

# Liver Diseases in the Hemochromatosis and Iron Overload Screening Study

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**Background & Aims:** The Hemochromatosis and Iron Overload Screening (HEIRS) Study screened 101,168 primary care participants for iron overload with serum transferrin saturation (TS), ferritin, and C282Y and H63D mutations of the *HFE* gene. **Methods:** All C282Y homozygotes and participants with an increased TS (>45% women, >50% men) and serum ferritin level (>200 µg/L women, >300 µg/L men) were recalled for a clinical history and physical examination, and blood tests including alanine transaminase (ALT) and aspartate transaminase levels. Hepatitis B surface antigen and anti-hepatitis C virus were measured if the ALT level was increased (>31 IU/L in women, >40 IU/L in men). **Results:** In the group of participants selected to return for clinical examination because of increased TS and ferritin levels, ALT increases and anti-hepatitis C virus were found in 95 of 284 (33%) African Americans, 50 of 466 (11%) Asian and Pacific Islanders, 21 of 120 (18%) Hispanics, and 40 of 477 (8.4%) Caucasians. ALT increases and hepatitis B surface antigen were detected in 24 of 466 (5%) Asian and Pacific Islanders, 10 of 284 (3.5%) African Americans, 3 of 120 (2.5%) Hispanics, and 2 of 477 (.42%) Caucasians. Of 86 liver biopsy specimens obtained for clinical purposes, 53 were reviewed by a single study pathologist. Liver fibrosis (stage 3 or 4) was present in 2 of 11 (18.2%) C282Y homozygotes that underwent central review and 2 of 302 (.66%) C282Y homozygotes attending the clinical examination. **Conclusions:** Screening for iron overload with ferritin and TS detects persons with viral hepatitis and other types of liver

disease. A minimum of .66% C282Y homozygotes have liver fibrosis.

Iron overload can be associated with a wide range of genetic and environmental factors and can lead to parenchymal organ damage. Homozygosity for the C282Y mutation of the *HFE* gene is a common genetic trait that increases susceptibility to iron overload in .3% to .5% of Caucasians of northern European descent.<sup>1,2</sup> Iron overload also can occur in non-Caucasians and may be related to yet undiscovered genetic mutations and/or environmental factors.<sup>3–9</sup>

The Hemochromatosis and Iron Overload Screening (HEIRS) study was a population screening study for iron overload in a multicenter, multi-ethnic, primary care-based sample of 101,168 adults who were at least 25 years of age.<sup>10</sup> Because the screening tests used for iron overload (transferrin saturation [TS] and serum ferritin) can be affected by liver disease, they also detect participants with liver diseases of diverse causes.

## Methods

A detailed description of the HEIRS study has been reported previously.<sup>10,11</sup> Participants were recruited over a 2-year period at 5 field centers (Washington, DC; Birming-

**Abbreviations used in this paper:** ALT, alanine transaminase; AST, aspartate transaminase; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HEIRS, Hemochromatosis and Iron Overload Screening; TS, transferrin saturation.

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1542-3565/06/\$32.00  
doi:10.1016/j.cgh.2006.04.013

ham, AL; Irvine, CA; Portland, OR—Honolulu, HI; and London, Ontario, Canada). Our sample was drawn from a primary care population, identified through primary care clinics and medical blood-drawing laboratories. Both patients and other persons accompanying the patient were potential participants. No advertising was performed to recruit participants. Eligibility criteria included age of at least 25 years and an ability to understand the written informed consent. Participants were asked how they heard about the study and whether they had been diagnosed previously with iron overload or hemochromatosis. Control participants were without C282Y and H63D mutations and had a normal TS and serum ferritin level. They were frequency matched for age within field centers.

Measurements included spectrophotometric serum iron levels and unsaturated iron binding capacity, turbidometric immunoassay of serum ferritin level (Hitachi 911; Roche Applied Science, Indianapolis, IN), and calculated TS on nonfasting blood samples. *HFE* C282Y and H63D alleles were determined from blood spots using a modification of the Invader assay (Third Wave Technologies, Madison, WI) that increases the allele-specific fluorescent signal by including 12 cycles of locus-specific polymerase chain reaction before the cleavage reaction.<sup>12</sup>

Participants with increases in TS and ferritin levels (TS: >45% women, >50% men; ferritin levels: >200 ug/L women, >300 ug/L men) and all patients with C282Y homozygotes were invited to participate in a clinical examination.

