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The natural history of *HFE* simple heterozygosity for C282Y and H63D: a prospective twelve year study

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Abstract

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Background and Aim—The risk of hemochromatosis-related morbidity for *HFE* simple heterozygosity for either the C282Y or H63D substitutions in the HFE protein was assessed using a prospective community –based cohort study.

Methods—*HFE* genotypes were measured for 31,192 persons of northern European descent, aged between 40 and 69 years when recruited to the Melbourne Collaborative Cohort Study, and who were followed for an average of 12 years. For a random sample of 1438 participants stratified according to *HFE* genotype two sets of biochemical iron indices performed 12 years apart and, at follow-up only, the presence/absence of six disease features associated with hereditary hemochromatosis were obtained. Summary measures for 257 (139 female) C282Y participants and 123 (74 female) H63D participants were compared with 330 (181 female) controls with neither *HFE* mutation.

Results—At baseline mean TS (95% confidence interval) and prevalence of TS > 55% were 35.14% (33.25,37.04) and 3/112(3%); 33.03% (29.9,36.15) and 0/39(0%); and 29.67% (27.93,31.4) and 3/135(2%) for C282Y, H63D and wild-type male participants, respectively. At follow-up, mean TS levels remained similar to baseline levels for both men and women irrespective of simple heterozygosity for either mutation. No *HFE* C282Y or H63D simple heterozygotes had documented iron overload (based on hepatic iron measures or serum ferritin greater than 1000mg/L at baseline with documented therapeutic venesection).

Conclusion—No documented iron overload was observed for *HFE* simple heterozygotes for either C282Y or H63D, and morbidity for both *HFE* simple heterozygote groups was similar to that of the *HFE* wild-type participants.

Keywords

liver disease; hereditary disease; iron overload-related disease; serum ferritin; transferrin saturation

Introduction

Hereditary hemochromatosis (HH) is a genetic disease disorder that can lead to iron overload which, if not prevented or treated, increases the risk of diseases including liver cirrhosis, arthritis, fatigue and diabetes (1). Two mutations in the *HFE* gene, C282Y and H63D, are associated with the majority of clinical cases of iron overload (2). In particular two *HFE* genotypes, C282Y homozygosity and C282Y/H63D compound heterozygosity, confer an increased risk of iron overload-related disease (2, 3). These *HFE* genotypes have been studied extensively in both community and clinical studies, and their epidemiological profile is well established (4-6).

Two *HFE* genotypes that have received less attention are simple heterozygotes for either the C282Y or the H63D mutation. In populations of northern European descent, H63D simple heterozygosity is more prevalent (23.6% to 31.1%) (7-9) than C282Y simple heterozygosity (8.6% to 11.9%) (5, 7, 8, 10). Despite these high prevalences, the population risk of *HFE* C282Y simple heterozygotes and *HFE* H63D heterozygotes developing HH-associated clinical signs and symptoms or iron overload-related disease has not been widely examined. If this risk is increased compared with that of the general community, then it would have an

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immediate implication for population genetic screening for *HFE* mutations, since such screening would potentially label a large proportion of the population as being both at increased risk of disease, and as carriers of a disease causing mutation.

Large cross-sectional population-based studies show that, on average, serum ferritin concentration (SF) and transferrin saturation (TS) levels for C282Y simple heterozygotes are within their respective clinically normal reference ranges, but tend to be higher compared with individuals without C282Y or H63D mutations, designated as *HFE* wild-type, for both sexes (5, 7, 10, 11). Similarly, mean SF and TS levels for H63D simple heterozygotes are within their respective clinically normal ranges, and comparable to *HFE* wild-types for both males and females (7). Male C282Y simple heterozygotes have been reported to have a 0.81-fold decrease (95%CI: 0.71-0.94) in the odds of diabetes compared with *HFE* wild-types (7), although the prevalence of diabetes in this study (11.5%), and in our own cohort (2%) is low (5, 12). An Australian study found no evidence that the presence of the H63D mutation resulted in an increased risk of clinically significant iron overload (9). In the work-place setting, the prevalence of self-reported tiredness, abdominal pain, joint pain and previous diagnosis of diabetes, arthritis and liver disease in simple C282Y heterozygotes was comparable to the prevalence of these symptoms/diseases for *HFE* wild-type individuals (13).

