METABOLIC SYNDROME AND RELATED DISORDERS
Volume 14, Number 2, 2016

Pp. 94–101
DOI: 10.1089/met.2015.0123

ORIGINAL ARTICLES

Risk Factors for Insulin Resistance, Metabolic Syndrome, and Diabetes in 248 *HFE* C282Y Homozygotes Identified by Population Screening in the HEIRS Study

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Abstract

Background: We sought to identify risk factors for insulin resistance, metabolic syndrome (MetS), and diabetes mellitus in 248 non-Hispanic white *HFE* C282Y homozygotes identified by population screening.
Methods: We analyzed observations obtained prospectively in a postscreening examination: age; sex; body mass index (BMI); systolic/diastolic blood pressure; metacarpophalangeal (MP) joint hypertrophy; hepatomegaly; complete blood counts; alanine/aspartate aminotransferase levels; elevated C-reactive protein (>0.5 mg/dL); transferrin saturation; serum ferritin; homeostasis model assessment-insulin resistance (HOMA-IR); and MetS.
Results: Twenty-six participants (10.5%) had diabetes diagnoses. A significant trend across HOMA-IR quartiles was observed only for blood neutrophils. Logistic regression on HOMA-IR fourth quartile revealed positive associations: age ($P = 0.0002$); male sex ($P = 0.0022$); and BMI ($P < 0.0001$). HOMA-IR fourth quartile predicted MetS ($P < 0.0001$). Logistic regression on diabetes revealed positive associations: age ($P = 0.0012$); male sex ($P = 0.0068$); MP joint hypertrophy ($P = 0.0167$); neutrophils ($P = 0.0342$); and MetS ($P = 0.0298$). Serum ferritin did not predict HOMA-IR fourth quartile, MetS, or diabetes.
Conclusions: In screening C282Y homozygotes, age, male sex, and BMI predicted HOMA-IR fourth quartile. HOMA-IR fourth quartile alone predicted MetS. Diabetes was associated with greater age, male sex, MP joint hypertrophy, greater blood neutrophil counts, and MetS.

Introduction

HEMOCHROMATOSIS DUE TO homozygosity for the C282Y mutation of the *HFE* gene on chromosome 6p22.2 occurs in 0.3%–0.6% of persons of northwestern European descent.[1,2] Iron overload, especially if severe, may cause cirrhosis, primary liver cancer, diabetes mellitus (diabetes), other endocrinopathy, arthropathy, and cardiomyopathy.[3] After discovery of *HFE* in 1996,[1] more persons with abnormal iron phenotypes were confirmed to have hemochromatosis using a genetic criterion (*HFE* C282Y homozygosity)[2,4–6] than with the traditional diagnostic triad of hyperpigmentation, diabetes, and cirrhosis.[7] Nonetheless, many C282Y homozygotes neither have nor eventually develop iron overload.[2,8] In reports of the late 20th C, insulin resistance (IR) was common in hemochromatosis patients with diabetes.[9–11] In two case series from the 21st C,[4,5] diabetes prevalence was lower in persons with hemochromatosis phenotypes than was typically reported in the mid-20th C.[12,13] In a 2013 report, the prevalence of type 2 diabetes in adult nonscreening hemochromatosis probands with C282Y homozygosity was 14.5% and a history of diabetes in first-degree family members and body mass index (BMI) were the most significant predictors of diabetes.[6] This confirmed earlier postulates that factors other than siderosis of pancreatic islets increase diabetes risk in persons with hemochromatosis.[14,15]

We hypothesized that factors other than siderosis of pancreatic islets increase IR, metabolic syndrome (MetS), and diabetes risk in 248 non-Hispanic whites discovered to have *HFE* C282Y homozygosity in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. We compared 15 characteristics of 26 participants with and 222 participants without diabetes diagnoses at a single time point.[16] We evaluated these variables across homeostasis model assessment-IR (HOMA-IR)[17] quartiles. Using logistic regressions, we determined significant associations of HOMA-IR

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fourth quartile, MetS, and diabetes with appropriate independent variables. The present results are discussed in the context of previous reports of IR, MetS, and diabetes in persons with and without hemochromatosis.