Alanine transaminase (ALT) and aspartate transaminase (AST) levels were analyzed on the Hitachi 911 analyzer using a colorimetric method (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN). Hepatitis testing was performed for participants with an increased ALT level (>31 IU/L women, >40 IU/L men). Hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (HCV) were analyzed using an immunometric assay on the Vitros ECi (Ortho-Clinical Diagnostics, Inc., Raritan, NJ). Results were reported as positive or negative. Self-reported daily alcohol consumption was documented from a dietary history and was reported as grams of ethanol per day.

Liver biopsy examination was not performed under the HEIRS study protocol, but biopsy specimens obtained as part of a participant's clinical care and data from medical records were sought. HEIRS participants were notified by mail about abnormal results of blood tests and hepatitis serology, but a liver biopsy examination was performed at the discretion of their respective physicians. Information about liver biopsy examinations was obtained from these physicians with informed consent from participants. We requested that liver biopsy specimens be forwarded to a HEIRS study liver pathologist who was blinded to all clinical information. Liver biopsy specimens were graded for liver iron levels according to the 0–4 scale of Scheuer et al,<sup>13</sup> and if a sufficient sample was available (>1 mg dry weight) then the liver iron concentration was measured by atomic absorption spectrophotometry as previously described.<sup>14</sup> Liver tissue was removed from paraffin-embedded blocks by washing in xylene.<sup>15</sup> Liver fibrosis was scored on a scale from 0 (normal) to 4 (cirrhosis).<sup>16</sup> The

clinicopathologic diagnosis of the local pathologist was recorded for all biopsy specimens.

## Results

The participants included 63,550 women and 37,618 men. The median age was 50 years (range, 25–100 y). By self-identified race/ethnicity, the sample included 44% Caucasians, 27% African Americans, 13% Asians, 13% Hispanics, .7% Pacific Islanders, .7% Native Americans, and 2% mixed or unknown race. The genotypic and phenotypic characteristics of the HEIRS study group have been reported previously.<sup>10</sup>

A total of 302 of 333 (91%) C282Y homozygotes and 1384 of 1928 (72%) non-C282Y homozygotes with increased TS and serum ferritin levels underwent clinical examination. This included 75 C282Y homozygotes with a previous diagnosis of hemochromatosis.

An increased ALT level was found in 49 of 302 (16.2%) C282Y homozygotes, 500 of 1376 (36.3%) non-C282Y homozygotes, and 70 of 641 (10.9%) control participants. Results of ALT and AST measurements, tests for HBsAg, anti-HCV, and hepatic fibrosis scores are reported in Table 1.

In the group of participants selected to return for clinical examination because they had increased TS and ferritin levels, ALT increases and anti-HCV were found in 95 of 284 (33%) African Americans, 50 of 466 (11%) Asian and Pacific Islanders, 21 of 120 (18%) Hispanics, and 40 of 477 (8.4%) Caucasians. Increased ALT and HBsAg levels were detected in 24 of 466 (5%) Asian and Pacific Islanders, 10 of 284 (3.5%) African Americans, 3 of 120 (2.5%) Hispanics, and 2 of 477 (.42%) Caucasians. Many participants reported a past history of hepatitis and other liver diseases (Table 2) so they were not newly discovered to have these conditions as a result of this screening study.

Twenty-two of the 302 C282Y homozygotes and 64 of the 1384 non-C282Y homozygotes who presented for clinical evaluation had diagnostic liver biopsy specimens or results made available to the study. Of these 86 liver biopsy specimens, 53 were available for review by our study pathologist. The indications for liver biopsy examination were not exclusively to document iron overload and included biopsy examinations performed to assess viral hepatitis and to evaluate causes of increased liver enzyme levels. Liver iron concentration was measured in 38 participants, including 8 from an outside laboratory. Four centrally processed liver biopsy specimens submitted for liver iron concentration measurement were considered insufficient to be reliable (<1 mg dry weight), and these values were deleted. The median ferritin level (range) at the clinical examination in C282Y homozy-

**Table 1.** Liver Disease Testing

Patients	ALT % > upper limit of reference range	AST % > upper limit of reference range	HBsAg+/ tested <sup>a</sup>
C282Y homozygotes <sup>b</sup>	49/302 (16.2%)	35/302 (11.6%)	0/48 (0)
Non-C282Y homozygotes <sup>c</sup>	500/1376 (36.3%)	468/1376 (34.0%)	38/497 (8.0%)
Controls <sup>d</sup>	70/641 (10.9%)	48/641 (7.5%)	1/71 (1.4%)

<sup>a</sup>Only participants with an increased ALT level were tested for hepatitis (31 IU/L women, >40 IU/L men).

