

A GENETIC AND EPIDEMIOLOGIC STUDY OF CARDIOVASCULAR DISEASE IN ALASKA NATIVES (GOCADAN): DESIGN AND METHODS

Barbara V. Howard ¹, Richard B. Devereux ², Shelley A. Cole ³, Michael Davidson ¹, Bennett Dyke ³, Sven O. E. Ebbesson ¹, Stephen E. Epstein ¹, David R. Robinson ¹, Betty Jarvis ¹, David J. Kaufman ¹, Sandra Laston ³, Jean W. MacCluer ³, Peter M. Okin ², Mary J. Roman ², Terry Romenesko ⁴, Giacomo Ruotolo ⁵, Michael Swenson ⁴, Charlotte R. Wenger ³, Sarah Williams-Blangero ³, Jianhui Zhu ¹, Clarence Saccheus ⁴, Richard R. Fabsitz ⁶, David C. Robbins ⁷

¹ MedStar Research Institute, Washington, DC

² Weill Medical College of Cornell University, New York, NY

³ Southwest Foundation for Biomedical Research, San Antonio, TX

⁴ Norton Sound Health Corporation, Nome, AK

⁵ University Vita-Salute San Raffaele, Milan, Italy

⁶ National Heart, Lung, and Blood Institute, Rockville, MD

⁷ Eli Lilly and Company, Indianapolis, IN

Received 9 November 2004, Accepted 16 March 2005

ABSTRACT

This article is a report of the design and methods of the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) Study. This longitudinal, population-based study was initiated to investigate the genetic determinants of cardiovascular disease and its risk factors. Between October 2000 and April 2004, this family study enrolled 1,214 Eskimos from several coastal villages in the Norton Sound region of Western Alaska. Examinations included a physical, laboratory determinations, and measures of subclinical disease. This study will generate a genome-wide scan for loci influencing cardiovascular disease-related traits. Relations between subclinical atherosclerosis and markers of inflammation will be examined using historic and newly drawn samples. The study will provide data on CVD prevalence, risk factors and the relative contribution of genetic and environmental determinants in Alaska Native peoples. Data from this study will contribute to the delivery of health-care and prevention of CVD in Alaska Eskimos and other populations. (*Int J Circumpolar Health* 2005;64(3):206-221.)

Keywords: Hypertension, epidemiology, heart disease, vascular disease, diabetes, genetics, Inuit Eskimo

INTRODUCTION

It is commonly believed that rates of mortality from coronary heart disease (CHD) are low among Eskimos (1), an idea reinforced by findings that fish oils are associated with reduced rates of coronary artery disease (CAD) (1). Between 1955 and 1965, coronary heart disease (CHD) mortality rates were 12-23% lower in Eskimos than in non-Native persons living in Alaska, and lower than those of Whites throughout the United States (2). Since this 1955-1965 survey, relative increases in CHD mortality have been observed in Eskimos. A comparison of disease trends suggests that, from 1979-1998, CHD mortality rates were stable, or increased among Eskimos, while rates declined among U.S. Whites (3). Younger Eskimos have more westernized diets and lower rates of physical activity than their elders did at the same age. The influence of lifestyle, especially the traditional fish-based diet, on CVD may be complex, because marine oils may reduce thromboses, but may not influence the atherosclerotic process. Moreover, regional differences in CVD mortality and incidence have been observed in Alaskan Eskimos (2-4); rates in the Norton Sound region are two-fold higher than in neighboring areas. Studies have suggested higher than expected prevalences of diabetes, hypertension, obesity and dyslipidemia (5-13). Studies in closely related Inuit populations in Greenland and Canada have proposed a similar evolution of risk factors and CVD (14-21). Changes in risk factors and lifestyle probably contribute to increasing CVD among Eskimos (9,22,23).

Although the pathogenesis of CVD probably involves interactions among environmental, behavioral and genetic factors, evidence

for susceptibility genes and gene-environment interactions remain inconclusive. One approach to understanding the genetics of heart disease is to develop genome-wide screens of well-characterized families. This approach treats CVD risk factors as phenotypes related to specific chromosomal regions. The availability of a relatively homogenous population, that has limited geographic mobility and large, reliably described pedigrees, is a significant asset in this type of study. In this article, we describe the objectives, design and methods of the Genetics of Coronary Artery Disease in Alaska Natives (GOCADAN) study, which has been undertaken to elucidate genetic and environmental factors contributing to CVD.

The goal of the GOCADAN Study is to identify cardiovascular disease risk factor phenotypes showing the strongest evidence for underlying genetic susceptibility in Eskimos. The following phenotypes were assessed: subclinical atherosclerosis (defined by ECG and carotid ultrasonography); diabetes, glycemic control, insulin concentrations; amount and distribution of body fat; blood pressure; lipids, apolipoproteins, HDL protein particle size concentration, and other measures of dyslipidemia; and inflammatory markers, including CRP, fibrinogen and homocysteine.

Additional questions to be addressed include: (a) what are the levels of CVD risk factors in the population; (b) are there differences in lifestyle and risk factors between older and younger persons; (c) do genes exist that influence both body fat and diabetes susceptibility; (d) is carotid atherosclerosis related to prior or persistent infection with *Chlamydia pneumoniae* (*C. pneumoniae*), cytomegalovirus (CMV), *Helicobacter pylori* (*H. pylori*), hepatitis A and hepatitis C viruses, and herpes

simplex types 1 and 2; and (e) do genetic variants in mannose-binding lectin, or apoE genes contribute to the relationships of infection and inflammation with coronary disease?

