Design of a Family Study Among High-Risk Caribbean Hispanics: The Northern Manhattan Family Study

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Stroke continues to kill disproportionately more Blacks and Hispanics than Whites in the United States. Racial/ethnic variations in the incidence of stroke and prevalence of stroke risk factors are probably explained by both genetic and environmental influences. Family studies can help identify genetic predisposition to stroke and potential stroke precursors. Few studies have evaluated the heritability of these stroke risk factors among non-White populations, and none have focused on Caribbean Hispanic populations. The aim of the Northern Manhattan Family Study (NOMAFS) is to investigate the gene-environment interaction of stroke risk factors among Caribbean Hispanics. The unique recruitment and methodologic approaches used in this study are relevant to the design and conduct of genetic aggregation studies to investigate complex genetic disorders in non-White populations. The aim of this paper is to describe the NOMAFS and report enrollment and characteristics of the participants. The NOMAFS will provide a data resource for the exploration of the genetic determinants of highly heritable stroke precursor phenotypes that are less complex than the stroke phenotype. Understanding the gene environment interaction is the critical next step toward the development of new and unique approaches to disease prevention and interventions. (*Ethn Dis*. 2007;17:351–357)

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Background

Stroke continues to kill disproportionately more Blacks and Hispanics than Whites in the United States.[1–4] Racial/ethnic variations in the incidence of stroke and prevalence of stroke risk factors are probably explained by both genetic and environmental influences. Some variation in well-known and highly heritable risk factors for stroke, including hypertension, diabetes, and lipids, can be explained by lifestyle factors, such as diet or lack of physical activity. Family studies can help identify genetic predisposition to stroke and stroke precursors.

Given the extreme complexity of genetic and non-genetic contributions to stroke, the evaluation of risk factors (ie, intermediate phenotypes for stroke) may reduce complexity and facilitate gene discovery. Subclinical markers, such as carotid intima media thickness (IMT), carotid distensibility (CD), and left ventricular mass (LVM), are risk factors for stroke and vascular disease. These markers may be less complex than the stroke phenotype. Few studies have evaluated the heritability of stroke risk factors among non-White populations, and none have focused on Caribbean Hispanic populations, the second largest subgroup of Hispanics in the United States.[5–9] Caribbean Hispanics, including Cubans, Dominicans, and Puerto Ricans, account for 15% of the 35 million US Hispanics and are the fastest growing ethnic group in the northeastern United States.[10–11]

The aim of this manuscript is to describe NOMAFS and to report enrollment and characteristics of the participants.

The main goals of Northern Manhattan Family Study (NOMAFS) are to evaluate high-risk Caribbean Hispanic families to: 1) systematically measure stroke precursor phenotypes; 2) determine heritability of these phenotypes; 3) collect DNA in families for preliminary genome-wide linkage analysis; and 4) identify new quantitative trait loci linked to these phenotypes. The unique recruitment and methodologic approaches are relevant to the design and conduct of genetic aggregation studies to investigate complex genetic disorders in non-White populations. The aim of this manuscript is to describe NOMAFS and to report enrollment and characteristics of the participants.

Methods

Enrollment of the Caribbean Family Cohort

The Northern Manhattan Family Study (NOMAFS) cohort was derived from the 1727 Caribbean Hispanic probands already enrolled in the multiethnic, community-based Northern Manhattan Study (NOMAS).[12] The Northern Manhattan Study (NOMAS) was assembled between 1993 and 2001 from a population-based random sample by using random digit dialing methods. At baseline, the cohort was characterized as: 1) White, Black, or

Hispanic residents of Northern Manhattan; 2) randomly derived from a household with a telephone; 3) age ≥40 years; and 4) no baseline history of stroke.

Eligibility

High-risk Caribbean Hispanic probands were identified from NOMAS according to the following criteria: 1) reporting a sibling with a history of myocardial infarction or stroke; or 2) having two of three quantitative risk phenotypes (maximal carotid plaque thickness, left ventricle mass or homocysteine level) ≥ 75th percentile in NOMAS. Families of the high-risk probands were considered eligible if the proband could provide a family history and had at least three primary relatives willing to participate. Children <18 years old were not eligible because of the low prevalence of risk factors.

