HFE C282Y/H63D Compound Heterozygotes Are at Low Risk of Hemochromatosis-Related Morbidity

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The risk of hemochromatosis-related morbidity is unknown among *HFE* compound heterozygotes (C282Y/H63D). We used a prospective population-based cohort study to estimate the prevalence of elevated iron indices and hemochromatosis-related morbidity for compound heterozygotes. In all, 31,192 subjects of northern European descent were genotyped for *HFE* C282Y and H63D. An *HFE*-genotype stratified random sample of 1,438 subjects, followed for an average of 12 years to a mean age of 65 years, completed questionnaires and gave blood. Clinical examinations were blinded to *HFE* genotype. A total of 180 (84 males) clinically examined C282Y/H63D participants were compared with 330 (149 males) controls with neither *HFE* mutation; 132 (65 males) and 270 (122 males), respectively, had serum iron measures at both timepoints. Mean serum ferritin (SF) and transferrin saturation (TS) were significantly greater for male and female compound heterozygotes than for wild-types at baseline and follow-up (all $P < 0.02$) except for females who were premenopausal at baseline, where SF was similar in both genotype groups. For subjects with serum measures from both baseline and follow-up, mean SF and TS levels did not change significantly for men or for postmenopausal women, but for premenopausal women SF levels increased from 43 to 109 μg/L for compound heterozygotes and from 35 to 64 μg/L for wild-types (both $P < 0.001$). Male and female compound heterozygotes had a similar prevalence of hemochromatosis-related morbidity to wild-types. One of 82 males and zero of 95 females had documented iron overload-related disease. *Conclusion:* For male compound heterozygotes, mean iron indices do not change during middle age but for female compound heterozygotes menopause results in increased mean SF. Although compound heterozygotes might maintain elevated iron indices during middle age, documented iron overload-related disease is rare. (HEPATOLOGY 2009;50:94-101.)

Hereditary hemochromatosis (HH) is a condition characterized by iron overload and which is both treatable and preventable. Iron overload increases the risk of disease such as liver cirrhosis, arthritis, fatigue, and diabetes.[1] Mutations in the *HFE* gene are responsible for the majority of clinical cases of iron overload.[2] Two *HFE* genotypes have been commonly described in cases of iron overload, C282Y homozygosity and C282Y/H63D compound

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HH, hereditary hemochromatosis; MCCS, Melbourne Collaborative Cohort Study; MCP, metacarpophalangeal; SF, serum ferritin; TS, transferrin saturation.

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heterozygosity.[2] Estimates of the prevalence of compound heterozygotes in populations of people of northern European descent have ranged from 1.7% to 4.1%, with the average 2% prevalence being four times that of C282Y homozygotes, which has a prevalence of one in 200.[3-8]

Little is known about the population risk of *HFE* compound heterozygotes developing HH-associated clinical signs and symptoms or iron overload-related disease. Evidence from large cross-sectional population studies has established that compound heterozygotes have a higher mean serum ferritin (SF) than other *HFE* genotypes, with the exception of C282Y homozygotes.[3,4,7,8]

Although most cases of clinical iron overload and iron overload-related disease are C282Y homozygotes, a small proportion of cases are compound heterozygotes.[1,4,9-13] It has been assumed from these case series that compound heterozygotes have a lower risk of progression to disease.

Walsh et al.[14] found that compound heterozygotes referred for clinical assessment had higher iron indices than those identified through family screening and that this group developed disease only in the presence of comorbid factors such as significant alcohol intake or obesity. To date there have been no prospective longitudinal data from a population cohort on the risk of disease for compound heterozygotes.

Our study examines *HFE* compound heterozygotes and wild-type (those with neither the C282Y nor the H63D mutation) individuals who were followed over a 12-year period and at ages (from 40 to 69 years at baseline to 54 to 83 years at follow-up) when those at risk of iron overload would have been expected to develop iron overload-related disease. We describe the natural history of serum iron indices and iron overload-related disease signs and symptoms using this large population-based sample of well-characterized subjects.

