

# The genetic response to short-term interventions affecting cardiovascular function: Rationale and design of the Heredity and Phenotype Intervention (HAPI) Heart Study

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**Background** The etiology of cardiovascular disease (CVD) is multifactorial. Efforts to identify genes influencing CVD risk have met with limited success to date, likely because of the small effect sizes of common CVD risk alleles and the presence of gene by gene and gene by environment interactions.

**Methods** The HAPI Heart Study was initiated in 2002 to measure the cardiovascular response to 4 short-term interventions affecting cardiovascular risk factors and to identify the genetic and environmental determinants of these responses. The measurements included blood pressure responses to the cold pressor stress test and to a high salt diet, triglyceride excursion in response to a high-fat challenge, and response in platelet aggregation to aspirin therapy.

**Results** The interventions were carried out in 868 relatively healthy Amish adults from large families. The heritabilities of selected response traits for each intervention ranged from 8% to 38%, suggesting that some of the variation associated with response to each intervention can be attributed to the additive effects of genes.

**Conclusions** Identifying these response genes may identify new mechanisms influencing CVD and may lead to individualized preventive strategies and improved early detection of high-risk individuals. (*Am Heart J* 2008;155:823-8.)

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Family studies have consistently demonstrated the importance of genetic factors in contributing to cardiovascular disease (CVD) susceptibility, acting independently as well as through known CVD risk factors.<sup>1-6</sup> However, identification of the specific genes involved has proved challenging. Although a number of single genes influencing CVD susceptibility have been identified to date, they collectively account for only a small proportion of the population attributable risk for CVD. The difficulties in identifying the genes influencing CVD have been ascribed to the very small individual effects of many gene variants and the presence of gene by gene and gene by environment interactions that lead to context-dependent penetrance of susceptibility alleles. In recognition of the importance that gene by environment interactions may play in influencing cardiovascular health, the National Heart, Lung, and Blood Institute of the National Institutes of Health funded the PROGRAM for GENetic Interaction (PROGEND) network. This network includes 5 independent but complementary studies designed to assess the genetics of the cardiovascular response to controlled

short-term perturbations that mimic long-term exposures known to affect cardiovascular health, including stress, diet, and antiplatelet, and lipid-lowering therapy.

The HAPI Heart Study was initiated in 2002. We chose to study the Old Order Amish as we hypothesized that genetic effects of complex phenotypes might be easier to discern in this genetically homogeneous population. Four interventions were performed including (1) a high-fat challenge, after which we measured the post-prandial excursion in triglycerides; (2) a cold pressor stress test (CPT), for which we measured changes in blood pressure and endothelial function; (3) a dietary salt intervention, involving 6-day ingestion of a high-salt diet, followed by 6-day ingestion of a low-salt diet, during which we measured changes in blood pressure; and (4) 14 days of low-dose aspirin therapy, during which we measured changes in platelet function. These interventions were performed in a highly controlled and consistent manner and the relevant CVD-related responses were accurately measured. We selected these interventions for study because it has been established that they induce responses in CVD risk factors, and there is large interindividual variation in these responses (some of which are likely to be determined by genetic factors). Because a large set of baseline and response cardiovascular phenotypes were concurrently obtained, additional study goals were to determine the relationship of these response phenotypes to baseline CVD traits and to each other and to identify genes influencing individual phenotypes or sets of phenotypes.

