HFE Cys282Tyr Homozygotes With Serum Ferritin Concentrations Below 1000 μg/L Are at Low Risk of Hemochromatosis

Katrina J. Allen,[1,2,3] Nadine A. Bertalli,[1,4] Nicholas J. Osborne,[1,2,4] Clare C. Constantine,[4,5] Martin B. Delatycki,[1,2,6] Amy E. Nisselle,[1,2] Amanda J. Nicoll,[7] Dorota M. Gertig,[8] Christine E. McLaren,[5] Graham G. Giles,[9] John L. Hopper,[4] Gregory J. Anderson,[10] John K. Olynyk,[11,12] Lawrie W. Powell,[10,13] Lyle C. Gurrin,[4] and for the HealthIron Study Investigators*

Hemochromatosis gene (*HFE*)-associated hereditary hemochromatosis (HH) is a genetic predisposition to iron overload and subsequent signs and symptoms of disease that potentially affects approximately 80,000 persons in Australia and almost 1 million persons in the United States. Most clinical cases are homozygous for the Cys282Tyr (C282Y) mutation in the *HFE* gene, with serum ferritin (SF) concentration >1000 μg/L as the strongest predictor of cirrhosis. The optimal treatment regimen for those with SF concentrations above the normal range but <1000 μg/L is unknown. We assessed *HFE* mutations in a prospective cohort of 31,192 participants of northern European descent, aged 40-69 years. An *HFE*-stratified random sample of 1438 participants including all C282Y homozygotes with iron studies 12 years apart were examined by physicians blinded to participants' *HFE* genotype. All previously undiagnosed C282Y homozygotes (35 male, 67 female) and all *HFE* wild-types (131 male, 160 female) with baseline and follow-up SF concentrations <1000 μg/L were assessed for HH-associated signs and symptoms including abnormal second/third metacarpophalangeal joints (MCP2/3), raised liver enzymes, hepatomegaly, and self-reported liver disease, fatigue, diabetes mellitus, and use of arthritis medication. The prevalence of HH-associated signs and symptoms was similar for C282Y homozygotes and *HFE* wild-types for both normal and moderately elevated SF concentrations. The maximum prevalence difference between *HFE* genotype groups with moderately elevated SF was 11% (MCP2/3, 95% confidence interval = −6%, 29%; $P = 0.22$) and for normal SF was 6% (arthritis medicine use, 95% confidence interval = −3%, 16%; $P = 0.11$). *Conclusion:* Previously undiagnosed C282Y homozygotes with SF concentrations that remain below 1000 μg/L are at low risk of developing HH-associated signs and symptoms at an age when disease would be expected to have developed. These observations have implications for the management of C282Y homozygotes. (HEPATOLOGY **2010;52:925-933**)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; C282Y, Cys282Tyr mutation; CI, confidence interval; H63D, His63Asp mutation; HFE, hemochromatosis; HH, hereditary hemochromatosis; MCCS, Melbourne Collaborative Cohort Study; MFIS, Modified Fatigue Impact Scale; MCP2/3, second/third metacarpophalangeal joints; SF, serum ferritin.

From the [1]Murdoch Childrens Research Institute, Melbourne, Australia; [2]Department of Pediatrics and [3]Department of Gastroenterology, Royal Children's Hospital, Melbourne, Australia; [4]Centre for MEGA Epidemiology, School of Population Health, The University of Melbourne, Melbourne, Australia; [5]Department of Epidemiology, University of California Irvine, Irvine, CA; [6]Austin Health, Melbourne, Australia; [7]Royal Melbourne Hospital, Melbourne, Australia; [8]Victorian Cytology Service, Inc., Melbourne, Australia; [9]Cancer Epidemiology Centre, Cancer Council Victoria, Melbourne, Australia; [10]Queensland Institute of Medical Research and University of Queensland, Brisbane, Australia; [11]Department of Gastroenterology, Fremantle Hospital, Fremantle, Australia; School of Medicine and Pharmacology, University of Western Australia, Perth, Australia; and Western Australian Institute of Medical Research, Perth, Australia; [12]Curtin Health Innovation Research Institute, Perth, Australia; and [13]University of Queensland, Brisbane, Australia; Royal Brisbane and Women's Hospital, Brisbane, Australia.

Received April 7, 2010; accepted May 25, 2010.

**The HealthIron Study Investigators also include: M. Bahlo (The Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia), C. D. Vulpe (University of California Berkeley, Berkeley, CA), S. M. Forrest (Australian Genome Research Facility, Melbourne, Australia), M. Southey (Department of Pathology, The University of Melbourne, Melbourne, Australia), and A. Fletcher (Department of Human Services, Melbourne, Australia).*

Hereditary hemochromatosis (HH) refers to symptoms and signs of disease that result from an inherited predisposition to iron overload. Iron overload is preventable, but can lead to significant health problems, including arthritis, hepatic cirrhosis, hepatocellular carcinoma, fatigue, and diabetes mellitus, if it is left untreated.[1] More than 80% of patients presenting with symptomatic iron overload[2,3] are homozygous for the 845G→A mutation in the hemochromatosis (*HFE*) gene, which leads to the Cys282Tyr (C282Y) substitution in the HFE protein.[4] The prevalence of C282Y homozygotes is at least 1 in 200 for people of northern European descent.[5,6] The majority of C282Y homozygotes have elevated iron indices[7,8] but the serum ferritin (SF) concentration threshold at which there is an increased risk of developing HH-associated signs and symptoms other than cirrhosis is not known.

