Potential Nonresponse Bias in a Clinical Examination After Initial Screening Using Iron Phenotyping and *HFE* Genotyping in the Hemochromatosis and Iron Overload Screening Study

Mark Speechley,¹ James C. Barton,² Leah Passmore,³ Helen Harrison,⁴ David M. Reboussin,³ Emily L. Harris,⁵ Charles A. Rivers,⁶ Margaret Fadojutimi-Akinsiku,⁷ Lari Wenzel,⁸ and Sharmin Diaz⁷

Background: Little is known about the factors affecting participation in clinical assessments after HEMochromatosis and IRon Overload Screening. **Methods:** Initial screening of 101,168 primary care patients in the HEMochromatosis and IRon Overload Screening study was performed using serum iron measures and hemochromatosis gene (*HFE*) genotyping. Using iron phenotypes and *HFE* genotypes, we identified 2256 cases and 1232 controls eligible to participate in a clinical examination. To assess the potential for nonresponse bias, we compared the sociodemographic, health status, and attitudinal characteristics of participants and nonparticipants using adjusted odds ratios (ORs) and 95% confidence interval (CI). **Results:** Overall participation was 74% in cases and 52% in controls; in both groups, participation was highest at a health maintenance organization and lowest among those under 45 years of age (cases: OR = 0.68; 95% CI 0.53, 0.87; controls: OR = 0.59; 95% CI 0.44, 0.78). In controls only, participation was also lower among those over 65 years of age than the reference group aged 46–64 (OR = 0.64; 95% CI 0.47, 0.88). Among cases, participation was higher in *HFE* C282Y homozygotes (OR = 3.98; 95% CI 2.60, 6.09), H63D homozygotes (OR = 2.79; 95% CI 1.23, 6.32), and C282Y/H63D compound heterozygotes (OR = 1.82; 95% CI 1.03, 3.22) than in other genotypes, and lower among non-Caucasians and those who preferred a non-English language than in Caucasians and those who preferred English ($p < 0.0001$). **Conclusions:** Subjects with greatest risk to have iron overload (C282Y homozygotes; cases ≥ 45 years; Caucasians) were more likely to participate in a postscreening clinical examination than other subjects. We detected no evidence of strong selection bias.

Introduction

HEMOCHROMATOSIS in Western European whites is typically associated with homozygosity for C282Y, a missense mutation in the hemochromatosis gene (*HFE*) on chromosome 6p21.3 (Feder *et al.*, 1996). Approximately 0.44–0.48% of non-Hispanic whites in North America are C282Y homozygotes (Beutler *et al.*, 2000; Adams *et al.*, 2005). Some persons with hemochromatosis absorb excessive quantities of dietary iron and develop iron overload and consequent hepatic injury, cirrhosis, diabetes mellitus, arthropathy, and

hypogonadotropic hypogonadism that typically become manifest in middle age (Witte *et al.*, 1996; Barton *et al.*, 1998). Early detection of hemochromatosis or iron overload could permit treatment to prevent injury of target organs by excessive iron. Accordingly, two large-scale screening programs in North America have used iron phenotyping and *HFE* genotyping (Beutler *et al.*, 2000; Adams *et al.*, 2005). Nonetheless, little is known about the willingness of subjects with abnormalities detected in initial screening to undergo postscreening assessments of possible hemochromatosis and iron overload and to receive management recommendations.

¹Department of Epidemiology & Biostatistics, University of Western Ontario, London, Canada.

²Southern Iron Disorders Center, Birmingham, Alabama.

³Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, North Carolina.

⁴London Health Sciences Centre, London, Canada.

⁵National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland.

⁶Microbiology/Immunogenetics Program, University of Alabama at Birmingham, Birmingham, Alabama.

⁷Howard University, Washington, District of Columbia.

⁸Department of Medicine, Center for Health Policy Research, University of California, Irvine, California.

Selection bias is a form of systematic error caused by differences in characteristics between those who do and those who do not participate in a study. Selection bias is important scientifically because it can invalidate conclusions and generalizations from studies in which it occurs (Last, 2001). For example, samples of patients from tertiary care settings may be biased because the participants have more severe or intractable disease than all those affected in the population. Selection bias due to self-selection occurs due to differences between people who choose to participate and those who do not. Self-selection bias can occur in cross-sectional and case-control studies, and at each point in a longitudinal study. In cross-sectional studies and case-control studies, and at the baseline of longitudinal investigations, it is a component of "nonresponse" bias. In longitudinal studies, it is one source of biased follow-up (Greenland, 1977).

The HEMochromatosis and IRon Overload Screening (HEIRS) study is a multiracial, multiethnic, primary care-based screening study of hemochromatosis and iron overload conducted at five field centers in the United States and Canada (McLaren *et al.*, 2003; Adams *et al.*, 2005). Some general results from the initial screening phase have been reported (Adams *et al.*, 2005; Barton *et al.*, 2005, 2006; Acton *et al.*, 2006a, 2006b). The purpose of the present analyses was to address the potential for nonresponse bias at the second (clinical examination) phase by comparing characteristics between those who did and did not participate, separately for a group of cases designated as at higher risk for hemochromatosis and iron overload, and a matched control group.

Materials and Methods

Study design and approval

The design and methodology of the HEIRS study has been reported in detail elsewhere (McLaren *et al.*, 2003). The local Institutional Review Board of each field center approved the study protocol (McLaren *et al.*, 2003; Adams *et al.*, 2005). Briefly, five HEIRS study field centers recruited participants ≥ 25 years of age who were able to give informed consent from primary care practices and blood-drawing laboratories. There were no other inclusion or exclusion criteria. The HEIRS study had two principal phases: initial screening and subsequent clinical examination (McLaren *et al.*, 2003). In the present analyses, we sought to evaluate the observed differences between participants and nonparticipants at the clinical examination phase.

