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Iron-Overload–Related Disease in *HFE* Hereditary Hemochromatosis

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ABSTRACT

BACKGROUND

Most persons who are homozygous for C282Y, the *HFE* allele most commonly associated with hereditary hemochromatosis, have elevated levels of serum ferritin and transferrin saturation. Diseases related to iron overload develop in some C282Y homozygotes, but the extent of the risk is controversial.

METHODS

We assessed *HFE* mutations in 31,192 persons of northern European descent between the ages of 40 and 69 years who participated in the Melbourne Collaborative Cohort Study and were followed for an average of 12 years. In a random sample of 1438 subjects stratified according to *HFE* genotype, including all 203 C282Y homozygotes (of whom 108 were women and 95 were men), we obtained clinical and biochemical data, including two sets of iron measurements performed 12 years apart. Disease related to iron overload was defined as documented iron overload and one or more of the following conditions: cirrhosis, liver fibrosis, hepatocellular carcinoma, elevated aminotransferase levels, physician-diagnosed symptomatic hemochromatosis, and arthropathy of the second and third metacarpophalangeal joints.

RESULTS

The proportion of C282Y homozygotes with documented iron-overload–related disease was 28.4% (95% confidence interval [CI], 18.8 to 40.2) for men and 1.2% (95% CI, 0.03 to 6.5) for women. Only one non-C282Y homozygote (a compound heterozygote) had documented iron-overload–related disease. Male C282Y homozygotes with a serum ferritin level of 1000 μg per liter or more were more likely to report fatigue, use of arthritis medicine, and a history of liver disease than were men who had the wild-type gene.

CONCLUSIONS

In persons who are homozygous for the C282Y mutation, iron-overload–related disease developed in a substantial proportion of men but in a small proportion of women.

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Hereditary hemochromatosis is an inherited condition of dysregulated iron absorption that can lead to total-body iron overload with secondary tissue damage in a wide range of organs. Left untreated, this condition can result in diseases such as hepatic cirrhosis and hepatocellular carcinoma.[1,2] Disease can be prevented by decreasing the body's iron stores, through either regular blood donation or therapeutic venesection.[3,4] Cirrhosis most commonly occurs in patients with a homozygous substitution of tyrosine for cysteine at position 282 (C282Y) in the HFE protein who have serum ferritin levels of more than 1000 μg per liter.[4,5] Patients with hereditary hemochromatosis can have a normal life expectancy if treatment is started before cirrhosis occurs.[6]

Persons who are homozygous for the C282Y mutation are at increased risk for iron overload. C282Y homozygotes account for 82 to 90% of clinical diagnoses of hereditary hemochromatosis among persons of northern European descent.[7] C282Y homozygotes can be characterized by the stage of progression as follows: a genetic predisposition without abnormalities, iron overload without symptoms, iron overload with symptoms (e.g., arthritis and fatigue), and iron overload with organ damage — in particular, cirrhosis.[8]

The prevalence of C282Y homozygosity is approximately 1 case per 200 persons, with elevated levels of serum ferritin and transferrin saturation occurring in 40 to 60% of female homozygotes and in 75 to 100% of male homozygotes.[9-13] An elevated serum ferritin level is a necessary but not sufficient prerequisite for the diagnosis of iron overload, which can be objectively documented either by liver biopsy with determination of quantitative hepatic iron levels or by quantitative phlebotomy.[14]

The presence of cirrhosis, severe fibrosis, hepatocellular carcinoma, or arthropathy of the second and third metacarpophalangeal joints in the context of documented iron overload and C282Y homozygosity constitutes iron-overload–related disease in patients with hereditary hemochromatosis.[15-17] It is not known whether other complications that are associated with the disease (e.g., fatigue, abdominal pain, and diabetes) can be attributed to an abnormal *HFE* genotype, particularly since these conditions are common and nonspecific. Population estimates of both documented disease related to iron overload and conditions associated with hereditary hemochromatosis in C282Y homozygotes have been hindered by the absence of clinical assessment before knowledge of the patient's genetic status or by an inability to account for the long lead time of preclinical iron-overload status.