We have recently shown that at least 28% of male C282Y homozygotes develop iron overload–related disease (as defined by both the presence of documented iron overload[9] and one of the following five objective HH features: hepatocellular carcinoma, cirrhosis/fibrosis, physician-diagnosed symptomatic HH, elevated liver enzymes, or evidence of HH-associated arthritis),[7] with onset in the majority by age 55 years. Other studies have shown that individuals with SF concentrations >1000 μg/L are at significantly increased risk of cirrhosis.[10,11] Assessment of HH-associated signs and symptoms for C282Y homozygotes has largely been limited to clinical case series where the sample sizes were greater for those with both SF concentrations >1000 μg/L and symptoms compared with those with only moderately elevated SF concentrations (i.e., above the upper limit of the normal range but below 1000 μg/L).[10–12] Several studies have reported prevalence estimates for C282Y homozygotes who were identified through cascade screening of relatives of a hemochromatosis-affected proband.[13,14] The relatedness of individuals, however, could lead to within-family correlation between both iron indices and the risk of disease, which has the potential to bias prevalence estimates of HH-associated signs and symptoms for C282Y homozygotes.

Several population-based studies have demonstrated that the majority (60%-80%) of untreated C282Y homozygotes develop SF concentrations that are elevated but below the threshold of 1000 μg/L.[8] Assuming a C282Y homozygosity prevalence of 0.44%[8] and a white population of 223,965,009,[15] we estimate that in the United States alone there are almost 700,000 C282Y homozygotes who will develop SF concentrations that are elevated but below 1000 μg/L[8] and almost 55,000 of these individuals in Australia. Given the greater prevalence of *HFE* mutations in the northern European population, the corresponding figure for the United Kingdom is likely to exceed 200,000. However, there is currently no population-based evidence from any country for the risk of developing HH-associated signs and symptoms for those individuals with moderately elevated SF. Such data would have implications for both clinical practice and population-based genetic screening for HH.[16]

We used an *HFE* genotype–stratified random sample of participants in a cohort study prospectively sampled and followed over a 12-year time period to assess the prevalence of HH-associated signs and symptoms for C282Y homozygotes with SF concentrations <1000 μg/L and to compare this with the corresponding prevalence for controls with neither the C282Y nor His63Asp (H63D) mutation using data collected when both participants and physicians were blinded to *HFE* genotype. Our findings on the prevalence of HH-associated signs and symptoms for C282Y-H63D compound heterozygotes and the other *HFE* genotype groups have been published elsewhere.[7,17]

Patients and Methods

Study Methods. The present study, known as HealthIron, is a substudy of the Melbourne Collaborative Cohort Study (MCCS).[18] Between 1990 and 1994, 41,514 people (24,469 of whom were women) with a target age range of 40-69 years were enrolled in the MCCS. Participants were recruited via the electoral roll (voting is compulsory in Australia), advertisements, and

This study was funded by National Institute of Diabetes, Digestive and Kidney Diseases (USA) (1 RO1 DK061885-01 A2) and the National Health and Medical Research Council (Australia) (grants no. 251668, 209057). Cohort recruitment was funded by VicHealth and The Cancer Council Victoria. The National Health and Medical Research Council also provided funding for the following authors: L.C.G. and K.J.A. (Career Development Awards), M.B.D. and J.K.O. (Practitioner Fellowships), G.J.A. (Senior Research Fellowship) and J.L.H. (Australia Fellowship).

Address reprint requests to: Katrina J. Allen, F.R.A.C.P., Ph.D., Murdoch Childrens Research Institute, The University of Melbourne Department of Paediatrics, Department of Gastroenterology, Royal Children's Hospital, Flemington Road, Parkville 3052, VIC, Australia. E-mail: katie.allen@rch.org.au; fax: +61 3 9345 6240.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.23786

Potential conflict of interest: Nothing to report.

community announcements in local media. The majority of participants gave a blood sample at baseline, which was aliquoted as blood spots on Guthrie cards and stored at room temperature. In addition, 1 mL samples of buffy coats and plasma were stored in liquid nitrogen.

For the HealthIron study, the DNA samples from a subsample of participants were extracted from Guthrie cards (n = 23,484) using Chelex reagent or from frozen buffy coats (CorProtocol 14102; Corbett, Sydney, Australia) (n = 7708) and genotyped for the nucleotide changes that correspond to the amino acid substitutions C282Y and H63D in the HFE protein, using TaqMan (Applied Biosystems, Carlsbad, CA) real-time polymerase chain reaction (PCR) probes as previously described.[7] Only samples from participants actively participating in the cohort who reported being born in Australia, the United Kingdom, Ireland, or New Zealand were processed. Participants born in southern Europe (Italy, Greece, or Malta) were excluded due to the lower prevalence of the *HFE* C282Y mutation in populations from that region.

