

Undiagnosed diabetes and impaired fasting glucose in *HFE* C282Y homozygotes and *HFE* wild-type controls in the HEIRS Study

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ABSTRACT

Objective: To determine prevalences and predictors of undiagnosed diabetes mellitus (UDM) and impaired fasting glucose (IFG) in non-Hispanic whites with *HFE* p.C282Y homozygosity and controls without common *HFE* mutations identified in population screening.

Research design and methods: We analyzed these observations in a postscreening examination: age; sex; body mass index; systolic/diastolic blood pressure; metacarpophalangeal joint hypertrophy; hepatomegaly; blood neutrophils; alanine and aspartate aminotransferase; elevated C reactive protein; transferrin saturation; serum ferritin; and Field Center.

Results: There were 223 p.C282Y homozygotes and 449 controls without diagnosed diabetes (43.9% men). Mean age of p.C282Y homozygotes was 52±13 years (controls 57±14 years; $p<0.0001$). Mean transferrin saturation in p.C282Y homozygotes was 67±26% (controls 34±14%; $p<0.0001$). Mean serum ferritin in p.C282Y homozygotes was 607 pmol/L (95% CI 497 to 517; controls 274 pmol/L (247 to 301); $p<0.0001$). Overall prevalences of UDM (4.0% vs 4.2%) and IFG (23.8% vs 25.6%) did not differ significantly between p.C282Y homozygotes and wt/wt controls, respectively. In logistic regressions, male sex, body mass index, and alanine aminotransferase were significantly associated with UDM. ORs were 2.7 (1.2 to 2.8); 1.0 (1.0 to 1.1); and 1.0 (1.0 to 1.0), respectively. Age, male sex, and body mass index were significantly associated with IFG. ORs were 1.0 (1.0 to 1.1); 2.8 (1.9 to 4.2); and 1.0 (1.0 to 1.1), respectively.

Conclusions: Prevalences of UDM and IFG were similar in p.C282Y homozygotes and controls in a postpopulation screening examination. Male sex was the strongest predictor of UDM and IFG.

HFE hemochromatosis is an autosomal recessive condition^{1 2} due to homozygosity for the p.C282Y mutation of the *HFE* gene on chromosome 6p21.3.³ *HFE* hemochromatosis occurs in 0.3–0.6% of persons of European descent.^{3–5} p.C282Y homozygosity accounts for ~90% of ‘classical’ hemochromatosis iron phenotypes in whites.^{3 4} Iron overload in p.C282Y homozygotes, especially if severe, may cause diabetes mellitus,

Significance of this study

What is already known about this subject?

- There are no previous reports of the prevalence of undiagnosed diabetes mellitus and impaired fasting glucose in *HFE* p.C282Y homozygotes identified by screening.

What are the new findings?

- The respective prevalences of undiagnosed diabetes mellitus and impaired fasting glucose were similar in non-Hispanic white p.C282Y homozygotes and matched controls without common *HFE* mutations in this cross-sectional, postpopulation screening examination. In multivariable analyses, male sex was the strongest independent variable associated with both undiagnosed diabetes mellitus and impaired fasting glucose. Serum ferritin level was not significantly associated either with undiagnosed diabetes mellitus or impaired fasting glucose.

How might these results change the focus of research or clinical practice?

- Factors other than serum ferritin or iron overload contribute to undiagnosed diabetes mellitus and impaired fasting glucose risks in p.C282Y homozygotes. Longitudinal studies of other cohorts may provide information about the incidence of undiagnosed diabetes mellitus and impaired fasting glucose in p.C282Y homozygotes.

other endocrinopathies, cirrhosis, primary liver cancer, and cardiomyopathy.⁶

Diabetes was diagnosed in ~80% of patients with hemochromatosis reported from the late 19th century to the mid-20th century.⁷ Most patients with hemochromatosis and diabetes also had heavy liver iron loading and cirrhosis.^{7 8} Early investigators attributed diabetes in persons with hemochromatosis to iron-induced fibrosis of the pancreatic acini and islets of Langerhans^{7 8} and specificity of iron deposition for the β cells of the islets.^{9 10} The results of population screening studies of the 21st century revealed that the prevalence of

type 2 diabetes in p.C282Y homozygotes and persons without common *HFE* mutations is similar.^{11–14} Today, increased type 2 diabetes risk in persons with *HFE* hemochromatosis is associated with one or more factors, including severe iron overload,^{7 8 15} decreased insulin secretion,^{15 16} cirrhosis,⁶ history of diabetes in first-degree relatives,^{17–19} increased body mass index,^{19–22} insulin resistance,²¹ and metabolic syndrome.²²