In this 12-year study involving a cohort of 31,192 subjects, we prospectively assessed the prevalence of iron-overload–related disease, along with morbidity and mortality, for C282Y homozygotes, as compared with three groups of subjects: those with compound heterozygosity for C282Y and the substitution of aspartic acid for histidine at position 63 (H63D), C282Y heterozygotes, and subjects with neither *HFE* variant.

METHODS

STUDY POPULATION

From 1990 to 1994, a total of 41,528 subjects (including 24,479 women) between the ages of 27 and 75 years (of whom 99% were between the ages of 40 and 69 years) were enrolled in the Melbourne Collaborative Cohort Study,[18] which is a prospective longitudinal study of diet and other lifestyle factors and the influence of these factors on the development of common chronic diseases. Subjects were recruited through the Australian Electoral Roll (voting is compulsory in Australia), advertisements, and community announcements in local media. After recruitment, subjects visited a study center, where they were interviewed about a range of lifestyle and dietary factors, underwent physical measurement, and provided a blood sample.

For this study (known as HealthIron), subjects who were born in southern Europe (Italy, Greece, and Malta) were excluded, owing to the low prevalence of the C282Y variant in those populations. This exclusion left 31,192 subjects who reported having been born in Australia, the United Kingdom, Ireland, or New Zealand (i.e., of northern European ancestry), with a mean (±SD) age of 55.3±8.9 years. At baseline, 7270 of 17,951 women (40.5%) reported that they were premenopausal; the menopausal status of 1313 women was unknown. All subjects were included in the analysis of mortality. For analyses of other outcomes, a random sample of 1438 subjects who were stratified according to *HFE* genotype and included all C282Y homozygotes were invited to undergo a clinical assessment as part of the HealthIron study. Deaths that occurred before December 31, 2004,

were identified through linkage to death records of the state of Victoria and the Australian National Death Index.

PRELIMINARY *HFE* GENOTYPING

For 23,484 subjects, DNA from stored baseline samples was extracted from Guthrie cards with the use of Chelex reagent. For 7708 subjects, DNA was extracted with the use of buffy-coat CorProtocol 14102 (Corbett Life Science) from frozen blood fractions stored in liquid nitrogen. All samples were genotyped for nucleotide changes that correspond to the amino acid substitution C282Y and H63D in *HFE* with the use of the Taqman real-time polymerase-chain-reaction (PCR) assay (Applied Biosystems), as described previously.[11,19] Owing to initial difficulties with the assay of DNA samples extracted from Guthrie cards, genotyping for the H63D variant was performed only among subjects who were heterozygous for the C282Y variant. Genotyping of 29,676 of the 31,192 samples (95.1%) was successful.

HEALTHIRON CLINICS

From 2004 to 2006, letters of invitation to participate in the HealthIron study were sent to a sample of 1438 subjects that included all C282Y homozygotes and a stratified random sample of subjects from the remaining groups with the *HFE* genotype. Investigators were unaware of subjects' *HFE* genotype until all clinical assessments had been completed. Before being interviewed and undergoing clinical examination, subjects were asked by the clinic manager not to reveal to the study physicians any previous diagnosis of iron overload or hemochromatosis.

Subjects completed a computer-assisted personal interview and provided a cheek-brush sample for confirmatory *HFE* genotyping. Blood samples were collected for measurement of iron indexes and liver enzyme levels with the use of automated assays (Roche Diagnostics) and were paired for analysis with the corresponding stored baseline serum samples. Blood samples were collected in the morning at both baseline and follow-up.

Study investigators performed a physical examination of the abdomen and metacarpophalangeal joints. Previously unidentified C282Y homozygotes were referred to a HealthIron follow-up clinic for ongoing treatment and a liver biopsy, if clinically indicated. Results of previous liver biopsy were obtained from physicians of C282Y homozygotes whose condition had been diagnosed before or during the course of the study. The definitions of iron overload and iron overload–related disease are listed in Table 1.

All subjects gave written informed consent to participate in both the Melbourne Collaborative Cohort Study and the HealthIron study. Both study protocols were approved by the ethics committee at the Cancer Council Victoria.

STATISTICAL ANALYSIS

Prevalences were estimated as observed proportions and compared by calculating prevalence ratios and 95% confidence intervals. Genotype groups were compared by analysis of variance for continuous measures, with serum ferritin analyzed on the natural logarithm scale, or by chi-square tests for proportions. Cox regression with age as the time axis was used to estimate the hazard ratio for the association between the *HFE* genotype and death. The proportional-hazards assumption was assessed by means of plots of the Nelson–Aalen estimate of the cumulative hazard and formal tests based on Schoenfeld residuals.