A comprehensive active follow-up of MCCS participants began in 2003 and was completed in June 2007. Letters of invitation to participate in the HealthIron study were sent to a sample of 1438 participants that included all C282Y homozygotes identified in the MCCS (n = 203) and a stratified random sample of approximately equal numbers of participants from each of the other five *HFE* genotype groups. All participants gave written, informed consent to participate in both MCCS and the HealthIron study. Both study protocols were approved by the Human Research Ethics Committee of the Cancer Council of Victoria. Participants attending a study center completed a computer-assisted personal interview (that included questions on medical history, blood donation history, and venesection), provided a cheekbrush DNA sample for confirmatory *HFE* genotyping using real-time PCR assay with TaqMan probes (Applied Biosystems), and underwent a clinical examination of the abdomen and metacarpophalangeal (MCP) joints by study physicians blinded to *HFE* genotype. Blood samples were collected for measurement of iron indices, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations using Roche automated assays (Roche Diagnostics, Indianapolis, IN) and were paired for analysis with stored baseline plasma samples for each participant. Blood samples were usually collected in the morning at both baseline and follow-up, and participants were requested to fast.

Definitions and Exclusion Criteria. We defined sex-specific and menopause-specific SF upper limit of normal thresholds to be >300 μg/L for men and postmenopausal women and >200 μg/L for premenopausal women. We categorized participants according to their baseline SF concentration. Those below the threshold at baseline were defined as "normal SF" and those above the threshold but below 1000 μg/L at baseline were defined as "moderately elevated SF."

We investigated the prevalence of eight outcomes (with examining physicians blinded to genotype) known or suspected from previous research to be associated with primary iron overload. These included: abnormality (bony spur, effusion, or tenderness) of the second and third MCP joints on either hand (MCP2/3), raised AST (>45 IU/L) concentration or raised ALT (>40 IU/L) concentration, a liver span of 13 cm or more (hepatomegaly), self-reported liver disease, self-reported fatigue, self-reported fatigue using the Modified Fatigue Impact Scale (MFIS),[19] self-reported diabetes mellitus, and self-reported use of arthritis medication. The MFIS, a shortened version of the Fatigue Impact Scale,[20] is a measure of self-reported fatigue based on 21 questions in three domains (physical, cognitive, and psychosocial) and scored on a scale of 0-84 (where a higher score indicates a greater impairment of daily activities due to fatigue). With the exception of diabetes mellitus and use of arthritis medication, which were recorded at baseline, all outcomes were measured at follow-up.

We excluded from the analyses those participants who had baseline SF concentrations >1000 μg/L, who had been diagnosed and treated for HH prior to baseline, or who were missing baseline SF concentrations and therefore could not be categorized. We also excluded participants with follow-up SF concentrations >1000 μg/L because current evidence suggests treatment should be recommended due to the high risk of irreversible cirrhosis.

Participants with neither the C282Y nor H63D mutation (referred to as "*HFE* wild-types") were the control group for comparison with C282Y homozygotes. Participants from all other *HFE* genotype groups except C282Y homozygotes were excluded.

Statistical Methods. The prevalence of HH-associated signs and symptoms, stratified by sex, *HFE* genotype (C282Y homozygote or *HFE* wild-type), and normal/moderately elevated SF, was estimated as the observed proportion. Confidence intervals (CIs) for prevalence differences and *P* values for two-sample comparison of proportions were generated by assuming the normal approximation to the underlying binomial

Table 1. Participant Demographics at Baseline as Per Inclusion Criteria for Prevalence of HH-Associated Signs and Symptoms Analysis*

	C282Y Homozygote		*HFE* Wild-type	
	Male *n = 35*	Female *n = 67*	Male *n = 131*	Female *n = 160*
Age (years), mean (SD)	55.6 (9.2)	54.4 (8.3)	53.7 (9.2)	53.5 (9.1)
BMI (kg/m^2), mean (SD)†	24.9 (2.9)	25.0 (3.9)	26.9 (4.2)	26.4 (4.8)
Alcohol (g/day), mean (SD)	12.6 (11.1)	6.1 (8.1)	21.1 (30.0)	7.0 (11.8)
Blood donation at baseline, number (%)				
Never	18 (51%)	39 (58%)	60 (46%)	87 (54%)
Former	9 (26%)	18 (27%)	44 (33%)	42 (26%)
Current	8 (23%)	10 (15%)	27 (21%)	31 (20%)

*Inclusion criteria: baseline and follow-up SF<1000 μg/L; undiagnosed and untreated prior to baseline.
†Male genotype comparison $P < 0.05$; Female genotype comparison $P = 0.05$.

distribution to quantify sampling variability. For statistical analyses of SF concentrations, the values were (natural) log-transformed. SF concentrations were summarized using the geometric mean and were compared between groups by using the SF ratio, which is calculated by exponentiating the difference of the mean log SF values.[21] Values for transferrin saturation and the MFIS were summarized using the sample mean and compared between groups using the two-sample *t* test.