<sup>b</sup>C282Y homozygotes with a normal TS and ferritin level were eligible for a clinical examination.

<sup>c</sup>Non-C282Y homozygotes became eligible for a clinical examination because of an increase in TS and serum ferritin levels.

<sup>d</sup>Control participants had no C282Y or H63D mutations of the *HFE* gene and a normal TS and ferritin level.

gotes undergoing liver biopsy examination was 941  $\mu\text{g/L}$  (19, 3717) compared with 352  $\mu\text{g/L}$  (<15, 5300) among those who did not have a biopsy examination. Liver iron concentration was available for 14 C282Y homozygotes and 20 non-C282Y homozygotes. The median (range) liver iron concentration in C282Y homozygotes was 105.5 (15.1, 279.4)  $\mu\text{mol/g}$  and in non-C282Y homozygotes was 25.7 (7.6, 365.4)  $\mu\text{mol/g}$  (normal, 0–36  $\mu\text{mol/g}$ ).

Thirty-three of the 302 C282Y homozygotes in the study had a serum ferritin level of more than 1000  $\mu\text{g/L}$  at the clinical examination, but only 10 of these were known to have had a liver biopsy examination performed. A noninvasive estimate of cirrhosis in C282Y homozygotes has been validated in 2 countries in which a ferritin level of more than 1000  $\mu\text{g/L}$ , an AST level of more than 40 IU/L, and platelet levels of less than 200,000  $\text{mm}^3$  predicted the presence of cirrhosis in 80% of cases.<sup>17,18</sup> This constellation of findings was present in 4 C282Y homozygotes in the present study, but biopsy examination information was available for only 1 cirrhotic participant. Of 11 C282Y homozygotes who underwent a liver biopsy examination and had a central reading, this

was the only one in whom cirrhosis was identified. None of the 7 non-C282Y homozygotes with complete laboratory data and a central reading indicating cirrhosis met the noninvasive criteria (Table 3). There were 7 C282Y homozygotes, 50 non-C282Y homozygotes, and 2 control participants who reported a history of cirrhosis. The self-reported liver diseases for all participants who attended the clinical examination are shown in Table 2.

Eighteen non-C282Y homozygotes had centrally determined fibrosis of stage 3 or 4. The local pathology reports on these indicated hepatitis C in 12, hepatitis B in 1, alcoholic liver disease in 3 (of whom 2 also had hepatitis C), and nonalcoholic steatohepatitis in 1 patient. Three had none of these diagnoses. The 2 C282Y homozygotes with centrally determined fibrosis of stage 3 or 4 had none of these diagnoses. A minimum estimate of stage 3 or 4 fibrosis of .66% was determined by the number of C282Y homozygotes with stage 3 or 4 fibrosis ( $n = 2$ ), divided by the total number of C282Y homozygotes ( $n = 302$ ).

Although 12 of 14 C282Y homozygotes whose hepatic iron concentration was quantified had increased levels, only 7 of 20 non-C282Y homozygotes had in-

**Table 2.** Self-Reported Liver Diseases

Liver disease	Patients		
	C282Y homozygotes	Non-C282Y homozygotes	Controls <sup>a</sup>
Total	N = 302	N = 1376	N = 641
Liver disease reported	n = 32	n = 303	n = 35
Fatty liver	6 (2.0%)	48 (3.5%)	4 (.6%)
Cirrhosis	7 (2.3%)	50 (3.6%)	2 (.3%)
Liver cancer	1 (.3%)	2 (.1%)	0
Hepatitis A	6 (2.0%)	21 (1.5%)	9 (1.4%)
Hepatitis B	3 (1.0%)	71 (5.2%)	7 (1.1%)
Hepatitis C	3 (1.0%)	154 (11.2%)	6 (.9%)
Hepatitis-other	4 (1.3%)	17 (1.2%)	4 (.6%)
Alcohol (g/day)	3.6 $\pm$ 8.4	14.1 $\pm$ 42.5	11.0 $\pm$ 21.7
No liver disease reported <sup>b</sup>	n = 270	n = 1073	n = 606

<sup>a</sup>Control participants had no C282Y or H63D mutations of the *HFE* gene and a normal TS and ferritin level.