Selection of study subjects

The HEIRS study screened 101,168 primary care patients over a 2-year period (February 2001–March 2003). As part of the informed consent process, the study provided background information on hemochromatosis and iron overload to all potential participants. All sites had information available in English; in addition, specific sites had written material and recruiters fluent in Mandarin, Spanish, and Vietnamese. Some initial screening participants were subsequently designated as cases or controls. Cases ($n = 2256$) were defined as (1) homozygous for the *HFE* C282Y mutation ($n = 333$), with ($n = 174$) or without ($n = 159$) elevated levels of serum ferritin (SF $>300 \mu\text{g/L}$ for men and $>200 \mu\text{g/L}$ for women) or transferrin saturation (TS $>50\%$ for men and $>45\%$ for women) or

(2) not homozygous for *HFE* C282Y but with the same elevated levels of SF and TS ($n = 1923$). Potential controls were eligible if they lacked *HFE* C282Y or H63D, and had non-elevated TS and SF values as defined above. Selected controls ($n = 1247$) were frequency matched to the cases at a 1:1 ratio on field center, age group (25–44, 45–64, and >65), and date of initial screening.

Case participants were informed of their TS and SF levels and *HFE* genotype by letter and invited by telephone to participate in a clinical examination. They were able to ask questions about their test results during the telephone call in their preferred language (English, Mandarin, Spanish, or Vietnamese). The content of the letters sent to cases was tailored to their specific genotype and phenotype. For example, cases not homozygous for *HFE* C282Y but with elevated iron values were "recommended" to share their results with their physician and "talk to a genetics counselor about risks to your family members" (Anderson *et al.*, 2006). Cases also received an information pamphlet. Control participants received a letter informing them that no phenotype or genotype abnormalities were detected, that they were "welcome" to share the information with their physician, and were invited by telephone to participate in the clinical examination as control subjects. Cases and controls were reimbursed for their out-of-pocket expenses such as parking and child care.

Screening laboratory methods

Phenotype measurements. Methodology and quality control used for measurements of TS and SF are described elsewhere (Barton *et al.*, 2005). The HEIRS study defined these initial screening phenotypes to be elevated: TS $>50\%$ for men and TS $>45\%$ for women; SF $>300 \mu\text{g/L}$ for men and SF $>200 \mu\text{g/L}$ for women (McLaren *et al.*, 2003).

Genotype analyses. *HFE* C282Y and H63D were detected using buffy coat samples from whole-blood ethylenediaminetetraacetic acid samples and a modification of the Invader assay (Third Wave Technologies, Madison, WI) as described in detail elsewhere (Barton *et al.*, 2005). Participants without C282Y or H63D were designated as having *HFE* wild-type genotype (wt/wt).

Statistical considerations

The primary outcome variable was participation in the clinical examination (yes = 1 and no = 0). Potential explanatory factors were drawn from initial screening data and included age, sex, race/ethnicity, recruiting field center, self-report of chronic conditions associated with iron overload (arthritis, diabetes, liver disease or liver cancer, heart failure, fertility problems, or impotence) and "too much iron in your body, iron overload, or hemochromatosis," self-rated health status, and attitudes about genetic testing and the perceived role of lifestyle in health. For cases only, participation was compared across six specific *HFE* genotypes: C282Y homozygosity; H63D homozygosity; C282Y/H63D compound heterozygosity; C282Y heterozygosity; H63D heterozygosity; and *HFE* wt/wt in participants who had elevated screening iron measures. By selection, all controls had the genotype *HFE* wt/wt.

We compared characteristics of participants and nonparticipants and analyzed data from cases and controls separately.

We used Pearson's chi-squared statistic to assess independence between strata of binary variables and across all strata for variables with multiple categories. Crude (unadjusted) odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to estimate the strength of association between participation and each variable. For variables with more than two levels, an OR and 95% CI were calculated for each stratum, compared to a reference category (OR = 1.0). These multiple-level variables included several questions about health beliefs and attitudes about genetic testing that were asked using Likert-type response options. Health beliefs (e.g., "My health is excellent") ranged from "Definitely true" to "Definitely false"; psychological effect (e.g., "During the past 4 weeks, have you felt downhearted and blue?") ranged from "All of the time" to "None of the time"; attitudes toward genetic testing (e.g., "In general, I think genetic testing to find out about disease is a good idea") ranged from "Strongly Agree" to "Strongly Disagree"; and self-rated health response options were Excellent, Good, Average, Fair, and Poor.

Because these variables were measured on an ordinal scale, the stratum-specific OR permitted examination of linear trends in the strength of association across levels of the variable. Specifically, the hypotheses being tested were that if self-rated health, or the strength of agreement with an attitude, were associated with participation in the clinical examination, then there should be a linear trend of increasing (or decreasing) OR across the ordinal response categories for these variables.

Multiple logistic regression was used to identify covariates that independently predicted participation, controlling for other factors in the model. Because there were different eligibility criteria and potential motivations for participation, logistic modeling was done separately for case and control strata. A univariate model for each covariate available in the dataset was fitted, and the *p*-values were examined. Covariates that yielded *p*-values < 0.10 in these univariate models were considered further in the model building, and those with *p*-values ≥ 0.10 were not considered further. The models were built sequentially by adding covariates, resulting in final models in which all remaining covariates were deemed statistically significant at *p* < 0.05. For brevity, only variables with Pearson's chi-squared *p*-values < 0.05 were included in the univariate results.