METHODS

Study population

The GOCADAN study population includes residents of several villages in the Norton Sound region, a largely roadless area of 23,000 square miles on the Northwest coast of Alaska. The region extends from the village of Shishmaref on the northern shore of the Seward Peninsula, to Stebbins on the southern coast of the Norton Sound, and includes 13 villages on the

mainland, two communities on St. Lawrence Island, and one community on Little Diomede. The area contains 570 miles of coastline and includes all of the Norton Sound and portions of the Bering Sea and Arctic Ocean, extending to latitude $66^{\circ}, 30'$ south of the Arctic Circle. A map of the region (Figure 1) gives an overview of the area. The Norton Sound Health Corporation (NSHC) is an Alaska Native-operated organization that serves as the primary health-care delivery system for the region. We recruited families from the villages of Brevig Mission, Teller, Golovin, White Mountain, Elim, Koyuk, Shaktoolik and Unalakleet, and the town of Nome. The population of the region is about 9,050, including approximately 7,700 Alaskan Eskimos. The villages are more



Figure 1. Norton Sound Region of Western Alaska.

than 90 percent Eskimo, compared with 51 percent in Nome.

Family members of 18 years old, or more, were recruited, with the goal of recruiting 1,200 residents of the villages, most of whom are members of large extended pedigrees. The population is predominately Inupiat in ethnicity. In keeping with the preference of study participants, we will refer to study participants as Alaskan Eskimos of Norton Sound, rather than Alaskan Natives, to distinguish the GOCADAN participants from other Alaskan Natives.

Study organization

Lab work is conducted at MedStar Research Institute (Washington, DC), genetic analyses at the Southwest Foundation for Biomedical Research (SFBR) (San Antonio), and cardiovascular evaluation at Cornell University Medical Center (New York). To ensure community input and appropriate implementation of the field exams, the study contracted with the NSHC to conduct field activities. The Institutional Review Board (IRB) for GOCADAN (MedStar Research Institute) reviews protocols and ancillary proposals. The NSHC formed a Scientific Advisory Board primarily comprised of local representatives to monitor study progress and review study publications. Personnel hired by the NSHC work closely with investigators in the field. Regularly scheduled Steering Committee meetings, teleconferences and site visits unite the institutions to manage the study.

Participant selection and recruitment

Each village is visited by two experienced GOCADAN investigators, and field workers familiar with the villages. In each village, a

local resident is hired to assist with recruiting and translation. An attempt is made to recruit all families in the villages chosen for study. Investigators first meet with the Village Council, where the rationale for a family study and the operations of the field clinic are explained, and a resolution of approval from the council is requested. If approval is given, investigators visit each household to explain the study. Written informed consent is requested and obtained for all study participants. Consenting household members ≥ 18 years old complete an interview, including the name, gender, date and place of birth of all household members; relationships among household members; and the parents and grandparents of each. Efforts are made to validate family relationships during the interview. These household survey data are transmitted to the SFBR, where they are computerized and cross-checked to further validate parent-offspring relationships. This is done for all identified family members, including those who are deceased and those not participating in GOCADAN. Extended pedigrees, which generally span multiple villages, are then constructed from reported relationships.

Pedigrees. Due to the relative isolation of the villages, populations of each locale approximate a single large extended family, with relations to other villages. Using the preliminary family trees sent to the Field Centers for verification, family members to be targeted in future recruitment efforts are identified. Attention is paid to relatives, whose data would complete, or link, large pedigrees, including parents of each descendant of participants, regardless of Alaskan Eskimo heritage.

Cohort examination. Participant examinations involve a physical and diagnosis of clinical events. Examination team members carry out these tasks in each village.

Clinical examination. In addition to a physical examination, the clinical survey collects demographic information, data on health habits, dietary intake, quality-of-life, physical activity, and medical and reproductive histories. Trained staff perform the clinical examinations. Procedures for the distribution and disposal of collected blood and urine samples are determined by NSHC.

Anthropometric measurements. Anthropometric measurements performed with the participant fasting and with an empty bladder, include height, waist and hip circumferences, measured to the nearest quarter inch, and weight, measured to the nearest tenth pound. Measurements have been converted to SI units. On the right side of the body, skin folds are measured to the nearest mm at two sites, triceps and subscapular (24). Body composition and fat-free mass are measured using the Impedance Meter # B1A101 (RJL Equipment Company).

Blood pressure measurements. Sitting blood pressure is measured on the right brachial artery using peak three measurements with a Baum mercury sphygmomanometer (W.A. Baum, Copiague, NY). Means of the second and third measurements are used as the figures for blood pressure. Systolic pressure is measured in the right brachial artery and both ankles by the Imex Mascot Doppler (Imex Medical Systems, Inc., Golden, CO). Ankle systolic blood pressure is measured in the

supine position. Right arm pressure is used to calculate ankle/brachial index for both legs.

Glucose tolerance test. Initially, participants are screened for diabetes by self-report and a measure of fasting glucose (Accu-Chek Advantage). Hemoglobin is also measured (HemoCue Hemoglobin Test). A random urine sample is collected and blood is taken by venipuncture at fasting and two hours after consuming a 75-g glucose load (Glutol, Paddock Laboratory, Inc., Minneapolis, MN). Participants are excluded from the glucose tolerance test if they

- a) Are insulin-requiring diabetics.
- b) Use oral agents, with records indicating two random blood glucose values > 250 mg/dl.
- c) Have fasting glucose of ≥ 225 mg/dl.

Expired air CO determination. Exhaled CO level, a biochemical validation of self-reported non-smoking status, is measured using a Vitalograph EC50 CO Monitor. A false negative result may occur if the participant has not smoked during the previous 24 hours. A false positive result occurs if the participant was recently exposed to excessively high levels of CO.