Results

One hundred sixty-one C282Y homozygotes (75 male and 86 female) and 336 *HFE* wild-types (153 male and 183 female) completed at least one of the following components of the HealthIron study: the HealthIron follow-up questionnaire, attendance at a follow-up clinic, or provision of a blood sample at either baseline or follow-up. Thirty-one C282Y homozygotes (27 male and 4 female) and one male *HFE* wild-type were excluded due to having baseline SF concentrations >1000 μg/L or being diagnosed and treated for HH prior to baseline. Data on these participants have been published previously.[7] We further excluded 21 homozygotes (10 male and 11 female) and 38 *HFE* wild-types (17 male and 21 female) who were missing baseline SF concentration and five C282Y homozygotes (two male and three female) and one male *HFE* wild-type who had SF concentration >1000 μg/L at follow-up. After applying these exclusion criteria, 102 C282Y homozygotes (35 male and 67 female) and 291 *HFE* wild-types (131 male and 160 female) remained. Although data from those participants with missing baseline SF concentrations or SF concentrations >1000 μg/L at follow-up are included in Table 2 for completeness, they were removed for all comparative analyses of the prevalence of HH-associated signs and symptoms.

Not all participants contributed data for each outcome, explaining the variation in denominators for the calculation of prevalence statistics. The majority of participants completed the HealthIron follow-up questionnaire (143/161 [88%] C282Y homozygotes and 320/336 [95%] *HFE* wild-types) or provided a blood sample at follow-up (134/161 [83%] C282Y homozygotes and 309/336 [92%] *HFE* wild-types). A lower proportion attended the follow-up clinics (109/161 [68%] C282Y homozygotes and 260/336 [77%] *HFE* wild-types).

Participant Demographics and Health-Related Characteristics. Summary statistics for age, body mass index, alcohol consumption, and blood donation at baseline are displayed in Table 1.

Table 2 presents sample sizes and the prevalence for five HH-associated signs and symptoms, stratified by *HFE* genotype, sex, baseline SF, and follow-up SF, including data from participants with missing baseline SF concentration. Although no formal analysis of the prevalence of HH-associated signs and symptoms for these participants was undertaken, expression in this group was low for both C282Y homozygotes and *HFE* wild-types, and it is unlikely that their exclusion would alter the conclusions of our analyses.

Table 3 displays the prevalence of HH-associated signs and symptoms and summary measures of iron indices for participants with SF concentrations <1000 μg/L at baseline, stratified by sex and *HFE* genotype. Despite significantly higher mean SF and transferrin saturation in C282Y homozygotes compared with *HFE* wild-type controls, the prevalence of HH-associated signs and symptoms was similar in these two groups for both sexes with the exception of male C282Y homozygotes for whom the prevalence of

Table 2. Prevalence of HH-Associated Signs and Symptoms Categorized by *HFE* Genotype, Baseline Serum Ferritin (SF) Concentration, and Sex

						Prevalence of HH-Associated Signs and Symptoms*									
						MCP 2/3		Raised AST or ALT		Fatigue Self-Report		Hepatomegaly		Liver disease Self-report	
Baseline SF	M n	F n	Follow-Up SF	M n	F n	M	F	M	F	M	F	M	F	M	F
C282Y Homozygotes															
Treated before baseline†,‡	4	2													
SF>1000 μg/L	23	2													
Moderately elevated	26	36	SF>1000 μg/L	2	2	0/2	0/0	0/2	1/2	1/2	1/2	0/2	0/0	0/2	0/2
			SF<1000 μg/L	12	22	3/8	2/17	2/12	0/22	0/11	3/21	0/8	0/17	0/12	2/21
			missing SF	4	4	0/0	0/0	0/0	0/0	0/1	0/2	0/0	0/0	0/1	0/2
			treated§,‖	8	8	2/5	1/7	2/7	0/8	3/8	1/8	0/5	0/7	1/7	0/8
			total	26	36	5/15	3/24	4/21	1/32	4/22	5/33	0/15	0/24	1/22	2/33
Normal	11	34	SF>1000 μg/L	0	1	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/1	0/0	0/1
			SF<1000 μg/L	6	24	1/4	3/20	0/6	3/24	0/6	5/22	1/4	1/20	0/6	3/22
			missing SF	3	6	0/0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1
			treated§	2	3	0/2	0/3	0/2	0/3	0/2	1/3	0/2	0/3	0/2	0/3
			total	11	34	1/6	3/24	0/8	4/28	0/8	6/27	1/6	1/24	0/8	3/27
Missing SF¶	10	11	SF>1000 μg/L	4	1	2/4	0/1	2/4	0/1	1/4	0/1	0/3	0/1	1/4	0/1
			SF<1000 μg/L	4	5	0/3	0/5	0/4	0/5	0/4	0/5	0/2	0/5	0/4	0/5
			treated§	2	5	0/2	0/3	0/2	0/5	0/2	0/5	0/2	0/3	0/2	0/4
			total	10	11	2/9	0/9	2/10	0/11	1/10	2/11	0/7	0/9	1/10	0/10
HFE Wild-Types															
SF>1000 μg/L	1	0													
Moderately elevated	32	4	SF>1000 μg/L	0	0										
			SF<1000 μg/L	30	4	3/25	0/4	4/29	0/4	4/30	0/4	3/24	0/4	2/30	0/4
			missing SF	2	0	0/0	0/0	0/0	0/0	0/1	0/0	0/0	0/0	0/1	0/0
			total	32	4	3/25	0/4	4/29	0/4	4/31	0/4	3/24	0/4	2/31	0/4
Normal	100	156	SF>1000 μg/L	1	0	0/1	0/0	0/1	0/0	1/1	0/0	0/1	0/0	0/1	0/0
			SF<1000 μg/L	91	144	14/79	12/121	14/91	3/144	8/89	28/142	2/78	2/115	2/88	10/142
			missing SF	8	12	0/0	0/1	0/1	0/0	0/5	2/8	0/0	0/1	0/5	0/7
			total	100	156	14/80	12/122	14/93	3/144	9/95	30/150	2/79	2/116	2/94	10/149
Missing SF¶	17	21	SF>1000 μg/L	0	0										
			SF<1000 μg/L	17	21	1/11	4/18	1/17	2/21	2/15	3/20	0/10	1/15	0/14	0/20
			total	17	21	1/11	4/18	1/17	2/21	2/15	3/20	0/10	1/15	0/14	0/20