## Methods

### Study population

Participants for the HAPI Heart Study were recruited from the Amish community of Lancaster County, PA. The Amish immigrated from central Europe (mainly Switzerland) to the United States to escape religious persecution over a 50-year period beginning in 1727.<sup>7</sup> The earliest immigrants settled in Lancaster County, PA, whereas later groups settled in the Midwest. Approximately 25 000 Old Order Amish currently live in Lancaster County today,<sup>8</sup> and virtually all can trace their ancestry back to the approximately 200 original founding individuals.<sup>9,10</sup>

Subjects were initially identified through prior participation in one of our previous studies, word of mouth, advertisements, a community-wide mailing, and referrals from local physicians. Only subjects aged 20 years and older were eligible to participate and age-eligible family members of all participating subjects were encouraged to enroll. In the planning stages of the study, support was obtained from the Amish community by the principal investigator (Dr Shuldiner) presenting the study objectives and procedures to community leaders and gaining their support to proceed. The study was approved by the Institutional Review Board of the University of Maryland, Baltimore, and other participating institutions and was monitored by an external Data Safety and Monitoring Board. Informed consent, including permission to contact relatives, was obtained before participation.

**Table 1.** Exclusion criteria for HAPI Heart Study

#### Study-wide exclusion criteria:

- Age < 20 yrs
- Non-Amish descent
- Currently pregnant or postpartum < 6 m
- Blood pressure at the time of screening > 180/105 (SBB/DBP) mm Hg
- Prescription medication use potentially affecting outcomes and vitamin or over-the-counter remedies that cannot be willingly or safely discontinued from 1 week before protocol initiation and until the end of the study (ie,  $\beta$ -blockers; calcium channel antagonists; ACE inhibitors; diuretics; lipid-lowering agents; nitrates; systemic glucocorticoids; adrenergic or cholinergic-acting agents, including cold formulas and antidepressants; and diet-weight loss agents)
- Coexisting malignancy
- Serum creatinine > 2.0 mg/dL
- AST or ALT > twice the upper limit of normal
- Hematocrit < 32%
- TSH < 0.4 or > 5.5 mIU/L

#### Intervention-specific exclusion criteria:

- Cold pressor stress test: history of Raynaud's disease
- High-fat challenge: malabsorption disorders, lactose intolerance, symptoms of gallbladder disease, and/or history of pancreatitis
- Dietary salt intervention: stage III or greater congestive heart failure and/or allergies to foods in the diet
- Aspirin intervention: history of bleeding disorder, gastrointestinal bleeding, blood pressure at the time of screening > 160/95 mm Hg, current use of aspirin for a condition that would place the subject at increased risk if it were to be discontinued for 14 days before protocol initiation (eg, history of unstable angina, myocardial infarction, angioplasty, coronary artery bypass grafting, atrial fibrillation, stroke or transient ischemic attack, type 2 diabetes, or deep vein thrombosis/other thrombosis), polycythemia (hematocrit > 52%), thrombocytosis (platelet count > 500 000), thrombocytopenia (platelet count < 75 000), surgery within the last 6 months, aspirin allergy, current breastfeeding, and/or aggregation with collagen 5  $\mu$ g/mL < 6.65  $\Omega$  or > 26  $\Omega$  or no aggregation at baseline with arachidonic acid

ACE, Angiotensin-converting enzyme; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TSH, thyroid-stimulating hormone.

### Eligibility criteria and recruitment

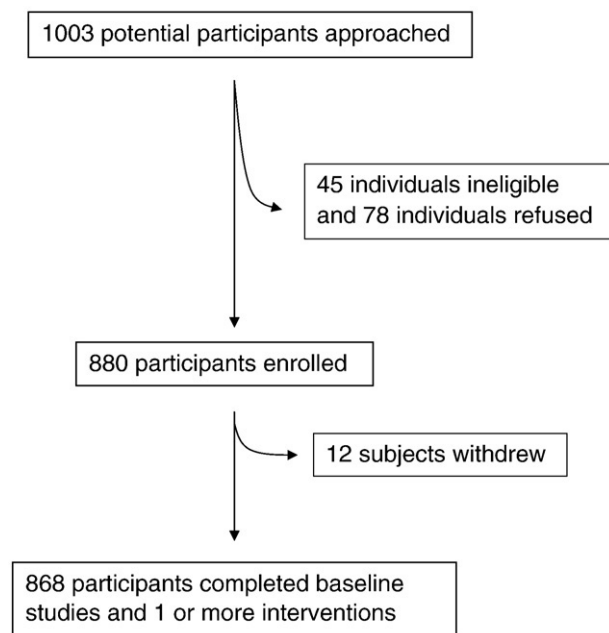
Before enrollment, potential study participants were visited at home by a study nurse and Amish liaison team, who performed a screening examination to determine eligibility. Although most subjects qualified for all interventions, there were separate exclusion criteria for each to enable subjects to participate in only those interventions for which they qualified. A summary of the global exclusion criteria and intervention-specific exclusion criteria is provided in [Table 1](#).