Table 1. Definitions of Categories of Iron Overload and Iron-Overload–Related Disease.*

Variable	Clinical Finding or Laboratory Measure
Iron overload	
Documented	At least one of the following: increased iron content shown by hepatic iron staining 3 or 4, iron concentration >90 μmol per gram, or hepatic iron index >1.9 (Whitlock et al.[14]) or serum ferritin >1000 μg per liter at baseline with documented therapeutic venesection
Provisional	Elevated serum ferritin (>300 μg per liter for men and postmenopausal women, >200 μg per liter for premenopausal women) in association with elevated transferrin saturation (>55% for men and >45% for women)
No evidence	Either normal or elevated serum ferritin with normal transferrin saturation
Iron-overload–related disease	At least one of the following plus documented iron overload: hepatocellular carcinoma, cirrhosis or fibrosis on percutaneous liver biopsy, tenderness or effusion of the second and third metacarpophalangeal joints, elevated levels of serum aspartate aminotransferase (>45 IU per liter) or serum alanine aminotransferase (>40 IU per liter), or diagnosis by a physician owing to symptoms associated with hereditary hemochromatosis

* Documented iron-overload–related disease was considered to be present if subjects had both documented iron overload and evidence of iron-overload–related disease.

Statistical analyses were performed with the use of Stata software, version 9.1 (Stata).

RESULTS

WHOLE-COHORT ANALYSIS

Among 29,676 subjects of northern European ancestry, 203 were homozygous for the C282Y mutation, which corresponded to an estimated prevalence of 1 case per 146 subjects, or 0.68% (95% confidence interval [CI], 0.59 to 0.78). The presence of homozygosity for the C282Y mutation was confirmed in an independent laboratory from either a follow-up cheek swab or a baseline serum sample.

There were 3295 subjects (11.1%) who were heterozygous for the C282Y mutation only. An additional 719 subjects (2.4%) were heterozygous for both the C282Y and H63D mutations (compound heterozygotes). The observed number of both simple (C282Y) and compound (C282Y–H63D) heterozygotes in the whole cohort was lower than expected on the basis of Hardy–Weinberg equilibrium (chi-square=6.25, P=0.01). With the exception of C282Y homozygotes, this baseline genotyping was not repeated with a second, independent method.

DEATH FROM ANY CAUSE

During an average of 11.4 years of follow-up, 2488 subjects (8.0%) died, including 19 of 203 C282Y homozygotes (9.4%), 59 of 719 compound heterozygotes (8.2%), and 295 of 3295 C282Y heterozygotes (9.0%). The hazard ratio for death from any cause among the C282Y homozygotes, as compared with subjects who had no C282Y mutation, was 1.04 (95% CI, 0.67 to 1.62; P=0.87).

HEALTHIRON SUBJECTS

Of the 1438 subjects who were invited to participate in the HealthIron study, 1054 (73.3%) completed at least two of the four components: a questionnaire, confirmatory *HFE* genotyping, blood sampling, and physical examination; 937 subjects (65.2%) completed all components, 58 (4.0%) completed three components (excluding physical examination), and 59 (4.1%) completed only the questionnaire and genotyping. Of the 384 subjects who did not complete any of the four components of the final study, 113 had died, 197 were no longer actively participating in the study, and 74 could not be contacted. A total of 1054 of the 1325 subjects who were still alive participated (79.5%). The male:female ratio and median ages at baseline and follow-up did not differ significantly among the *HFE* genotype groups (Table 2).