Patients and Methods

Melbourne Collaborative Cohort Study (MCCS). Between 1990 and 1994, the Melbourne Collaborative Cohort Study (MCCS) recruited 41,514 people (24,469 females) aged between 27 and 75 years (99% were aged 40 to 69 years) through the Australian Electoral Roll, advertisements, and community announcements in local media. The aim of the project was to prospectively investigate the role of diet and other lifestyle factors in causing common chronic diseases and to investigate possible associations between these exposures and common genetic variants.[15] At baseline, participants attended a study center where they were interviewed and completed a questionnaire about dietary and lifestyle factors, underwent a physical examination, and provided a sample of blood.

The HealthIron Study. Beginning in 2004, 31,192 MCCS participants of northern European descent (born in Australia, the United Kingdom, Ireland, or New Zealand) were genotyped for the C282Y *HFE* mutation using stored baseline blood samples. Participants of southern European descent (n = 10,336) were excluded due to the low prevalence of *HFE* mutations. Those with one copy of the C282Y mutation were then genotyped for H63D to determine whether they were simple (one copy of the C282Y mutation) or compound heterozygotes (one copy of each of the C282Y and H63D mutations).

All participants homozygous for the C282Y mutation (n = 203) plus a random sample stratified by *HFE* genotype including 242 compound heterozygotes and 361 participants who were wild-type for both *HFE* mutations were selected for invitation to attend follow-up clinics between 2004 and 2006 as part of the HealthIron study. Of the 1,438 people invited to participate in the HealthIron study, 107 were deceased and 279 were lost to follow-up, leaving 1,052 who participated. The overall participation by those invited was 73.2% (79.0% excluding those already deceased) with no significant variation in participation when stratified by genotype (data not shown).

At baseline, participants had a fasting blood sample taken and completed questionnaires that included information about diet, alcohol intake, and medical history. Follow-up clinics were held between 2004 and 2006. As part of the study, participants completed a computer-assisted personal interview that included information about medical history, blood donation, had a fasting

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This article is dedicated to the memory of Ernest Beutler, who did much in recent years to stimulate interest in the natural history of hemochromatosis.

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blood sample taken for iron studies and liver enzymes, were examined by a medical practitioner blinded to genotype, and had a cheekbrush swab taken to confirm the original *HFE* genotype from their baseline blood sample.

All participants gave written informed consent to participate in both the MCCS and the HealthIron substudy. Both protocols were approved by the Cancer Council Victoria's Human Research Ethics Committee.

Analysis. Elevated SF was defined for males and postmenopausal females as >300 μg/L and for premenopausal females >200 μg/L. Elevated transferrin saturation (TS) was defined for males as >55% and for females >45%. When examining the prevalence of disease by elevated iron indices, elevated SF was defined as elevated SF (based on the thresholds above) on at least one occasion. We defined normal SF as having values below these thresholds at both baseline and follow-up.

We investigated the prevalence of six disease features associated with HH: abnormal (i.e., presence of bony spur, effusion, or tenderness) second and third metacarpophalangeal (MCP) joints on either hand (MCP 2/3), use of arthritis medication, self-reported fatigue, raised aspartate aminotransferase or raised alanine aminotransferase levels (raised AST/ALT), self-reported history of liver disease, and hepatomegaly. With the exception of the use of arthritis medication, all disease features were measured at follow-up. Some subjects did not participate in all components, and as a result did not contribute data to the prevalence calculation for every disease feature.

Iron overload-related disease was defined as per Allen et al.[3] with one of the following five features: hepatocellular carcinoma, liver cirrhosis or fibrosis, abnormal 2/3 MCP, raised aminotransferases, or physician-diagnosed HH due to symptoms in the context of either provisional or documented iron overload (see footnotes to Table 6 for definition).

Menopausal status for women was measured at baseline and classified as premenopausal or postmenopausal. Blood donation history was classified at baseline as never, former (ceased before baseline), or current (still donating at baseline).

Participants who were diagnosed and treated for HH and those with any SF >1,000 μg/L were included in the analysis. Their inclusion avoids a downward bias of the estimated prevalence of disease features at follow-up due to the exclusion of cases with clinical symptoms.