A total of 1003 individuals were identified for recruitment and received home visits to establish eligibility. Of these, 78 (7.8%) refused to participate and 45 (4.5%) did not meet one or more of the global eligibility criteria. Twelve eligible and consented individuals withdrew before completing any of the interventions, leaving a final sample size of 868 participants ([Figure 1](#)).

### Study protocol and interventions

The study protocol required a 4-week period to complete all 4 interventions (see [Figure 2](#)). After obtaining permission from their prescribing physicians, all medications, vitamins, and supplements were discontinued for 7 days before Clinic Visit 1 and for the duration of the study. As Amish do not drive cars,

**Figure 1**



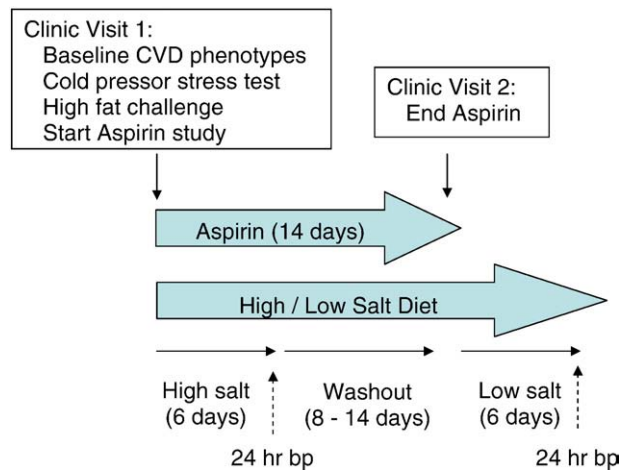
Recruitment of subjects into the HAPI Heart Study.

transportation to the Amish Research Clinic (Lancaster, PA) was provided. Subjects were instructed to fast for 12 hours before their appointment, to abstain from excessive physical activity on the morning of the visit, and to bring a first morning void urine sample. Clinic Visit 1 lasted for 8 to 10 hours, during which time the following were performed: physical examination, urine pregnancy test, fasting blood sample for pre-aspirin analyses of platelet aggregation and inflammatory markers, blood pressure monitoring, CPT, high-fat challenge, electrocardiogram, ultrasound measurement of carotid intima media thickness, measurement of pulse wave velocity and ankle-brachial index (ABI), and echocardiogram. All blood samples were obtained through an indwelling angiocatheter.

The CPT was conducted by having the subjects immerse their right hand and wrist in ice water for a period of 2½ minutes. Before immersion, blood pressure measurements were taken every 5 minutes for at least 20 minutes. Nine additional blood pressure measurements were taken during and after the cold pressor stimulus at minutes 1, 2, 3, 4, 5, 7.5, 10, 15, and 20 after immersion. A brachial artery reactivity test was performed during the course of the cold pressor stimulus to measure changes in brachial artery diameter during and after the CPT stimulus.

