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HFE C282Y 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], and **Lyle C Gurrin**[4] **for the HealthIron Study Investigators***

[1] Murdoch Childrens Research Institute, Melbourne, Australia

[2] Department of Pediatrics, The University of Melbourne, Royal Children's Hospital, Melbourne, Australia

[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, USA

[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, Brisbane, Australia; University of Queensland, Brisbane, Australia

[11] Department of Gastroenterology, Fremantle Hospital, Fremantle, Australia; School of Medicine and Pharmacology, University of Western Australia, Perth, Australia; Western Australian Institute of Medical Research, Perth, Australia

[12] Curtin Health Innovation Research Institute, Perth, Australia

[13] University of Queensland, Brisbane, Australia; Royal Brisbane and Women's Hospital, Brisbane, Australia

Abstract

HFE-associated hereditary hemochromatosis (HH) is a genetic predisposition to iron overload and subsequent signs and symptoms of disease potentially affecting around 80,000 in Australia and almost one million people in the USA. Most clinical cases are homozygous for the C282Y mutation in the *HFE* gene, with serum ferritin (SF) concentration >1000 μg/L the strongest predictor of cirrhosis. The optimal treatment regimen for those with SF concentrations above the

Corresponding author: Associate Professor Katrina Allen, FRACP, PhD, Murdoch Childrens Research Institute, The University of Melbourne Department of Paediatrics, Department of Gastroenterology, Royal Children's Hospital, Flemington Rd Parkville, 3052, VIC, Australia, katie.allen@rch.org.au.

*The HealthIron Study Investigators also include: M Bahlo (The Walter & Eliza Hall Institute for Medical Research, Melbourne, Australia), CD Vulpe (University of California, Berkeley, CA, USA), SM 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).

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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 *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 (MCP 2/3), 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% (MCP 2/3 95% CI (−6%, 29%), p=0.22) and for normal SF was 6% (arthritis medicine use, 95% CI (−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.

Keywords

hemochromatosis; iron overload; C282Y homozygotes; serum ferritin

Introduction

Hereditary hemochromatosis (HH) refers to symptoms and signs of disease that result from an inherited predisposition to iron overload. Iron overload is preventable, but if left untreated can lead to significant health problems including arthritis, hepatic cirrhosis, hepatocellular carcinoma (HCC), fatigue, and diabetes mellitus.1 More than 80% of patients presenting with symptomatic iron-overload 2, 3 are homozygous for the 845G>A mutation in the *HFE* gene, which leads to the 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 indices7, 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 overload9 and one of the following five objective HH features: HCC, cirrhosis/fibrosis, physician-diagnosed symptomatic HH, elevated liver enzymes or evidence of HH-associated arthritis)7 – 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 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 symptomsfor 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 223,965,00915 we estimate that in the USA alone there are almost 700,000 C282Y homozygotes who will develop SF concentrations that are elevated but below 1000 μg/L8 and almost 55,000 in Australia. Given the greater prevalence of *HFE* mutations in the northern European population, the corresponding figure for the UK 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 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 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

Methods

Study methods

The present study, known as HealthIron, is a sub-study of the Melbourne Collaborative Cohort Study (MCCS).18 Between 1990 and 1994, 41,514 people (24,469 women) with a target age range 40–69 years were enrolled in the MCCS. Participants were recruited via the electoral roll (voting is compulsory in Australia), advertisements and 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 sub-sample 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, California, USA) real-time 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 Cancer Council of Victoria's Human Research Ethics Committee. Participants attending a study centre 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, Carlsbad, California, USA) 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, USA) 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- and menopause-specific SF upper limit of normal thresholds to be >300 μg/L for men and post-menopausal women and >200 μg/L for pre-menopausal women. We categorised 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 (raised AST or ALT), a liver span 13cm 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, 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 since 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 for prevalence differences and p-values for two-sample comparison of proportions were generated by assuming the normal approximation to the underlying binomial distribution to quantify sampling variability. For statistical analyses of SF concentrations, the values were (natural) log transformed. SF concentrations were summarised 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 (TS) and the MFIS were summarised using the sample mean and compared between groups using the two-sample t-test.

Results

One hundred and 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 four 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 (BMI), 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 TS 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 abnormal MCP2/3 was increased compared with male *HFE* wild-types (32% vs 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 MCP 2/3, 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 MCP 2/3 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 BMI>30 kg/m^2 or high alcohol consumption (>60 g/d men and >40 g/d women) (classified according to Australian National Health and Medical Research Council guidelines 2001)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.