Results

Univariate results

Table 1 shows the distributions of characteristics between participants and nonparticipants separately for cases and controls for variables associated with participation at *p* < 0.05. Participation was 74% for cases and 52% for controls (*p* < 0.001).

Table 1 also shows stratum-specific OR and 95% CIs for strata of the above factors. For genotype variables (cases only), compared to those with *HFE* wt/wt, participation was significantly greater in C282Y homozygotes (OR = 4.17), H63D homozygotes (OR = 2.62), and C282Y/H63D compound heterozygotes (OR = 2.19). With respect to phenotype (elevated iron measures, cases only), participants with elevated iron parameters were 2.4 times more likely to participate than those with normal iron values (OR = 2.44).

In cases, the relative odds of participation were 27% higher for women (OR = 1.27); in controls, there was no significant

difference in the proportions of men and women who participated. Age group was associated with participation in cases (*p* = 0.002) and in controls (*p* < 0.001). In cases, there was a linear trend in OR reflecting greater participation in older age groups than in younger age groups. No linear trend in participation by age group was observed in control subjects, but controls < 45 years of age were 41% less likely to participate than in older participants (OR = 0.59).

Non-Caucasian cases were 35–39% less likely than Caucasian cases to participate in the clinical examination (*p* < 0.001); non-Caucasian controls were 22–43% less likely to participate than Caucasian controls (*p* = 0.008). Cases recruited from the four field centers that were not based at a health maintenance organization (HMO) were 64–73% less likely to participate than those recruited at the Kaiser Permanente Field Center (*p* < 0.001); non-HMO controls were 31–60% less likely to participate (*p* < 0.001). Cases with a language preference other than English were 36–52% less likely to participate than those who preferred English (*p* < 0.001). Among controls, language preference overall was associated with participation (*p* = 0.002); controls who preferred Spanish were 56% less likely to participate than those who preferred another language (OR = 0.44; 95% CI 0.28, 0.71).

Health conditions associated with participation in cases were self-report of arthritis (*p* = 0.045), heart failure (*p* = 0.030), and infertility/impotence (*p* = 0.003). Those who reported "no" or "not sure" about infertility/impotence were 66% less likely to participate than those who reported these conditions.

Several psychosocial variables, and attitudes about genetic testing, were significantly associated with participation. In participants who qualified as cases, these included the beliefs that genetic information should be shared with family members (*p* = 0.016); genetic testing is generally a good idea (*p* = 0.029); genetic testing is good because the results allow one to change to a healthier lifestyle (*p* < 0.002); genetic testing is bad because it may affect one's insurance (*p* < 0.001); lifestyle is an important factor in health (*p* = 0.047); and responses to the statement "I am a happy person" (*p* = 0.047). In controls, participation was associated with self-rated health (*p* < 0.001); the belief that lifestyle is an important factor in health (*p* = 0.018); and responses to the statements "My health is excellent" (*p* = 0.024) and "I am a happy person" (*p* = 0.015).

When ORs were examined for trends among ordinal Likert-type response options, cases who rated their health as other than excellent were consistently less likely to participate, but there was not a linear trend in OR for cases or controls. A linear trend in OR was seen for only one variable, in cases only: compared to those who strongly agreed with the view that "genetic testing is a good idea because it enables a change to a healthier lifestyle," those who agreed were 16% less likely to participate. Those who disagreed were 62% less likely, and those who strongly disagreed were 85% less likely to participate. A partial trend in ORs was seen in controls, in whom the odds of participation increased with the frequency of being happy. Compared to those who were happy "none of the time," participation increased by 80% in those happy "a little of the time," by 126% for "some of the time," by 147% for "a good bit of the time," and by over 300% for those happy "most of the time." Variables not associated (*p* > 0.05; not shown) with participation in either cases or controls were

TABLE 1. UNADJUSTED ODDS RATIOS FOR CHARACTERISTICS OF CASES AND CONTROLS WHO DID AND DID NOT PARTICIPATE IN THE CLINICAL EXAMINATION PHASE OF THE HEMOCHROMATOSIS AND IRON OVERLOAD SCREENING STUDY, 2001–2003, BY CASES AND CONTROLS