The high-fat challenge was administered 1 hour after completion of the CPT. Before the beginning of the fat challenge, blood was drawn to measure fasting lipids and brachial artery flow-mediated vasodilation was measured to assess fasting endothelial function. The high-fat challenge, prepared in the form of a whipping cream milk shake, was standardized to consist of 782 calories per m<sup>2</sup> of body surface

**Figure 2**



Schema of HAPI Heart Study intervention schedules.

with 77.6% of calories from fat, 19.2% from carbohydrate, and 3.1% from protein. After ingestion, blood was drawn at 1, 2, 3, 4, and 6 hours to assess the triglyceride excursion. Brachial artery flow-mediated vasodilations were also performed at 2, 4, and 6 hours after the fat challenge to assess post-prandial effects on endothelial function. The subject rested and remained fasting during the 6 hours post-fat challenge.

The aspirin intervention was begun the day after Clinic Visit 1; for the next 14 consecutive days the subject took 81 mg aspirin every day. One to 3 days before the second clinic visit a nurse and liaison performed a home visit to ensure compliance. On the 14th day, the subjects took their aspirin shortly before arriving at the Amish Research Clinic for Clinic Visit 2. Fasting blood was drawn to measure post-aspirin platelet aggregation. The subjects also collected and brought their first morning urine sample for measurement of 11-dehydrothromboxane B2. To assess compliance, a pill count was performed and each subject kept a diary. The subjects were permitted to miss up to 4 aspirin doses over the 2-week period and still be included in the final analysis, provided that they took the aspirin for at least 3 consecutive days before Clinic Visit 2. The aspirin intervention could be extended for up to 3 days (17 days total) to meet this criterion.

The Monday after Clinic Visit 1, subjects began 6 days of a high-salt diet, followed by a 6- to 14-day washout period, and then 6 days of a low-salt diet. The 6-day course for the high-/low-salt interventions was chosen so that the special diet would not interfere with their Sunday church meal. All meals were culturally appropriate and prepared by an Amish caterer and provided to the subjects by home delivery. The high- and low-salt diets contained 280 and 40 meq sodium per day, respectively. Both diets contained 140 meq potassium per day. The diets provided approximately 2900 kcal/d with 55% from carbohydrates, 15% from protein, and 30% from fats. On the sixth day of each diet, the subjects wore an ambulatory blood pressure monitor to assess their blood pressure during a 24-hour period. A nurse and Amish liaison visited the subjects on the third and fifth days of each diet to ensure that they were not

adversely affected by the diets and to check their food diary for compliance. In addition, the subjects were instructed to collect the first morning urine sample on days 4, 5, and 6 for measurement of sodium and creatinine as an independent assessment of compliance to the salt diets. The 3-day means of the urine sodium/creatinine ratios were  $162.1 \pm 60.8$  meq/mg (range 20.4-444.1) and  $33.5 \pm 18.6$  meq/mg (range 6.9-164.9) for the high- and low-salt diets, respectively, indicating a >4-fold decrease in urinary sodium excretion from high- to low-salt diets. During the high- and low-salt interventions, subjects also recorded into their diaries the times they went to bed and woke up to define active and inactive periods. Direct measurements of physical activity were obtained during this period by having subjects wear accelerometers (Actical, Mini Mitter/Respironics, Bend, OR) for 6 consecutive days.

### DNA collection protocol and genotyping

Genomic DNA was isolated from whole blood for genetic analyses on all participants. The quality of DNA was assessed by single SNP TaqMan genotyping (ABI) as well as by agarose gel electrophoresis as part of the protocol for whole genome genotyping with Affymetrix 500K SNP chips.

### Description of genetic analysis approaches

The primary phenotypes collected in this study are listed in Table II. The primary outcomes are the responses to the different interventions. These can be parameterized in a number of ways. For example, biologically relevant parameters that can be derived from the CPT include maximal blood pressure change from baseline, total blood pressure excursion (incremental area under the curve [iAUC]), or divided into reactivity (iAUC during the first 2 minutes) and recovery (iAUC from 3 to 5 minutes).<sup>11</sup> More detailed descriptions of response phenotypes will be the subject of future reports. Three types of genetic analyses will be carried out to detect the genetic effects of response phenotypes in the HAPI Heart Study: heritability analysis to quantify the contribution of unmeasured additive genetic effects,<sup>11</sup> linkage analysis to detect genetic effects attributable to specific measured loci,<sup>12</sup> and association analyses to detect measured genotype-phenotype associations. Power to detect these types of genetic effects in the HAPI Heart Study sample was estimated by simulating genotypes for founding members of the pedigrees, dropping alleles down through the pedigrees according to Mendel's laws, and assigning phenotypic values for the 868 examined individuals under various genetic models.<sup>12,13</sup>