Materials and Methods

Participants and initial screening

The National Heart, Lung, and Blood Institute/National Human Genome Research Institute HEIRS Study evaluated the prevalence, genetic, and environmental determinants, and potential clinical, personal, and societal impacts of hemochromatosis and iron overload in a multiethnic, primary care-based sample of 101,168 adults enrolled during the interval 2001–2003 at four field centers in the United States and one in Canada.[18] The Study was conducted in accordance with the principles of the Declaration of Helsinki. Participants, at least 25 years of age and able to give informed consent, were recruited from a health maintenance organization, diagnostic blood collection centers, and public and private primary care offices in ambulatory clinics associated with the field centers.[18] Initial screening of participants included iron phenotyping and genotyping for the *HFE* C282Y and H63D alleles.[18]

Clinical examination

Invitations to participate in a postscreening clinical examination (CE) were extended to all C282Y homozygotes (regardless of iron phenotype).[16] The present study only includes observations in participants who identified themselves as non-Hispanic whites.[16] The prevalence of C282Y homozygosity in race/ethnicity groups other than non-Hispanic whites is extremely low.[19,20] The median interval between initial screening and CE participation was 8 months. At the CE, eligible participants were informed of their initial screening transferrin saturation (TS), serum ferritin (SF), and *HFE* genotype. The CE included a questionnaire addressing medical history and medications completed by the participant and a focused physical examination performed by a HEIRS Study physician.[16] Hypertrophy of the second and third metacarpophalangeal (MP) joints was evaluated because it is the most distinctive characteristic of hemochromatosis arthopathy.[18,20] Obtaining detailed accounts of previous diagnoses of hemochromatosis or phlebotomy therapy was beyond the scope of the HEIRS Study.

At CE, a morning fasting blood sample was obtained to confirm the *HFE* genotype[16] and perform complete blood counts (Beckman Coulter GenS; Beckman/Coulter, Fullerton, CA), measurements of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, serum C-reactive protein (CRP), TS and SF (Hitachi 9/11 Analyzer; Roche Applied Science, Indianapolis, IN), serum glucose (Hitachi 9/11 Analyzer; Roche Applied Science, Madison, WI), and serum insulin (DPC Immulite Analyzer; Diagnostic Products, Los Angeles, CA).[2,16] Using control specimens that represented normal ranges of SF, the total coefficient of variation for the Hitachi 9/11 Analyzer was 5.82%–6.78%. For higher range SF standards, the total CV was 5.98%–8.24%.[21] All testing was performed at the HEIRS Study Central Laboratory (Fairview-University Medical Center Clinical Laboratory, University of Minnesota, Fairview, MN). In participants with elevated ALT activities, reflex testing for hepatitis B surface antigen and hepatitis C antibody was performed (Vitros ECi; Ortho-Clinical Diagnostics Incorporated, Raritan, NJ). Reference ranges are displayed in table footnotes. BMI was computed as kg/m^2. Probands with BMI 25.0–29.9 kg/m^2 and BMI ≥30.0 kg/m^2 were defined as overweight and obese, respectively.

Participant exclusions

Thirty-seven participants were excluded because they fasted <8 hr or some of their data were missing.

Homeostasis model assessment-IR

Use of HOMA-IR to quantify IR and its relationship to MetS and diabetes in HEIRS Study CE participants was approved prospectively. IR was estimated using HOMA-IR [(serum glucose [mg/dL] x serum insulin [mIU/L]) ÷ 405].[17] HOMA-IR values were divided as quartiles, yielding corresponding HOMA-IR ranges. Participants in the fourth quartile (HOMA-IR ≥3.06) were defined as having IR.

Definition of MetS

MetS was defined as the concurrence of each of these three attributes: BMI ≥30 kg/m^2; systolic blood pressure (SBP) ≥130 mm Hg or diastolic blood pressure (DBP) ≥85 mm Hg; and fasting serum glucose ≥100 mg/dL.[22–24] Impaired glucose metabolism is an almost universal component of diverse MS definitions.[25] We used the fasting glucose ≥100 mg/dL criterion supported by the American Diabetes Association and other Study groups.[26–28] We used BMI instead of a central obesity measure.[22,24] We grouped positivity for these three attributes into a dichotomous MetS variable.