Characteristic	Cases ^a			Controls ^b		
	Invited to clinical examination			Invited to clinical examination		
	(n = 2256)			(n = 1232)		
	Attended clinical examination			Attended clinical examination		
	No	Yes	OR (95% CI)	No	Yes	OR (95% CI)
	No. (%)	No. (%)		No. (%)	No. (%)	
	578 (25.6)	1678 (74.4)		591 (48.0)	641 (52.0)	
Sex		0.0148 ^c			0.4379 ^c	
Female	224 (23.0)	748 (77.0)	1.27 (1.05, 1.54)	357 (47.1)	401 (52.9)	1.10 (0.87, 1.38)
Age group		0.0016 ^c			0.0006 ^c	
<45	186 (30.4)	425 (69.6)	0.76 (0.61, 0.94)	195 (56.0)	153 (44.0)	0.59 (0.45, 0.77)
46–64	292 (24.9)	879 (75.1)	1.0 ^d	241 (43.0)	320 (57.0)	1.0 ^d
>65	100 (21.1)	374 (78.9)	1.24 (0.96, 1.61)	155 (48.0)	168 (52.0)	0.82 (0.62, 1.07)
Race/ethnicity		0.0001 ^c			0.0079 ^c	
Caucasian	197 (20.5)	762 (79.5)	1.0 ^d	282 (43.5)	367 (56.5)	1.0 ^d
African-American	122 (29.8)	288 (70.2)	0.61 (0.47, 0.79)	110 (54.5)	92 (45.5)	0.64 (0.47, 0.88)
Asian/Pacific Islander	198 (29.8)	466 (70.2)	0.61 (0.48, 0.76)	109 (49.5)	111 (50.5)	0.78 (0.58, 1.06)
Hispanic	50 (28.4)	126 (71.6)	0.65 (0.45, 0.94)	76 (57.6)	56 (42.4)	0.57 (0.39, 0.83)
Other/unknown	11 (23.4)	36 (76.6)	0.85 (0.42, 1.69)	14 (48.3)	15 (51.7)	0.82 (0.39, 1.73)
Field Center		<0.0001 ^c			<0.0001 ^c	
Kaiser	52 (11.3)	407 (88.7)	1.0 ^d	137 (36.7)	236 (63.3)	1.0 ^d
Howard	102 (30.5)	232 (69.5)	0.29 (0.20, 0.42)	31 (45.6)	37 (54.4)	0.69 (0.41, 1.17)
London	157 (27.0)	425 (73.0)	0.35 (0.25, 0.49)	183 (48.7)	193 (51.3)	0.61 (0.46, 0.82)
UAB	81 (26.4)	226 (73.6)	0.36 (0.24, 0.52)	121 (56.8)	92 (43.2)	0.44 (0.31, 0.62)
UCI	186 (32.4)	388 (67.6)	0.27 (0.19, 0.37)	119 (58.9)	83 (41.1)	0.40 (0.29, 0.57)
Specific genotype		<0.0001 ^c				
+/+	382 (30.0)	892 (70.0)	1.0 ^d			
H63D/+	104 (31.0)	231 (69.0)	0.95 (0.73, 1.23)			
C282Y/+	33 (23.1)	110 (76.9)	1.43 (0.95, 2.14)			
C282Y/H63D	17 (16.3)	87 (83.7)	2.19 (1.29, 3.74)			
H63D/H63D	9 (14.1)	55 (85.9)	2.62 (1.28, 5.35)			
C282Y/C282Y	31 (9.3)	302 (90.7)	4.17 (2.83, 6.15)			
Zygosity		<0.0001 ^c				
Heterozygote	163 (25.2)	483 (74.8)	1.27 (1.02, 1.57)			
Homozygote	31 (9.3)	302 (90.7)	4.17 (2.83, 6.15)			
Phenotype		0.0002 ^c				
Elevated TS and SF	557 (26.6)	1539 (73.4)	2.40 (1.50, 3.83)			
Preferred language		<0.0001 ^c			0.0024 ^c	
English	419 (23.3)	1379 (76.7)	1.0 ^d	495 (46.1)	579 (53.9)	1.0 ^d
Spanish	40 (32.3)	84 (67.7)	0.64 (0.43, 0.94)	54 (65.9)	28 (34.1)	0.44 (0.28, 0.71)
Mandarin	14 (38.9)	22 (61.1)	0.48 (0.24, 0.94)	13 (61.9)	8 (38.1)	0.53 (0.22, 1.28)
Vietnamese	105 (35.2)	193 (64.8)	0.56 (0.43, 0.73)	29 (53.7)	25 (46.3)	0.74 (0.43, 1.28)
Arthritis		0.0446 ^c			0.1809 ^c	
Yes	137 (22.6)	470 (77.4)	1.0 ^d	186 (50.5)	182 (49.5)	1.0 ^d
No	386 (27.2)	1032 (72.8)	0.78 (0.62, 0.97)	352 (45.9)	415 (54.1)	1.20 (0.94, 1.55)
Not sure	32 (21.5)	117 (78.5)	1.07 (0.69, 1.65)	37 (54.4)	31 (45.6)	0.86 (0.51, 1.44)
Diabetes		0.8105 ^c			0.0562 ^c	
Yes	77 (26.1)	218 (73.9)	1.0 ^d	83 (55.0)	68 (45.0)	1.0 ^d
No	446 (25.2)	1327 (74.8)	1.05 (0.79, 1.39)	468 (46.4)	541 (53.6)	1.41 (1.00, 1.99)
Not sure	28 (27.7)	73 (72.3)	0.92 (0.55, 1.53)	25 (58.1)	18 (41.9)	0.88 (0.44, 1.74)
Heart failure		0.0303 ^c			0.7170 ^c	
Yes	28 (28.9)	69 (71.1)	1.0 ^d	32 (52.5)	29 (47.5)	1.0 ^d
No	483 (24.7)	1469 (75.3)	1.23 (0.79, 1.94)	513 (47.1)	576 (52.9)	1.24 (0.74, 2.08)
Not sure	40 (35.4)	73 (64.6)	0.74 (0.41, 1.33)	21 (47.7)	23 (52.3)	1.21 (0.56, 2.63)
Fertility problems/impotence		0.0027 ^c			0.1090 ^c	
Yes	11 (10.8)	91 (89.2)	1.0 ^d	25 (39.7)	38 (60.3)	1.0 ^d
No	509 (26.0)	1452 (74.0)	0.34 (0.18, 0.65)	520 (47.7)	570 (52.3)	0.72 (0.43, 1.21)
Not sure	20 (26.0)	57 (74.0)	0.34 (0.15, 0.77)	20 (62.5)	12 (37.5)	0.39 (0.16, 0.95)

(continued)

TABLE 1. (CONTINUED)