In the US National Health and Nutrition Examination Survey (NHANES) 1999–2002, approximately one-third of participants with diabetes had previously undiagnosed diabetes mellitus (UDM).²³ Impaired fasting glucose (IFG) occurred in more than 25% of non-Hispanic whites in NHANES 1999–2002.²³ In a previous report, we identified risk factors for diagnosed diabetes in p.C282Y homozygotes who participated in a postscreening clinical examination of the Hemochromatosis and Iron Overload Screening (HEIRS) Study.²² We also postulated that the respective prevalences of UDM and IFG in p.C282Y homozygotes and control subjects who lacked common missense in *HFE* (wt/wt) do not differ significantly. To learn more about UDM and IFG in p.C282Y homozygotes, we evaluated observations from non-Hispanic white HEIRS Study participants without diagnosed diabetes who participated in a postscreening examination. We determined prevalences and independent predictors of UDM and IFG in *HFE* p.C282Y homozygotes and control participants without common *HFE* mutations. We compared our observations with those of UDM and IFG in populations of non-Hispanic whites in NHANES 1999–2002²³ and discuss the implications of our results for understanding diabetes risk in persons with *HFE* hemochromatosis.

RESEARCH DESIGN AND METHODS

Participants and initial screening

The National Heart, Lung, and Blood Institute/National Human Genome Research Institute HEIRS Study investigators 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, cross-sectional sample of 101 168 adults enrolled during the interval 2001–2003 at four Field Centers in the USA and one in Canada.²⁴ The Study was conducted in accordance with the principles of the Declaration of Helsinki. Participants ≥ 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.²⁴ Initial screening of participants, performed in 2000–2002, included iron phenotyping and genotyping for *HFE* p.C282Y and p.H63D alleles.²⁴

Clinical examination

In accordance with the HEIRS Study design,¹⁴ clinical examination participants designated as cases included

all p.C282Y homozygotes and other participants with high-iron phenotypes, regardless of *HFE* genotype.¹⁴ Before the clinical examination, control subjects in three broad age groups (25 to 45, >45 to 65, and >65 years) were selected in a 1:1 ratio with all p.C282Y homozygotes identified at each of the five Field Centers. Control subjects were selected from participants with *HFE* wt/wt, without elevations in transferrin saturation (TS) and serum ferritin (SF), and with TS and SF within the middle half of the respective gender-specific distributions.²⁴ The present study evaluated postscreening clinical examination observations in non-Hispanic white p.C282Y homozygotes and wt/wt control participants.

The median interval between initial screening and clinical examination participation was 8 months. The clinical examination included a questionnaire addressing medical history and medications that was completed by the participant and a focused physical examination performed by a HEIRS Study physician.¹⁴ Body mass index was computed as kg/m². Systolic and diastolic blood pressures were measured using the auscultatory method and a mercury or aneroid sphygmomanometer. Participants were evaluated for hypertrophy of the second and third metacarpophalangeal joints, the most distinctive characteristic of hemochromatosis arthropathy.^{6 24} Obtaining accounts of previous diagnoses of hemochromatosis or phlebotomy therapy was beyond the scope of the HEIRS Study.

At clinical examination, a morning blood sample was obtained after an overnight fast of ≥ 8 hours for: confirmatory *HFE* genotyping;¹⁴ complete blood counts (Beckman Coulter GenS, Beckman/Coulter, Fullerton, California, USA); serum alanine and aspartate aminotransferase activities; serum C reactive protein; TS and SF (Hitachi 9/11 Analyzer, Roche Applied Science, Indianapolis, Indiana, USA); and serum glucose (Hitachi 9/11 Analyzer, Roche Applied Science, Madison, Wisconsin, USA).^{14 25} 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 coefficient of variation was 5.98–8.24%.²⁶ All testing was performed at the HEIRS Study Central Laboratory (Fairview-University Medical Center Clinical Laboratory, University of Minnesota, Fairview, Minnesota, USA). In participants with elevated alanine aminotransferase, reflex testing for hepatitis B surface antigen and hepatitis C antibody was performed (Vitros ECI, Ortho-Clinical Diagnostics Incorporated). Reference ranges for these analytes are displayed in the footnotes of table 1.