Definition of diabetes

Participants were classified by self-reports of diabetes diagnoses at initial screening and confirmed at CE by review of medications and medication lists. Three participants reported treatment with insulin. We defined undiagnosed diabetes according to the criteria of the American Diabetes Association.[29] Participants with undiagnosed diabetes were not included in the diabetes subgroup of the present study.

Statistical analyses

There were complete observations in 248 nonpregnant participants. Distributions of SBP, DBP, and TS values were normal (d'Agostino's test). We used natural log (ln) transformation to normalize other data (age, sex, neutrophils, lymphocytes, ALT, AST, and SF). Each mean ln-transformed datum was converted to an anti-ln [95% CI]. Dichotomous variables included the following: sex; diabetes; hypertrophy of MP joints; hepatomegaly; elevated CRP; HOMA-IR fourth quartile values; and MetS. We performed respective regressions on HOMA-IR values, HOMA-IR fourth quartile, MetS, and diabetes using independent variables.

Analyses were performed with (SAS v. 9.1, SAS Institute, Inc., Cary, NC), Excel 2000® (Microsoft Corp., Redmond, WA), and GB-Stat® (v. 10.0; Dynamic Microsystems, Inc., Silver Spring, MD). Descriptive data are displayed as enumerations, percentages, mean ± 1 SD, or mean [95% CI]. Means were compared using Student's *t*-test (two-tailed). Proportions were compared using Pearson's X^2 test or

Fisher's exact test, as appropriate. We computed Pearson's r and value of P for linear regressions on the independent variable HOMA-IR quartiles 1–4 versus the respective dichotomous or continuous variables as a measure of trend. Odds ratios (ORs) [95% CI] are displayed for some logistic regressions. We defined nominal values of $P < 0.05$ to be significant. Bonferroni corrections were applied to control the type I error rate at 0.05 for multiple comparisons of continuous and dichotomous data, as appropriate.

Results

Characteristics of 248 HFE *C282Y homozygotes*

Participants with diabetes were significantly older, more likely to be men, had higher SBP, and were more likely to have hypertrophy of MP joints, higher blood neutrophil counts, elevated CRP, HOMA-IR fourth quartile, and MetS than participants without diabetes (Table 1). The proportions of participants with and without diabetes who were overweight or obese or who reported that they had cirrhosis did not differ significantly (data not shown). Seven participants (2.8%) had fasting glucose levels >126 mg/dL and thus may have had undiagnosed diabetes.[29] The mean SF in 107 men was 418 μg/L [325, 536] and in 141 women was 191 μg/L [153, 228] ($P < 0.0001$).

Characteristics of participants in first and fourth HOMA-IR quartiles

After Bonferroni corrections (significant $P < 0.0031$), there were significant differences in these variables: age; male sex; BMI; SBP; DBP; neutrophils; lymphocytes; ALT; AST; diabetes; and MetS (Table 2).

Trends of values across HOMA-IR quartiles

After Bonferroni corrections (significant $P < 0.0031$), a significant trend across HOMA-IR quartiles was observed only in neutrophils (Table 2).

SF, HOMA-IR fourth quartile, MetS, and diabetes

There were no significant differences in proportions of probands with and without an elevated SF who had HOMA-IR fourth quartile, MetS, or diabetes (Table 3).

Regression on HOMA-IR values

We used these independent variables: age; male sex; diabetes; BMI; SBP/DBP; MP joint hypertrophy; hepatomegaly; ALT/AST; elevated CRP; neutrophils; lymphocytes; TS; and SF. Multiple regression on HOMA-IR values revealed positive associations: diabetes ($P < 0.0001$) and BMI ($P < 0.0001$). Significance of ANOVA for this regression was $P < 0.0001$. This model accounted for 28.7% of the variance in HOMA-IR values.