We examined the influence of comorbid factors on liver enzymes by conducting separate analyses, excluding participants with a body mass index (BMI) greater than 30 kg/m^2 or alcohol intake greater than 60 g/d for men and greater than 40 g/d for women when calculating the prevalence of raised AST/ALT. Increased BMI and alcohol intake are common causes of raised iron indices and abnormal serum transaminase levels.

Table 1. Participant Information at Baseline: Mean Age, BMI, and Alcohol Intake Stratified by Genotype and Sex

	n	Age (years) Mean (SD)	BMI (kg/m^2) Mean (SD)	Alcohol (g/day) Mean (SD)
Compound heterozygote				
Male	84	54 (9)	27 (4)	19 (20)
Female	96	54 (9)	25 (4)	8 (12)
HFE wild-type				
Male	149	54 (9)	27 (4)	19 (23)
Female	181	54 (9)	26 (5)	8 (13)

Statistical Analysis. Prevalences of elevated iron indices and disease features were estimated as the observed proportions at a single timepoint and presented with 95% confidence intervals (CIs) calculated using the binomial distribution. For all analyses SF levels were (natural) log transformed. Comparisons of mean log SF and TS measurements between groups at either baseline or follow-up were made using the two-sample *t*-test and comparisons within groups comparing baseline and follow-up were made using the paired *t*-test. Two-sided *P*-values are presented.

Results

In all, 31,192 participants of northern European descent were recruited to the MCCS. *HFE* genotyping was successful for 29,676 (95%), of whom 719 (2.4%) were heterozygous for both the C282Y and H63D mutation. Of these compound heterozygotes, 242 were selected for invitation to the HealthIron study, of which 180 (84 men; 96 women) attended the follow-up clinic (75% response). A total of 621 participants without the C282Y mutation were selected for invitation to the HealthIron study, of whom 459 attended the follow-up clinic (74% response). Of those attending, 330 (149 men and 181 women) did not have the H63D mutation (so were *HFE* wild-type) and form the control group in this study. More than half the women were postmenopausal at baseline: 52 (54%) compound heterozygotes and 107 (59%) *HFE* wild-types.

For both men and women the mean age, BMI, and daily alcohol intake were similar for the two genotype groups (Table 1). The only exception was mean BMI for females, which was lower by 1.3 kg/m^2 for compound heterozygotes ($P = 0.03$).

Iron Indices. Male and postmenopausal female compound heterozygotes had higher mean SF and TS at baseline and follow-up (Table 2a) compared with *HFE* wild-types. For women premenopausal at baseline, there was

Table 2a. Mean Iron Indices Stratified by *HFE* Genotype, Sex, and, for Women, Menopause Status

	Baseline Iron Indices		
	n	Baseline SF (μg/L)* Geometric Mean (95% CI)	Baseline TS (%)† Mean (95% CI)
Men			
Compound heterozygote	69	219.7 (169.9–284.1)	41.5 (38.7–44.3)
HFE wild-type	130	152.1 (129.3–179.0)	29.8 (28.1–31.5)
P		0.01	<0.001
Premenopausal women			
Compound heterozygote	33	44.3 (30.6–64.2)	34.8 (28.7–40.9)
HFE wild-type	67	35.2 (28.0–44.3)	22.4 (20.2–24.6)
P		0.27	<0.001
Postmenopausal women			
Compound heterozygote	39	134.8 (140.8–173.5)	39.7 (36.5–43.0)
HFE wild-type	91	83.1 (68.2–101.3)	26.4 (24.8–27.9)
P		0.01	<0.001
	Follow-up iron indices		
	n	Follow-up SF (μg/L)‡,§ Geometric Mean (95% CI)	Follow-up TS (%) | Mean (95% CI)
Men			
Compound heterozygote	78	186.5 (148.9–233.6)	40.1 (37.1–43.0)
HFE wild-type	140	134.2 (113.2–158.4)	29.1 (27.5–30.8)
P		0.02	<0.001
Women‖			
Compound heterozygote	91	120.4 (100.6–144.0)	38.9 (36.5–41.3)
HFE wild-type	169	80.1 (69.3–92.5)	24.9 (23.6–26.3)
P		<0.001	<0.001

*27 (9 compound heterozygotes [5 males, 3 premenopausal females and 2 postmenopausal females] and 18 *HFE* wild-types [8 males, 3 premenopausal females and 7 postmenopausal females]) had SF measures available at baseline but not follow-up.