Assessment of daily activity (pedometer). The Digiwalker pedometer counts the number of steps taken and is worn for seven days. Each night, participants record the number of steps taken and amount of time the pedometer was worn.

Chemistry lab procedures

Samples of whole blood, plasma, serum and urine are stored at -70°C . Measurements

using these samples include a lipid panel by a conventional enzymatic chemistry analyzer (total cholesterol, total triglyceride, high-density lipoprotein [HDL] cholesterol, low-density lipoprotein [LDL] cholesterol), and detailed lipoprotein subclassification (type, size and concentration) by nuclear magnetic resonance (NMR) spectroscopy (25). Additional measures include apolipoprotein A1 (apoA1), apolipoprotein B (apoB), glucose, urine (micro) albumin, urine creatinine, hemoglobin A1C, fibrinogen, high-sensitivity C-reactive protein (CRP), homocysteine, lipoprotein(a) (Lp(a)), and apolipoprotein E (apoE) genotype. Fasting insulin and thyroid-stimulating hormone levels (TSH) are measured using chemiluminescence (26). Plasma samples and buffy coats are stored at -70°C and then sent to SFBR for DNA isolation, genotyping and analysis.

Cardiovascular examination

Atherosclerosis of the carotid artery system is assessed by ultrasound, using an established protocol (27,28). Combining ultrasound evidence of discrete atherosclerotic plaques with the intimal-medial thickness (IMT) of the common carotid incorporates measures of both atherosclerosis and arteriosclerosis (27,28). Two-dimensional (B-Mode) and 2-D guided M-mode images and Doppler recordings identify and grade the severity of stenotic lesions. Standard electrocardiograms using computerized electrocardiography (GE Medical Systems) are processed and reviewed for evidence of myocardial infarction, ischemia and left ventricular hypertrophy (LVH), using the GE Marquette Electronics MUSE system and a program to derive Minnesota Codes from digital ECG tracings (29). Carotid

ultrasound results and ECGs are transmitted to Cornell Medical Center for review.

Markers of infectious disease

Based on evidence for an association between CVD and multiple infectious agents, the following measures are made.

Infectious serology. IgG, IgA and IgM antibody to *C. pneumoniae* are determined by microimmunofluorescence, using formalin-fixed whole elementary bodies of *C. pneumoniae* strain AR-39 as an antigen (30). Serum IgG antibodies to other pathogens are determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits from Wampol (CMV and *H. pylori*), Focus (HSV1 and HSV2), DiaSorin Inc. (HAV) and Ortho (HCV).

Mannose-binding protein (MBP). Genotyping is used to assess four functional coding sequence polymorphisms of the MBP gene, associated with variation in circulating levels of mannose-binding lectin and susceptibility to infection. These sequence variants are (a) G to A transition in exon 1, causing replacement of glycine by aspartic acid at amino acid 54; (b) G to A transition, causing replacement of glycine by glutamic acid at amino acid 57; (c) C to T transition, causing replacement of arginine by cysteine at amino acid 52, and (d) a potentially functional G to C transition at nucleotide position -550 of the MBP promoter, previously correlated with CVD in a small sample of Native Alaskans (31).

Participant referral

Individuals participating in the clinical and laboratory examinations receive assistance in securing medical care for significant medical

conditions uncovered during the exams, as well as education about a healthier lifestyle aimed at preventing cardiovascular disease. GOCADAN uses established guidelines for

medical referrals, based on findings from clinical and laboratory examinations, which are summarized in Table I. The liaison NSHC physician for clinical evaluation receives a

Table I. Clinical referral guidelines for GOCADAN participants, 2001-2004.

Type of Referral	M.D. visit scheduled within:	Indications	Actions
Emergency	Immediately	Systolic BP > 260 mm Diastolic BP > 130 mm Abnormal ECG w/ acute cardiac symptoms Symptom suggesting life-threatening angina or edema	Refer to village paramedic personnel Phone/radio consult Nome NSHC physician Air evacuation if needed
Immediate	1 day	Fasting glucose > 400 mg/dl Systolic BP 200-259 mm Diastolic BP 105-129 mm Diabetic foot ulcer Angina in last day Neurologic symptoms in past week Carbon monoxide reading >125	Notify participant's physician or NSHC Arrange for appointment and transport Provide NSHC referral
Urgent	1 week	Suspected heart failure Inappropriate medication use One Touch glucose > 200 mg/dl (absent diabetes) Active TB symptoms Carbon Monoxide reading 20-124 (non-smoker) or 50-124 (smoker) Untreated neurological symptoms 1 week to 6 months ago	Arrange for appointment and transport Provide NSHC referral
Routine	1 month or first convenient	Systolic BP 140-199 mm Diastolic BP 90-104 mm Old MI (Rose Questionnaire) Previously unrecognized neurological problem (i.e. stroke) Previously unrecognized claudication Both pedal pulses missing in one extremity Doppler ankle/arm pressure ratio < 0.8 Hemoglobin <10 (women) or <12 (men)	Arrange for appointment and transport Provide NSHC referral

BP, blood pressure; ECG, electrocardiogram; TB, tuberculosis; MI, myocardial infarction; NSHC, Norton Sound Health Corporation

summary of laboratory findings as soon as they are available.

Surveillance and follow-up

As part of the baseline survey, we have characterized prevalent disease by reviewing and adjudicating events reported by participants. Cohort surveillance will define incidence rates for fatal and non-fatal CVD outcomes by reviewing deaths as they are discovered and reported events after participants are re-examined. Review procedures and criteria for the definition of events have followed recent AHA/NHLBI guidelines (32).