Regression on HOMA-IR fourth quartile

We used these independent variables: age; male sex; diabetes; BMI; SBP/DBP; MP joint hypertrophy; hepatomegaly; ALT/AST; elevated CRP; neutrophils; lymphocytes; TS; and SF. Logistic regression on HOMA-IR fourth quartile (dichotomous) revealed these associations: age ($P = 0.0002$); male sex ($P = 0.0022$); and BMI ($P < 0.0001$). ORs for these variables were 1.0 [1.0–1.1]; 3.2 [1.5, 6.6]; and 1.2 [1.2, 1.3], respectively. Significance of this regression was $P < 0.0001$. This model accounted for 26.2% of the variance in HOMA-IR fourth quartile.

TABLE 1. CHARACTERISTICS OF 248 *HFE* C282Y HOMOZYGOTES

Characteristic[a]	*Diabetes (n = 26)*[b]	*No diabetes (n = 222)*	P[c]
Mean age, year	62 [58, 67]	50 [48, 51]	<0.0001
Male, % (*n*)	65.4 (17)	40.5 (90)	0.0155
Mean BMI, kg/m^2	29.6 [27.6, 31.9]	27.6 [26.9, 28.3]	0.0876
Mean SBP, mm Hg	139 ± 21	124 ± 17	0.0021
Mean DBP, mm Hg	83 ± 11	77 ± 11	0.0227
MP joint hypertrophy, % (*n*)	42.3 (11)	10.6 (24)	<0.0001
Hepatomegaly, % (*n*)	19.2 (5)	8.0 (18)	0.0717
Mean blood neutrophils × 10^3/μL	4.2 [3.6, 4.9]	3.5 [3.3, 3.7]	0.0465
Mean blood lymphocytes × 10^3/μL	1.4 [1.2, 1.7]	1.6 [1.6, 1.7]	0.1708
Mean serum ALT, IU/mL	25 [20, 31]	21 [19, 23]	0.2142
Mean serum AST, IU/mL	24 [20, 29]	22 [21, 24]	0.3892
CRP >0.5 mg/dL, % (*n*)	50.0 (13)	31.5 (70)	0.0155
Mean transferrin saturation, %	61 ± 29	67 ± 25	0.2788
Mean serum ferritin, μg/L	355 [212, 595]	259 [216, 310]	0.2807
HOMA-IR fourth quartile, % (*n*)	76.9 (20)	18.0 (42)	<0.0001
Metabolic syndrome, % (*n*)	19.2 (5)	5.9 (13)	<0.0282

Results are displayed as mean ± 1 SD, mean [95% CI], or percentage (*n*), as appropriate.

[a]Reference ranges: white blood cells 4.0–11.0 × 10^3/μL; absolute neutrophils 1.6–8.3 × 10^3/μL; absolute lymphocytes 0.8–5.3 × 10^3/μL; ALT 0–31 IU/L (F) and 0–40 IU/L (M); AST 0–31 IU/L (F) and 0–37 IU/L (M); and CRP 0–0.5 mg/dL. ALT activity <4 IU/L was imputed as 3 IU/L. CRP <0.3 mg/dL was imputed as 0.2 mg/L. Elevated CRP levels were defined as >0.5 mg/dL. Reference ranges for iron-related analytes included the following: serum iron concentration 45–160 μg/dL (M) and 30–160 μg/dL (F); serum total iron-binding capacity 228–428 μg/dL; and TS 15%–50%; SF 20–300 μg/L (M); SF 10–120 μg/L (F 15–45 year); and SF 10–300 μg/L (F > 45 year). TS <15% was imputed as 7.5%. Reference ranges for serum glucose and serum insulin were 60–115 mg/dL and 0–20 mIU/L, respectively.

[b]Three participants (11.5%) reported taking insulin.

[c]Nominal values of *P*. Bonferroni correction for 16 comparisons yielded a revised *P* for significance of <0.0031.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance; MP, metacarpophalangeal; SBP, systolic blood pressure.