Characteristic	Cases ^a			Controls ^b		
	Invited to clinical examination			Invited to clinical examination		
	(n = 2256)			(n = 1232)		
	Attended clinical examination			Attended clinical examination		
	No	Yes	OR (95% CI)	No	Yes	OR (95% CI)
	No. (%)	No. (%)		No. (%)	No. (%)	
Health	578 (25.6)	1678 (74.4)		591 (48.0)	641 (52.0)	
Poor	30 (28.0)	77 (72.0)	0.62 (0.35, 1.09)	29 (64.4)	16 (35.6)	0.48 (0.24, 0.97)
Fair	103 (25.8)	297 (74.3)	0.70 (0.45, 1.08)	107 (59.1)	74 (40.9)	0.61 (0.39, 0.95)
Average	178 (28.6)	445 (71.4)	0.60 (0.40, 0.91)	156 (48.0)	169 (52.0)	0.95 (0.64, 1.42)
Good	208 (23.7)	670 (76.3)	0.78 (0.52, 1.17)	221 (42.7)	296 (57.3)	1.17 (0.80, 1.71)
Excellent	34 (19.4)	141 (80.6)	1.0 ^d	64 (46.7)	73 (53.3)	1.0 ^d
My health is excellent		0.6834 ^c			0.0239 ^c	
Definitely true	79 (23.2)	262 (76.8)	1.23 (0.84, 1.78)	106 (48.2)	114 (51.8)	1.66 (1.01, 2.74)
Mostly true	239 (24.7)	727 (75.3)	1.12 (0.82, 1.54)	253 (43.9)	323 (56.1)	1.97 (1.25, 3.11)
Do not know	85 (27.7)	222 (72.3)	0.97 (0.66, 1.40)	80 (51.0)	77 (49.0)	1.48 (0.88, 2.52)
Mostly false	67 (25.4)	197 (74.6)	1.09 (0.73, 1.61)	75 (52.4)	68 (47.6)	1.40 (0.82, 2.39)
Definitely false	68 (27.0)	184 (73.0)	1.0 ^d	54 (60.7)	35 (39.3)	1.0 ^d
Been a happy person		0.0474 ^c			0.0155 ^c	
All the time	91 (29.1)	222 (70.9)	0.76 (0.42, 1.36)	93 (54.7)	77 (45.3)	1.93 (0.71, 5.26)
Most of the time	226 (22.5)	779 (77.5)	1.07 (0.62, 1.85)	277 (43.6)	358 (56.4)	3.01 (1.14, 7.94)
Some of the time	66 (23.3)	217 (76.7)	1.02 (0.56, 1.85)	69 (50.7)	67 (49.3)	2.26 (0.82, 6.24)
A little of the time	40 (29.9)	94 (70.1)	0.73 (0.38, 1.39)	35 (56.5)	27 (43.5)	1.80 (0.61, 5.30)
None of the time	18 (23.7)	58 (76.3)	1.0 ^d	14 (70.0)	6 (30.0)	1.0 ^d
Overall, genetic testing is a good idea		0.0291 ^c			0.1955 ^c	
Strongly agree	238 (22.2)	836 (77.8)	1.0 ^d	291 (46.8)	331 (53.2)	1.0 ^d
Agree	267 (27.4)	707 (72.6)	0.75 (0.62, 0.92)	253 (47.5)	280 (52.5)	0.97 (0.77, 1.23)
Disagree	12 (33.3)	24 (66.7)	0.57 (0.28, 1.16)	10 (55.6)	8 (44.4)	0.70 (0.27, 1.81)
Strongly disagree	2 (22.2)	7 (77.8)	1.00 (0.21, 4.83)	6 (85.7)	1 (14.3)	0.15 (0.02, 1.22)
Genetic testing is good because it allows change to healthier lifestyle		0.0020 ^c			0.1106 ^c	
Strongly agree	228 (22.8)	773 (77.2)	1.0 ^d	258 (44.1)	327 (55.9)	1.0 ^d
Agree	259 (26.1)	735 (73.9)	0.84 (0.68, 1.03)	286 (51.3)	272 (48.7)	0.75 (0.59, 0.95)
Disagree	14 (43.8)	18 (56.3)	0.38 (0.19, 0.77)	10 (43.5)	13 (56.5)	1.03 (0.44, 2.38)
Strongly disagree	4 (66.7)	2 (33.3)	0.15 (0.03, 0.81)	2 (50.0)	2 (50.0)	0.79 (0.11, 5.64)
Genetic testing is bad because of trouble getting/keeping insurance		0.0004 ^c			0.7483 ^c	
Strongly agree	40 (25.0)	120 (75.0)	1.0 ^d	54 (47.8)	59 (52.2)	1.0 ^d
Agree	97 (19.1)	411 (80.9)	1.41 (0.93, 2.15)	160 (45.1)	195 (54.9)	1.12 (0.73, 1.70)
Disagree	272 (28.5)	684 (71.5)	0.84 (0.57, 1.23)	232 (48.8)	243 (51.2)	0.96 (0.64, 1.45)
Strongly disagree	63 (20.7)	241 (79.3)	1.28 (0.81, 2.01)	81 (48.2)	87 (51.8)	0.98 (0.61, 1.58)
Lifestyle as a factor in disease		0.0469 ^c			0.0178 ^c	
Very important	385 (24.5)	1185 (75.5)	1.0 ^d	415 (45.5)	498 (54.5)	1.0 ^d
Somewhat important	120 (25.2)	356 (74.8)	0.96 (0.76, 1.22)	135 (55.3)	109 (44.7)	0.67 (0.51, 0.89)
Not important	21 (42.0)	29 (58.0)	0.45 (0.25, 0.80)	11 (52.4)	10 (47.6)	0.76 (0.32, 1.80)
Not sure	14 (26.9)	38 (73.1)	0.88 (0.47, 1.64)	13 (65.0)	7 (35.0)	0.45 (0.18, 1.14)

^aCases met either or both genotypic or phenotypic criteria for the clinical examination.^bControls met neither genotypic nor phenotypic criteria and were matched to the controls on age group, field center, and date of initial screening visit.^cChi-squared *p*-value for the 2×*n* table for that characteristic. Only variables with *p*-values <0.05 in cases or controls are presented.^dReferent group.