Logistics of field exam implementation

Conducting research in remote Alaskan villages offers unique logistic challenges. Before any research activity begins in a study village, a project investigator meets with village leaders to describe the research, answer questions and obtain their permission to proceed with the study. Next, the household survey is conducted and the research team rents a home to be used for processing blood and urine specimens, conducting physical exams, and administering questionnaires. Once a clinic site is secured, the team sets up local and long-distance phone services, assures the site has proper electrical wiring, hires a fuel delivery person, and sets up an account in the native store for supplies. A recruiter and field technician (usually a retired health aide) are hired from the village based on suggestions from local leaders and health clinic staff.

A Norton Sound air cargo carrier is hired to transport project equipment between study villages. The equipment is the seated snow mobile, or a four-wheeler, because, for seven months of the year, roads are rarely cleared to

permit vehicle use in the villages. The project has a four-wheeler used for participant transportation. Staff transport on passenger bush planes must be arranged separately from the cargo flights.

Once the equipment reaches the new village, staff from the previous study village help to establish the new clinic and train incoming village team members hired to help with the study. Once field examinations begin, weekly flights to Nome assure the transport of specimens and equipment needing repair. Staff residing outside of the study villages live at the village GOCADAN clinic during the work week.

Alaskan climate and culture dictate that recruitment and exams be scheduled around seasonal activities. After the spring ice break-up, most villagers are engaged in subsistence fishing, while hunting activities take precedence during the fall and winter. Long summer days mean late sleep schedules, limiting morning exams, and presenting a challenge for individuals fasting for evening clinics. The most critical determinant of air transport in Alaska is “weather permitting” – all study shipments and travel depend on this condition.

Staff training

Clinic staff are certified in data collection according to the standards described in the GOCADAN Operations Manual (33). Staff members are certified for each component of the examination, using checklists contained in the manual. This ensures uniform training and methodology across villages. A detailed set of quality assurance checks is covered for each examination item. The manual details protocols for measurements, including blood pres-

tures, impedances and electrocardiograms, personal interview techniques, and recruitment.

An experienced, certified research project coordinator initially traveled to the field site in Alaska to conduct training before the GOCADAN field examinations. The training included lectures, interactive discussions, demonstrations and return demonstrations. In addition to the manual, other visual aids were used, including videotapes and handouts. All equipment and supplies for the examination were used during training. The entire field staff, the principal investigator and selected co-investigators attended the week-long training.

In addition to formal skills training, time was allotted to address such issues as recruitment, clinic flow, assignment of participant ID numbers, quality control and data management. To ensure quality control, site visits are conducted and duplicate measurements and laboratory specimens are processed.

Genotyping methods

Sample collection and DNA isolation. Buffy coats isolated from fasting blood samples are stored in a -20°C freezer in the village clinic, transported to Nome within a week for storage at -80°C at the NSHC Hospital, and transported regularly from Nome to the Penn Medical Laboratories, where buffy coats are forwarded to the Genetics Core Laboratory at SFBR for DNA extraction. One buffy coat sample from each GOCADAN participant remains stored at the NSHC Hospital.

Genotyping short tandem repeats. Each GOCADAN participant is genotyped using the ABI PRISM Linkage Mapping Set-MD10 Version 2.5 (Applied Biosystems), which

consists of PCR primers for microsatellite loci (34-36) spaced an average of 10-centiMorgans (cM) apart (range 2.4 to 24.1 cM). Markers are amplified in separate PCR reactions to avoid preferential amplification, using True Allele PCR Premix (Applied Biosystems), and Applied Biosystems 9700 thermocyclers, according to the manufacturer's specifications. Each individual's PCR reaction products are pooled, fluorescently labeled, and loaded into an ABI PRISM 3100 Genetic Analyzer for automated genotyping. Genotypes are assigned using the Genotyper software package (Applied Biosystems).

High-resolution mapping and positional candidate gene analysis

For chromosomal regions in which we identify quantitative trait loci (QTLs) influencing variation in disease risk factors, we will type additional loci to obtain a higher resolution genetic map of that region. Candidate genes in such regions are genotyped for any previously identified single nucleotide polymorphisms (SNPs) in our study population, and linkage/disequilibrium analysis for association with the phenotype is performed.

Genotype and pedigree data cleaning. To clean the genotypic data for linkage analysis, we must eliminate pedigree errors (pedigree optimization), eliminate Mendelian and double recombinant errors, estimate marker allele frequencies, construct a genetic map, and calculate multipoint identity-by-descent (IBD) matrices. PEDSYS (37), the genetic analysis package SOLAR (38), PREST (39,40), SimWalk2 (41), CRIMAP (42), and LOKI are all used to this end (43). For some tasks, we use the computer ranch at the Southwest Foun-

dation in San Antonio, which includes 1500 processors operating in parallel.

Validating the accuracy of the pedigrees is an ongoing process that continues even after recruitment has been completed. Using the PEDSYS (37) program, family data were then translated into pedigree drawings and returned to field staff for verification by participants wherever reported relationships were unclear, or inconsistent. During the second stage of verification, pedigree structures and genetic marker data are used to check one another based on the markers' consistency with Mendelian laws of segregation. Pedigree errors are corrected by changing, or deleting problematic parent-offspring links, whenever an individual link can be unambiguously identified as discrepant. When more than one individual may be the source of the discrepancy, the SimWalk2 (41) program is used to make decisions about any genotypes to exclude. Questions about family relationships must be asked with sensitivity to each family member's background, particularly when a family member is known to be adopted, or where questions exist about the identity of a person's biological mother, or father. Caution was used in any case where the interviewer might have information of which the family member might be unaware. All parental relationships indicated by marker data that were undisclosed, or unknown by family members are not verified in the community, in order to protect the privacy of individuals.