†2 male *HFE* wild-types had a baseline TS measure but no baseline SF measure.

‡44 (23 compound heterozygotes [13 males, 10 premenopausal and 13 postmenopausal females] and 38 HFE wild-types [17 males, 7 premenopausal and 14 postmenopausal females]) had SF measures available at follow-up but not baseline.

§Those who were therapeutically venesected (3 male compound heterozygotes) were included.

| 1 female compound heterozygote and 1 male *HFE* wild-type had a follow-up TS measure but no follow-up SF measure.

‖All women had become postmenopausal by follow-up.

little difference in the mean baseline SF for the compound heterozygote and *HFE* wild-type groups.

There was little change in mean SF or TS between baseline and follow-up within genotype groups except for women premenopausal at baseline. In this subgroup, the geometric mean SF increased from 42.5 μg/L at baseline to 109.3 μg/L at follow-up ($P < 0.001$) for compound heterozygotes and from 35.0 μg/L to 64.4 μg/L ($P < 0.001$) for *HFE* wild-types (Table 2b). For men, 41/65 (63%) of compound heterozygotes and 72/122 (59%) of *HFE* wild-type men had lower SF at follow-up than baseline. For women postmenopausal at baseline 17/37 (46%) compound heterozygotes and 43/84 (51%) *HFE* wild-types had lower SF at follow-up than baseline. For women premenopausal at baseline 28/30 (93%) compound heterozygotes and 46/64 (72%) *HFE* wild-types had higher SF at follow-up than baseline. Compound

Table 2b. Mean Iron Indices Stratified by *HFE* Genotype, Sex, and, for Women, Menopause Status, in Participants with Both Baseline and Follow-up Iron Measures

	n	Baseline SF (μg/L) Geometric Mean (95% CI)	Follow-up SF (μg/L) geometric mean (95% CI)	P
Men				
Compound heterozygote	65	215.8 (166.4–279.8)	177.9 (137.8–229.9)	0.13
HFE wild-type	122	149.8 (126.8–176.9)	134.7 (112.2–161.8)	0.20
Premenopausal women*				
Compound heterozygote	30	42.5 (28.5–63.5)	109.3 (78.2–152.8)	<0.001
HFE wild-type	64	35.0 (27.8–44.2)	64.4 (49.5–83.9)	<0.001
Postmenopausal women†				
Compound heterozygote	37	134.5 (103.1–175.5)	126.2 (95.1–167.6)	0.62
HFE wild-type	84	83.2 (67.6–102.4)	85.4 (70.8–103.0)	0.79
	n	Baseline TS (%) mean (95% CI)	Follow-up TS (%) mean (95% CI)	p
Men				
Compound heterozygote	65	41.7 (38.9–44.5)	38.8 (35.6–42.0)	0.11
HFE wild-type	125	29.7 (27.9–31.45)	29.3 (27.5–31.1)	0.67
Premenopausal women*				
Compound heterozygote	30	34.2 (27.7–40.7)	37.6 (33.0–42.2)	0.27
HFE wild-type	64	22.4 (20.1–24.7)	23.3 (21.1–25.6)	0.50
Postmenopausal women†				
Compound heterozygote	38	39.9 (36.7–43.2)	38.7 (34.9–42.6)	0.53
HFE wild-type	84	26.8 (25.2–28.4)	25.1 (23.3–26.9)	0.12

*Women classified as premenopausal at baseline.

†Women classified as postmenopausal at baseline.

Table 2c. Prevalence of Elevated Baseline and Follow-up Iron Indices Stratified by *HFE* Genotype and Sex

	Elevated Baseline SF	Elevated Follow-up SF
Men		
Compound heterozygote	29/69 (42%)	29/78 (37%)
HFE wild-type	33/130 (25%)	29/139 (21%)
P	0.02	0.09
Women		
Compound heterozygote	7/72 (10%)	10/90 (11%)
HFE wild-type	4/158 (3%)	8/169 (5%)
P	0.02	0.02
	Elevated Baseline TS	**Elevated Follow-up TS**
Men		
Compound heterozygote	7/69 (10%)	6/78 (8%)
HFE wild-type	3/132 (2%)	2/140 (1%)
P	0.02	0.05
Women		
Compound heterozygote	22/72 (31%)	20/91 (22%)
HFE wild-type	1/158 (1%)	4/169 (2%)
P	<0.001	<0.001