## Results

### Composition and family structures of the HAPI Heart Study

A total of 868 subjects were enrolled into the HAPI Heart Study between May 2003 and August 2006. Participants included members of 184 sibships, ranging in size from 2 to 10, plus an additional 344 individuals with no siblings in the study. A total of 425 of these subjects could be combined into a single pedigree by adding in the parents and grandparents of all examined individuals. Relationship types among the examined

**Table II.** Phenotypic measurements in the HAPI Heart Study

Category	Measurements
Demographics	Age, sex, marital status, parity, occupation
Medical history	Hypertension, diabetes, cancer, myocardial infarction, stroke, heart or carotid surgery, medication use
Lifestyle	Smoking, physical activity
Anthropometry and resting BP and HR	Height, weight, waist circumference, BP, and HR (3 measures)
Baseline blood analyses	Complete blood count, fasting serum glucose, insulin, adiponectin, AST, ALT, TSH, BUN, creatinine, lipid profile and subfractions, lipid particle size, measures of inflammation
Baseline urine analyses	Sodium, creatinine
Other	Carotid intima media thickness, pulse wave velocity, ABI, echocardiogram
Intervention-specific:	
High-fat challenge	Lipids: fasting blood sample (just before the test) and at 1, 2, 3, 4, and 6 h after the test Brachial artery flow-mediated vasodilation: fasting and at 2, 4, and 6 h after the test
Cold pressor stress test	BP: stable baseline and during and after the test at 1, 2, 3, 4, 5, 7.5, 10, 15, and 20 min HR: along with BP measurements Brachial artery reactivity: before and during the test and after withdrawal from ice water
High-/low-salt diets	Ambulatory BP and HR monitoring: on the 6th day of each diet for 24 h Urine sodium and creatinine: first morning void collected on days 4, 5, and 6 of each diet
Aspirin	Platelet aggregation and platelet function assays: whole blood samples before and after 14 d of aspirin therapy using collagen, ADP, and arachidonic acid as agonists Urinary 11-dehydrothromboxane B2: first morning void before and after 14 d of aspirin therapy

ADP, Adenosine 5-diphosphate; BUN, blood urea nitrogen; HR, heart rate.

individuals included 633 sib pairs, 327 parent-offspring pairs, 443 avuncular (aunt/uncle-niece/nephew) pairs, and 191 first-cousin pairs.

### Clinical characteristics of HAPI Heart Study participants

Clinical characteristics of the 868 HAPI Heart Study participants are described in Table III. The mean age ( $\pm$ SD) of study subjects was 43.7 ( $\pm$  14.0) years with a range of 20 to 80 years. Mean body mass was 25.6 and 27.8 kg/m<sup>2</sup> in men and women, respectively. High-density lipoprotein cholesterol levels were relatively high and triglyceride levels low in this physically active Amish population.