CI, confidence interval; OR, odds ratio; TS, transferrin saturation; SF, serum ferritin; UAB, University of Alabama at Birmingham; UCI, University of California, Irvine.

self-reported diabetes, reporting that blood relatives have iron overload, the expectation that health will get worse, feeling downhearted and blue, and agreement with the belief that heredity is an important reason why people get sick.

Because hypothesized linear trends in OR across ordinal strata in most of the psychosocial and attitudinal questions were absent, statistically significant OR in specific strata should be interpreted with caution, particularly given the number of hypotheses tested herein.

Adjusted analyses

After multiple logistic modeling (Table 2), variables remaining as independent predictors of participation in cases were C282Y homozygosity (OR = 3.98), H63D homozygosity (OR = 2.79), or C282Y/H63D compound homozygosity (OR = 1.82), whereas age <45 years was associated with lower participation than age ≥45 years (OR = 0.68). Controls <45 years (OR = 0.59) or >65 years (OR = 0.64) were less likely to participate. Participation was lower among those recruited at non-HMO field centers for both groups, with statistically significant adjusted OR of 0.30–0.37 in cases and 0.38–0.62 in controls.

Discussion

In the present study, subjects selected as cases with greatest risk to have iron overload (*HFE* C282Y homozygotes, and phenotypically or genotypically defined cases ≥45 years) were more likely to participate in a postinitial screening clinical examination than other subjects. A major factor associated with study participation is the salience, or personal interest, that an eligible participant has in the specific topic

under investigation (Heberlein and Baumgartner, 1978). In case-control studies, participation rates of cases are typically higher than those of control subjects. Salience was detected in the present longitudinal study, wherein participation among cases, eligible for clinical examination on the basis of phenotype or genotype abnormalities, was 74%; participation was significantly lower (52%) among controls who were informed by letter that they had no identified abnormality. This is consistent with a review that reported median case-control participation rates of 81% and 74%, respectively, in 107 such studies published in 2003 (Morton *et al.*, 2006). Still, it is noteworthy that at least one half of the eligible controls participated in a clinical examination in spite of no elevated risk for the disease under investigation. Salience might also explain different participation rates within the present cases. Participation was highest in C282Y homozygotes, H63D homozygotes, and C282Y/H63D compound heterozygotes (84–91) and lowest in those with *HFE* genotypes C282Y/wt, H63D/wt, and wt/wt (69–71%). Similarly, hemochromatosis probands with C282Y homozygosity diagnosed during routine medical care were significantly more likely to comply with maintenance phlebotomy recommendations than probands with other *HFE* genotypes (Hicken *et al.*, 2003). It is difficult to directly ascertain the effect on participation played by participants' understanding of their results. However, in an analysis of a sample of non-C282Y homozygous HEIRS study participants who did not meet the criteria for the clinical exam and who received SF, TS, and *HFE* gene test results by mail only ($n = 1281$), Harrison *et al.* (2008) found that those with normal *HFE* test results, normal iron test results, and English-speaking participants understood test results and study recommendations better than those with one or two *HFE* mutations, out-of-range TS or SF results, and non-English-speaking participants, respectively. We also cannot test the influence—positive or negative—of participants' physicians and other care providers on the decision to attend the clinical examination. The content of the letters, specifically whether people were “welcome,” “suggested,” “encouraged,” or “recommended” to share their results with their MD, varied by phenotype and genotype (Anderson *et al.*, 2006). It is possible but unknown if some eligible participants followed up with their own physicians independent of further involvement in the HEIRS study.

We observed that factors primarily related to study design and sociodemographic characteristics have similar associations with clinical examination participation in both case and control groups. Specifically, the participation proportions of cases and controls recruited at the single HMO were uniformly greater than those of corresponding groups from other field centers. Participation was lower in both cases and controls among non-Caucasian groups than in Caucasians, and lower in those with language preference other than English than in those who preferred English. The direction and magnitude of potential bias in incidence estimates derived from the clinical examination data are more difficult to predict with factors such as field center and ethnicity than with age, because these factors are not as uniformly associated with disease incidence as with age.

Most other factors we analyzed, many of which were measured at an ordinal level, had no consistent relationship with participation by either cases or controls. Initial screening in the HEIRS study involved genotyping, but participation in

TABLE 2. ADJUSTED ODDS RATIOS FROM LOGISTIC REGRESSION FOR CHARACTERISTICS ASSOCIATED WITH PARTICIPATION IN THE CLINICAL EXAMINATION PHASE OF THE HEMOCHROMATOSIS AND IRON OVERLOAD SCREENING STUDY, BY CASES AND CONTROLS

Characteristic	Cases (n = 2256)		Controls (n = 1232)	
	OR	95% CI	OR	95% CI
Specific genotype				
+/+	1.0	^a		
H63D/+	0.88	0.65, 1.17		
C282Y/+	1.35	0.86, 2.13		
C282Y/H63D	1.82	1.03, 3.22		
H63D/H63D	2.79	1.23, 6.32		
C282Y/C282Y	3.98	2.60, 6.09		
Field Center				
Kaiser	1.0	^a	1.0	^a
Howard	0.37	0.23, 0.59	0.65	0.33, 1.26
London	0.35	0.24, 0.52	0.62	0.45, 0.86
UAB	0.30	0.19, 0.46	0.41	0.28, 0.59
UCI	0.30	0.20, 0.44	0.38	0.26, 0.56
Age group				
<45	0.68	0.53, 0.87	0.59	0.44, 0.78
46–64	1.0	^a	1.0	^a
>65	0.90	0.66, 1.22	0.64	0.47, 0.88

Adjusted for *HFE* genotype, field center, and age group in cases and for field center and age group in controls.