Of 142 C282Y homozygotes (66 of whom were men) who participated in follow-up assessments, 12 (8.5%) had received the diagnosis of hemochromatosis as part of usual care before the baseline assessment for the Melbourne Collaborative Cohort Study, 52 (36.6%) received the diagnosis after baseline but before the clinical assessments for the HealthIron study, and 78 (54.9%) were informed after undergoing the HealthIron follow-up assessments. C282Y homozygotes with a baseline or follow-up serum ferritin level of 1000 μg per liter or more were more likely to have received the diagnosis of hereditary hemochromatosis during routine care and before follow-up than were those with a serum ferritin level of less than 1000 μg per liter (23 of 37 subjects [62.2%] vs. 41 of 105 subjects [39.0%], P=0.02). Among the 64 subjects in whom hereditary hemochromatosis was diagnosed before follow-up, the diagnosis was the result of an assessment of symptoms for 18 subjects (28.1%), follow-up of family members for 20 subjects (31.2%), and routine blood tests for 13 subjects (20.3%); the reason was unknown for 13 subjects (20.3%).

SERUM FERRITIN AND TRANSFERRIN SATURATION

Of the 1438 subjects who participated in the HealthIron study, 850 (59.1%) were fasting when the baseline blood sample was obtained, and 941 of 1054 (89.3%) were fasting when the follow-up sample was obtained. At baseline, among C282Y homozygotes, the level of serum ferritin was more than 300 μg per liter in 45 of 55 men (81.8%) and more than 200 μg per liter (>300 μg per liter for postmenopausal subjects) in 36 of 65 women (55.4%). In the same group, transferrin saturation was more than 55% in 40 of 55 men (72.7%) and more than 45% in 45 of 65 women (69.2%). At least one serum ferritin value of 1000 μg per liter or more was recorded in 33 of 74 men (44.6%) and in 7 of 84 women (8.3%) (Table 2).

For men, the mean serum ferritin level decreased during the 12-year period from baseline to follow-up in all *HFE* genotype groups (Table 2), although there were large changes for only two subgroups of subjects: C282Y homozygotes, who had a mean reduction from 1195 μg per liter to 593 μg per liter, including 30% of subjects who had undergone therapeutic venesection, and compound heterozygotes, who had a mean reduction from

351 μg per liter to 264 μg per liter. In contrast, the mean serum ferritin level increased in women for all genotype groups during the study period (Table 2), except for C282Y homozygotes, of whom 20% had undergone therapeutic venesection. Male C282Y homozygotes (including those who underwent venesection) were the only group in which the mean transferrin saturation changed significantly between baseline and follow-up (a decrease from 73±3% to 63±3%) (P=0.03), although only 64% of male C282Y homozygotes were fasting at the time of the baseline sampling, as compared with 100% at follow-up.

Clinical Features According to HFE Genotype

Male C282Y homozygotes with serum ferritin level of 1000 μg per liter or more at baseline or follow-up had a higher prevalence of reported fatigue, liver disease, and use of arthritis medication than did subjects with the wild-type gene and C282Y heterozygotes combined. In addition, a higher proportion of male C282Y homozygotes had increased levels of alanine aminotransferase or aspartate aminotransferase in the absence of excess alcohol intake (Table 3).

The presence of abnormal second and third metacarpophalangeal joints was more common in male C282Y homozygotes than in control subjects, regardless of serum ferritin levels. In contrast, the use of arthritis medication and elevated levels of alanine aminotransferase or aspartate aminotransferase were the only features associated with hereditary hemochromatosis that were more commonly reported for female C282Y homozygotes with a serum ferritin level of more than 1000 μg per liter than for C282Y heterozygotes or those with the wild-type gene.

IRON OVERLOAD AND RELATED DISEASE

For C282Y homozygotes, 21 of 74 men (28.4%; 95% CI, 18.8 to 40.2) and 1 of 84 women (1.2%; 95% CI, 0.03 to 6.5) satisfied the criteria for documented disease related to iron overload (Tables 1 and 4). Of the 22 C282Y homozygotes with documented iron overload–related disease, 2 had hepatocellular carcinoma, 12 had fibrosis or cirrhosis, 6 had raised levels of alanine aminotransferase or aspartate aminotransferase, 5 had abnormal metacarpophalangeal joints, and 11 had received a previous diagnosis of hereditary hemochromatosis as a result of symptoms that prompted an evaluation.

For 40 C282Y homozygotes, liver biopsies were clinically indicated because of a serum ferritin level of 1000 μg per liter or more.[4,5] Of these subjects, 17 (including 16 men) underwent liver biopsy (42.5%); all subjects had an iron grade of 2 or more, and 12 had liver cirrhosis or fibrosis.