<sup>b</sup>Includes both "no" and "don't know" responses.

**Table 1 continued.** Liver Disease Testing

HBsAg+/examined	Anti-HCV+/tested <sup>a</sup>	Anti-HCV+/examined	Stage 3 or 4 fibrosis/ biopsied	Stage 3 or 4 fibrosis/ examined
0/302 (0)	1/48 (2.1%)	1/302 (.3%)	2/11 (18.2%)	2/302 (.66%)
40/1376 (2.9%)	209/497 (42.1%)	206/1376 (15%)	18/36 (50%)	18/1376 (1.3%)
1/641 (.16%)	7/71 (9.9%)	6/641 (1.1%)	No biopsy examination performed	No biopsy examination performed

creased hepatic iron concentrations (Figure 1). Three of the 7 non-C282Y homozygotes with increased liver iron concentration had a diagnosis of hepatitis B or C or nonalcoholic steatohepatitis, and 4 had no diagnosis of liver disease. The highest liver iron concentration was seen in a 60-year-old renal transplant recipient with chronic hepatitis B who had received intravenous iron for 12 years on hemodialysis. There was 1 Caucasian compound heterozygote (C282Y/H63D) with a mild increase of liver iron concentration (39  $\mu\text{mol/g}$ ). All the remaining 3 patients had iron concentrations greater than 40  $\mu\text{mol/g}$ . One was a Caucasian C282Y heterozygote, and 2 were Asians, 1 was a H63D heterozygote and 1 had no C282Y or H63D mutations. In this data set, 3 of 8

Asians and 1 of 2 African Americans who had liver iron concentrations measured had an increased liver iron concentration. Nine participants (non-C282Y homozygotes) had cirrhosis reported by local pathologists that were not centrally reviewed. Eight of these participants had hepatitis B or C, and 1 with no other diagnosis had no stainable iron.

## Discussion

The liver is the primary target organ in hemochromatosis and the major site of initial iron deposition. Liver disease is the most well established of the complications of hemochromatosis, and iron depletion therapy

**Table 3.** Liver Biopsy Examination Results in Centrally Reviewed C282Y Homozygotes and Cirrhotic Nonhomozygotes

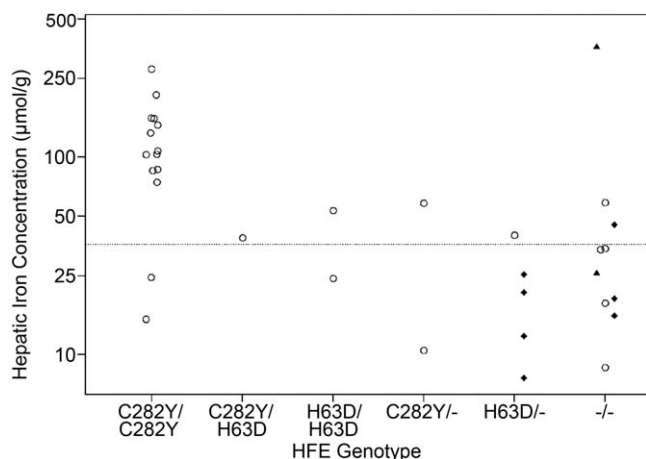
Age/sex	Genotype	Ferritin level	TS, %	ALT level	AST level	Hepatocyte iron grade (0–4)	Liver iron concentration	Fibrosis score: 0–4	Anti-HCV
30–35M	C282Y/C282Y	1650	100	61	33	3	87	1	–
30–35M	C282Y/C282Y	2230	69	133	67	3	157	1	–
35–40M	C282Y/C282Y	1970	95	79	46	3	75	2	–
40–45M	C282Y/C282Y	1660	100	56	45	3	207	1	–
50–55M	C282Y/C282Y	986	95	21	14	3	86	0	–
50–55M	C282Y/C282Y	1680	38	87	59	3	158	3	–
60–65M	C282Y/C282Y	5200	100	75	55	3	108	4	–
70–75M	C282Y/C282Y	1688	100	39	33	3	280	1	–
75–80M	C282Y/C282Y	2340	74	54	35	3	103	2	–
45–50 F	C282Y/C282Y	497	88	55	39	3		0	–
50–55 F	C282Y/C282Y	1960	96			3	145	0	–
50–55M	–/–	774	52	78	72	1	19	4	+
55–60 M <sup>a</sup>	–/–	1770	91	113	165	1	9	4	–
50–55M	–/–	411	65	158	166	2	N/A	4	+
50–55M	–/–	820	69	223	206	2	N/A	4	+
50–55 F	–/–	1563	99	39	135	2	N/A	4	+
60–65 F	–/–	760	91	60	70	3	N/A	4	+
45–50 F	–/–	563	54	67	131	2	N/A	4	+