These previous studies (4, 9-11) possess a number of shortcomings. None stratified by women's menopausal status (nor indeed recorded this parameter), and none have measured iron indices for the same participants at two or more time points. Furthermore, participants were examined by medical practitioners who were not blinded to their *HFE* genotype status. In some studies, *HFE*-associated signs, symptoms and features of disease were not examined, and only iron indices were assessed (10, 11). Findings from one study in the work-place setting may not be generalizable to the wider population due to the possibility of selection bias (13).

The “HealthIron” study, results of which we present in this paper, does not suffer from the deficiencies outlined in the previous paragraph. We examined clinical and epidemiological data from *HFE* C282Y and H63D simple heterozygotes and wild-type individuals who were followed over a 12-year period and at ages when those at risk of iron overload would have been expected to develop iron overload-related disease (from 40-69 years at baseline to 54-83 years at follow-up). We describe the natural history of serum iron indices and iron overload-related disease signs and symptoms using this large community-based sample of well-characterised subjects.

Methods

The Melbourne Collaborative Cohort Study

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) (14). At baseline, participants attended a study centre where they completed a questionnaire about dietary and lifestyle factors, underwent a physical examination and provided a blood sample.

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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 the other three *HFE* genotypes (C282Y/H63D compound heterozygote, C282Y simple heterozygote, no C282Y mutation), were selected for invitation to attend follow-up clinics between 2004 and 2006 as part of the HealthIron study. H63D simple heterozygotes were identified by genotyping of those with no C282Y mutation who attended the follow-up clinic. Of the 1,438 people invited to participate in the HealthIron study, 107 were deceased and 277 were lost to follow-up, leaving 1,054 who participated. The overall participation by those invited was 73.3 per cent (79.2 per cent 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 and blood donation, had a fasting 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 sub-study. Both protocols were approved by the Cancer Council Victoria's Human Research Ethics Committee.

Definitions of biochemical/clinical exposures and outcomes

Iron indices

Elevated SF was defined for males and post-menopausal females as >300 μg/L and for pre-menopausal females as >200 μg/L. Elevated transferrin saturation (TS) was defined for males as >55% and for females as >45%. When examining the prevalence of disease by elevated iron indices, SF was considered to be elevated if it exceeded the specified threshold on at least one occasion. We defined normal SF as having values below these thresholds at both baseline and follow-up.

Disease features, iron overload-related disease (IORD) and menopausal status

We investigated the prevalence of six disease features associated with hereditary hemochromatosis (HH): Abnormal (i.e. presence of bony spur, effusion or tenderness) second and/or third metacarpophalangeal (MCP) joints on either hand (MCP 2/3), use of arthritis medication, self-reported fatigue, raised aspartate aminotransferase or raised alanine

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aminotransferase levels (raised AST or raised ALT), self-reported history of liver disease and/or hepatomegaly. With the exception of the use of arthritis medication, which was assessed at baseline and follow-up, all disease features were measured at follow-up alone.

Iron overload-related disease (IORD) was defined as the presence of one of the following five features: hepatocellular carcinoma, liver cirrhosis or fibrosis, abnormal $2^{nd}/3^{rd}$ MCP joints, raised aminotransferases, or physician-diagnosed HH due to symptoms in the context of either provisional or documented iron overload (see footnotes to Supplementary Table 3 for definition) (5).

Menopausal status for women was measured at baseline and classified as pre-menopausal or post-menopausal

Statistical analysis of biochemical and clinical outcomes

The Mann-Whitney U test was used to detect differences in both location and spread of age, BMI and alcohol intake between groups. For all analyses, SF levels were natural log (ln) transformed. Comparisons of mean ln 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. No correction for multiple testing was made since we present a relatively small number of comparisons in the content of a genetic association study.