*Definitions of prevalence of HH-associated signs and symptoms headings:

MCP 2/3: defined as any bony spur, tenderness, effusion on both the second and third metacarpophalangeal joints on either hand as determined by physicians blinded to *HFE* genotype at follow-up.

Raised AST or ALT: defined as aspartate aminotransferase (AST) concentration >45 IU/L or alanine aminotransferase (ALT) concentration >40 IU/L at follow-up

Fatigue: self-reported fatigue (ever/never) at follow-up.

Hepatomegaly: defined as liver span of 13 cm or more as assessed by physicians blinded to genotype at follow-up.

Liver disease: self-reported liver disease at follow-up.

†Treated defined as therapeutically venesected before baseline;

‡one male C282Y homozygote treated before baseline also had baseline SF >1000 μg/L and is counted in both sections;

§treated defined as therapeutically venesected after baseline.

‖Two male and two female C282Y homozygotes treated after baseline maintained moderately elevated SF at follow-up.

¶Participants with SF missing at baseline groups are excluded from demographics in Table 1 and subsequent analyses but are included here for completeness of descriptive data. One male and two female C282Y homozygotes and three male and two female *HFE* wild-type participants with neither baseline nor follow-up SF are excluded from this table.

Table 3. Prevalence of HH-Associated Signs and Symptoms in C282Y Homozygotes and *HFE* Wild-Types with Serum Ferritin (SF) Concentration <1000 μg/L at Baseline, by Sex

Signs and Symptoms	SF < 1000 μg/L at Baseline: Male				SF < 1000 μg/L at Baseline: Female			
	C282Y Homozygotes n=35	*HFE* Wild-Types n=131	Difference (95% CI)	P*	C282Y Homozygotes n=67	*HFE* Wild-Types n=160	Difference (95% CI)	P*
Geometric mean SF (95% CI)	315.7 (203.9, 488.9)	149.6 (127.7, 175.2)	*2.1‡ (1.4, 3.1)*	*<0.001*	141.0 (93.8, 212.0)	58.6 (49.8, 68.8)	*2.4‡ (1.7, 3.5)*	*<0.001*
Mean TS (95% CI)	63 (55.3, 70.9)	29.6 (27.9, 31.4)	*33.4 (28.3, 38.7)*	*<0.001*	51.1 (45.5, 56.6)	24.6 (23.3, 25.9)	*26.5 (22.4, 30.5)*	*<0.001*
MCP 2/3 prevalence (%)	6/19 (32%)	17/104 (16%)	*16 (-7, 37)*	*0.117*	6/47 (13%)	12/126 (10%)	*3 (-8, 14)*	*0.534*
Raised AST or ALT prevalence (%)	4/27 (15%)	18/121 (15%)	*0 (-15, 15)*	*0.994*	3/57 (5%)	3/148 (2%)	*3 (-3, 9)*	*0.218*
Hepato-megaly prevalence (%)	1/19 (5%)	5/102 (5%)	*0 (-11, 11)*	*0.947*	1/47 (2%)	2/120 (2%)	*0 (-4, 5)*	*0.840*
Liver disease prevalence (%)	1/28 (4%)	4/124 (3%)	*0 (-7, 8)*	*0.926*	5/57 (9%)	10/153 (7%)	*2 (-6, 11)*	*0.576*
Fatigue: self-report prevalence (%)	3/28 (11%)	12/125 (10%)	*1 (-11, 14)*	*0.858*	10/57 (18%)	30/154 (19%)	*-2 (-14, 10)*	*0.750*
Fatigue: MFIS mean (SD)	19.5 (13.4)	20.8 (15.2)	*-1.3 (-7.7, 5.0)*	*0.685*	25.5 (17.0)	26.6 (16.1)	*-1.1 (-6.2, 4.0)*	*0.672*
Diabetes prevalence (%)	1/35 (3%)	3/131 (2%)	*1 (-6, 7)*	*0.846*	0/67 (0%)	1/160 (1%)	*-1 (-2, 1)*	*0.517*
Arthritis medicine prevalence (%)	2/35 (6%)	2/131 (2%)	*4 (-4, 12)*	*0.151*	6/67 (9%)	12/160 (8%)	*1 (-7, 9)*	*0.711*

*P value comparing geometric mean SF, transferrin saturation (TS) and the Modified Fatigue Impact Scale (MFIS) from the two-sample t test; P value comparing outcomes from chi-squared test;
‡SF ratio generated by exponentiating the difference in log SF between C282Y homozygotes and *HFE* wild-types.

abnormal MCP2/3 was increased compared with male *HFE* wild-types (32% versus 16%; prevalence difference = 16%; 95% CI = −7%, 37%; $P = 0.12$).