TABLE 2. CHARACTERISTICS OF 248 *HFE* C282Y HOMOZYGOTES BY HOMA-IR QUARTILES

	HOMA-IR quartiles					
Characteristic[a]	*1* (n = *62*)	*2* (n = *62*)	*3* (n = *62*)	*4* (n = *62*)	P (*quartile 1 vs. 4*)[b]	P (*Pearson's r for trend*)[b]
HOMA-IR, range	≤1.26	1.27–2.01	2.02–3.05	≥3.06	—	—
Mean age, year	50 ± 15	51 ± 13	51 ± 12	58 ± 13	0.0016	0.2413 (0.7587)
Male, % (*n*)	30.6 (19)	43.5 (27)	38.7 (24)	59.7 (37)	0.0010	0.1317 (0.8683)
Mean BMI, kg/m^2	24.4 [23.6, 25.5]	26.7 [25.5, 27.8]	26.7 [25.5, 27.8]	31.5 [29.9, 33.3]	<0.0001	0.0794 (0.9206)
Mean SBP, mm Hg	121 ± 20	124 ± 16	126 ± 19	133 ± 18	0.0005	0.0379 (0.9621)
Mean DBP, mm Hg	73 ± 11	78 ± 10	80 ± 11	81 ± 9	<0.0001	0.0569 (0.9431)
MP joint hypertrophy, % (*n*)	16.1 (10)	11.3 (7)	3.2 (2)	25.8 (16)	0.1856	0.7129 (0.2871)
Hepatomegaly, % (*n*)	4.8 (3)	4.8 (3)	6.5 (4)	21.0 (13)	0.0074	0.1736 (0.8264)
Mean neutrophils $\times 10^3/\mu L$	3.2 [2.9, 3.5]	3.5 [3.1, 3.9]	3.7 [3.4, 4.0]	4.0 [3.6, 4.4]	0.0020	0.0029 (0.9971)
Mean lymphocytes $\times 10^3/\mu L$	1.5 [1.3, 1.6]	1.7 [1.6, 1.8]	1.7 [1.5, 1.8]	1.7 [1.5, 1.9]	<0.0001	0.2254 (0.7746)
Mean ALT, IU/L	16 [14, 19]	20 [17, 23]	23 [19, 26]	29 [25, 34]	<0.0001	0.0101 (0.9899)
Mean AST, IU/L	21 [19, 22]	22 [20, 24]	23 [20, 26]	25 [23, 28]	<0.0001	0.0173 (0.9827)
CRP >0.5 mg/dL, % (*n*)	25.8 (16)	27.4 (17)	38.7 (24)	41.9 (26)	0.0578	0.0434 (0.9566)
Mean TS, %	73 ± 25	70 ± 25	57 ± 26	66 ± 25	0.1344	0.3686 (–0.6314)
Mean SF, μg/L	279 [205, 380]	284 [202, 401]	206 [141, 302]	313 [222, 441]	0.6266	0.8674 (–0.1326)
Metabolic syndrome, % (*n*)	0 (0)	0 (0)	4.8 (3)	24.2 (15)	0.0020	0.8671 (0.1329)
Diabetes, % (*n*)	4.8 (3)	1.6 (1)	3.2 (2)	32.2 (20)	<0.0001	0.2569 (0.7431)

Results are displayed as mean ±1 SD, mean [95% CI], or percentage (*n*), as appropriate.

[a]Reference ranges: white blood cells $4.0–11.0 \times 10^3/\mu L$; absolute neutrophils $1.6–8.3 \times 10^3/\mu L$; absolute lymphocytes $0.8–5.3 \times 10^3/\mu L$; ALT 0–31 IU/L (F) and 0–40 IU/L (M); AST 0–31 IU/L (F) and 0–37 IU/L (M); and CRP 0–0.5 mg/dL. ALT activity <4 IU/L was imputed as 3 IU/L. CRP <0.3 mg/dL was imputed as 0.2 mg/L. Elevated CRP levels were defined as >0.5 mg/dL. Reference ranges for iron-related analytes included the following: serum iron concentration 45–160 μg/dL (M) and 30–160 μg/dL (F); serum total iron-binding capacity 228–428 μg/dL; and TS 15%–50%; SF 20–300 μg/L (M); SF 10–120 μg/L (F 15–45 year); and SF 10–300 μg/L (F > 45 year). TS <15% was imputed as 7.5%. The reference ranges for serum glucose and serum insulin were 60–115 mg/dL and 0–20 mIU/L, respectively.

[b]Bonferroni correction for 16 comparisons yielded a revised *P* for significance of <0.0031.

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment-insulin resistance; MP, metacarpophalangeal; SBP, systolic blood pressure; SF, serum ferritin; TS, transferrin saturation.

