtype analyses in our study showed that 2 of the 3 SNP from *NOS3* that were associated with overall stroke (rs2070744 and rs3918166) were primarily associated with stroke of undetermined etiology. Although published data on *NOS3* and stroke subtypes are limited, 1 previous study reported an association between the intron 4 variant in *NOS3* and small-vessel ischemic stroke in white men and women who were 67 years of age (on average).[23]

With regard to migraine, 1 *NOS3* SNP (rs3918166) was significantly associated with migraine with visual aura, but this association did not mediate the affect of migraine with visual aura on stroke risk. Borroni et al[20] recently reported that *NOS3* polymorphism rs1799983 (Glu298Asp) was a risk factor for migraine with aura; however, we found no evidence of this association in our analyses.

ET-1, encoded by the *EDN1* gene, is the most powerful vasoconstrictor of vascular smooth muscle.[11] It increases vascular resistance and blood pressure, and may also mediate the vasoconstrictive phase in migraine attacks.[18,42,43] ET-1 has 2 actions; it induces vasoconstriction of blood vessels by binding to the endothelin-A (ETA) and endothelin-B (ETB) receptors in smooth muscle cells (encoded by the *EDNRA* and *EDNRB* genes, respectively), and it also mediates vasodilatation through acting on ETB receptors in the endothelium (Figure). Therefore, NO and ET-1 released from endothelial cells act reciprocally to maintain vascular tone. In most healthy arteries, vasodilation predominates so the net effect should be vasodilation.[11] However, if endothelial function or NO activity is impaired, the vasoconstrictor effect of ET-1 on smooth muscle receptors may remain unopposed,[44,45] potentially leading to disorders associated with endothelial dysfunction such as stroke. ET-1 has been associated with animal studies with cerebral blood flow reduction to levels that induce infarction[16] and, in human studies, to increases in plasma, cerebrospinal fluid, and cerebral tissue after an ischemic stroke.[46,47]

In this study we observed an association between *EDN* htSNP rs1800542 and stroke and between 2 *EDNRB* htSNP (rs4885493 and rs10507875) and stroke in whites. None of these associations was present among blacks. The exploratory subtype analyses indicated that the increased stroke risk associated with *EDN* was primarily for cardioembolic stroke, whereas that associated with *EDNRB* was for strokes of undetermined etiology. We did not find evidence of elevated risk for small-vessel disease associated with EDN or EDNRB in our subtype analysis, which is consistent with at least 1 other study.[48] Two SNP in *EDN* were associated with migraine but did not mediate the effect of migraine on stroke risk. At least 1 other study has assessed polymorphisms in *EDN* and history of migraine in a population-based study of men and women who were 69 years of age (on average), but did not find any association with *EDN*. However, an association between *EDNRA* variant (−231 A/G) and migraine was observed.[21]

Because of the ligand/receptor relation between ET-1 and ETB, we considered a possible interaction between *EDN*, which encodes for ET-1 and *EDNRB*, which encodes ETB. We tested rs1800542 from *EDN* and rs10507875 and rs4885493 from *EDNRB* for an interaction with stroke risk in whites and did not find any evidence of an interaction with stroke risk in our data. As mentioned, 1 reason for this may be that *EDN* was primarily associated with cardioembolic stroke in subtype analyses and *EDNRB* was primarily associated with stroke of undetermined etiology.

We observed associations with migraine and stroke in this analysis of polymorphisms from *NOS3*, *EDN*, and *EDNRB*. Potential explanations for our results include direct action of the polymorphism on disease risk; linkage disequilibrium with a nearby disease susceptibility allele; population stratification bias; or chance findings related to multiple testing. To test for population stratification, we genotyped 40 markers with no known association with stroke in our population and evaluated them for systematic differences in allele frequency between cases and controls using the program Structure[49] and found no evidence for population stratification within blacks or whites in our study.[33]

To test the global null hypothesis that there is no relationship between allele distribution and case status, we performed permutation analyses. The corrected *P* values for single SNP

analyses for *NOS3* and *EDNRB* were 0.02; therefore, with an alpha level of 0.05, we have good evidence in favor of rejecting the null hypothesis. However, the corrected *P* value for EDN was 0.2, which is not sufficient evidence to reject the null hypothesis that there is no association between allele distribution and case or control status, also indicating that it is possible that our results for rs1800542 are attributable to chance. Our sample size posed limitations on our ability to evaluate the relationship between the alleles, migraine, and stroke risk. Our power to detect the reported associations between EDN and EDNRB htSNP and stroke risk was ≈80%; however, a larger sample size would have been preferable because our power declined substantially to ≈60% after correction for multiple comparisons. Subsequently, stroke subtype analyses were performed on an exploratory or hypothesis-generating basis given the small samples sizes for most subtypes.

This study has several strengths. First, we used a population-based design, which is optimal for studying early-onset stroke attributable to the low incidence of stroke in this age range. Second, we examined multiple polymorphisms and had moderate to good coverage of 3 of our genes. Third, our study is the first to our knowledge to evaluate the association between variants in *NOS3*, *EDN*, and *EDNRB* with migraine and stroke in a group of young white and black women.

Endothelial damage, perhaps in combination with a genetic component, has been suggested as a plausible mechanism of association for migraine and stroke or cardiovascular disease.[50–52] Although we observed separate associations between tested polymorphisms and migraine and stroke in our study, we did not show that the association between migraine and stroke is mediated by the presence of SNP from our chosen candidate genes. Because this is the first study to our knowledge to test this hypothesis, replication of our findings and inclusion of other genes in this physiological pathway will be important. Because of the high linkage disequilibrium in all of our candidate genes, we could not determine from these analyses whether the functional alleles that may be causing the observed associations with stroke are in our candidate genes or are upstream or downstream on the chromosomes; therefore, regions outside of these genes should be considered in functional studies.

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