The prevalence of elevated iron indices and disease features was estimated as the observed proportions at a single time point, and comparisons between groups were made using Pearson's chi-squared test. If cell counts were less than 5, then Fisher's exact test was performed. Some subjects did not participate in all components and as a result did not contribute data to the prevalence calculation for every disease feature.

We examined the influence of co-morbid 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 60g/day for men and greater than 40g/day for women. Increased BMI and alcohol intake are common causes of raised iron indices and abnormal serum transaminase levels.

Participants who were diagnosed with and treated for HH and those with any SF>1000 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.

Results

HFE genotyping was successful for 29,676 of 31,192 (95%) subjects of whom 3295 (11.1%) were C282Y simple heterozygotes. Of these, 337 C282Y simple heterozygotes and 621 participants with no copies of the C282Y mutation were selected for invitation to the HealthIron study. Follow-up clinics were attended by 257 C282Y simple heterozygotes and 469 without the C282Y mutation (responses of 76.3% and 75.5% respectively). Subsequent

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genotyping revealed that of those without the C282Y mutation attending the clinic, 123 (74 women) were simple heterozygotes for H63D and 330 (181 women) were wild-type for *HFE*. The remaining 16 participants (10 women) attending the clinics were homozygous for the H63D mutation and were excluded from further consideration in this study. Close to or more than half of all women in each of the three *HFE* genotype groups were post-menopausal at baseline (63/139 (45%) C282Y simple heterozygotes, 44/74 (59%) H36D simple heterozygotes and 110/181 (61%) *HFE* wild-types).

For men and women, there was little difference in the median and IQR of age, BMI and daily alcohol intake for C282Y and H63D simple heterozygotes compared with *HFE* wild-types (Table 1). The Mann-Whitney U test identified a statistically significant difference in the median and spread of the distribution of daily alcohol intake and BMI for female C282Y simple heterozygotes. These differences were small in magnitude and, therefore, not clinically relevant. The blood donation history of HFE simple heterozygotes for either C282Y or H63D was comparable to wild-types for men, pre-menopausal women and post-menopausal women.

Iron indices

C282Y and H63D simple heterozygotes had, on average, normal serum ferritin and transferrin saturation (TS) levels at baseline, irrespective of sex and menopausal status (Table 2). At follow-up, both geometric mean SF levels and mean TS levels remained within the normal range and similar to baseline levels for men and post-menopausal women, regardless of whether they had the C282Y or H63D substitution in the HFE protein (Supplementary Table 1). For pre-menopausal women, the onset of menopause increased the geometric mean SF levels of C282Y simple heterozygotes (Baseline: 36.3μg/L (95% CI: 28.3, 46.5); Follow-up: 87.1μg/L (95% CI: 69.3,109.5)) and H63D simple heterozygotes (Baseline: 57.34μg/L (95% CI: 36.14,90.97); Follow-up: 80.4μg/L (95% CI: 51.45,125.64)). The majority of participants had similar or lower SF and TS at follow-up than at baseline. Only 7 participants had SF > 500 μg/L at follow-up who did not have a similarly elevated SF at baseline. The number of participants whose TS rose over the period of the study was also low (115 (only 12 patients' TS rose to greater than 50% from baseline to follow) out of 559 patients with both baseline and follow-up TS). For both C282Y and H63D simple heterozygotes, the prevalence of elevated SF or TS was comparable to *HFE* wild-types at both time points, and was particularly low for women (Supplementary Table 2).