SF for females is menopausal specific (premenopausal SF>200 μg/L, postmenopausal SF>300 μg/L).

heterozygotes of both sexes also had a higher prevalence of elevated iron measures compared with *HFE* wild-types at both timepoints (Table 2c). Seven male and six female compound heterozygotes and two male *HFE* wild-types had both elevated SF and TS values at either baseline or follow-up.

Approximately half of all men (41/84 [48%] compound heterozygotes and 78/149 (52%) *HFE* wild-types) had ever donated blood. Just over half of the premenopausal women (25/44 [57%] compound heterozygotes and 43/74 [58%] *HFE* wild-types) and about one third of the postmenopausal women (20/52 [38%] compound heterozygotes and 40/107 [37%] *HFE* wild-types) had ever donated blood (Table 3).

Fifteen (six men and nine women) compound heterozygotes and no *HFE* wild-types self-reported ever being told by a doctor that they had "too much iron in [their] body, iron overload or hemochromatosis." Diagnosis prior to follow-up was due to symptomatic HH for two men, follow-up of HH-affected family members for one premenopausal woman, genetic test for two postmenopausal women, routine blood tests for three men, three premenopausal, and two postmenopausal women, and the reason was unknown for one man and one premenopausal woman. Three of these compound heterozygotes had been treated with therapeutic venesection during the study period. Two male compound heterozygotes and no *HFE* wild-types had baseline SF >1,000 μg/L. Both had a BMI greater than 25 kg/m^2 and reported alcohol intake greater than 40 g/day.

Prevalence of Disease Features. The estimated prevalence of the six disease features for each sex and *HFE* genotype group are given in Table 4. After stratifying by sex the prevalence of disease features was similar for compound heterozygotes and *HFE* wild-types, although there was weak evidence of a difference between the two genotype groups in the prevalence of abnormal metacarpophalangeal joints in women (20% for compound heterozygotes compared with 11% for *HFE* wild-types, $P = 0.07$). Exclusion of individuals who were obese (BMI >30 kg/m^2) or who had high alcohol intake (>60 g/d for men or >40 g/d for women) had no effect on the comparison of the prevalence of abnormal liver enzymes between compound heterozygotes and *HFE* wild-types for either sex (5/60 [8%] compared with 15/105 [14%] [$P = 0.26$] for men and 1/78 [1%] compared with 2/136 [2%] [$P = 0.91$] for women).

The prevalence of disease for compound heterozygotes by SF level is shown in Table 5. For male and female participants the prevalence of disease was similar for those with elevated SF and those with normal SF. The only exception was male compound heterozygotes with elevated SF who had a greater prevalence of abnormal liver enzymes compared with those with normal SF (5/36 [14%] compared with 0/35 [0%], $P = 0.02$). There was little change in the observed prevalence of abnormal liver enzymes for compound heterozygotes after excluding those participants who were obese or with a heavy alcohol intake for men (4/24 [17%] for elevated SF compared with 0/29 [0%] for normal SF [$P = 0.02$]) or women

Table 3. Blood Donation History at Baseline Stratified by *HFE* Genotype, Sex, and, for Women, Menopause Status

	Compound Heterozygote Blood Donation at Baseline			Wild-Type Blood Donation at Baseline			
	Never	Former	Current	Never	Former	Current	*P*
Male	43 (51%)	20 (24%)	21 (25%)	71 (48%)	48 (32%)	30 (20%)	0.36
Female							
Premenopausal	19 (43%)	13 (30%)	12 (27%)	31 (42%)	24 (32%)	19 (26%)	0.95
Postmenopausal	32 (62%)	14 (27%)	6 (11%)	67 (63%)	26 (24%)	14 (13%)	0.92
	51	27	18	98	50	33	