^aReferent group.

the second, more involved, postscreening phase of the study was not associated with the strength of stated attitudes toward genetic testing. No psychosocial or attitudinal variables remained in the final logistic models.

Investigations of factors affecting participation in two-stage studies, the designs of which resemble that of the HEIRS study, have shown greater participation among those with specific health concerns (Cobb *et al.*, 1957). For example, those who participated in the examination phase of National Health and Nutrition Examination Survey II were more likely than nonparticipants to have a health problem they wished to discuss with a physician (Forthofer, 1983). In the present study, the ORs for controls who reported that it was "mostly true" that their health was going to get worse and those who felt this was "definitely true" were not statistically significant. Altogether, these observations suggest that health-related motives for participation in a phenotype and genotype screening study involving a postscreening clinical examination are complex and may not be predictable. Only knowledge of true exposure and outcome status in both participants and nonparticipants allows accurate estimate of the magnitude and direction of selection bias. This knowledge is usually only available in countries that have population-based registries of both risk factor and health outcome data and that permit investigators to sample from them. This situation does not exist in either the United States or Canada, the countries from which HEIRS study participants were recruited. Nonetheless, the unpredictability of most factors studied suggests that the clinical examination participation appears to be random with respect to these factors. The use of the OR as the measure of association, coupled with their cautious interpretation when they are close to the null, should yield valid inferences from the data collected during the clinical examination phase of the HEIRS study or similar screening programs.

Acknowledgments

Following are the participating HEIRS study investigators and institutions:

Field Centers

Birmingham, AL—University of Alabama at Birmingham

Dr. Ronald T. Acton (Principal Investigator), Dr. James C. Barton (Co-Principal Investigator), Ms. Deborah Dixon, Dr. Susan Ferguson, Dr. Richard Jones, Dr. Jerry McKnight, Dr. Charles A. Rivers, Dr. Diane Tucker, and Ms. Janice C. Ware.

Irvine, CA—University of California, Irvine

Dr. Christine E. McLaren (Principal Investigator), Dr. Gordon D. McLaren (Co-Principal Investigator), Dr. Hoda Anton-Culver, Ms. Jo Ann A. Baca, Dr. Thomas C. Bent, Dr. Lance C. Brunner, Dr. Michael M. Dao, Dr. Corey S. Jorgensen, Dr. Julie Kuniyoshi, Dr. Huan D. Le, Dr. Miles K. Masatsugu, Dr. Frank L. Meyskens, Dr. David Morohashi, Dr. Huan P. Nguyen, Dr. Sophocles N. Panagon, Dr. Chi Phung, Dr. Virgil Raymundo, Dr. Thomas Ton, Professor Ann P. Walker, Dr. Lari B. Wenzel, and Dr. Argyrios Ziogas.

London, Canada—London Health Sciences Center

Dr. Paul C. Adams (Principal Investigator), Ms. Erin Bloch, Dr. Subrata Chakrabarti, Ms. Arlene Fleischhauer, Ms. Helen Harrison, Ms. Bonnie Hogan, Ms. Kelly Jia, Dr. John Jordan, Ms. Sheila Larson, Dr. Edward Lin, Ms. Melissa Lopez,

MDS Laboratories, Dr. Godfrey Moses, Ms. Lien Nguyen, Ms. Corry Pepper, Dr. Tara Power, Dr. Mark Speechley, Dr. Donald Sun and Ms. Diane Woelfle.

Portland, OR and Honolulu, HI—Kaiser Permanente Center for Health Research, Northwest and Hawaii, and Oregon Health and Science University

Dr. Emily L. Harris (Principal Investigator), Dr. Mikel Aickin, Dr. Elaine Baker, Ms. Marjorie Erwin, Ms. Joan Holup, Ms. Carol Lloyd, Dr. Nancy Press, Dr. Richard D. Press, Dr. Jacob Reiss, Dr. Cheryl Ritenbaugh, Ms. Aileen Uchida, Dr. Thomas Vogt, and Dr. Dwight Yim.

Washington, DC—Howard University

Dr. Victor R. Gordeuk (Principal Investigator), Dr. Fitzroy W. Dawkins (Co-Principal Investigator), Ms. Margaret Fadojutimi-Akinsiku, Dr. Oswaldo Castro, Dr. Debra White-Coleman, Dr. Melvin Gerald, Ms. Barbara W. Harrison, Dr. Ometha Lewis-Jack, Dr. Robert F. Murray, Dr. Shelley McDonald-Pinkett, Ms. Angela Rock, Dr. Juan Romagoza, and Dr. Robert Williams.

Central Laboratory

Minneapolis, MN—University of Minnesota and Fairview-University Medical Center

Dr. John H. Eckfeldt (Principal Investigator and Steering Committee Chair), Ms. Catherine Leiendecker-Foster, Dr. Ronald C. McGlennen, Mr. Greg Rynders, and Dr. Michael Y. Tsai.

Coordinating Center

Winston-Salem, NC—Wake Forest University

Dr. David M. Reboussin (Principal Investigator), Dr. Beverly M. Snively (Co-Principal Investigator), Dr. Roger Anderson, Ms. Eleise Bostic, Ms. Brenda L. Craven, Ms. Shellie Ellis, Dr. Curt Furberg, Mr. Jason Griffin, Dr. Mark Hall, Mr. Darrin Harris, Ms. Leora Henkin, Dr. Sharon Jackson, Dr. Tamison Jewett, Mr. Mark D. King, Mr. Kurt Lohman, Ms. Laura Lovato, Dr. Joe Michaleckyj, Ms. Shana Palla, Ms. Tina Parks, Ms. Leah Passmore, Dr. Pradyumna D. Phatak, Dr. Stephen Rich, Ms. Andrea Ruggiero, Dr. Mara Vitols, Mr. Gary Wolgast, and Mr. Daniel Zaccaro.