Participant exclusions

The data set included observations on 1129 non-Hispanic whites, among whom were 285 p.C282Y homozygotes and 523 wt/wt control participants. We excluded 62 p.C282Y homozygotes (22 diagnosed diabetes or previously diagnosed hemochromatosis; 10

Table 1 Characteristics of 672 HEIRS Study participants without diagnosed diabetes*

Characteristic	HFE p.C282Y homozygotes (n=223)	HFE wt homozygotes (n=449)	Value of p†
Mean age, years	52±13	57±14	<0.0001
Male, percent (n)	40.3 (91)	45.4 (204)	0.2551
Mean body mass index, kg/m ²	28.2±5.7	28.3±5.8	0.8831
Mean systolic blood pressure, mm Hg	125±17	124±17	0.6755
Mean diastolic blood pressure, mm Hg	78±11	76±10	0.0838
Hepatomegaly, percent (n)	9.0 (20)	5.3 (24)	0.0965
Metacarpophalangeal joint hypertrophy, percent (n)	11.7 (26)	6.0 (27)	0.0144
Mean blood neutrophils×10 ⁹ /L	3.8±1.7	3.6±1.6	0.1344
Mean alanine aminotransferase, µkat/L	0.43±0.38	0.48±0.48	0.2898
Mean aspartate aminotransferase, µkat/L	0.42±0.32	0.48±0.47	0.0328
Elevated C reactive protein, percent (n)	32.7 (73)	29.0 (130)	0.3272
Mean transferrin saturation, percent	67±26	34±14	<0.0001
Mean serum ferritin, pmol/L	607 (497 to 517)	274 (247 to 301)	<0.0001
Undiagnosed diabetes, percent (95% CI) (n)‡	4.0 (9) (2.0 to 7.8)	4.2 (19) (2.6 to 6.7)	1.0000
Impaired fasting glucose, percent (95% CI) (n)‡	23.8 (53) (18.5 to 30.0)	25.6 (115) (21.7 to 30.0)	0.6369

*Means were compared using Student's t-test (two-tailed). Mean values are displayed ±1 SD. Proportions were compared using Fisher's exact test (two-tailed). Proportions are displayed as percentage (95% CI). Reference ranges: blood neutrophils 1.6–8.3×10⁹/µL; alanine aminotransferase 0–0.52 µkat/L (F) and 0–0.67 µkat/L (M); aspartate aminotransferase 0–0.52 µkat/L (F) and 0–0.62 µkat/L (M); and C reactive protein 0–47.6 nmol/L. Alanine aminotransferase <0.07 µkat/L was imputed as 0.05 µkat/L. C reactive protein <2.9 nmol/L was imputed as 1.9 nmol/L. Elevated C reactive protein was defined as >47.6 nmol/L.

Reference ranges for iron-related analytes included serum iron 8.0–28.6 µmol/L (M) and 5.4–28.6 µmol/L (F); serum total iron-binding capacity 40.8–76.6 µmol/L; transferrin saturation 15–50%; serum ferritin 44.9–674.1 pmol/L (M); serum ferritin 22.5–269.6 pmol/L (F 15–45 years); and serum ferritin 22.5–674.1 pmol/L (F >45 years). Transferrin saturation was defined as the quotient of serum iron by serum total iron-binding capacity. Transferrin saturation <15% was imputed as 7.5%. Reference range for serum glucose was 3.33–6.38 mmol/L.

†Nominal values of p. Bonferroni correction for 15 comparisons yielded a revised p for significance of <0.0033.

‡UDM: OR 0.95 (0.42 to 2.14); p=0.9048. Cohen's h statistic=0.01. IFG: OR 0.91 (0.62 to 1.2); p=0.6029. Cohen's h statistic=0.03.