We defined inclusion in terms of the feasibility for enrollment as living within a geographically favorable range to enable complete, in-person evaluation. Our commitment to recruit large Caribbean Hispanic families included enrollment in the New York metropolitan area and the Dominican Republic. We chose the Dominican Republic as our second enrollment site because most of our Hispanic cohort self-identified as Dominican, and we were most likely to enroll first-degree relatives there. However, our study continues to recruit other Caribbean Hispanic subpopulations. For this manuscript, the characteristics of our population include families enrolled in the New York metropolitan area. Family members living outside the New York area or the Dominican Republic were excluded unless they committed to visit New York at least once during the study.

Recruitment

Selected probands were reached primarily by phone and were asked to make the first contact with their family members. Probands were asked to refer relatives who lived within the New York area or the Dominican Republic and were offered the option of calling their relatives from our facilities or using a calling card that we provided to defray any charges they might incur. After initial family contact to obtain approval, we followed up with relatives to solicit participation. Subjects who could not be contacted by phone were sent letters requesting that they contact our office. Bilingual interviews were conducted by using structured questionnaires.

In-person appointments were scheduled. A letter confirming the appointment was sent reiterating the benefits of the study. Reminder phone calls were made the day before each visit. Efforts to encourage participation included round-trip transportation, financial compensation, breakfast, flexible scheduling including evening, Saturday appointments, group appointments for families who wished to come together, and home visits. The study was approved by the Columbia University Medical Center Institutional Review Board and the National Bioethics Committee in the Dominican Republic.

Participants enrolled in the Dominican Republic were phoned before the field team arrived and contacted again by phone the day before their appointment at Corazones Unidos. All travel expenses were reimbursed. Unlike families enrolled in New York, most families in the Dominican Republic arrived as a group.

Subjects were enrolled during a one-day, four-hour visit. Written informed consent was obtained from all participants. Data were obtained directly from the subjects, and interviews were conducted to assess demographics, social resources, and medical history including medications, vascular risk factors, family history, dietary intake.

Measurements

Primary phenotypes for this study included homocysteine levels, carotid IMT, CD, and LVM. All measurements were taken at baseline only. Interviews were conducted in English or Spanish. Our baseline data collection is summarized in Table 1. Standardized questions were adapted from the Behavioral Risk Factor Surveillance System by the Centers for Disease Control and Prevention regarding the following conditions: hypertension, diabetes, hypercholesterolemia, peripheral vascular disease, transient ischemic attack, cigarette smoking, alcohol use, physical activity, and cardiac conditions such as myocardial infarction, coronary artery disease, angina, congestive heart failure, atrial fibrillation, other arrhythmias, and valvular heart disease.[13] Questions regarding stroke and transient ischemic attack were adapted from the National Institute for Neurological Disorders and Stroke Data Bank and Asymptomatic Carotid Artery Stenosis Study surveillance instruments. Physical activity, alcohol use, family history, and other conventional stroke risk factors were measured as described previously.[12,14]

Blood pressure was measured with a calibrated standard aneroid sphygmomanometer (Omron; Vernon Hills, Illinois); height and weight were measured with calibrated scales; and hip and waist measurements used standard protocols.[15] Skinfold was measured in the right triceps and abdomen, and the mean of three measurements was calculated.[15] Dietary intake was obtained from the Brief Food Frequency Survey developed at the National Cancer Institute; this questionnaire is both reliable and valid. [16–18] It has been validated in Hispanic populations and covers foods, dietary habits, nutritional supplements, and specific traditional foods (eg, plantains, mango, rice).[18]

Homocysteine, cystathionine, methylmalonic acid, and methylcitrate levels were analyzed in serum samples at the University of Colorado's metabolite laboratory by using gas chromatography-mass spectrophotometry.[19–21] Plasma levels of cholesterol, high-density