Data handling and methods of analysis. Phenotypic and family history data are transferred to the PEDSYS program (37). To evaluate the genetic and environmental contributions to CVD risk factors, we use a variance

component approach implemented in SOLAR (38). The quantitative phenotype for an individual (y) is modeled as (44,45):

$$y = \mu + \sum \beta_j v_{ij} + g_i + e_i \quad [1]$$

where μ is the mean of the trait in males, β_j is the regression coefficient for the covariate j , v_{ij} is the value of covariate j in individual i , and g_i and e_i represent the deviations from μ for the individual i that are attributable to additive genetic effects and unmeasured environmental effects, respectively. g_i and e_i are assumed to be uncorrelated with one another and normally distributed with mean 0 and variances σ_g^2 and σ_e^2 .

Analyses of CVD risk factors uses combined data from all villages. Analysis of each phenotype is restricted to individuals for whom all relevant covariate data are complete. Any individual with a phenotypic value greater than four standard deviations from the mean is excluded. Individuals currently taking lipid-lowering medications are excluded from analyses of lipoprotein phenotypes. Similar exclusions are made for individuals taking anti-hypertensive medications in blood pressure analyses and for those taking anti-diabetic medications in the analysis of glucose and insulin phenotypes.

RESULTS

Table II shows means and ranges for selected CVD risk factors among the 1,214 participants, and compares men and women with respect to these factors, adjusting for age and village. Women had higher HDL levels and lower blood pressure than men; however, women had higher indices of glycemia and were more obese. In the initial heritability analysis, we include the following covariates: gender, age, age by

Table II. Distribution of phenotypes among initial 1,214 participants in GOCADAN, 2001-2004.

Phenotype	Males (n = 537)	Females (n = 677)	p-value
	Mean (range)	Mean (range)	
Obesity			
Height (m)	1.70 (1.45-1.91)	1.57 (1.30-1.73)	<.0001
Weight (kg)	76.6 (50.9-143.0)	71.0 (40.9-133.7)	<.0001
BMI (kg/m ²)	26.6 (17.46-51.83)	28.6 (17.13-54.88)	<.0001
Waist-hip ratio	0.86 (0.70-1.24)	0.84 (0.51-1.27)	<.0001
Triceps (mm)	12.9 (3-34)	21.3 (5.5-51)	<.0001
Subscapular (mm)	14.6 (3-49)	20.4 (4-53)	<.0001
Lipids[†]			
LDL-C (mg/dL)	117 (40-249)	115 (34-275)	0.24
HDL-C (mg/dL)	55 (13-170)	63 (29-126)	<.0001
Total cholesterol (mg/dL)	196 (118-339)	203 (118-389)	0.002
Triglyceride (mg/dL)	126 (39-561)	129 (27-1296)	0.81
ApoA-I (mg/dL)	147 (41-263)	164 (59-258)	<.0001
ApoB (mg/dL)	99 (30-196)	98 (30-193)	0.48
Lp(a) (mg/dL)	4.99(0.06-42.21)	5.36 (0.05-51.36)	0.29
Blood pressure[‡]			
SBP (mmHg)	121 (84-163)	118 (84-168)	<.0001
DBP (mmHg)	78 (56-105)	74 (47-114)	<.0001
% who are hypertensive	22.7%	20.2%	0.29
Diabetes[§]			
Fasting glucose (mg/dL)	101 (47-400)	101 (48-281)	0.56
2-hour glucose (mg/dL)	90 (36-248)	101 (24-341)	<.0001
Fasting insulin [#] (uU/mL)	9.44 (1.90-88.5)	11.15 (1.90-64.6)	0.001
2-hour insulin [#] (uU/mL)	26.20 (1.90-255)	41.80 (4.8-294)	<.0001
ACR ^{**} (mg/g)	42.7 (1.7-3825)	41.1 (2.1-7530)	0.97
Hemoglobin A1c (%)	5.5 (4.6-11.9)	5.4 (4.6-9.0)	0.14
Inflammatory markers			
Fibrinogen (mg/dL)	328 (110-895)	334 (109-813)	0.42
Homocysteine (umol/L)	8.0 (1.9-31.1)	6.9 (1.9-30.7)	<.0001
CRP ^{††} (mg/dL)	0.31 (0.019-5.04)	0.35 (0.019-29.9)	0.68
Carotid U/S			
Left DD (mm)	6.02 (3.7-8.9)	5.57 (3.9-8.0)	<.0001
Left SD (mm)	6.66 (4.1-9.7)	6.16 (4.2-8.4)	<.0001
Left IMT (mm)	0.62 (0.3-1.4)	0.59 (0.3-1.2)	<.0001
Right DD (mm)	6.04 (4-8.9)	5.60 (3.75-7.9)	<.0001
Right SD (mm)	6.69 (4.5-9.7)	6.21 (4.4-8.5)	<.0001
Right IMT (mm)	0.62 (0.3-1.2)	0.60 (0.3-1.4)	0.17

BMI, body mass index; WHR, waist/hip ratio; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; Lp(a), lipoprotein a, SBP, systolic blood pressure; DBP, diastolic blood pressure; ACR, albumin/creatinine ratio; CRP, C-reactive protein; DD, diastolic diameter; SD, systolic diameter; IMT, intimal medial thickness. P-values are adjusted for age and village.

[†] Individuals taking cholesterol-lowering medications were not included in lipid analyses.