Of the 868 subjects enrolled in the study, 466 completed all 4 interventions, 796 completed at least 3 interventions, 857 completed at least 2 interventions, and 867 completed at least 1 intervention. The salt intervention could not be offered throughout the entire study period, including during the wedding season each year (November-December); it was carried out in only

**Table III.** Clinical characteristics of HAPI Heart Study participants (n = 868), according to sex †

Characteristic	Men (n = 460)	Women (n = 408)
Age (y)	42.2 ± 0.6	45.4 ± 0.7***
BMI (kg/m <sup>2</sup> )	25.6 ± 0.1	27.8 ± 0.3***
TCHOL (mg/dL)	202.5 ± 2.1	215.7 ± 2.5**
HDL (mg/dL)	52.6 ± 0.6	59.5 ± 0.8***
TG (mg/dL)	63.9 ± 1.7	73.8 ± 2.3***
SBP (mm Hg)	121.5 ± 0.6	121.4 ± 0.8
DBP (mm Hg)	77.6 ± 0.4	75.8 ± 0.4***
Diabetes (%)	0.9	1.0
Current smokers (%) ‡	20.0	0.0***
% on lipid-lowering medications §	1.1	1.0
% on antihypertensive medications §	0.2	0.3

\*\*P < .01; \*\*\*P < .001, men vs women, adjusted for age.

†Values represent means ± SE (or percentiles).

‡Includes use of cigarettes, cigars, and pipes.

§Medication use assessed at the time of recruitment (before participants being asked to discontinue their medication use).

473 subjects. Table IV provides the mean (±SDs) for several of the primary response traits and their heritabilities. The traits shown include the TG excursions after the high-fat challenge, expressed as iAUC; the systolic (SBP) and diastolic blood pressure (DBP) responses to the CPT, computed as iAUC from 0 to 2 minutes during the CPT; the SBP and DBP responses to the high-salt diet during the active (awake) period, computed as the differences in the means between the high- and low-salt diets; and the percent change in platelet aggregation to a 1 µg/mL collagen agonist in response to 14 days of aspirin therapy. As expected, there is great interindividual variation in responses to the same standardized interventions. Significant differences in mean response between men and women were observed for the triglyceride response to the high-fat challenge (P < .001); there were no significant sex differences in the other response phenotypes.

The heritabilities of all response traits, after adjustment for age, sex, and body mass index (BMI), differed significantly from zero, with the exception of the SBP response to the high-salt diet during the active period. The heritabilities for the remaining traits ranged from 0.16 for the reactivity of SBP to the CPT (P = .007) to 0.38 for the triglyceride response to the high-fat challenge (P < .001). More detailed heritability analyses will be the subject of future studies.

#### Power to detect genetic effects

Power calculations indicated that this sample provides 69% power to detect a heritability of 10% and 89% power to detect a heritability of 15%, at α = .01. For linkage, our simulations indicated that we would have 57% power to detect an LOD score of 3.0 and 85% power to detect an LOD score of 2.0 for a quantitative trait locus accounting for 20% of the total trait variation. Power to detect associations in this sample is excellent, with our

**Table IV.** Mean (± SE) values and heritabilities of selected primary response phenotypes \*

Phenotype	Men (n = 460)	Women (n = 408)	Heritability ± SE
High-fat challenge			
Triglyceride	552.0 ± 15.9	369.7 ± 19.3 †	0.38 ± 0.08 †
iAUC (6 h)			
Cold pressor stress			
test reactivity			
SBP iAUC	27.3 ± 0.8	29.3 ± 1.2	0.16 ± 0.07 ‡
(2 min)			
DBP iAUC	19.9 ± 0.6	15.5 ± 0.9	0.24 ± 0.08 †
(2 min)			
Salt sensitivity			
(active period)			
SBP	0.5 ± 6.0	0.1 ± 0.5	0.08 ± 0.11
DBP	-1.9 ± 0.3	-1.4 ± 0.4	0.19 ± 0.12 §
Aspirin			
Collagen	6.8 ± 0.2	6.5 ± 0.3	0.19 ± 0.08 ‡
(1 µg/mL)			

\*Sex differences adjusted for age; all heritability analyses adjusted for age, sex, and BMI (cold pressor analyses additionally adjusted for baseline heart rate).

†P < .01.

‡P < .001.