NHLBI Project Office

Bethesda, MD—Ms. Phyliss Sholinsky (Project Officer), Dr. Ebony Bookman, Dr. Henry Chang, Dr. Richard Fabsitz, Dr. Cashell Jaquish, Dr. Teri Manolio, and Ms. Lisa O'Neill.

NHGRI Project Office

Bethesda, MD—Ms. Elizabeth Thomson.

Dr. Jean MacCluer, Southwest Foundation for Biomedical Research, also contributed to the design of this study.

Funding Support

HEIRS contract numbers

The HEIRS study was initiated and funded by NHLBI, in conjunction with NHGRI.

N01-HC-05185 (University of Minnesota)

N01-HC-05186 (Howard University)

N01-HC-05188 (University of Alabama at Birmingham)

N01-HC-05189 (Center for Health Research, Kaiser Permanente)

N01-HC-05190 (University of California, Irvine)
 N01-HC-05191 (London Health Sciences Centre)
 N01-HC-05192 (Wake Forest University)

Additional support was provided by the University of Alabama at Birmingham General Clinical Research Center (GCRC) grant M01-RR00032, Southern Iron Disorders Center (J.C.B.), Howard University GCRC grant M01-RR10284, Howard University Research Scientist Award UH1-HL03679-05 from the National Heart, Lung, and Blood Institute and the Office of Research on Minority Health (Dr. Victor R. Gordeuk), and grant UC Irvine M01 RR000827 from the General Clinical Research Centers Program of the National Center for Research Resources National Institutes of Health (Dr. Christine E. McLaren).

Disclosure Statement

No competing financial interests exist.

References

- Acton RT, Barton JC, Passmore LV, *et al.* (2006a) Relationships of serum ferritin, transferrin saturation, and *HFE* mutations and self-reported diabetes in the Hemochromatosis and Iron Overload Screening (HEIRS) study. *Diabetes Care* 29:2084–2089.
- Acton RT, Barton JC, Snively BM, *et al.* (2006b) Geographic and racial/ethnic differences in *HFE* mutation frequencies in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. *Ethn Dis* 16:815–821.
- Adams PC, Reboussin DM, Barton JC, *et al.* (2005) Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med* 352:1769–1778.
- Anderson RT, Wenzel L, Walker AP, *et al.* (2006) Impact of hemochromatosis screening in patients with indeterminate results: the hemochromatosis and iron overload screening study. *Genet Med* 8:681–687.
- Barton JC, Acton RT, Dawkins FW, *et al.* (2005) Initial screening transferrin saturation values, serum ferritin concentrations, and *HFE* genotypes in whites and blacks in the Hemochromatosis and Iron Overload Screening Study. *Genet Test* 9:231–241.
- Barton JC, Acton RT, Lovato L, *et al.* (2006) Initial screening transferrin saturation values, serum ferritin concentrations, and *HFE* genotypes in Native Americans and whites in the Hemochromatosis and Iron Overload Screening Study. *Clin Genet* 69:48–57.
- Barton JC, McDonnell SM, Adams PC, *et al.* (1998) Management of hemochromatosis. Hemochromatosis Management Working Group. *Ann Intern Med* 129:932–939.
- Beutler E, Felitti V, Gelbart T, *et al.* (2000) The effect of *HFE* genotypes on measurements of iron overload in patients attending a health appraisal clinic. *Ann Intern Med* 133:329–337.
- Cobb SC, King S, Chen E (1957) Differences between respondents and nonrespondents in a morbidity survey involving clinical examination. *J Chronic Dis* 6:95–108.
- Feder JN, Gnirke A, Thomas W, *et al.* (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 13:399–408.
- Forthofer RN (1983) Investigation of nonresponse bias in NHANES II. *Am J Epidemiol* 117:507–515.
- Greenland S (1977) Response and follow-up bias in cohort studies. *Am J Epidemiol* 106:184–187.
- Harrison HF, Harrison BW, Walker AP, *et al.* (2008) Screening for hemochromatosis and iron overload: satisfaction with results notification and understanding of mailed results in unaffected participants of the HEIRS study. *Genet Test* 12:491–500.
- Heberlein TA, Baumgartner R (1978) Factors affecting response rates to mailed questionnaires: a quantitative analysis of the published literature. *Am Sociol Rev* 43:447–462.
- Hicken BL, Tucker DC, Barton JC (2003) Patient compliance with phlebotomy therapy for iron overload associated with hemochromatosis. *Am J Gastroenterol* 98:2072–2077.
- Last J (2001) *A Dictionary of Epidemiology*, 4th edition. Oxford University Press, New York.
- McLaren CE, Barton JC, Adams PC, *et al.* (2003) Hemochromatosis and Iron Overload Screening (HEIRS) study design for an evaluation of 100,000 primary care-based adults. *Am J Med Sci* 325:53–62.
- Morton LM, Cahill J, Hartge P (2006) Reporting participation in epidemiologic studies: a survey of practice. *Am J Epidemiol* 163:197–203.
- Witte DL, Crosby WH, Edwards CQ, *et al.* (1996) Practice guideline development task force of the College of American Pathologists. Hereditary hemochromatosis. *Clin Chim Acta* 245:139–200.

Address correspondence to:

Mark Speechley, Ph.D.

Department of Epidemiology & Biostatistics

Room K201, Kresge Building

University of Western Ontario

London N6A 5C1

Ontario

Canada

E-mail: speechly@uwo.ca