NOTE. Participants' ages are presented as a range to preserve confidentiality. Reference ranges: serum ferritin level, 15–200  $\mu\text{g/L}$  women, 30–300  $\mu\text{g/L}$  men; TS, 20%–45% women, 20%–50% men; ALT level, 0–31 IU/L women, 0–40 IU/L men; AST level, 0–31 IU/L women, 0–40 IU/L men. All of these participants were negative for HBsAg. Indications for liver biopsy examination varied and included an assessment of iron overload and hepatitis C.

–/–, no C282Y or H63D mutation of the *HFE* gene.

<sup>a</sup>This participant reported a daily alcohol intake of 254 g of absolute ethanol.





**Figure 1.** Hepatic iron concentration by *HFE* genotype. The dotted line represents the upper limit of the reference range for liver iron concentrations (36  $\mu\text{mol/g}$  dry weight). Only participants with an increased ALT level were tested for HBsAg ( $\blacktriangle$ ) and anti-HCV ( $\blacklozenge$ ). Open circles represent participants without a positive test for hepatitis B or C.

by phlebotomy stabilizes the liver disease and prevents its progression to cirrhosis, which can affect long-term survival adversely.<sup>19,20</sup> Although patients with advanced liver disease from hemochromatosis are seen commonly in tertiary referral centers, the prevalence of liver disease in a primary care population screened for iron overload by serum ferritin, TS, and *HFE* genotyping is less well established.

Serum ferritin and TS both can be increased in inflammatory liver diseases such as hepatitis B and C.<sup>21,22</sup> Chronic viral hepatitis is more common than C282Y-linked hemochromatosis, particularly in certain ethnic/racial groups, and thus screening by iron testing detects persons with viral hepatitis as well. This study also has identified patients with alcoholic and nonalcoholic steatohepatitis and increased iron tests. The use of fasting TS may have improved the specificity for iron overload but was not feasible in such a large screening study.<sup>23,24</sup> In a previous study, nonalcoholic fatty liver was shown to be more common in Hispanics and to occur at a lower body mass index in Asians than in other races/ethnicities.<sup>25</sup> The preponderance of women in the HEIRS study also may have led to an underestimation of the clinical expression of liver disease. The highly selected subset of participants on whom we were able to obtain biopsy examination results could produce only prevalence estimates for the overall HEIRS study cohort that are highly uncertain. It is possible that we have underestimated the prevalence of hepatic fibrosis in this study. Increased ALT and AST levels were more common in all participants with increased iron tests, and the control population had a prevalence of increased ALT levels similar to a large population study (8%).<sup>26</sup> A recent study of

fibrosis in 672 hemochromatosis patients showed liver fibrosis (stages 2–4) in 18.4% of men and 5.4% of women.<sup>20</sup> Previous studies have shown that most test results of increased iron levels in patients with chronic viral hepatitis and steatohepatitis are not associated with significant iron overload, although mild to moderate iron overload can occur in these conditions.<sup>21,22,27,28</sup> These observations do not support the use of iron tests to screen for viral hepatitis because more direct testing is available readily and population screening for viral hepatitis has not been recommended widely.<sup>29</sup> The results do show the potential for the overestimation of the prevalence of iron overload in patients with viral hepatitis or steatohepatitis. Many patients with viral hepatitis will undergo liver biopsy examination as part of their diagnostic and prognostic assessment so that the presence of iron overload can be ascertained. In Caucasian patients who do not undergo liver biopsy examination, *HFE* genotyping has been a helpful diagnostic test to identify an additional risk factor for iron overload in a patient with chronic viral hepatitis.<sup>26</sup>

Current practice guidelines<sup>30</sup> recommend liver biopsy examination in C282Y homozygotes whose serum ferritin level is greater than 1000  $\mu\text{g/L}$  or when concomitant risk factors may exist. In a screening study of 65,238 Norwegians in whom 149 liver biopsy examinations were performed, cirrhosis was detected in 4% of the men and none of the women.<sup>31</sup> This contrasts with referral centers that have reported cirrhosis in 15%–30% of referred cases.<sup>9,19,32</sup> A major goal of screening for hemochromatosis is to detect iron overload and to initiate phlebotomy therapy before the development of cirrhosis. Not all C282Y homozygotes have progressive iron overload,<sup>33,34</sup> and it is difficult to ascertain how many of the C282Y homozygotes would have developed liver disease in the current study if they had not been detected and treated.