Only 25 and 13 participants has SF elevated above 500 μg/L at either baseline or follow-up, respectively, and those HFE simple heterozygotes with SF > 1000 μg/L at either time point are described further below. Two male C282Y simple heterozygotes (both with BMI > 30 kg/m^2) had baseline SF>1000 μg/L, and at follow-up, one male H63D simple heterozygote (BMI = 26 kg/m^2) had SF>1000 μg/L. The H63D simple heterozygote reported that they did not drink alcohol, while the C282Y simple heterozygotes reported alcohol intakes of 15 g/day and 22 g/day. All three of these participants had TS<55% at the same time as their SF was severely elevated. We note that HFE C282Y homozygotes with SF>1000 μg/L rarely have TS < 55% (5, 15) suggesting that severely elevated serum ferritin in HFE simple heterozygotes is attributable to co-morbid factors rather than iron overload.

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Prevalence of disease features

The estimated prevalence of the six disease features for each sex and *HFE* genotype group are given in Table 3. After stratifying by sex, the prevalence of disease features for both C282Y and H63D simple heterozygotes was similar to that for wild-types, with the exception of a lower prevalence of raised AST or ALT for male C282Y simple heterozygotes (5%) compared with wild-types (14%). After individuals who were obese (BMI>30 kg/m^2) or who had high alcohol intake (>60 g/day for men or >40 g/day for women) were excluded, abnormal liver enzymes were significantly less prevalent for male C282Y simple heterozygotes (3/93 (3.2%)) relative to wild-types (15/105 (14%)). For male and female C282Y and H63D simple heterozygotes, the observed prevalence of disease was similar for those with elevated SF and those with normal SF (Table 4). Excluding those participants who were obese or with a heavy alcohol intake did not alter this finding. The small absolute numbers suggest that this is not clinically relevant.

Supplementary Table 3 presents the prevalence of iron overload-related disease for C282Y and H63D simple heterozygotes. No male or female C282Y or H63D simple heterozygotes had documented iron overload-related disease. Two male C282Y simple heterozygotes (both elevated SF and TS at baseline; only one had follow-up measures for SF and TS, and this was normal), one female C282Y simple heterozygote pre-menopausal at baseline (elevated SF at baseline and follow-up; only elevated TS at follow-up), and one male H63D simple heterozygote (only had SF and TS measures at follow-up – both elevated) fitted the criteria of provisional iron overload (as defined in (5) and at the bottom of Supplementary Table 3), but had none of the six disease features associated with HH. These four patients were aged between 50 and 63 years old; baseline BMI ranged between 21.76 and 24.66; alcohol intake was high for the male H63D simple heterozygote (42.8 g/day) and one of the C282Y simple heterozygote (41.7 g/day), and was moderate for the other male C282Y simple heterozygote (18.5 g/day) and the female C282Y simple heterozygote (1.61 g/day). Two male HFE wild-types fitted the criteria of provisional iron overload, only one of whom reported experiencing disease features associated with HH; arthritis medication and fatigue.

Discussion

Our study is the first to describe longitudinally the natural history of serum iron indices, and to determine whether HH-associated features and iron overload-related disease develop in *HFE* C282Y and H63D simple heterozygotes. Iron indices remained stable, and, on average, within their clinically normal ranges in middle age for C282Y and H63D simple heterozygotes, so these individuals are unlikely to be candidates for therapeutic venesection for iron overload. Menopause resulted in increased mean SF for female C282Y and H63D simple heterozygotes and wild-type participants, but the iron indices, on average, remained within their normal ranges. No documented iron overload was observed for *HFE* simple heterozygotes for either C282Y or H63D, and iron overload related disease morbidity for both *HFE* simple heterozygote groups was similar to *HFE* wild-types.

We note that the thresholds used to define elevated SF and elevated TS differ between cross-sectional population studies, and some thresholds are sex dependent while others are not (7, 9-11). Our results on the prevalence of elevated iron indices are in line with those from other

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studies so it is unlikely that our conclusions are sensitive to the choice of threshold for the clinically normal range of iron indices such as TS and SF.