Excluding 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- 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. Further, 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 a higher prevalence of arthritis for male C282Y homozygotes compared with male *HFE* wild-types, when stratified by SF concentration rather than sex the association remained, suggesting that for C282Y homozygotes arthritis might occur independently of iron overload. 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 and 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 disease16 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 MRI 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 symptoms27

The association between iron indices and the risk of HH-associated signs and symptoms has also been examined among community-recruited participants in the 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 They found that the prevalence of chronic fatigue and MCP 2/3 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 for the present study and there were no longitudinal data on iron studies, so the results are not directly comparable with those presented in this paper.

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.

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Table 1

Participant demographics at baseline as per inclusion criteria for prevalence of HH-assoicated signs and symptoms analysis.*

	C282Y Homozygote		*HFE* Wild-type	
	Male *n = 35*	**Female *n = 67***	**Male *n = 131***	**Female *n = 160***
Age (yrs) 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/d) 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

Table 2

Prevalence of HH-associated signs and symptoms categorized by *HFE* genotype, baseline serum ferritin (SF) concentration, and sex.

C282Y homozygotes																
Baseline SF			**Follow-up SF**			**Prevalenceof HH-associated signs and symptoms[#]**										
						MCP 2/3		**raised AST or ALT**		**fatigue self-report**		**hepatomegaly**		**liver disease self-report**	
	M	**F**		**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	n	**n**		**n**	**n**										
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**																
Baseline SF			**Follow-up SF**			**Prevalence of HH-associated signs and symptoms[#]**										
						MCP 2/3		**raised AST or ALT**		**fatigue Self-report**		**hepatomegaly**		**liver disease self-report**	
	M	**F**		**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	n	**n**		**n**	**n**										
SF>1000 μg/L	1	0													
Moderately elevated	32	4	**SF>1000 μg/L**	0	0										

C282Y homozygotes															
Baseline SF			**Follow-up SF**			**Prevalenceof HH-associated signs and symptoms#**									
						MCP 2/3		**raised AST or ALT**		**fatigue self-report**		**hepatomegaly**		**liver disease self-report**	
	M	**F**		**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	n	**n**		**n**	**n**										
Normal	100	156	**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**
			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**

† treated defined as therapeutically venesected before baseline;

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

‡ treated defined as therapeutically venesected after baseline

^ 2 male and 2 female C282Y homozygotes treated after baseline maintained moderately elevated SF at follow-up

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 13cm or more as assessed by physicians blinded to genotype at follow-up
Liver disease: self-reported liver disease 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. 1 male and 2 female C282Y homozygotes and 3 male and 2 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.

	SF<1000 μg/L at baseline – male				SF<1000 μg/L at baseline – female			
Signs and symptoms	**C282Y homozygotes**	***HFE* wild-types**	**difference (95% CI)**	***p*#**	**C282Y homozygotes**	***HFE* wild-types**	**difference (95% CI)**	***p*#**
	n=35	***n=131***			***n=67***	***n=160***		
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 prev. (%)	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 prev. (%)	4/27 (15%)	18/121 (15%)	*0 (−15, 15)*	*0.994*	3/57 (5%)	3/148 (2%)	*3 (−3, 9)*	*0.218*
Hepatomegaly prev. (%)	1/19 (5%)	5/102 (5%)	*0 (−11, 11)*	*0.947*	1/47 (2%)	2/120 (2%)	*0 (−4, 5)*	*0.840*
Liver disease prev. (%)	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 prev. (%)	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 prev. (%)	1/35 (3%)	3/131 (2%)	*1 (−6, 7)*	*0.846*	0/67 (0%)	1/160 (1%)	*−1 (−2, 1)*	*0.517*
Arthritis medicine prev. (%)	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

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 v Normal SF for C282Y homs	
	C282Y homs*	*HFE* wild- types	difference (95% CI)	$p^{\#}$	C282Y homs&	*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 prev. (%)	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 prev. (%)	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 prev. (%)	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 prev. (%)	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 prev. (%)	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 prev. (%)	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 prev. (%)	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 8 male and 8 female C282Y homozygotes therapeutically venesected after baseline;

#p-value comparing outcomes from chi-squared test

&includes 2 male and 3 female C282Y homozygotes therapeutically venesected after baseline