The assessment of non-*HFE* iron overload in the present study also is difficult because the number of liver biopsy specimens was small. Most participants with an increased liver iron concentration had other risk factors for hepatic iron overload. Genotyping for new iron-related genes such as ferroportin and hemojuvelin and a genome-wide linkage scan are in progress in the HEIRS study and may add additional information about the causes of iron overload in non-C282Y homozygotes.

In summary, our results indicate that screening a primary care population with serum ferritin levels and TS to detect iron overload also will identify many participants with liver diseases of diverse causes. The detection of these conditions and appropriate treatment could

be considered a beneficial aspect of screening for iron overload. Although cirrhosis caused by iron overload is uncommon in this primary care population, prevention of cirrhosis is a major goal of screening.

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The Hemochromatosis and Iron Overload Screening Study was initiated and funded by the National Heart Lung and Blood Institute (NHLBI), in conjunction with the National Human Genome Research Institute (NHGRI). The study was supported by contracts N01-HC-05185 (University of Minnesota), N01-HC-05186 (Howard University), N01-HC-05188 (University of Alabama at Birmingham), N01-HC-05189 (Kaiser Permanente Center for Health Research), N01-HC-05190 (University of California, Irvine), N01-HC-05191 (London Health Sciences Centre), and N01-HC-05192 (Wake Forest University). Additional support was provided by the Howard University General Clinical Research Center (grant M01-RR10284) and from the NHLBI and the Office of Minority Health (UH1-HL03679-05), and the General Clinical Research Center, College of Medicine, University of California, Irvine, with funds provided by the National Center for Research Resources (5M01RR 00827-29), US Public Health Service. Additional support was provided to the Alabama Field Center by a General Clinical Research Center grant (M01-RR00032) awarded to the University of Alabama at Birmingham.

Centers and investigators who participated with the study are listed in the online version of this article at [www.cghjournal.org](http://www.cghjournal.org).

## Appendix

The authors would like to acknowledge those who participated at the following field centers: University of Alabama at Birmingham, Birmingham, AL: 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; University of California, Irvine, Irvine, CA: 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. Korey 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. Argrios Ziogas; London Health Sciences Center, London, Ontario, Canada: Dr. Paul C. Adams (Principal Investigator), Ms. Erin Bloch, Dr. Subrata Chakrabarti, Colin Bradley, Ms. Arlene Fleischhauer, Ms. Helen Harrison, Ms. Kelly Jia, Ms. Sheila Larson, Dr. Edward Lin, Ms. Melissa Lopez, Ms. Lien Nguyen, Ms. Corry Pepper, Dr. Tara Power, Dr. Mark Speechley, Dr. Donald Sun, and Ms. Diane Woelfle; Kaiser Permanente Center for Health Research, Northwest and Hawaii, and Oregon Health and Science University, Portland, OR and Honolulu, HI: 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; Howard University,

Washington, DC: 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; those who participated at the central laboratory: University of Minnesota and Fairview—University Medical Center, Minneapolis, MN: 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; those who participated at the coordinating center: Wake Forest University, Winston-Salem, NC: Dr. David M. Reboussin (Principal Investigator), Dr. Beverly M. Snively (Co-Principal Investigator), Dr. Roger Anderson, Ms. Eleese 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 Vitolsins, Mr. Gary Wolgast, and Mr. Daniel Zaccaro; those who participated at the NHLBI Project Office, Bethesda, MD: Ms. Phyliss Sholinsky (Project Officer), Dr. Ebony Bookman, Dr. Henry Chang, Ms. Kristiane Cooper, Dr. Richard Fabsitz, Dr. Cashell Jaquish, Dr. Teri Manolio, and Ms. Lisa O'Neill; those who participated at the NHGRI Project Office, Bethesda, MD: Ms. Elizabeth Thomson. Dr. Jean MacCluer (Southwest Foundation for Biomedical Research) also contributed to the design of this study.