A strength of our study is its longitudinal nature. There is only one other prospectively-recruited, community-based study that focussed specifically on hereditary haemochromatosis. Our findings are consistent with the results of that study, but extend their findings by reporting serial iron indices and clinical examination at follow-up more than a decade after recruitment. Nevertheless, our study does have a number of weaknesses. The majority of participants were recruited in middle age so our conclusions do not necessarily apply to younger age groups. Clinical examination for haemochromatosis-associated features was not conducted at baseline, so we relied on self-reported information from questionnaires and interviews. Data on magnetic resonance imaging or liver biopsy to quantify hepatic iron content were not part of the protocol for clinical examination at follow-up, and these procedures were was only performed when clinically indicated by the participants' physician independently of their involvement in this study. However, in a separate study we have shown that such subjects do not have iron loading above general population levels (16). For participants who had therapeutic venesection or who engaged in voluntary (or altruistic) blood donation, quantitative phlebotomy was self-reported, although, where possible, records were linked to the Australian Red Cross Blood Service to establish the validity of the self-reported number of episodes of blood removal (17).

In conclusion, *HFE* simple heterozygotes for either C282Y or H63D are at low risk of developing iron overload in middle age, and rarely exhibit any of the symptoms of HH. We found no evidence that *HFE* simple heterozygotes would benefit from more frequent medical examination than the general population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Participant information at baseline: age, BMI and alcohol intake stratified by genotype and sex

	n	Age (years) mean (sd) [median;IQR†]	p‡	BMI (kg/m^2) mean (sd) [median;IQR]	p	Alcohol (g/day) mean (sd) [median;IQR]	p
Male							
C282Y Simple Heterozygote	118	53.7 (9.2) [52.5;45.5-61.5]	0.67	26 (3.2) [25.6;23.8-27.3]	0.17	18.2 (17.8) [13.9;2.5-29.8]	0.76
H63D Simple Heterozygote	49	54.4 (9.2) [52.8;46.9-63.9]	0.91	27.4 (4.1) [26.7;24.6-28.9]	0.32	15.2 (17.4) [12.6;.2-19.6]	0.29
***HFE* Wild-type**	149	54.2 (9.1) [54.2;45.3-61]		26.8 (4.2) [25.9;24.3-28.3]		19.4 (23.3) [13.5;3-24.2]	
Female							
C282Y Simple Heterozygote	139	52.5 (8.8) [50.4;44.6-59.9]	0.29	25.1 (4.1) [24.5;22.1-27.1]	0.01	11 (14.1) [4.3;0-16.5]	<0.01
H63D Simple Heterozygote	74	55.2 (9.9) [56;44.1-64.4]	0.25	25.3 (4) [24.8;22.6-27.2]	0.15	7.6 (11.9) [1.7;0-12.9]	0.90
***HFE* Wild-type**	181	53.7 (9.1) [53.5;45.2-61.7]		26.3 (4.6) [25.3;23.2-28.5]		7.4 (12.9) [1;0-8.6]	

†IQR – inter-quartile range

‡Two-sided p-value derived from Mann-Whitney U test comparing C282Y simple heterozygotes to *HFE* wild-types, and H63D simple heterozygotes to *HFE* wild-type

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Table 2

Geometric mean serum ferritin (SF) and mean transferrin saturation (TS) values stratified by *HFE* genotype and sex. Baseline SF and TS for women are stratified according to menopausal status. All women had become post-menopausal by follow-up