Four of the liver biopsies were performed before baseline, with grade 1 fibrosis observed in a 22-year-old subject, grade 0 in a 42-year-old subject, grade 3 in a 64-year-old subject, and grade 0 in a 67-year-old subject. Seven liver biopsies were performed after baseline and before follow-up, with grade 2 fibrosis observed in both a 57-year-old subject and a 58-year-old subject with self-reported alcoholic liver disease, grade 1 in a 58-year-old subject, and grade 0 in a 59-year-old subject; cirrhosis in a 62-year-old subject and a 71-year-old subject; and grade 3 fibrosis in a 76-year-old subject who consumed more than 100 g of alcohol per day. Six biopsies were performed in subjects who were referred to a hepatologist after they had undergone follow-up assessments at the HealthIron study clinic, with grade 0 fibrosis observed in a 54-year-old subject and a 55-year-old subject and grade 1 in three 56-year-old subjects and one 59-year-old subject.

No subjects with biopsy results reported having had viral hepatitis, and only one (as noted above) reported having a high baseline level of alcohol consumption (>40 g per day for women and >60 g per day for men). Only one subject who was not a C282Y homozygote (a compound heterozygote) had documented iron-overload–related disease.

PROVISIONAL IRON OVERLOAD AND RELATED DISEASE

Provisional iron overload (defined in Table 1) occurred in six compound heterozygotes, in two C282Y heterozygotes, in one H63D heterozygote, and in two subjects with neither mutation. Only one of the two subjects with neither mutation had evidence of iron-overload–related disease. In comparison, 83 C282Y homozygotes (both men and women) had provisional iron overload; of these subjects, 24% had objective evidence of iron-overload–related disease (Table 4).

DISCUSSION

In a prospective cohort study with more than 12 years of follow-up, for C282Y homozygotes, documented iron-overload–related disease developed in 28.4% of men but only 1.2% of women. C282Y homozygotes with a serum ferritin level of 1000 μg

Table 2. Demographic and Clinical Characteristics of 1290 Subjects, According to *HFE* Genotype.*

Variable	C282Y Homozygote	Compound Heterozygote	C282Y Heterozygote	H63D Heterozygote or Homozygote	No C282Y or H63D Mutation	P Value†
Subjects in HealthIron study — no.‡	203	242	337	147	361	
Median age at follow-up — yr	65.2	64.6	63.2	66.6	66.0	0.33
Male sex — no. (%)	95 (46.8)	117 (48.3)	161 (47.8)	61 (41.5)	164 (45.4)	0.74
Serum ferritin§						
At baseline						
No. of men	55	69	100	42	130	
Value — μg/liter	1195±182	351±41	250±21	266±27	216±15	<0.001
No. of women	65	72	112	71	158	
Value — μg/liter	374±52	124±13	93±9	128±16	90±7	<0.001
At follow-up						
No. of men	61	78	107	52	139	
Value — μg/liter	593±90	264±22	229±16	193±28	199±16	0.001
No. of women	73	90	125	76	169	
Value — μg/liter	355±38	159±11	136±11	139±14	118±9	<0.001
Transferrin saturation						
At baseline						
No. of men	55	69	100	43	132	
Value — %	73±3	42±1	35±1	34±2	30±1	<0.001
No. of women	65	72	113	72	158	
Value — %	53±3	37±2	30±1	28±1	25±1	<0.001
At follow-up						
No. of men	61	78	107	52	140	
Value — %	63±3	40±1	33±1	32±2	29±1	<0.001
No. of women	73	91	127	76	169	
Value — %	54±2	39±1	31±1	27±1	25±1	<0.001
Any serum ferritin ≥1000 μg per liter — no./total no. (%)¶						
Men	33/74 (44.6)	2/90 (2.2)	2/129 (1.6)	1/54 (1.9)	2/150 (1.3)	<0.001
Women	7/84 (8.3)	0/98	0/144	0/84	0/181	<0.001
Blood donation						
Any donation — no./total no. (%)						
Men	35/66 (53.0)	43/84 (51.2)	67/117 (57.3)	28/52 (53.8)	78/142 (54.9)	0.92
Women	36/77 (46.8)	46/95 (48.4)	66/139 (47.5)	41/79 (51.9)	85/176 (48.3)	0.97
Average lifetime donations (excluding therapeutic venesection)‖						
No. of men	32	40	61	27	74	
No. of donations	39.6±5.9	50.6±6.3	40.6±7.0	25.2±5.1	47.2±5.2	0.42
No. of women	35	39	56	33	76	
No. of donations	28.2±6.1	27.3±4.2	26.7±3.8	27.9±4.6	27.2±3.2	0.95

Table 2. (Continued.)