Regression on MetS

We used these independent variables: age; male sex; diabetes; MP joint hypertrophy; hepatomegaly; ALT/AST; elevated CRP; neutrophils; lymphocytes; TS; SF; and HOMA fourth quartile. Logistic regression on MetS revealed one positive association: HOMA-IR fourth quartile [$P < 0.0001$; OR 24.7 (5.5, 111.4)]. Significance of this regression was $P < 0.0001$. This model accounted for 31.5% of the variance of MetS.

Regression on diabetes

We used these independent variables: age; male sex; MP joint hypertrophy; hepatomegaly; ALT/AST; elevated CRP; neutrophils; lymphocytes; TS; SF; and MetS. Logistic regression on diabetes revealed these positive associations and ORs: age [$P = 0.0012$; 1.1 (1.0, 1.1)]; male sex [$P = 0.0068$; 4.2 (1.5, 11.8)]; hypertrophy of MP joints [$P = 0.0167$; 3.4 (1.2, 9.5)]; blood neutrophils [$P = 0.0342$; 1.3 (1.0, 1.6)]; and MetS [$P = 0.0298$; 4.2 (1.1, 15.6)]. There was a negative association with TS [$P = 0.0482$; OR 0.9 (0.9, 1.0)]. Significance of this regression was $P < 0.0001$. This model accounted for 25.8% of the variance in diabetes.

TABLE 3. SERUM FERRITIN IN 248 *HFE* C282Y HOMOZYGOTES

Characteristic	*Serum ferritin elevated* (n = *155*)	*Serum ferritin not elevated* (n = *93*)	P
HOMA-IR fourth quartile, % (*n*)	24.5 (38)	25.8 (24)	0.8203
Metabolic syndrome, % (*n*)	7.1 (11)	7.5 (7)	0.8810
Diabetes, % (*n*)	11.6 (18)	8.6 (8)	0.4537

Regressions on HOMA-IR, HOMA-IR fourth quartile, and diabetes in 72 nonobese participants

We used appropriate independent variables as described above. Regression on HOMA-IR revealed these positive associations: diabetes ($P < 0.0001$) and DBP ($P = 0.0006$); and this negative association: hepatomegaly ($P < 0.0001$). Significance of ANOVA for this regression was $P < 0.0001$. This model accounted for 53.3% of the variance in HOMA-IR values. Regression on HOMA-IR fourth quartile revealed a significant positive association with diabetes ($P = 0.0003$). Significance of ANOVA for this regression was 0.0003. This model accounted for 16.8% of the variance in HOMA-IR fourth quartile values. Regression on diabetes revealed no significant predictors. Regression on MetS was not possible because nonobese participants did not meet BMI criteria for having MetS.

Discussion

Risk factors for IR in the present *HFE* C282Y homozygotes, defined as HOMA-IR fourth quartile, revealed these significant associations, after adjustment for other variables: age; male sex; and BMI. These results are consistent with observations in persons not selected for hemochromatosis diagnoses or C282Y homozygosity. In nonobese men, aging was associated with decreased insulin sensitivity in one study,[30] but not in another report.[31] The numbers of insulin receptors in adipose cells removed from older subjects are reduced.[32,33] In men, higher IR may be related to greater amounts of visceral and hepatic adipose tissue and lack of a possible protective effect of estrogen.[34,35] Downregulation of muscle phosphatase and tensin homolog could explain greater retention of insulin sensitivity with higher adiposity in women than men.[36] IR in Spanish men increased as BMI increased.[37] In Swedish men, BMI was the strongest predictor of insulin sensitivity after adjustment for other metabolic factors.[38] In healthy adult volunteers without diabetes, BMI was independently associated with IR.[39]

IR was the only positive predictor of MetS after adjustment for other variables in the present study. IR is the central component of MetS, a constellation of cardiovascular and metabolic abnormalities for which definitions vary.[22,23,40–42] IR is relatively common in hemochromatosis patients with diabetes.[9–11] Although cirrhosis of any cause contributes to IR,[43] the prevalence of cirrhosis in C282Y homozygotes in the HEIRS Study was low.[44]

Type 2 diabetes predominated in the present cohort because only 3 of 26 (11.5%) participants with diagnosed diabetes reported using insulin. Based on a fasting serum glucose criterion,[29] 3% of the present participants without reports of diabetes diagnoses and treatment may also have had diabetes. Diagnosis of diabetes, measurements of HbA1c, evaluation of diabetes complications, and follow-up of diabetes diagnoses, management, and complications were beyond the scope of the HEIRS Study.