Table 4 displays the prevalence of HH-associated signs and symptoms in C282Y homozygotes compared with *HFE* wild-types, stratified by baseline SF. There was little difference in the prevalence of HH-associated signs and symptoms for C282Y homozygotes compared with *HFE* wild-types, or for C282Y homozygotes with moderately elevated SF compared with those with normal SF. The two exceptions were abnormal MCP2/3, which occurred more frequently for C282Y homozygotes with moderately elevated SF than for *HFE* wild-types with moderately elevated SF (prevalence difference = 11%; 95% CI = −6%, 29%; $P = 0.22$) and hepatomegaly, which was less common for C282Y homozygotes than *HFE* wild-types (prevalence difference = −11%; 95% CI = −22%, 0%; $P = 0.04$). Similar results for MCP2/3 and hepatomegaly were observed when comparing C282Y homozygotes with moderately elevated SF to those homozygotes with normal SF.

Sensitivity Analysis. We conducted a sensitivity analysis, excluding participants with body mass index >30 kg/m^2 or high alcohol consumption (>60 g/day for men and >40 g/day for women) (classified according to the Australian National Health and Medical Research Council guidelines)[22] from the calculation of prevalence statistics for raised AST or ALT, hepatomegaly, and self-reported liver disease. This allowed us to assess the sensitivity of the results to these additional exclusion criteria, which are based on known risk factors for elevated liver enzymes and liver disease.

Exclusion of participants with heavy alcohol consumption and/or obesity changed the prevalence of raised AST or AST, hepatomegaly, and self-reported liver disease by less than 3% for each sex-specific and SF concentration-specific *HFE* genotype group.

Discussion

We found little evidence that C282Y homozygotes with SF concentrations below 1000 μg/L at either baseline or follow-up 12 years later were at increased risk of HH-associated signs and symptoms compared with *HFE* wild-types, despite having, on average, significantly greater SF at baseline. Furthermore, C282Y homozygotes with moderately elevated SF concentrations were not at increased risk of HH-associated signs and symptoms compared with those C282Y homozygotes with normal SF concentrations at baseline, after an average of 12 years follow-up. Although we observed

Table 4. Prevalence of HH-Associated Signs and Symptoms in C282Y Homozygotes and *HFE* Wild-Types, by SF Concentration at Baseline

	Moderately Elevated SF at Baseline				Normal SF at Baseline				Moderately Elevated SF vs. Normal SF for C282Y Homozygotes	
	C282Y Homozygotes*	*HFE* Wild-Types	Difference (95% CI)	*P*†	C282Y Homozygotes‡	*HFE* Wild-Types	Difference (95% CI)	*P*†	Difference (95% CI)	*P*†
Male (n)	24	32			11	99				
Female (n)	34	4			33	156				
Total (n)	58	36			44	255				
Signs and symptoms										
MCP 2/3 prevalence (%)	8/37 (22%)	3/29 (10%)	*11 (–6, 29)*	*0.22*	4/29 (14%)	26/201 (13%)	*1 (–13, 14)*	*0.90*	*8 (–10, 26)*	*0.41*
Raised AST or ALT prevalence (%)	4/49 (8%)	4/33 (12%)	*–4 (–17, 10)*	*0.55*	3/35 (9%)	17/236 (7%)	*1 (–8, 11)*	*0.77*	*–1 (12, 12)*	*0.95*
Hepatomegaly prevalence (%)	0/37 (0%)	3/28 (11%)	*–11 (–22, 0)*	*0.04*	2/29 (7%)	4/194 (2%)	*5 (–5, 14)*	*0.13*	*–7 (–16, 2)*	*0.11*
Liver disease prevalence (%)	3/51 (6%)	2/35 (6%)	*0 (–10, 10)*	*0.97*	3/34 (9%)	12/242 (5%)	*4 (–6, 14)*	*0.35*	*–3 (–14, 9)*	*0.60*
Fatigue: self-report prevalence (%)	7/51 (14%)	4/35 (11%)	*2 (–12, 16)*	*0.75*	6/34 (18%)	38/244 (16%)	*2 (–12, 16)*	*0.76*	*–4 (–20, 12)*	*0.62*
Fatigue: MFIS mean (SD)	22.6 (15.8)	23.1 (12.8)	*–0.5 (–7.2, 6.2)*	*0.88*	25.0 (16.6)	24.2 (16.3)	*0.8 (–5.1, 6.7)*	*0.79*	*–2.4 (–9.7, 4.8)*	*0.51*
Diabetes prevalence (%)	1/28 (2%)	1/36 (3%)	*–1 (–7, 5)*	*0.71*	0/44 (0%)	3/255 (1%)	*–1 (–2, 0)*	*0.47*	*2 (–2, 5)*	*0.38*
Arthritis medicine prevalence (%)	3/58 (5%)	1/36 (3%)	*2 (–5, 10)*	*0.58*	5/44 (11%)	13/255 (5%)	*6 (–3, 16)*	*0.11*	*–6 (–17, 5)*	*0.25*