[‡] Individuals taking antihypertensive medications were not included in blood pressure analyses.

[§] Individuals taking diabetes-related medications were not included in diabetes analyses.

[#] Insulin – used 1.99 in calculations for results reported as < 2.

^{**} ACR – used 5.7 in calculations for results reported as ≤5.7

^{††} CRP – used 0.019 in calculations for results reported as < 0.02

gender interaction, diabetes status, percent Inupiat heritage, village, education, estrogen use, alcohol consumption, smoking status, and temperature and hours of daylight on the day of the exam. Covariates whose effects are significant at the $p \leq 0.10$ level in the initial analysis are retained in subsequent analyses. Table III lists covariates retained in each heritability analysis. Some potentially important covariates are excluded, because they may also be genetically mediated. Including such variables could substantially reduce the heritabilities if pleiotropic genes affecting both covariates and risk factors are present. Further analyses to detect and characterize such pleiotropy are planned. Table IV lists heritabilities of selected phenotypes for the initial 954 persons examined, measured as the proportion of residual phenotypic variance due to the additive effect of genes. The proportion of phenotypic variance due to genes should be viewed as the minimum genetic contribution to the phenotype, because it only represents the additive genetic component. The residual variance almost certainly contains additional, non-additive genetic effects, meaning that the estimate of heritability of each trait (the proportion of variance in the trait due to genes) is likely to be a conservative one. With the exception of fat-free mass, fat mass, percent body fat, and homocysteine phenotypes, covariates accounted for at most 31% of the variance in the CVD risk factors. With the exception of insulin, all heritabilities were significant at $p \leq 0.05$. Obesity-related traits showed consistent substantial effects of genes. Heritabilities for lipid phenotypes were between 16% and 42%, with the exception of Lp(a), which had a heritability of 77%. Diabetes-related phenotypes showed relatively small genetic effects.

These data demonstrate that genetic effects explain a significant proportion of variability for several CVD risk factors in the GOCADAN population, suggesting that linkage analysis to search for the source of these signals is warranted.

DISCUSSION

GOCADAN provides a unique opportunity to characterize prevalence of cardiovascular disease and its associated risk factors, and to quantify the genetic contributions to CVD in the genetically isolated Alaskan Eskimo population, which is subject to increasing transition from a traditional to a Western lifestyle. With this lifestyle change has come increased prevalence of obesity and other CVD risk factors. In most U.S. populations, widespread dispersal of relatives makes identification and recruitment of extended families difficult. However, the GOCADAN participants make up a few very large families. For genetic analysis of common complex traits, the extended family sampling strategy is a powerful approach for the localization of susceptibility genes (e.g., 35-37,46-48). The relative homogeneity of the Norton Sound Eskimo communities also increases the likelihood of identifying genetic determinants of disease risk. Genes that interact with lifestyle factors to bring about disease may exist in high frequency in this small isolated population, which is highly susceptible to both founder effects and genetic drift. Enhancement of risk allele frequency by such forces would make these genes easier to detect.

Through the partnership established to conduct this study, important goals will be achieved. Community input will provide the

Table III. Non-genetic covariates retained in heritability analyses of cardiovascular disease phenotypes, initial 952 GOCADAN participants, 2000-2004.

Phenotype	Sex	Age	Age x sex	Estrogen	Center	Drinking	Smoking	Diabetes status	% Inupiat heritage	Education
Obesity										
Weight	x	x			x		x	x	x	
Body mass index	x				x		x	x		
Waist circumference		x			x			x		
Waist / hip ratio	x	x	x		x			x		x
Subscapular skin fold	x		x	x	x		x	x		
Triceps skin fold	x			x	x		x	x		x
Fat free mass	x	x			x		x	x	x	
Fat mass	x				x			x	x	
% body fat	x				x		x	x		
Lipids										
ApoA I	x	x	x	x	x				x	
ApoB		x	x		x			x		
Lp(a)								x	x	
Total cholesterol	x	x	x		x	x			x	
HDL	x	x	x		x				x	
LDL		x	x		x	x				
Triglycerides ^a			x	x		x				
small LDL	x	x	x		x		x	x		
medium LDL	x								x	
large LDL	x	x							x	
LDL size	x	x			x	x		x	x	
small HDL			x	x	x	x				
intermediate HDL		x		x	x	x		x		
large HDL	x	x			x	x			x	
HDL size	x	x			x				x	
Blood pressures										
Systolic	x	x	x		x		x	x		x
Diastolic	x		x	x	x		x			
Diabetes										
Fasting glucose		x			x			x		
Fasting insulin ^a	x		x		x	x		x		
A1C	x	x	x		x			x		
Clotting factors										
Fibrina		x	x		x					
Homocysteine	x	x			x			x	x	x
Iron parameters										
Ferritin	x	x	x		x			x	x	
Hb	x	x	x	x	x		x	x	x	

^a log transformed

LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; Lp(a), lipoprotein a; Hb, hemoglobin; A1C, hemoglobin A1c

Table IV. Proportion of variance of CVD phenotypes due to genes and other covariates, initial 952 GOCADAN participants, 2000-2004.