Diabetes risk in the present C282Y homozygotes was greater with age after adjustment for other variables, although the diabetes OR associated with age was relatively low. In the Behavioral Risk Factor Surveillance System survey of 195,005 adults and in 97,470 HEIRS Study participants, diabetes prevalence increased with age.[45,46] In US nonscreening C282Y homozygotes, age was not significantly associated with type 2 diabetes risk in multivariable analyses.[6] Men in the present study had greater diabetes risk than women. In 97,470 HEIRS Study participants, diabetes prevalence was greater in men.[46] In contrast, male gender was not a significant independent risk factor for type 2 diabetes in US nonscreening C282Y homozygotes in multivariable analyses.[6] In US non-Hispanic whites ≥20 years of age, the prevalence of diagnosed diabetes did not differ significantly between men and women.[47]

MP joint hypertrophy was positively associated with diabetes in the present participants in both univariable and logistic regression analyses. The HEIRS Study selected MP joint hypertrophy as a proxy CE physical examination indicator of hemochromatosis hand arthropathy.[3,16,48] Iron overload alone would not explain the present observations because neither SF in the present study nor SF and iron removed to achieve iron depletion in nonscreening hemochromatosis probands with C282Y homozygosity[6] were significant independent risk factors for diabetes. Noniron arthropathy associated with diabetes that mimics hemochromatosis hand arthropathy[48–50] could explain the association we observed. Although most C282Y heterozygotes do not have or develop iron overload,[2] C282Y heterozygosity was more prevalent in adults with osteoarthritis of the hand documented radiographically.[51] Accordingly, C282Y may be linked to determinants of MP or hand arthropathy.

Blood neutrophil count was positively associated with diabetes in the present study. In studies of persons unselected for hemochromatosis diagnoses, blood neutrophil counts were higher in those with IR[52] or incident diabetes,[53] after correction for other variables. In a cross-sectional study of 30,793 Korean subjects in hospital, blood neutrophil counts were significantly higher in patients with diabetes.[54] These observations suggest that the pathogenesis of diabetes in C282Y homozygotes involves inflammation as it does in persons with type 2 diabetes without hemochromatosis diagnoses.[55]

MetS, as defined herein, was an independent risk factor for diabetes in the present participants. MetS is also a risk factor for type 2 diabetes in persons unselected for hemochromatosis diagnoses.[25,29,52]

There was a negative association of TS with diabetes in the present regression analyses, although the OR associated with TS was only slightly below unity. Analysis of combined data from the National Health and Nutrition Examination Survey (NHANES) I (1971–1974) and the NHANES I Epidemiologic Follow-up Study (1992) revealed that TS >55% was not associated with an increased risk to develop diabetes.[56]

The present results demonstrate that SF is not a significant predictor of HOMA-IR fourth quartile, MetS, or diabetes in screening C282Y homozygotes, although many studies report a positive association of SF with these metabolic abnormalities in persons unselected for hemochromatosis, iron overload, or *HFE* genotypes.[46,57–59] IR and MetS are common in patients with hemochromatosis,[9–11,60] but we found no previous reports of statistical associations of SF and risk for HOMA-IR fourth quartile or MetS in subjects with hemochromatosis or C282Y homozygosity. On the other hand, neither SF nor iron removed by phlebotomy to achieve iron depletion was significantly associated with type 2 diabetes present at nonscreening diagnosis of hemochromatosis and C282Y homozygosity after correction for other variables.[6] *HFE* C282Y and H63D alleles were significantly associated with SF, but not with diabetes risk in an analysis of combined data in US adults from the Nurses' Health Study and the Health Professionals Follow-up Study (2591 subjects with diabetes, 3052 controls).[61] In screening C282Y homozygotes, there is a significant correlation of SF and iron burdens.[62] These observations substantiate that increased iron storage is the major determinant of SF in C282Y homozygotes with or without diabetes.