Table 4. Prevalence of Disease Features Stratified by *HFE* Genotype and Sex

	Male			Female		
	Compound Heterozygote	*HFE* Wild-Type	*P*	Compound Heterozygote	*HFE* Wild-Type	*P*
MCP 2/3*	13/64 (20%)	18/116 (16%)	0.42	16/80 (20%)	16/144 (11%)	0.07
Arthritis medicine†	2/84 (2%)	3/149 (2%)	0.85	7/96 (7%)	14/181 (8%)	0.84
Fatigue‡	9/83 (11%)	15/143 (10%)	0.93	14/93 (15%)	33/176 (19%)	0.45
Raised AST/ALT§	6/78 (8%)	19/139 (14%)	0.19	1/91 (1%)	5/169 (3%)	0.34
Liver disease |	6/83 (7%)	4/141 (3%)	0.12	4/92 (4%)	10/175 (6%)	0.63
Hepatomegaly‖	5/62 (8%)	5/113 (4%)	0.32	1/77 (1%)	3/135 (2%)	0.64

*Presence of bony spur, tenderness or effusion of the 2nd and 3rd MCP joints on either hand. Examination conducted by physicians blinded to genotype and HH status.

†Self-reported answer to the questions "Has a doctor ever told you that you have arthritis or rheumatism?" followed by "If you have arthritis or rheumatism, do you take aspirin?"

‡Aspartate aminotransferase > 45 IU/L or alanine aminotransferase >40 IU/L

§Self-reported answer to the question "Have you ever sought medical attention because of fatigue?"

| Self-reported answer to the question "Has a doctor ever told you that you have liver disease?"

‖Liver enlargement defined as a liver span of 13cm or more. Examination conducted by physicians blinded to genotype and HH status.

(0/11 [0%] for elevated SF compared with 1/51 [2%] for normal SF [$P = 0.64$]).

Table 6 presents the prevalence of iron overload-related disease for compound heterozygotes. One male (1/82 = 1.22%, 95% CI 0.03%, 6.61%) and no females (0/95 = 0.00%, 95% CI 0.00%, 3.10%) had documented iron overload-related disease. Two male *HFE* wild-types fit the criteria of provisional iron overload, only one of whom had HH-associated disease.

Discussion

This article is the first to describe the natural history of serum iron indices and the development of both HH-associated features and iron overload–related disease for *HFE* compound heterozygotes compared with *HFE* wild-types. *HFE* compound heterozygotes were more likely than *HFE* wild-type subjects to develop elevated iron indices in middle age, but in our population-based cohort study only one in 82 men and no women had documented iron overload-related disease. This prevalence of iron overload-related disease is considerably lower than for male C282Y homozygotes in this cohort, of whom 28% had disease by the same mean age of 65 years.[3] For both compound heterozygotes and C282Y homozygotes, therefore, iron overload-related disease occurs primarily in individuals with SF levels greater than 1,000 μg/L.[3]

Our study confirms previous reports that compound heterozygotes have an increased prevalence of elevated SF compared with wild-types,[7,8,10] although mean SF levels at baseline and follow-up were within the normal range. We extended these observations by showing that SF does not rise significantly for male or postmenopausal female compound heterozygotes after middle age. The onset of menopause, however, does increase SF levels for female compound heterozygotes, presumably due to the cessation of regular physiological blood loss associated with menstruation. As found in previous cross-sectional population studies,[4] mean SF levels were much lower in compound heterozygotes at baseline and follow-up than for C282Y homozygotes,[3] although the iron-regulatory mechanisms underpinning this genotypic difference are poorly understood.

The prevalence of HH-associated features for *HFE* compound heterozygotes was no greater than for *HFE* wild-type controls. Nor were HH-associated features more common for compound heterozygotes with elevated

Table 5. Prevalence of Disease in Compound Heterozygotes by Serum Ferritin (SF) Level and Sex

	Male Compound Heterozygotes			Female Compound Heterozygotes		
	Elevated SF*	Normal SF	*P*	Elevated SF	Normal SF	*P*
MCP 2/3[1]	5/31 (16%)	7/30 (23%)	0.48	2/13 (15%)	9/47 (19%)	0.76
Arthritis medicine[2]	1/38 (3%)	1/35 (3%)	0.98	1/15 (7%)	5/55 (9%)	0.77
Fatigue[3]	4/38 (11%)	4/34 (12%)	0.87	2/15 (13%)	12/54 (22%)	0.45
Raised AST/ALT[4]	5/36 (14%)	0/35 (0%)	0.02	0/15 (0%)	1/55 (2%)	0.60
Liver disease[5]	3/38 (8%)	2/34 (6%)	0.74	1/15 (7%)	3/53 (6%)	0.88
Hepatomegaly[6]	3/30 (10%)	1/29 (3%)	0.31	0/12 (0%)	1/46 (2%)	0.61

*Seven male and six female compound heterozygotes had elevated SF and elevated TS.