Variable	C282Y Homozygote	Compound Heterozygote	C282Y Heterozygote	H63D Heterozygote or Homozygote	No C282Y or H63D Mutation	P Value†
Alcohol consumption						
Nondrinkers at baseline — no./total no. (%)						
Men	7/67 (10.4)	15/84 (17.9)	17/118 (14.4)	14/55 (25.5)	21/149 (14.1)	0.19
Women	19/79 (24.1)	27/96 (28.1)	38/139 (27.3)	34/84 (40.5)	73/181 (40.3)	0.01
Consumption at baseline						
No. of men	60	69	101	41	128	
Grams per day	17±2	23±2	21±2	21±2	23±2	0.39
No. of women	60	69	101	50	108	
Grams per day	11±2	11±1	15±1	13±2	12±1	0.26
Obesity (BMI >30) at baseline — no./total no. (%)**						
Men	4/95 (4.2)	23/117 (19.7)	19/161 (11.8)	11/61 (18.0)	28/164 (17.1)	0.07
Women	14/108 (13.0)	20/125 (16.0)	22/176 (12.5)	9/86 (10.5)	36/197 (18.3)	0.40

* Plus–minus values are means ±SD.
† P values are for comparisons of genotype groups by analysis of variance for means or chi-square tests for proportions.
‡ An additional 148 subjects were selected to participate in the study but did not undergo full genotyping for *HFE*: 132 had no copies of C282Y but did not undergo genotyping for H63D, and 16 did not undergo genotyping for either locus.
§ At baseline, 4 male and 1 female C282Y homozygotes received the diagnosis of hereditary hemochromatosis and started therapeutic venesection, as did 25 male and 17 female C282Y homozygotes during the study period.
¶ Among subjects with a serum ferritin level of 1000 μg per liter or more, all the C282Y homozygotes, one male compound heterozygote, and one male subject with neither mutation also had an elevated transferrin saturation.
‖ In Australia, one blood donation usually consists of 450 ml (i.e., 1 unit) of blood.
** The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters.

per liter or more were at higher risk for symptoms and disease associated with the *HFE* gene than were either C282Y homozygotes with a serum ferritin level of 1000 μg per liter or less or subjects with other *HFE* genotypes. This finding confirms and extends the observation of Guyader and colleagues[5] that a serum ferritin level of 1000 μg per liter or more is associated not only with cirrhosis but also with symptomatic hereditary hemochromatosis in C282Y homozygotes. Arthropathy, as defined by clinically abnormal metacarpophalangeal joints, was unrelated to serum ferritin levels in homozygotes, a finding that confirmed results reported previously.[17]

Investigators who conducted clinical examinations were not aware of the genotype of the study subjects. Our estimate of disease penetrance (the probability of expression of a genotype) is conservative, since we used strictly objective criteria on the basis of liver biopsy, liver-enzyme levels, clinical examination, or physicians' diagnosis in the context of documented iron overload. Subjective criteria such as a history of fatigue or liver disease were excluded from our assessment of disease penetrance. Detailed data on lifestyle factors were collected as part of the study. Because male C282Y homozygotes were not significantly more likely to be obese and did not have greater alcohol intake than male subjects who were not C282Y homozygotes, these factors are unlikely to explain the increased prevalence of elevated levels of alanine aminotransferase or aspartate aminotransferase among male C282Y homozygotes.