Phenotype	Proportion of variance due to covariates (%)	Proportion of variance due to genes (%)	SE
Obesity			
Weight	11	58	0.08
Body mass index	11	55	0.08
Waist circumference	11.3	49	0.08
Waist / hip ratio	20.7	35	0.08
Subscapular skin fold	19.8	49	0.08
Tricep skin fold	33	45	0.08
Fat free mass	74.4	47	0.09
Fat mass	39.1	55	0.08
% body fat	73.4	44	0.08
Lipids*			
ApoA1	23	42	0.08
ApoB	12.5	24	0.07
Lp(a)	5	77	0.12
Total cholesterol	19.4	31	0.07
HDL-C	20.2	44	0.08
LDL-C	11	26	0.07
Total triglycerides	6.4	31	0.08
small LDL	10.1	16	0.09
medium LDL	2.8	32	0.11
large LDL	16.2	30	0.11
LDL size	15.2	23	0.11
small HDL	9.8	40	0.1
intermediate HDL	15.1	30	0.13
large HDL	18.9	41	0.11
HDL size	18.5	33	0.1
Blood pressures†			
Systolic	27	25	0.09
Diastolic	8.9	35	0.08
Diabetes‡			
Fasting glucose	24	16	0.07
Fasting insulin§	8	9	0.08
A1C	27.4	36	0.09
Clotting factors			
Fibrin	18.1	24	0.07
Homocysteine	39.9	28	0.09
Iron parameters			
Ferritin	24.9	21	0.08
Hb	31.6	15	0.07

* after exclusion of subjects with cholesterol lowering medications

† after exclusion of subjects with anti-hypertensive medications

‡ after exclusion of subjects with anti-diabetic medications

§: not significant at 0.05; all other heritabilities are significant

LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; Lp(a), lipoprotein a; Hb, hemoglobin; A1C, hemoglobin A1c

guidance needed to collect the villages' data properly and interpret them wisely. Inclusion of the communities is intended to create direct involvement of the people of Norton Sound, treating them as true participants in GOCADAN. Incorporating the NSHC, which provides expertise for local participant referrals, clinical confirmation of research findings, and access to historical and incident medical records, assures that research findings will be translated directly into the institution providing medical care to study participants. Finally, the academic institutions provide the research expertise, analytic capability, and access to funding. We anticipate that this partnership will contribute to the greater understanding of cardiovascular problems and to better ways of addressing those problems among both Norton Sound Eskimos and the larger population.

Acknowledgment

This research is supported by grant U01 HL64244 from the National Heart, Lung, and Blood Institute.

REFERENCES

1. Kromhout D, Bosschieter EB, Coulander CL. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985;312:1205-09.
2. Davidson M, Bulkow LR, Gellin BG. Cardiac mortality in Alaska's indigenous and non-native residents. *Int J Epidemiol* 1993;22:62-71.
3. Lanier AP, Ehrsam G, Sardidge J. Alaska Native mortality 1989-1998. Office of Alaska Native Health Research, Division of Community Health Services, Alaska Native Tribal Health Consortium, 2002.
4. Middaugh JP. Cardiovascular deaths among Alaskan Natives 1980-86. *Am J Public Health* 1990;80:282-85.
5. Nobmann ED, Ebbesson SOE, White RG, et al. Dietary intakes among Siberian Yupiks of Alaska and implications for cardiovascular disease. *Int J Circumpolar Health* 1998;57:4-17.
6. Ebbesson SOE, Schraer DC, Nobmann ED, Ebbesson LOE. Lipoprotein profiles in Yupik Eskimos: the Alaska-Siberia Project. *Arctic Med Res* 1996;55:165-73.
7. Schraer CD, Ebbesson SOE, Adler AI, Cohen JS, Boyko EJ, Nobmann ED. Glucose tolerance and insulin resistance syndrome among St. Lawrence Island Eskimos: The Alaska-Siberia Project. *Arct Med Res* 1998;57:4-17.
8. Ebbesson SOE, Schraer CD, Adler A, et al. 1998. Diabetes mellitus and impaired glucose tolerance in three Alaskan Eskimo populations: The Alaska-Siberia Project. *Diabetes Care* 1998;21:563-69.
9. Ebbesson SOE, Kennish J, Ebbesson LOE, Go O, Yeh J. Diabetes is related to fatty acid imbalance in Eskimos. *Int J Circumpolar Health* 1998;58:108-19.
10. Schraer CD, Ebbesson SOE, Boyko EJ, Nobmann ED, Adler AI, Cohen J. Hypertension and diabetes among Siberian Yupik Eskimos of St. Lawrence Island, Alaska. *Public Health Reports, Vol. III, Supplement, 1996*; 42-4.
11. Schraer CD, Risica PM, Ebbesson SOE, Go, OT. Low fasting insulin levels in Eskimos compared to American Indians: Are Eskimos less insulin resistant? *Int J Circumpolar Health* 1999;58:272-80.
12. Risica PM, Schraer C, Ebbesson SOE, Nobmann ED, Caballero B. Overweight and obesity in Alaskan Eskimos of the Bering Straits Region: The Alaska Siberia Project. *Int J Obes Relat Metab Disord* 2000;24:939-44.
13. Risica PM, Ebbesson SOE, Schraer CD, Nobmann ED, Caballero B. Body fat distribution in Alaskan Eskimos of the Bering Straits Region: The Alaska Siberia Project. *Int J Obes Relat Metab Disord* 2000; 24:171-179.
14. Young TK, Moffatt ME, O'Neil JD. Cardiovascular diseases in a Canadian Arctic population. *Am J Public Health* 1993;83:881-7.
15. Hegele RA, Young TK, Connelly PW. Are Canadian Inuit at increased genetic risk for coronary heart disease? *J Mol Med* 1997;75:364-70.
16. Boudreau DA, Scheer WD, Malcom GT, Mulvad G, Pedersen HS, Jul E. Apolipoprotein E and atherosclerosis in Greenland Inuit. *Atherosclerosis* 1999; 145: 207-19.
17. Pederson HS, Mulvad G, Newman WP 3rd, Boudreau DA. Atherosclerosis in coronary arteries and aorta among Greenlanders: an autopsy study. *Atherosclerosis* 2003;170:93-103.
18. Bjerregaard P, Young TK, Hegele RA. Low incidence of cardiovascular disease among the Inuit—what is the evidence? *Atherosclerosis* 2003; 166:351-7.
19. Hegele, RA. Genetic prediction of atherosclerosis: lessons from studies in native Canadian populations. *Clin Chim Acta* 1999;286:47-61.
20. Bjerregaard P, Young TK, Dewailly E, Ebbesson SO. Indigenous health in the Arctic: an overview of the circumpolar Inuit population. *Scand J Public Health* 2004;32:390-5.
21. Hegele RA, Cao H, Harris SB, Hanley AJ, Zinman B. The hepatic nuclear factor-1alpha G319S variant is associated with early-onset type 2 diabetes in Canadian Oji-Cree. *J Clin Endocrinol Metab* 1999; 84: 1077-82.