*Includes eight male and eight female C282Y homozygotes therapeutically venesected after baseline;

†*P* value comparing outcomes from chi-squared test;

‡Includes two male and three female C282Y homozygotes therapeutically venesected after baseline.

a higher prevalence of arthritis for male C282Y homozygotes compared with male *HFE* wild-types, the association remained when patients were stratified by SF concentration rather than sex, which suggests arthritis might occur independently of iron overload for C282Y homozygotes. This hypothesis is supported by the clinical observation that arthritis has often been present in patients for an extended period prior to diagnosis of HH,[3,23] and reports that it does not respond well to venesection treatment.[3] However, the suggestion that the lack of treatment is causally related to the development of arthritis requires further scrutiny.

Our study has several strengths. It is the largest sample of C282Y homozygotes followed prospectively over a long period.[24,25] Data were collected with both physicians and participants blinded to *HFE* genotype, limiting recall bias. Data on modifying factors such as heavy alcohol consumption and obesity were also recorded prospectively, although, due to their low prevalence, we were unable to assess the extent to which they contributed to the prevalence of HH-associated signs and symptoms for C282Y homozygotes.

One limitation of our study is that the majority of participants were recruited after 45 years of age; therefore, our findings do not necessarily apply to younger C282Y homozygotes. However, previous population studies of hemochromatosis where the average age of participants was much younger have not found a high prevalence of disease.[16] Moreover, the prevalence of C282Y homozygosity observed in our sample was larger than established estimates of this prevalence from large cross-sectional studies,[2] a scenario that is unlikely if an appreciable fraction of eligible C282Y homozygotes declined to participate due to ill health. Data on the use of magnetic resonance imaging scanning or liver biopsies to quantify liver iron content were not collected systematically, and therefore we are unable to exclude the presence of cirrhosis or fibrosis. However, in a consecutive clinical series of 672 C282Y homozygotes, cirrhosis was not detected in any patient with SF < 1000 μg/L.[10]

Treated C282Y homozygotes were included in this study for completeness. We cannot infer that they were more or less likely to have HH-associated signs and symptoms. Some were ascertained through presentation with symptoms (and therefore more likely to have HH-associated signs and symptoms), but further data on the reasons for diagnosis are not available. Others were ascertained through cascade or other opportunistic screening and were asymptomatic. We note that one previous study that excluded treated C282Y homozygotes from the analysis concluded that most C282Y homozygotes do not develop iron overload–related disease.[26] This approach is likely to have underestimated the prevalence of HH-associated signs and symptoms.[27]

The association between iron indices and the risk of HH-associated signs and symptoms has also been examined among community-recruited participants in

the Hemochromatosis and Iron Overload Screening (HEIRS) study, which is the largest cross-sectional population-based study of iron indices in C282Y homozygotes to date. HEIRS assessed the prevalence of HH-associated signs and symptoms after participants were informed of both their iron and *HFE* genotype status, and the examining physicians were not blinded to genotype.[8,28] The HEIRS authors found that the prevalence of chronic fatigue and MCP2/3 was greater for C282Y homozygotes either previously diagnosed or newly diagnosed with any elevated SF, compared with *HFE* genotype controls. However, they did not stratify based on SF concentrations <1000 μg/L, as in the present study, and there were no longitudinal data on iron studies, so the results are not directly comparable with those presented here.

Our results raise the question as to whether C282Y homozygotes with SF concentrations <1000 μg/L should be managed aggressively or simply monitored to prevent SF rising over the critical threshold of 1000 μg/L. Further evaluation of the clinical benefits of therapeutic venesection should be undertaken to definitively confirm our suggestion that careful observation is a viable alternative to venesection therapy of such subjects. Ideally, a randomized controlled trial of phlebotomy versus a "wait and watch" approach for C282Y homozygotes with SF < 1000 μg/L would be mounted, although the follow-up period required for such a study to produce definitive results may be prohibitively long. If such a trial demonstrated that phlebotomy therapy was not superior, then the "wait and watch" approach would save many thousands of C282Y homozygotes worldwide from unnecessary venesection.

Acknowledgment: Dr. Sue Forrest from the Australian Genome Research Facility, Melbourne, supervised *HFE* genotyping of the cohort samples. Andrea A. Tesoriero with Dr. Melissa C. Southey (both at The University of Melbourne) supervised DNA extraction. Ashley Fletcher provided assistance with study coordination and sample retrieval. This study was made possible by the contribution of many people, including the original investigators and the diligent team who recruited the participants and who continue working on follow-up. We would like to express our gratitude to the many thousands of Melbourne residents who continue to participate in the study.