F, female; HEIRS, Hemochromatosis and Iron Overload Screening; IFG, impaired fasting glucose; M, male; UDM, undiagnosed diabetes mellitus.

pregnancy; 30 missing data, overnight fast <8 hours, report of cirrhosis, or positivity for hepatitis B or C) and 74 control participants (27 diagnosed diabetes; 8 pregnancy; 39 missing data, overnight fast <8 hours, report of cirrhosis, or positivity for hepatitis B or C). We excluded participants with reports of cirrhosis because we could not substantiate these reports. Individual prevalences of diagnosed diabetes, pregnancy, missing data, overnight fast <8 hours, report of cirrhosis, or hepatitis B or C positivity did not differ significantly between p.C282Y homozygotes and wt/wt control participants (data not shown).

Definitions of diagnosed diabetes, UDM, and IFG

Diabetes diagnoses in participants classified by self-reports at initial screening were confirmed at clinical examination by questionnaire and reviews of medications and medication lists. We defined UDM as fasting serum glucose ≥6.99 mmol/L in participants without diabetes diagnoses.^{27 28} We defined IFG as an elevated fasting serum glucose ≥5.55 and <6.99 mmol/L in participants without diabetes diagnoses.^{27 28}

Statistics

The data set consisted of complete observations on 223 p.C282Y homozygotes and 449 wt/wt control participants without diagnosed diabetes from five Field Centers which were pooled for analysis. To summarize diabetes, UDM, and IFG prevalence in HEIRS Study

clinical examination participants, previously published observations on 22 p.C282Y homozygotes and 27 wt/wt controls with diagnosed diabetes²² are included in the last of the present tables. Distributions of age, systolic/diastolic blood pressures, neutrophils, lymphocytes, alanine and aspartate aminotransferase activities, and TS values were normal. We used natural log (ln) transformation to normalize SF data. Each mean ln-transformed datum was converted to an anti-ln (95% CI). Dichotomous variables included HFE genotype; sex; hypertrophy of metacarpophalangeal joints; hepatomegaly; elevated C reactive protein; and an entry for each of the five Field Centers.

We performed regressions on UDM and IFG in 672 participants using these independent variables: age; sex; body mass index; systolic/diastolic blood pressures; hepatomegaly; metacarpophalangeal joint hypertrophy; blood neutrophils; levels of alanine and aspartate aminotransferases; elevated C reactive protein; TS; and SF; and Field Center. The models were run unconditional.

Analyses were performed with SAS V.9.1 (SAS Institute, Cary, North Carolina, USA), Excel 2000 (Microsoft Corp, Redmond, Washington, USA), and GB-Stat (V.10.0, Dynamic Microsystems, Silver Spring, Maryland, USA). Descriptive data are displayed as enumerations, proportions (95% CI), mean±1 SD, or mean (95% CI). Means were compared using Student's t-test (two-tailed). Proportions were compared using Fisher's

exact test (two-tailed). We computed ORs and Cohen's h statistic as estimates of effect sizes to enhance interpretation of comparisons of proportions involving UDM and IFG prevalence. We performed analyses of covariance to determine whether there were significant interactions of the independent variables age and sex on the respective dependent variables UDM and IFG. ORs (95% CI) are also displayed for significant variables from 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

General characteristics

Mean age of the 672 participants was 55 ± 14 years, 295 (43.9%) of whom were men (table 1). There were 223 p.C282Y homozygotes and 449 wt/wt control participants without diagnosed diabetes. p.C282Y homozygotes had lower mean age and higher TS and SF than wt/wt control participants, after Bonferroni correction (table 1).

Age, UDM, and IFG

The prevalence of UDM was greater in participants aged ≥ 65 than < 65 years in p.C282Y homozygotes and wt/wt control participants, but the differences were not significant (table 2). The prevalence of IFG was significantly greater in participants aged ≥ 65 years in both genotype groups (table 2).

Sex, UDM, and IFG

The prevalence of UDM was greater in men than women, although the difference was significant only in wt/wt control participants (table 3). The prevalence of IFG was significantly greater in men than women in both genotype groups (table 3).