Table 1. Baseline data in NOMFAS

Data Type	Instrument	Data collected
Demographics	Demographics	Gender, age, race-ethnicity
	Social resources	Education, insurance, acculturation, social isolation
Medical	CDC Behavioral Risk Factor Surveillance	Vascular risk factors, cognition
	NCI Block Diet Survey	Diet
	Family pedigree	Family
Physical measures	Blood pressure	Blood pressure
	Anthropometrics	Skinfold, height, weight
Bloods	Buffy coat collection	DNA
	Homocysteine	Homocysteine and metabolites
	Lipid panel	HDL, LDL
	Chemistry	Blood glucose
Imaging	Carotid doppler	Carotid IMT
		Carotid distensibility
	Echocardiogram	Left ventricular hypertrophy

NOMFAS=Northern Manhattan Family Study.

lipoproteins, and triglycerides were determined using standardized enzymatic procedures (Boehringer, Mannheim, Germany). DNA was extracted from whole blood by using a DNA purification kit (Geantra Systems, Minneapolis, Minn). DNA samples were sent to the Center for Inherited Disease Research for genotyping. Genome-wide linkage analyses were performed at Columbia University to identify potential loci linked to vascular risk factors of interest.

Carotid Ultrasound and Echocardiography Studies

Carotid IMT is an excellent, reproducible, marker of subclinical atherosclerosis and was assessed by high-resolution B-mode carotid ultrasound according to a standardized scanning and reading protocol.[22] Measurements of IMT were performed offline by using the automatic computerized edge tracking system.[23] Carotid IMT was calculated as a composite measure of 12 sites.

Carotid distensibility (CD) has been introduced as a new measure of subclinical disease and a risk factor for cardiovascular disease and stroke. Carotid distensibility (CD) was performed in the 10-mm segment of the right common carotid artery (CCA) below the origin of the carotid bulb according to a standardized ultrasound protocol.[24] Brachial artery blood pressure measurements were taken with a semi-automated oscillometric blood pressure recorder (Dinamap Pro 100, Criticon, LLC, Tampa, Fla) twice, before and after each ultrasound exam, and averaged. The diameters for the right CCA were measured from 5 B/M-mode registrations and averaged.

Left ventricular mass (LVM) was assessed by two-dimensional transthoracic echocardiography. Standard echocardiography, including color-Doppler flow study was performed with patients in a left lateral decubitus position. Left ventricular mass (LVM) was calculated according to the modified American Society of Echocardiographers formula.[24,25] The LVM was then divided by the body surface area to obtain an index used in the assessment of LVM phenotype.

The results of blood analysis, echocardiogram, and carotid Doppler ultrasound were mailed to each participant to continue interest in participation and alert them to any major findings. Significant abnormal findings were referred for medical followup and were monitored closely by the research team according to a predetermined safety protocol. A safety plan was in place to detect elevated blood pressure. Dangerously elevated blood pressure was triaged to the emergency room, and all other blood pressure elevations were discussed with the patients' physicians.

Statistical Methods

Genetic analyses were performed to estimate the heritability of each phenotype of interest (homocysteine, carotid IMT, distensibility, and LVH), and genome-wide linkage as well as family-based association analyses were conducted to search for susceptibility loci. Our power estimate was based on our projected sample size of 1455 family members, and this estimate hinges on the heritability of the phenotypes of interest. For example, if the true heritability of the phenotype is 30%, our sample size will give us 92% power to estimate the heritability in the range of .22 to .38.

Simulation studies were performed to evaluate the power to detect linkage on the basis of the same family data (1502 subjects in 114 families). We considered the configurations in which the total heritability of the phenotype ranged from 30%–50%. We calculated the power to detect linkage in our sample assuming a single quantitative trait loci accounting for 20%, 40%, and 60% of the total heritability. For example, the simulation results indicate that for a phenotype with 50% heritability and a quantitative trait loci accounting for 40% of the heritability, the power to detect linkage at the significant level is almost 90%.[26,27] These simulation studies suggest that the family data will provide excellent power to detect linkage.