Baseline iron indices:						
	n	**Baseline SF (μg/L)† geometric mean (95% CI)**	**p‡**	**n**	**Baseline TS (%)§ mean (95% CI)**	**p**
Men						
C282Y Simple Heterozygote	112	179.11 (151.31,212.02)	0.14	112	35.14 (33.25,37.04)	<0.01
H63D Simple Heterozygote	38	214.01 (168.66,271.53)	0.03	39	33.03 (29.9,36.15)	0.07
***HFE* Wild-type**	133	150.81 (128.59,176.86)		135	29.67 (27.93,31.4)	
Pre-menopausal women						
C282Y Simple Heterozygote	71	37.23 (29.49,46.99)	0.73	72	29.97 (27.16,32.78)	<0.01
H63D Simple Heterozygote	26	53.36 (35.3,80.66)	0.06	27	26.82 (22.49,31.14)	0.05
***HFE* Wild-type**	65	35.16 (27.93,44.26)		65	22.48 (20.27,24.69)	
Post-menopausal women						
C282Y Simple Heterozygote	51	109.18 (86.73,137.45)	0.09	51	29.1 (26.24,31.96)	0.04
H63D Simple Heterozygote	37	100.38 (73.27,137.52)	0.31	37	28.16 (25.58,30.75)	0.16
***HFE* Wild-type**	95	83.01 (68.3,100.9)		95	26.06 (24.51,27.62)	
Follow-up iron indices:						
		Follow-up SF (μg/L)¶ geometric mean (95% CI)			**Follow-up TS (%)†† mean (95% CI)**	
Men						
C282Y Simple Heterozygote	107	159.65 (131.53,193.79)	0.18	107	33.04 (30.7,35.38)	0.01
H63D Simple Heterozygote	46	141.17 (107.22,185.88)	0.76	46	31.61 (28.33,34.89)	0.15

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Baseline iron indices:						
	n	**Baseline SF (μg/L)[†] geometric mean (95% CI)**	**p‡**	**n**	**Baseline TS (%)[§] mean (95% CI)**	**p**
***HFE* Wild-type**	139	134.16 (113.63,158.38)		140	29.14 (27.5,30.78)	
Women[‡‡]						
C282Y Simple Heterozygote	125	97.61 (83.71,113.82)	0.07	127	31.13 (29.28,32.98)	<0.01
H63D Simple Heterozygote	66	87.36 (66.81,114.22)	0.54	66	26.83 (24.66,29)	0.14
***HFE* Wild-type**	169	80.08 (69.29,92.55)		169	24.94 (23.62,26.26)	

[†] 46 (19 C282Y simple heterozygotes (10 males, 6 pre-menopausal females and 3 post-menopausal females), 9 H63D simple heterozygotes (1 male, 3 pre-menopausal females and 5 post-menopausal) and 18 *HFE* wild-types (8 males, 3 pre-menopausal females and 7 post-menopausal females)) had SF measures available at baseline but not follow-up.

[‡] p-values comparing C282Y simple heterozygotes to *HFE* wild-types, and H63D simple heterozygotes to *HFE* wild-types.

[§] 1 pre-menopausal female C282Y simple heterozygote, 1 pre-menopausal female H63D simple heterozygote, 1 male H63D simple heterozygote and 2 male *HFE* wild-types had a baseline TS measure but no baseline SF measure.

[¶] 99 (39 C282Y simple heterozygotes (17 males, 6 pre-menopausal and 16 post-menopausal females) 22 H63D simple heterozygotes (11 males, 4 pre-menopausal females and 7 post-menopausal females) and 38 *HFE* wild-types (17 males, 6 pre-menopausal and 15 post-menopausal females)) had SF measures available at follow-up but not baseline.

[††] 2 female C282Y simple heterozygotes and 1 male *HFE* wild-type had a follow-up TS measure but no follow-up SF measure.

[‡‡] 3 C282Y simple heterozygotes, 1 H63D simple heterozygote and 3 *HFE* wild-type females were pre-menopausal at follow-up.