Interaction of sex and age on UDM and IFG

Using UDM as the dependent variable and sex and age as the independent variables, the F-value was 5.4210 ($p = 0.0200$) in an analysis of covariance. Using IFG as the dependent variable and sex and age as the independent variables in another analysis of covariance, the F-value was 28.6394 ($p < 0.0001$). These results suggest that there are significant interactions of age and sex on UDM and IFG.

Table 2 Prevalence of undiagnosed diabetes and impaired fasting glucose by age*

HFE p.C282Y/p.C282Y	Age <65 years (n=184)	Age ≥ 65 years (n=39)	p Value	OR (95% CI)	Cohen's h statistic
Undiagnosed diabetes, percent	3.3 (1.3 to 7.3) (6)	7.7 (2.0 to 22.0) (3)	0.1945	0.40 (0.10 to 1.7); $p = 0.2152$	0.20
Impaired fasting glucose, percent	20.7 (15.2 to 27.4) (38)	38.5 (23.8 to 55.3) (15)	0.0230	0.42 (0.20 to 0.86); $p = 0.0199$	0.87
HFE wt/wt	Age <65 years (n=317)	Age ≥ 65 years (n=132)	p Value		
Undiagnosed diabetes, percent	3.5 (1.8 to 6.3) (11)	6.8 (3.4 to 12.9) (9)	0.1338	0.49 (0.20 to 1.2); $p = 0.1239$	0.15
Impaired fasting glucose, percent	22.1 (17.7 to 27.1) (70)	34.1 (26.2 to 42.9) (45)	0.0092	0.56 (0.36 to 0.88); $p = 0.0115$	0.27

*Estimates based on observations in 672 participants without diagnosed diabetes. Proportions were compared using Fisher's exact test (two-tailed). Proportions are displayed as percentage (95% confidence limits) (n).

Table 3 Prevalence of undiagnosed diabetes and impaired fasting glucose in men and women*

HFE p.C282Y/p.C282Y	Men (n=91)	Women (n=132)	Value of p	OR (95% CI)	Cohen's h statistic
Undiagnosed diabetes, percent	6.6 (2.7 to 14.3) (6)	2.3 (0.6 to 7.0) (3)	0.1644	3.0 (0.7 to 12.5); $p = 0.1235$	0.22
Impaired fasting glucose, percent	34.1 (24.7 to 44.8) (31)	16.7 (11.0 to 24.4) (22)	0.0038	2.6 (1.4 to 4.8); $p = 0.0032$	0.41
HFE wt/wt	Men (n=204)	Women (n=245)	p Value		
Undiagnosed diabetes, percent	6.4 (3.6 to 10.9) (13)	2.9 (1.3 to 6.1) (7)	0.1059	2.3 (0.9 to 5.9); $p = 0.0797$	0.21
Impaired fasting glucose, percent	35.8 (29.3 to 42.8) (73)	17.1 (15.3, 26.8) (42)	0.0001	2.7 (1.7 to 4.2); $p < 0.0001$	0.43

*Estimates based on observations in 672 participants without diagnosed diabetes. Proportions were compared using Fisher's exact test (two-tailed). Proportions are displayed as percentage (95% confidence limits) (n).

Regression on UDM

In a logistic regression model, there were three significant positive associations: male sex ($p=0.0220$); body mass index ($p=0.0009$); and alanine aminotransferase ($p=0.0303$). The OR were 2.7 (1.2 to 2.8); 1.0 (1.0 to 1.1); and 1.0 (1.0 to 1.0), respectively. The p value of this regression was 0.0002. The regression model accounted for 10.4% of the deviation of UDM.

Regression on IFG

In a logistic regression model, there were three significant positive associations: age ($p=0.0005$); male sex ($p<0.0001$); and body mass index ($p<0.0001$). The ORs were 1.0 (1.0 to 1.1); 2.8 (1.9 to 4.2); and 1.0 (1.0 to 1.1), respectively. The p value of this regression was $p<0.0001$. The regression model accounted for 10.7% of the deviation of IFG.

Prevalence of diagnosed diabetes, UDM, and IFG

The prevalence of diagnosed diabetes was greater in p.C282Y homozygotes than in wt/wt control participants, although this difference was not significant (table 4). Likewise, the prevalence of UDM and IFG did not differ significantly between p.C282Y homozygotes and wt/wt control participants (table 4).