RESULTS

Characteristics of our Caribbean Hispanic Population

Approximately 38% (n=662) of the 1727 Caribbean Hispanics in NOMAS met high-risk criteria. We contacted 406 probands and found 361 eligible families; 27 families were too small, and 18 probands were cognitively impaired

Table 2. Demographic characteristics of Caribbean Hispanics in Northern Manhattan Family Study

	Males (*n*=250)		Females (*n*=420)		Total (*N*=670)	
	n	**%**	***n***	**%**	***n***	**%**
Age (years)						
18 to 34	73	11	101	15	174	26
35 to 44	49	7	76	11	125	19
45 to 54	49	7	80	12	129	19
55 to 64	37	6	61	9	98	15
65 to 74	23	3	65	10	88	13
75 to 84	18	3	24	4	42	6
>85 years	1	0	13	2	14	2
Race						
White	42	6	51	7.	93	14
Black	49	7	29	4	78	12
American Indian	20	3	26	4	46	7
Asian or Pacific Islander	0	0	1	0	1	0
More than one race	159	24	293	43	452	67
>High school education	141	56	188	44	329	49
Living in New York (>5 years)	228	91	372	88	600	89
Birthplace						
United States	39		67		106	
Dominican Republic	202		339		541	
Puerto Rico	2		7		9	
Cuba	0		2		2	
Nicaragua	1		0		1	
Ecuador	2		2		4	
Columbia	4		0		4	

The advantages of the family study method include large extended pedigrees, known high vascular risk, sharing of environmental confounders, and ability to enroll US and Dominican Republic residents.

and unable to provide a reliable family history. Of eligible families, 200 were interested; another 62 families refused, 12 have not been contacted to date, and 87 were ineligible because of scattered geographic distribution.

To date, 87 families with a total of 670 subjects over the age of 18 have had a complete baseline assessment in New York. We have also enrolled 70 subjects from these families in the Dominican Republic (data not shown). No one has refused to participate because of issues pertaining to collection of genetic material. The mean age of the cohort is 48 years (±18 years). Among those enrolled, most (81%) were born in the Dominican Republic but have lived in New York >5 years (Table 2). Almost 51% did not complete high school. Our Caribbean Hispanic families are large, with a mean family size to date of 7.7 (±6.8) participants, and 59 families have at least five members.

The distribution of vascular risk factors is shown in Table 3. We report a high prevalence of hypertension (42%), diabetes (13%), elevated low-density lipoprotein, and high body mass index in our families. Distribution of vascular phenotypes is shown in Table 4.

DISCUSSION

This manuscript is one of the first to describe the methodologic issues involved with the recruitment, enrollment, and sampling of genetic material among Caribbean Hispanics living in and outside of the United States. Few epidemiologic studies have determined the heritability of stroke risk factors in Caribbean Hispanics. The advantages of the family study method include large extended pedigrees, known high vascular risk, sharing of environmental confounders, and ability to enroll US and Dominican Republic residents.

A number of family studies have examined the genetics of cardiovascular disease, primarily among White populations.[28–37] Little data exist among Caribbean Hispanics. The National Heart, Lung, and Blood Institute (NHLBI) family study recruited from three epidemiologic studies: the Framingham Heart Study, the Atherosclerosis Risk in Communities Study, and the Utah Health Family Tree Study.[34] The NHLBI population was primarily White with some data collection on African American families. The Detroit Project investigates the genetics of blood pressure among African Americans and Whites, while the Honolulu Heart Study examined cardiovascular disease and heritability among Japanese American families.[28,37] The San Antonio Family Heart Study is a population-based study investigating the genetics of heart disease in Mexican Americans from San Antonio and is composed of 42 extended families (1431 subjects).[32]

Our success in the enrollment of an immigrant sample from urban and rural areas provides evidence that familial aggregation studies can be conducted in vulnerable minority populations. Studies have suggested that minority populations may be more reluctant to participate in studies because they mistrust the healthcare system.[4] We suggest

Table 3. Distribution of vascular risk factors in Northern Manhattan Family Study