Table 6. Prevalence of Iron-Overload-Related Disease in *HFE* Compound Heterozygotes

	Male			Female		
	HH-Associated Disease	No HH-Associated Disease	Total	HH-Associated Disease	No HH-Associated Disease	Total
Documented iron overload[1]	1	0	1	0	0	0
Provisional iron overload[2]	0	6	6	1	5	6
No iron overload[3]	18	57	75	16	73	89
Total	19	63	82	17	78	95

Iron overload is categorized as one of the following:

1. Documented iron overload: Increased iron content shown by hepatic iron staining 3 or 4, iron concentration >90 μmol/g, or HII >1.9 (Whitlock, 14) or SF >1000 μg/L at baseline with documented therapeutic venesection.
2. Provisional iron overload: Raised SF (>300 μg/L for males and postmenopausal women, >200 μg/L premenopausal women) in association with raised TS (>55% males, >45% females).
3. No evidence of iron overload: Normal SF or elevated SF but in the context of normal TS during study period.

Iron overload-related disease is defined as occurrence of at least one of the following five conditions in the context of documented iron overload as defined above:

1. Hepatocellular carcinoma.
2. Cirrhosis or fibrosis on percutaneous liver biopsy.
3. Bony, tenderness or effusion of both of the second and third metacarpophalangeal joints on examination by study physician blinded to genotype.
4. Raised serum aspartate aminotransferase (AST > 45 IU/L) or serum alanine, aminotransferase (ALT > 40 IU/L).
5. Physician diagnosis due to presentation with HH-associated symptoms.

Documented iron overload-related disease was considered present if participants had BOTH documented iron overload AND evidence of iron overload-related disease.

SF than for those compound heterozygotes with normal serum ferritin. It must be noted that there were few participants in either *HFE* genotype group with SF >1,000 μg/L and we are unable to determine whether SF >1,000 μg/L alone is a risk factor for HH-associated disease as it is for C282Y homozygotes[3] or whether for compound heterozygotes HH-associated features requires the presence of comorbid factors such as high alcohol intake and obesity. We are unable to compare baseline iron indices between participants and nonparticipants because no blood samples were available for those lost to follow-up. The possibility that those with higher baseline iron indices participated less frequently in follow-up cannot be discounted, despite the good overall response.

Results from liver biopsies were available only if this investigation had been undertaken based on usual clinical protocols[16,17] and were only requested from physicians of C282Y homozygotes. This may have led us to underestimate the prevalence iron overload-related disease, because the presence of fibrosis and cirrhosis are one of the disease criteria contributing to the definition of iron overload-related disease. There is, however, good evidence from studies of C282Y homozygotes that the risk of cirrhosis is low when SF is less than 1,000 μg/L.[11]

One further limitation of this study is that we are unable to examine the influence of comorbid factors on the development of iron overload-related disease due to the low prevalence of SF >1,000 μg/L in this population-recruited cohort. These factors may act synergistically with iron overload for both C282Y homozygotes and C282Y/H63D compound heterozygotes to produce disease.[18] The increased prevalence of raised AST/ALT for male compound heterozygotes compared with *HFE* wild-types might be due to the effects of alcohol intake or excess body fat,[14] although the effect remained even when those participants who drank daily more than 60 grams of alcohol (men) or more than 40 grams of alcohol (women) or had a BMI over 30 kg/m^2 were excluded during our sensitivity analysis.

In conclusion, *HFE* compound heterozygotes are more likely to have elevated iron indices in middle age compared with people with neither *HFE* mutation, but the prevalence of iron overload-related disease is rare.

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