The high prevalence of C282Y homozygosity in this study may be attributable to a high rate of British immigration in this age group in Melbourne.[20] This high prevalence of C282Y homozygosity makes it unlikely that patients who had received the diagnosis of hereditary hemochromatosis previously were underrepresented in our initial recruitment, and linkage to the National Death Index minimized selective mortality bias. Thirty-

Table 3. Prevalence of Clinical Features Associated with Hereditary Hemochromatosis, According to *HFE* Genotype.*

Variable	C282Y Homozygote		Compound Heterozygote	H63D Homozygote or Heterozygote	C282Y Heterozygote or Wild Type	C282Y Homozygote with Serum Ferritin >1000 μg per Liter versus Wild-Type or C282Y Heterozygote	
	Serum Ferritin ≥1000 μg/liter	Serum Ferritin <1000 μg/liter				Prevalence Ratio (95% CI)	P Value
No. of subjects							
Men	33	62	117	60	325		
Women	7	101	125	86	373		
			no./total no. (%)				
Fatigue†							
Men	7/30 (23.3)	5/35 (14.3)	9/84 (10.7)	8/52 (15.4)	24/259 (9.3)	2.52 (1.19–5.34)	0.02
Women	1/7 (14.3)	14/69 (20.3)	15/94 (16.0)	14/80 (17.5)	58/315 (18.4)	0.78 (0.12–4.83)	0.78
Abnormal metacarpophalangeal joints‡							
Men	6/25 (24.0)	7/24 (29.2)	13/64 (20.3)	8/45 (17.8)	29/210 (13.8)	1.74 (0.80–3.77)	0.18§
Women	1/ 4 (25.0)	6/56 (10.7)	16/80 (20.0)	9/63 (14.3)	24/247 (9.7)	2.57 (0.45–14.65)	0.31
Use of arthritis medicine at baseline¶							
Men	3/33 (9.1)	7/63 (11.1)	2/117 (1.7)	2/60 (3.3)	7/325 (2.2)	4.22 (1.15–15.55)	0.02
Women	5/7 (71.4)	9/101 (8.9)	12/125 (9.6)	9/86 (10.5)	25/373 (6.7)	10.66 (5.83–19.47)	<0.001
AST >45 IU/liter or ALT >40 IU/liter‖							
Men	6/27 (22.2)	4/33 (12.1)	6/74 (8.1)	5/50 (10.0)	21/233 (9.0)	2.47 (1.09–5.57)	0.03
Women	2/5 (40.0)	3/66 (4.5)	1/89 (1.1)	0/74	12/287 (4.2)	9.57 (2.86–32.02)	<0.001
Hepatomegaly**							
Men	0/23	1/23 (4.3)	5/62 (8.1)	0/45	8/204 (3.9)	0 (0–0.05)	0.33
Women	0/4	1/56 (1.8)	1/77 (1.3)	2/61 (3.3)	4/233 (1.7)	0 (0–0.09)	0.79
History of liver disease†							
Men	5/30 (16.7)	1/35 (2.9)	6/84 (7.1)	4/52 (7.7)	7/258 (2.7)	6.14 (2.08–18.15)	<0.001
Women	0/7	5/68 (7.4)	4/93 (4.3)	5/79 (6.3)	23/315 (7.3)	0 (0–0.01)	0.46
Diabetes††							
Men	1/33 (3.0)	2/63 (3.2)	4/117 (3.4)	3/60 (5.0)	9/325 (2.8)	1.09 (0.14–8.37)	0.93
Women	0/7	0/101	2/125 (1.6)	4/86 (4.7)	4/373 (1.1)	0 (0–0.07)	0.78

* Among C282Y homozygotes with a serum ferritin level of 1000 μg per liter or more at baseline or follow-up, only two men and one woman had three or more features; among those who did not have a serum ferritin level of 1000 μg per liter or more, one woman had three or more features. ALT denotes alanine aminotransferase, AST aspartate aminotransferase, and CI confidence interval.

† Data are for subjects who reported having this condition at follow-up.

‡ This condition was defined as a bony spur or tenderness or effusion on both the second and third metacarpophalangeal joints on either hand, as determined by a physician who was unaware of genotype at follow-up.

§ For the comparison of combined C282Y homozygotes with combined C282Y heterozygotes and subjects with a wild-type gene, the prevalence ratio was 1.92 (95% CI, 1.08 to 3.42; P=0.03).

¶ At baseline, the use of arthritis drugs was established by the subject's response to the question "Has the doctor ever told you that you have had arthritis or rheumatism?" followed by "If you have arthritis or rheumatism, do you take aspirin?"

‖ Excluded from this category were subjects with a daily alcohol intake of more than 60 g for men or more than 40 g for women.