	Males (*N* =250)		Females (*N* =420)		Total (*N* =670)	
	n	%	*n*	%	*n*	%
Hypertension	94	38	189	45*	283	42
Diabetes mellitus	38	15	50	12	88	13
Coronary artery disease	26	10	46	11	72	11
No physical activity	124	50	232	55	356	53
Moderate alcohol intake (>0 to <2 drinks per day)	142	57	140	33*	282	42
Ever smoked	113	45	144	34*	257	38
Current smoker	38	15	56	13	94	14
	Mean ± SD		Mean ± SD		Mean ± SD	
Body mass index	28.6 ± 5.4		29.6 ± 6.1		29.2 ± 5.9	
Waist:hip ratio	.93 ± .08		.92 ± .62		.95 ± .49	
Skinfold thickness (mm)	22.3 ± 11.3		32.1 ± 10.5*		28.4 ± 11.8	
Fasting glucose (mg/dL)	90.9 ± 43.9		85.9 ± 27.3		87.8 ± 34.4	
Total cholesterol (mg/dL)	186.4 ± 43.7		186.9 ± 37.8		186.7 ± 40.0	
HDL (mg/dL)	42.7 ± 11.6		51.8 ± 14.4*		48.4 ± 14.1	
LDL (mg/dL)	115.2 ± 35.9		111.6 ± 35.6		112.9 ± 40.0	
Triglycerides (mg/dL)	144.1 ± 127.7		116.8 ± 72.0*		126.8 ± 97.1	

* $P<.05$ between males and females.

that probands' introducing the study to their family members may increase trust. Additionally, careful wording in the consent form may decrease confusion regarding the collection of genetic material. The use of Spanish-speaking interviewers who share cultural identification with the study population also improved our enrollment rate. Our ability to be flexible in terms of scheduling has also contributed to our successful recruitment.

Critical to the discernment of genetic and environmental factors for vascular disease is the rigor by which the phenotype is defined. Further, key to future genetic studies is the identification of phenotypes with high heritability.[38] We have systematically measured phenotypes among the members of our high-risk Caribbean Hispanic families to determine heritability of putative stroke risk factors. Our standardized, validated protocols have helped reduce concern over measurement variability and precision.

Although the focus of this study is the genetic contribution to subclinical vascular risk factors, we collected extensive data on nativity, education, and other social determinants of health. We believe that controlling for proper and adequate environmental factors is critical to the determination of heritability. Factors including acculturation will be examined within the heritability model.

Since the probands of this family study were selected from the NOMAS study, we will have available data for association gene mapping to replicate and identify the susceptibility locus once we complete linkage analysis. Our cohorts provide data obtained by the same protocols for both association and linkage mapping. Moreover, we have the ability to cross-validate genetic findings by using association study designs in the parent NOMAS studies. This combined approach has been considered an important method to detect susceptibility genes for a particular disease.[38]

Table 4. Distribution of phenotypes in Northern Manhattan Family Study

	Males		Females		Total	
	n	Mean ± SD	*n*	Mean ± SD	*N*	Mean ± SD
Intima-media thickness	228	.83 ± .07	377	.81 ± .07	605	.82 ± .08
Carotid distensibility	228	1.74 ± 1.04	375	1.61 ± 1.03	603	1.66 ± 1.04
Left ventricular mass	140	106.9 ± 29.82	272	94.2 ± 25.4	412	98.5 ± 27.6
Homocysteine(μmol/L)	106	9.56 ± 4.71	222	7.15 ± 2.41	328	7.93 ± 3.51

Limitations of the study include incomplete ascertainment of families due to geographical distribution. However, unlike other types of epidemiological studies, linkage analysis can effectively compensate for incomplete families, especially when family size is large.[39] Other strengths include the efficiency of integrating a family study in the setting of NOMAS and the strengths of capitalizing on existing collaborations that will enhance the feasibility of examining family members.

The NOMAFS will provide a data resource for the exploration of the genetic determinants of highly heritable stroke precursor phenotypes that are less complex than the stroke phonotype. Understanding the gene and environment interactions is the critical next step toward the development of new and unique approaches to disease prevention and interventions.

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