In total, our analyses revealed that UDM accounted for 29.0% of diabetes in p.C282Y homozygotes and 41.3% of diabetes in wt/wt control participants ($p=0.2723$). Approximately one-quarter of the participants in each genotype group had IFG.

DISCUSSION

In the present HEIRS Study participants without diagnosed diabetes, the prevalence of UDM as defined by the American Diabetes Association^{27 28} was 3.7% in p.C282Y homozygotes and 4.0% in wt/wt controls. The mean age of p.C282Y homozygotes was significantly lower than that of wt/wt control participants. The prevalence of UDM in participants <65 years of age and ≥ 65 years of age did not differ significantly. Likewise, age was not a significant independent predictor of

UDM. In non-Hispanic white participants in NHANES 1999–2002, the crude prevalence of UDM defined by the same criterion²⁷ was 2.9% and increased from 0.4% (0.1% to 3.1%) in participants aged 20–29 years to 6.0% (4.1% to 8.6%) in participants ≥ 65 years of age.²³

The prevalence of UDM was higher in the present men than women, although the difference was significant only in wt/wt control participants (6.4% vs 2.9%, respectively). In regression analyses, male sex was a significant positive predictor for UDM (OR 2.7). In non-Hispanic white NHANES 1999–2002 participants, the standardized prevalence of UDM was significantly higher in men than women (3.5% vs 1.9%, respectively).²³ The prevalence of UDM increased from 0.4% (0.1% to 3.1%) in men aged 20–29 years to 6.0% (4.1% to 8.6%) in participants ≥ 65 years of age.²³

The prevalence of IFG in the present participants without diagnosed diabetes as defined by the American Diabetes Association^{27 28} was 21.6% in p.C282Y homozygotes and 24.2% in wt/wt controls. The prevalence of IFG was significantly greater in p.C282Y homozygotes and wt/wt control participants ≥ 65 years of age than participants <65 years of age in corresponding genotype groups. There was a significant positive association of age with IFG, although the OR was relatively low. McClain *et al*¹⁵ evaluated records of homozygous hemochromatosis probands aged >40 years who underwent glucose measurements after an overnight fast at the University of Utah in the interval 1975–1998. In 101 Utah probands without diabetes, the prevalence of IFG was 17.8%.¹⁵ This prevalence is lower than that in the present p.C282Y homozygotes, possibly due to the more stringent IFG criterion used in the Utah study (fasting glucose 6.00–6.99 mmol/L).^{15 29} In non-Hispanic white participants in NHANES 1999–2002, the crude prevalence of IFG defined by the same criterion used here was 27.0% and ranged from 15.3% (11.9% to 19.3%) in participants aged 20–29 years to 40.0% (36.2% to 43.9%) in participants ≥ 65 years of age.²³

The prevalence of IFG was higher in men than women with UDM in the present p.C282Y homozygotes (34.1% vs 16.7%, respectively) and wt/wt control

Table 4 Diabetes and impaired fasting glucose in 721 HEIRS Study participants*

Classification	HFE p.C282Y/p.C282Y (n=245)	HFE wt/wt (n=476)	p Value	OR (95% CI)	Cohen's h statistic
Diagnosed diabetes, percent (n)	9.0 (5.8 to 13.5) (22)	5.7 (3.8 to 8.3) (27)	0.1644	1.6 (0.9 to 2.9); $p=0.0974$	0.13
Undiagnosed diabetes, percent (n)	3.7 (1.8 to 7.1) (9)	4.0 (2.5 to 6.3) (19)	1.0000	0.9 (0.4 to 2.1); $p=0.8342$	0.02
Impaired fasting glucose, percent (n)	21.6 (16.8 to 27.4) (53)	24.2 (20.4 to 28.3) (115)	0.4587	0.9 (0.6 to 1.3); $p=0.4473$	0.06