** Liver enlargement was defined as a liver span of 13 cm or more as assessed during blinded clinical examination at follow-up.

†† Data are for subjects who reported that they had physician-diagnosed diabetes at baseline.

Table 4. Categorization of Iron Overload and Iron-Overload–Related Disease among C282Y Homozygotes.*

Variable	Disease Associated with Hereditary Hemochromatosis	No Disease Associated with Hereditary Hemochromatosis	Total
	no. (%)		
Documented iron overload			
Men	21 (28.4)	6 (8.1)	27
Women	1 (1.2)	2 (2.4)	3
Provisional iron overload			
Men	10 (13.5)	22 (29.7)	32
Women	10 (11.9)	41 (48.8)	51
No evidence of iron overload			
Men	2 (2.7)	13 (17.6)	15
Women	6 (7.1)	24 (28.6)	30
Total			
Men	33	41	74
Women	17	67	84

* Complete definitions of iron-overload and iron-overload–related disease are listed in Table 1. Percentages are for 158 C282Y homozygotes (74 men and 84 women) for whom at least one serum ferritin measurement was available. Of those subjects, 142 underwent follow-up assessments, 14 died during the study, and 2 did not undergo follow-up assessments for other reasons.

seven percent of C282Y homozygotes were identified during follow-up, which may have modified the natural history of disease progression for these subjects and resulted in an underestimation of disease penetrance. Our study did not address the association of *HFE* mutations with less commonly reported features of hereditary hemochromatosis, such as impotence and cardiomyopathy.

Two other longitudinal studies have attempted to characterize the penetrance of disease related to hereditary hemochromatosis with the use of population-based data.[12,21] Andersen and colleagues[12] retrospectively assessed 23 C282Y homozygotes (including 16 women) during a 25-year period and found no evidence of liver disease associated with hereditary hemochromatosis. However, the study was potentially compromised by selective mortality bias, owing to the high rate of attrition in the cohort (53%), the fact that 35% of the members of the original cohort were not genotyped for *HFE* mutations, and the fact that 3 of the 23 C282Y homozygotes died before they could be examined. Olynyk and colleagues,[21] who retrospectively assessed 10 C282Y homozygous patients (including 6 women) during a 17-year period, reported that of the 6 patients who underwent liver biopsy, 3 had cirrhosis or fibrosis. However, not all the patients in that study were at an age when symptoms of disease would have been expected. In a systematic review, Whitlock and colleagues[14] estimated, after accounting for patients who were lost to follow-up, that disease would eventually develop in 25 to 60% of C282Y homozygotes.

On the basis of a cross-sectional population study of subjects between the ages of 20 and 80 years (an age range that was wider than that of our study and that included a greater proportion of subjects under the age of 50 years), Beutler and colleagues[10] suggested that disease attributable to hereditary hemochromatosis occurred in less than 1% of all C282Y homozygotes, regardless of sex. However, Beutler et al. did not perform clinical examinations or liver biopsies, and a quarter of the C282Y homozygotes were excluded on the basis that they had received a previous diagnosis of hereditary hemochromatosis. This exclusion would be expected to reduce the estimate of clinical penetrance of C282Y homozygosity.[22,23] Contrary to our results, Beutler et al. found no association between C282Y homozygosity and the presence of fatigue or arthritis, although they reported an association with a history of liver disease. The exclusion of subjects in whom hereditary hemochromatosis had already been diagnosed might account

for this finding, since our study showed that subjects with a serum ferritin level of 1000 μg per liter or more were more likely to present with symptoms than were those with a level of less than 1000 μg per liter.

The increased prevalence of iron-overload–related disease in C282Y homozygous men, as compared with that in women, is frequently ascribed to recurrent physiologic blood loss and the resultant slower accumulation of iron in women. However, disparate frequencies of HLA A*03B*07 haplotypes in men and women have been reported in hereditary hemochromatosis probands,[24] which may be relevant to sex-specific phenotypic expression of this disease. Studies of iron regulatory pathways in African Americans have also suggested that serum ferritin levels may be genetically determined by sex differences as well as environmental factors.[25,26]

In conclusion, disease related to iron overload commonly develops in men (but not in women) who are homozygous for the C282Y mutation, especially when serum ferritin levels are 1000 μg per liter or more.

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