22. Nobmann ED, Ebbesson SOE, White RG, Bulkow LR, Schraer CD. Association between dietary factors and plasma lipids related to cardiovascular disease among Siberian Yupiks of Alaska. *Int J Circumpolar Health* 1999;58:254-71.
23. Biery AJ, Ebbesson SOE, Shuldiner AR, Boyer BB. The b3-adrenergic receptor TRP64ARG polymorphism and obesity in Alaskan Eskimos. *Int J Obes* 1997; 21:1176-79.
24. Lohman TG, Roche AF. Anthropometric standardization reference. Visby, Sweden: Books on Demand, 1988.
25. Otvos JD, Jeyarajah EJ, Bennett DW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin Chem* 1992; 38:1632-8.
26. Burtis CA, Ashwood ER, editors. Tietz textbook of clinical chemistry. 2nd ed. Philadelphia, PA: WB Saunders, 1994:943-44.
27. Roman MJ, Saba PS, Pini R, et al. Parallel cardiac and vascular adaptation in hypertension. *Circulation* 1992;86:1909-18.
28. Roman MJ, Pickering TG, Schwartz JE, Pini R, Devereux RB. The association of carotid atherosclerosis and left ventricular hypertrophy. *J Am Coll Cardiol* 1995;25:83-90.
29. Okin PM, Devereux RB, Howard B, Fabsitz RR, Lee ET, Welyt TJ. Assessment of QT interval and QT dispersion for prediction of all-cause and cardiovascular mortality in American Indians: The Strong Heart Study. *Circulation* 2000;101:61-66.
30. Davidson M, Cho-Chou K, Middaugh JP, Campbell LA, Wang SP, Newman WP. Confirmed previous infection with *C. pneumoniae* (TWAR) and its presence in early coronary atherosclerosis. *Circulation* 1998;98:628-33.
31. Jacobsen S, Baslund B, Madsen HO, Tvede N, Svejgaard A, Garred P. Mannose-binding lectin variant alleles and HLA-DR4 alleles are associated with giant cell arteritis. *J Rheumatol* 2002;29:2148-53.
32. Luepker R, Apple F, Christenson R, et al. Case Definitions for Acute Coronary Heart Disease in Epidemiology and Clinical Research Studies. *Circulation* 2003;108:2543-2549.
33. GOCADAN Operations Manual. San Antonio, TX: Population Genetics Laboratory, Southwest Foundation for Biomedical Research, 2000-2004.
34. Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, et al. A second-generation linkage map of the human genome. *Nature* 1992;359:794-801.
35. Gyapay G, Morissette J, Vignal A, et al. The 1993-1994 Genethon human genetic linkage map. *Nat Genet* 1994;7:246-339.
36. Dib C, Faure S, Fizames C, et al. A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 1996;380:152-54.
37. Dyke B. PEDSYS, a pedigree data management system user's manual. Population Genetics Laboratory Technical Report No. 2. second edition. San Antonio, TX: Southwest Foundation for Biomedical Research, 1993.
38. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* 1998;162:1198-1211.
39. McPeck MS, Sun L. Statistical tests for detection of misspecified relationships by use of genome-screen data. *Am J Hum Genet* 2000;66:1076-94.
40. Sun L, Wilder K, McPeck MS. Enhanced pedigree error detection. *Hum Hered* 2002;54:99-110.
41. Sobel E, Lange K. Descent graphs in pedigree analysis: applications to haplotyping, location scores, and marker sharing statistics. *Am J Hum Genet* 1996; 58:1323-37.
42. Green P, Falls K, Crooke S. Documentation for CRIMAP, version 2.4. 1990 (<http://www.cbi.pku.edu.cn/Docback/Embnetut/Crimap/manualp.txt>).
43. Heath SC. Markov chain segregation and linkage analysis for oligogenic models. *Am J Hum Genet* 1997;61:748-60.
44. Falconer DS. Introduction to quantitative genetics, third edition. Essex, UK: Longman, 1989.
45. Amos CI. Robust variance-components approach for assessing genetic linkage in pedigrees. *Am J Hum Genet* 1994;54:535-43.
46. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980;32:314-31.
47. Amos CI, Zhu DK, Boerwinkle E. Assessing genetic linkage and association with robust components of variance approaches. *Ann of Hum Genet* 1996; 60:143-60.
48. Williams JT, Duggirala R, Blangero J. Statistical properties of a variance-components method for quantitative trait linkage analysis in nuclear families and extended pedigrees. *Genet Epidemiol* 1997;14:1065-70.

Dr. Barbara Howard
 6495 New Hampshire Avenue, Suite 201
 Hyattsville, MD 20783
 USA
 Email: Barbara.v.howard@medstar.net