References

1. Pietrangelo A. Hereditary hemochromatosis–a new look at an old disease. N Engl J Med 2004;350:2383-2397.
2. Adams PC, Barton JC. Haemochromatosis. Lancet 2007;370:1855-1860.
3. McDonnell SM, Preston BL, Jewell SA, Barton JC, Edwards CQ, Adams PC, et al. A survey of 2,851 patients with hemochromatosis: symptoms and response to treatment. Am J Med 1999;106:619-624.
4. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399-408.
5. Merryweather-Clarke AT, Pointon JJ, Jouanolle AM, Rochette J, Robson KJ. Geography of HFE C282Y and H63D mutations. Genet Test 2000;4:183-198.
6. Steinberg KK, Cogswell ME, Chang JC, Caudill SP, McQuillan GM, Bowman BA, et al. Prevalence of C282Y and H63D mutations in the hemochromatosis (HFE) gene in the United States. JAMA 2001;285:2216-2222.
7. Allen KJ, Gurrin LC, Constantine CC, Osborne NJ, Delatycki MB, Nicoll AJ, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. N Engl J Med 2008;358:221-230.
8. Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769-1778.
9. Whitlock EP, Garlitz BA, Harris EL, Beil TL, Smith PR. Screening for hereditary hemochromatosis: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med 2006;145:209-223.
10. Powell LW, Dixon JL, Ramm GA, Purdie DM, Lincoln DJ, Anderson GJ, et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med 2006;166:294-301.
11. Morrison ED, Brandhagen DJ, Phatak PD, Barton JC, Krawitt EL, El-Serag HB, et al. Serum ferritin level predicts advanced hepatic fibrosis among U.S. patients with phenotypic hemochromatosis. Ann Intern Med 2003;138:627-633.
12. Crawford DH, Jazwinska EC, Cullen LM, Powell LW. Expression of HLA-linked hemochromatosis in subjects homozygous or heterozygous for the C282Y mutation. Gastroenterology 1998;114:1003-1008.
13. Bulaj ZJ, Ajioka RS, Phillips JD, LaSalle BA, Jorde LB, Griffen LM, et al. Disease-related conditions in relatives of patients with hemochromatosis. N Engl J Med 2000;343:1529-1535.
14. McCune CA, Ravine D, Carter K, Jackson HA, Hutton D, Hedderich J, et al. Iron loading and morbidity among relatives of HFE C282Y homozygotes identified either by population genetic testing or presenting as patients. Gut 2006;55:554-562.
15. U.S. Census Bureau, Population Data. 2009. Cited December 2, 2009. http://factfinder.census.gov/home/saff.
16. Delatycki MB, Allen KJ, Nisselle AE, Collins V, Metcalfe S, du Sart D, et al. Use of community genetic screening to prevent HFE-associated hereditary haemochromatosis. Lancet 2005;366:314-316.
17. Gurrin LC, Bertalli NA, Dalton GW, Osborne NJ, Constantine CC, McLaren CE, et al. HFE C282Y/H63D compound heterozygotes are at low risk of hemochromatosis-related morbidity. HEPATOLOGY 2009;50:94-101.
18. Giles GG, English DR. The Melbourne Collaborative Cohort Study. IARC Sci Publ 2002;156:69-70.
19. Ritvo PG, Fischer JS, Miller D, Andrews H, Paty HW, LaRocca NG. Multiple Sclerosis Quality of Life Inventory (MSQLI): A User's Manual. New York: National Multiple Sclerosis Society; 1997.
20. Fisk JD, Ritvo PG, Ross L, Haase DA, Marrie TJ, Schlech WF. Measuring the functional impact of fatigue: initial validation of the fatigue impact scale. Clin Infect Dis 1994;18(Suppl. 1):S79-S83.
21. Gurrin LC, Osborne NJ, Constantine CC, McLaren CE, English DR, Gertig DM, et al. The natural history of serum iron indices for HFE C282Y homozygosity associated with hereditary hemochromatosis. Gastroenterology 2008;135:1945-1952.
22. National Health and Medical Research Council (Australia). Australian Alcohol Guidelines: Health Risks and Benefits. Canberra, Australia: NHMRC; 2001.

23. Ines LS, da Silva JA, Malcata AB, Porto AL. Arthropathy of genetic hemochromatosis: a major and distinctive manifestation of the disease. Clin Exp Rheumatol 2001;19:98-102.
24. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 1999;341:718-724.
25. Andersen RV, Tybjaerg-Hansen A, Appleyard M, Birgens H, Nordestgaard BG. Hemochromatosis mutations in the general population: iron overload progression rate. Blood 2004;103:2914-2919.
26. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G–> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-218.
27. Allen KJ, Warner B, Delatycki MB. Clinical haemochromatosis in HFE mutation carriers. Lancet 2002;360:412-413; author reply 413–414.
28. McLaren GD, McLaren CE, Adams PC, Barton JC, Reboussin DM, Gordeuk VR, et al. Clinical manifestations of hemochromatosis in HFE C282Y homozygotes identified by screening. Can J Gastroenterol 2008;22:923-930.