*Proportions were compared using Fisher's exact test (two-tailed). Proportions are displayed as percentage (95% confidence limits; n). Observations on 721 participants include 672 participants from the present report and 49 HEIRS Study clinical examination participants with diagnosed diabetes described in a previous report (22). Overall prevalences in 721 participants: diagnosed diabetes 6.5% (4.9% to 8.5%); undiagnosed diabetes 3.9% (2.6% to 5.6%); and impaired fasting glucose 23.3% (20.3% to 26.6%). HEIRS, Hemochromatosis and Iron Overload Screening.

participants (35.7% vs 17.1%, respectively). Male sex was a significant positive independent predictor of IFG (OR 2.8). In NHANES 1999–2002 non-Hispanic white participants, the standardized prevalence of IFG was significantly higher in men than women (33.1% vs 19.6%, respectively).²³

HFE genotypes and SF levels were not significantly associated with UDM or IFG in the present study. Likewise, SF and quantities of iron removed by phlebotomy to achieve iron depletion were not significantly associated with type 2 diabetes in p.C282Y homozygotes in a non-screening venue¹⁹ or in the HEIRS Study.²² The prevalence of type 2 diabetes did not differ significantly between p.C282Y homozygotes and corresponding control participants in four population screening studies.^{30–33}

The prevalence of diabetes in non-Hispanic white p.C282Y homozygotes diagnosed in screening is similar to that in age-matched and sex-matched *HFE* wt/wt control subjects,^{11–13 24} although there are no previous substantive reports of UDM and IFG prevalence in p.C282Y homozygotes identified in screening. At the outset of the present clinical examination substudy, we postulated that there were no meaningful differences in the corresponding prevalence proportions of UDM and IFG between *HFE* genotype subgroups. Previously, when the HEIRS Study was formulated (1998–1999), there were few published estimates of *HFE* genotype frequencies and no estimates of UDM and IFG in p.C282Y homozygotes identified in screening in the USA. Numbers of HEIRS Study participants and their race/ethnicity were specified by the National Heart, Lung, and Blood Institute/National Human Genome Research Institute and could not be changed. Thus, ideal numbers of participants invited to the clinical examination could not be ascertained a priori (ie, before screening) and those who would attend the clinical examination could not be predicted. Statistically non-significant differences between overall UDM and IFG prevalences (failure to reject the null hypothesis) in the present study are not necessarily equivalent to insufficient power. Post hoc power calculations based on data already gathered and analyzed represent little more than p value transformations.^{34–37}

UDM and IFG increase the risk for the development of type 2 diabetes.^{38–41} Whereas the HEIRS Study is a cross-sectional study, longitudinal studies of other p.C282Y cohorts may provide information about the incidence of UDM and IFG in p.C282Y homozygotes. The prevalences of UDM and IFG in the present non-Hispanic white HEIRS Study participants and in non-Hispanic white participants in the contemporaneous NHANES 1999–2002²³ are similar. Regardless, the ages of HEIRS Study participants were ≥ 25 years²⁴ whereas the ages of NHANES 1999–2002 participants were ≥ 20 years.²³ There were differences in methods and sites used to recruit and evaluate participants for these respective studies. The present wt/wt control participants were selected because they had initial screening

TS and SF levels between the 25th and 75th centiles of sex-specific distributions. Thus, wt/wt control participants with extremes of TS and SF phenotypes were not represented in our data. Since the present cohort consisted predominantly of middle age and older participants, putative effects associated with younger participants may have been undetected. Hardy-Weinberg analyses of HEIRS Study initial screening data revealed that predicted numbers of p.C282Y homozygotes recruited to the Study were not decreased,²⁵ suggesting that there was no selective survivor bias among p.C282Y homozygotes. It is unlikely that having greater numbers of participants in the present study would have revealed meaningful differences not already discovered. The results of our regression analyses indicate that other factors not evaluated in this study also influence the occurrence of UDM and IFG.

CONCLUSIONS

Prevalences of UDM and IFG were similar in p.C282Y homozygotes and wt/wt control participants in a postpopulation screening examination. Male sex was the strongest predictor of UDM and IFG.

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Contributors JaCB conceived the study, evaluated participants, acquired data, and performed data analyses. JCIB acquired data and performed data analyses. PCA conceived the study, acquired data, and evaluated participants. RTA conceived the study, acquired data, and performed data analyses. All authors drafted the manuscript and approved its final form. JaCB and RTA take responsibility for the contents of the article.

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