§P < .05.

simulations indicating 80% power to detect a quantitative trait locus explaining 1% to 2% of phenotypic variation at α = .01, 2% to 3% of phenotypic variation at α = .0001, and 4% to 5% of phenotypic variation at α = .000001.

## Discussion

The HAPI Heart Study was designed to facilitate the mapping of genes that influence response to 4 different environmental perturbations relevant to CVD. Our design is an efficient way of studying gene by environment interactions as the between-person variation across different environments is eliminated. Indeed, the heritability analyses indicate that for nearly all traits, there is statistical evidence that some of this variation can be attributed to the additive effects of genes. Furthermore, as the 4 interventions were performed in the same individuals, we may discern genes that have pleiotropic effects, for example, blood pressure response to CPT and blood pressure response to a high-salt diet.

The Amish in Lancaster County are predominantly Old Order Amish, the most conservative of the Amish sects in terms of adherence to strict social norms and resistance to using modern technologies. All Amish are rural living and most earn their living by farming. Marriages between Amish and those of non-Amish descent are rare. Because of their unique ancestral background, there is a high degree of consanguinity. First-cousin marriages are uncommon, but on average, any 2 Amish individuals are more closely related than second cousins, but less related than first cousins once removed, who share 1/64 and 1/32 of their genes in common, respectively.

This Amish population has several unique characteristics that make it particularly well suited for genetic studies. First, the population is a closed founder population that is genetically homogeneous. There are extensive genealogical records spanning 12 to 14 generations that allow researchers to trace all living Amish to their founding ancestors in the mid-1700s with minimal pedigree errors and/or nonpaternity.<sup>9,14</sup> Second, Amish families are characterized by very large sibships averaging 7 to 9 siblings each who are geographically localized. This, combined with extraordinary cooperation from the Amish community, greatly facilitates high rates of participation, remarkable adherence to complicated intervention protocols, and reliable follow-up. Third, the homogeneity of socioeconomic status and lifestyle (eg, minimal smoking and alcohol consumption, abundant and consistent physical activity, limited medication use, and similar dietary routines) reduces sources of nongenetic variation. Despite the unique ancestral background of the Amish, it seems reasonable to expect that most common disease-predisposing alleles found in other European white populations will also be found in the Amish. Indeed, classical population genetic theory and our<sup>15</sup> and others' empirical data show that populations with a modest number of founders like the Amish have allele frequencies (on average) that are similar to those in the ancestral population, particularly for common alleles (eg, minor allele frequencies >5%).

The family-based design of the HAPI Heart Study provides additional opportunities for analyses that are not possible in conventional population-based studies. For example, heritability and linkage analysis contrast phenotypic similarities between related and unrelated individuals. Family-based tests of association that test for evidence of association in the presence of linkage, such as the TDT or FBAT, are also possible, although the added value of such tests is debatable in genetically homogeneous isolate populations with minimal population stratification, such as the Amish. Lastly, the unique family structure of the HAPI Heart Study cohort facilitates investigations of genetic imprinting, maternal effects, and other nonclassical genetic phenomenon that might play a role in complex diseases.

Conventional genetic association tests can also be carried out in families, with the caveat that the nonindependence of family members must be taken into account as failure to do so will inflate the variance of the genotypic effect size.<sup>13</sup> Analytic approaches for doing this include accounting for family structure as a random effect in a mixed effect model or through generalized estimating equations modeling or by specifying explicitly the covariance structure as a random effect using variance component modeling. Because of the unique ancestral background of this population with its relatively small number of founders, an additional analytic approach

potentially useful in the Amish is to map recessively acting genes with rare alleles through homozygosity by descent mapping.<sup>16</sup>

In conclusion, through its use of standardized interventions relevant to CVD, the HAPI Heart Study uses a powerful approach for assessing how genes modify the response to particular controlled environments. Identifying these response genes may identify new mechanisms influencing the regulation of cardiovascular health, leading eventually to improved and individualized preventive strategies and treatments and to improved early identification of high-risk individuals.

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