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Table 3

Prevalence of disease features for simple heterozygotes and *HFE* wild-types by sex

		Men	p[†]	Women	p
MCP 2/3[‡]	**C282Y Simple Heterozygote**	11/94(12%)	0.43	8/103(8%)	0.38
	H63D Simple Heterozygote	7/41(17%)	0.81	9/55(16%)	0.32
	***HFE* Wild-type**	18/116(16%)		16/144(11%)	
Arthritis medicine[§]	**C282Y Simple Heterozygote**	4/161(2%)	0.72¶¶	10/176(6%)	0.46
	H63D Simple Heterozygote	2/54(4%)	0.6¶¶	9/76(12%)	0.27
	***HFE* Wild-type**	3/164(2%)		15/197(8%)	
Fatigue[¶]	**C282Y Simple Heterozygote**	9/115(8%)	0.47	25/139(18%)	0.86
	H63D Simple Heterozygote	7/46(15%)	0.38	11/70(16%)	0.57
	***HFE* Wild-type**	15/144(10%)		33/176(19%)	
Raised AST/ALT[††]	**C282Y Simple Heterozygote**	5/107(5%)	0.02	9/127(7%)	0.11¶¶
	H63D Simple Heterozygote	4/46(9%)	0.38	0/66(0%)	0.33¶¶
	***HFE* Wild-type**	19/139(14%)		5/169(3%)	
Liver disease[‡‡]	**C282Y Simple Heterozygote**	3/116(3%)	1.00¶¶	13/140(9%)	0.23
	H63D Simple Heterozygote	4/46(9%)	0.1¶¶	5/69(7%)	0.65
	***HFE* Wild-type**	4/142(3%)		10/175(6%)	
Hepatomegaly[§§]	**C282Y Simple Heterozygote**	3/91(3%)	0.73¶¶	1/98(1%)	0.64¶¶
	H63D Simple Heterozygote	0/41(0%)	0.32¶¶	2/54(4%)	0.63¶¶
	***HFE* Wild-type**	5/113(4%)		3/135(2%)	

[†] p-values comparing C282Y simple heterozygotes to *HFE* wild-types, and H63D simple heterozygotes to *HFE* wild-types.

[‡] 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?”

[¶] Self reported answer to the question “Have you ever sought medical attention because of fatigue?”

[††] Aspartate aminotransferase > 45 IU/L or alanine aminotransferase >40 IU/L

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[##] 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.

[¶¶] Fisher's exact test

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Table 4

Prevalence of disease for male simple heterozygotes with elevated iron indices compared with those with non-elevated iron indices

	Men			Women		
	Elevated SF†	Normal SF	p‡	Elevated SF†	Normal SF	p
C282Y Simple Heterozygotes						
MCP 2/3	5/37(14%)	4/48(8%)	0.49	1/9(11%)	5/76(7%)	0.5
Arthritis medicine	0/48(0%)	3/56(5%)	0.25	1/12(8%)	6/95(6%)	0.58
Fatigue	6/43(14%)	3/54(6%)	0.18	2/12(17%)	12/94 (13%)	0.66
Raised AST/ALT	2/41(5%)	3/56(5%)	1.00	2/11(18%)	5/95(5%)	0.15
Liver disease	1/43(2%)	0/55(0%)	0.44	1/12(8%)	12/94 (13%)	1.00
Hepatomegaly	0/34(0%)	3/48(6%)	0.26	0/8(0%)	0/74(0%)	-
H63D Simple Heterozygotes						
MCP 2/3	2/13(15%)	4/20(20%)	1.00	2/6(33%)	5/39(13%)	0.23
Arthritis medicine	2/15(13%)	0/22(0%)	0.16	2/10(20%)	5/46(11%)	0.6
Fatigue	1/14(7%)	4/22(18%)	0.63	1/10(10%)	6/44(14%)	1.00
Raised AST/ALT	1/14(7%)	2/22(9%)	1.00	0/10(0%)	0/46(0%)	-
Liver disease	2/14(14%)	1/22(5%)	0.55	0/10(0%)	3/43(7%)	1.00
Hepatomegaly	0/13(0%)	0/20(0%)	-	0/6(0%)	1/38(3%)	1.00

† 6 male C282Y simple heterozygotes had elevated SF and elevated TS at either baseline or follow-up.

‡ p-values comparing C282Y simple heterozygotes to *HFE* wild-types, and H63D simple heterozygotes to *HFE* wild-types. All calculated using Fisher's exact test.