We propose that SF was not a significant predictor of HOMA-IR fourth quartile, MetS, or diabetes in the present C282Y homozygotes, in part, because hyperferritinemia was due predominantly to increased iron storage, not to inflammation, neoplasms, or other causes. SF is a mixture of iron-rich ferritin and apoferritin.[63,64] Iron-rich SF is in equilibrium with body iron stores.[65,66] The iron composition of SF is increased in hemochromatosis and other iron overload disorders, consistent with the function of ferritin as an iron storage protein.[63,67] Because the liver is a major iron storage organ, the iron content of SF in noniron liver disorders associated with

hepatocellular necrosis is also increased, presumably due to the release of intracellular iron-rich ferritin into the blood.[63,64,67] Ferritin released into the blood as an acute-phase reactant due to inflammation, anemia of chronic disease, or malignancy is typically apoferritin and has low iron content,[63,64,67] especially ferritin molecules released from cells or tissues that do not typically store iron.[68–71] Interleukin-1 and ethanol enhance the production and secretion of apoferritin.[72,73]

In adult subjects without common *HFE* alleles, SF is a significant predictor of IR, MetS, and diabetes.[74,75] In a study that excluded C282Y homozygotes, the mean SF was greater in subjects with MetS.[76] Diabetes risk among participants without *HFE* C282Y/C282Y or C282Y/H63D was greater in those with higher SF in the Busselton Health Study.[59] In the HEIRS Study, SF was significantly associated with diabetes after adjusting for age, sex, racial/ethnic group, *HFE* genotype, and field center.[46] The mean SF in subjects with or without diabetes in cohorts that excluded participants with *HFE* hemochromatosis genotypes[59,76] is much lower than the mean SF of untreated persons with hemochromatosis and C282Y homozygosity.[2,77]

The efficacy of phlebotomy therapy in decreasing IR or increasing insulin sensitivity in persons with hemochromatosis is variable.[10,78,79] Obtaining detailed accounts of previous diagnoses of hemochromatosis or phlebotomy therapy of initial screening or CE participants was beyond the scope of the HEIRS Study. Therefore, if IR were reduced and diabetes were prevented by previous diagnosis and phlebotomy therapy in some of the present C282Y homozygotes, our estimates of IR and diabetes prevalence are conservative.

Conclusions

In screening C282Y homozygotes, age, male sex, and BMI predicted HOMA-IR fourth quartile. HOMA-IR fourth quartile alone predicted MetS. Diabetes was associated with greater age, male sex, MP joint hypertrophy, greater blood neutrophil counts, and MetS. Many of the significant predictors of HOMA-IR fourth quartile, MetS, and diabetes we identified in the present participants are also significant predictors of the corresponding abnormalities in persons unselected for C282Y homozygosity.

Acknowledgments

The authors recognize and appreciate the contributions of all HEIRS Study investigators and participants. The HEIRS Study (January 2000–2006) was initiated and funded by the National Heart, Lung, and Blood Institute in conjunction with the National Human Genome Research Institute. The study was supported by contracts N01-HC05185 (University of Minnesota); N01-HC05186, N01-CM-07003-74, and the Minority Community Clinical Oncology Program (Howard University); N01-HC05188 (University of Alabama at Birmingham); N01-C05189 (Kaiser Permanente Center for Health Research); N01-HC05190 (University of California, Irvine); N01-HC05191 (London Health Sciences Centre); and N01-C05192 (Wake Forest University). Additional support was provided by University of Alabama at Birmingham General Clinical Research Center grant M01-RR00032 and the Southern Iron Disorders Center (to J.C.B., R.T.A.). The present work was supported, in part, by Southern Iron Disorders Center.

Author Disclosure Statement

No competing financial interests exist.

Author Contributions

Ja.C.B. conceived the study, evaluated participants, acquired data, and performed data analyses. R.T.A. conceived the study, acquired data, and performed data analyses. J.Cl.B. acquired data and performed data analyses. P.C.A. conceived the study, acquired data, and evaluated participants. All authors drafted the article and approved its final form.

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