Clinical manifestations of hemochromatosis in *HFE* C282Y homozygotes identified by screening

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BACKGROUND: Patients with hemochromatosis may suffer organ damage from iron overload, often with serious clinical consequences.

OBJECTIVE: To assess prevalences of self-reported symptoms and clinical signs and conditions in persons homozygous for the hemochromatosis gene (*HFE*) mutation (C282Y) identified by screening.

METHODS: Participants were adults 25 years of age or older enrolled in the Hemochromatosis and Iron Overload Screening (HEIRS) Study. C282Y homozygotes (n=282) were compared with control participants without the *HFE* C282Y or H63D alleles (ie, wild type/wild type; n=364).

RESULTS: Previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated serum ferritin levels had higher prevalences of certain symptoms such as chronic fatigue (OR 2.8; 95% CI 1.34 to 5.95, and OR 2.0; 95% CI 1.07 to 3.75, respectively), and had more hyperpigmentation on physical examination (OR 4.7; 95% CI 1.50 to 15.06, and OR 3.7; 95% CI 1.10 to 12.16, respectively) and swelling or tenderness of the second and third metacarpophalangeal joints (OR 4.2; 95% CI 1.37 to 13.03, and OR 3.3; 95% CI 1.17 to 9.49, respectively) than control subjects. Joint stiffness was also more common among newly diagnosed C282Y homozygotes with elevated serum ferritin than among control subjects (OR 2.7; 95% CI 1.38 to 5.30). However, the sex- and age-adjusted prevalences of self-reported symptoms and signs of liver disease, heart disease, diabetes and most other major clinical manifestations of hemochromatosis were similar in C282Y homozygotes and control subjects.

CONCLUSIONS: Some symptoms and conditions associated with hemochromatosis were more prevalent among C282Y homozygotes identified by screening than among control subjects, but prevalences of most outcomes were similar in C282Y homozygotes and controls in this primary care-based study.

Key Words: Complications; Cross-sectional study; Hemochromatosis; *HFE*; Iron overload; Prevalence

Les manifestations cliniques de l'hémochromatose chez des homozygotes C282Y *HFE* repérés par dépistage

HISTORIQUE : Les parents atteints d'hémochromatose peuvent souffrir d'une atteinte des organes causée par surcharge en fer, entraînant souvent de graves conséquences cliniques.

OBJECTIF : Évaluer la prévalence de symptômes déclarés par les patients ainsi que des signes cliniques et des pathologies des personnes homozygotes à la mutation (C282Y) du gène d'hémochromatose (*HFE*) repérées par dépistage.

MÉTHODOLOGIE : Les participants étaient des adultes de 25 ans et plus qui participaient à l'étude HEIRS sur le dépistage de l'hémochromatose et de la surcharge en fer. On a comparé les homozygotes C282Y (n=282) aux sujets témoins sans allèles C282Y ou H63D *HFE* (c'est-à-dire type sauvage/type sauvage; n=364).

RÉSULTATS : Les homozygotes C282Y déjà diagnostiqués et les homozygotes nouvellement diagnostiqués ayant des taux élevés de ferritine sérique présentaient une plus forte prévalence de certains symptômes, tels que la fatigue chronique (RRR 2,8; 95 % IC 1,34 à 5,95, et RRR 2,0; 95 % IC 1,07 à 3,75, respectivement), plus d'hyperpigmentation à l'examen physique (RRR 4,7; 95 % IC 1,50 à 15,06 et RRR 3,7; 95 % IC 1,10 à 12,16, respectivement) et un œdème ou une sensibilité des deuxième et troisième articulations métacarpo-phalangiennes (RRR 4,2; 95 % IC 1,37 à 13,03 et RRR 3,3; 95 % IC 1,17 à 9,49, respectivement) que les sujets témoins. La raideur articulaire était également plus courante chez les homozygotes C282Y nouvellement diagnostiqués ayant une ferritine sérique élevée que chez les sujets témoins (RRR 2,7; 95 % IC 1,38 à 5,30). Cependant, la prévalence de symptômes déclarés par le patient et de signes de maladie hépatique, de maladie cardiaque, de diabète et de la plupart des autres principales manifestations cliniques d'hémochromatose, rajustés selon le sexe et l'âge, était similaire chez les homozygotes C282Y et les sujets témoins.

CONCLUSIONS : Certains symptômes et certaines pathologies associés à l'hémochromatose étaient plus prévalents chez les homozygotes C282Y repérés par dépistage que chez les sujets témoins, mais la prévalence de la plupart des issues était similaire chez les homozygotes C282Y et les sujets témoins dans le cadre de la présente étude menée en soins primaires.

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Hemochromatosis is an autosomal recessive disorder characterized by an increased risk of iron overload attributable to excessive dietary iron absorption (1-3). Left untreated, progressive iron overload can damage the liver, heart, pancreas, anterior pituitary gland and joints (1-5). Hemochromatosis is common among Caucasian populations of northern European origin. In the United States, 80% to 90% of Caucasian patients diagnosed with hemochromatosis are homozygous for a mutation in the hemochromatosis (*HFE*) gene (exon 4, nt845G→A; cys282tyr, C282Y) (6,7). Homozygosity for the C282Y mutation is found in approximately five of every 1000 persons of northern European descent (8-10). A second common mutation of *HFE* (exon 2, nt187C→G; his63asp, H63D) is more widely distributed in other populations; C282Y/H63D compound heterozygotes comprise a proportion of hemochromatosis patients (3) but exhibit milder phenotypic manifestations (2,9,11).

The major complications of iron overload in hemochromatosis patients can be prevented by phlebotomy therapy to remove excess iron, and patients treated before the onset of organ damage have a normal life expectancy (12). This has stimulated interest in early detection (3,13,14) and recent studies (15-19) have suggested that manifestations of severe iron overload are relatively uncommon in C282Y homozygotes identified by screening.

In the current study, we compared the prevalence of symptoms and clinical conditions among non-Hispanic Caucasian C282Y homozygotes identified in the multicentre Hemochromatosis and Iron Overload Screening (HEIRS) Study with the prevalence among control subjects from the same population. We hypothesized that symptoms and clinical conditions typically associated with iron overload are more prevalent in previously diagnosed C282Y homozygotes and in newly diagnosed C282Y homozygotes with elevated serum ferritin (SF) levels than in control participants. The investigators also postulated that the prevalence of symptoms and clinical conditions in newly diagnosed C282Y homozygotes with normal SF levels is not greater than in control participants.

METHODS

Subjects

The HEIRS Study is evaluating the prevalence, genetic and environmental determinants, and potential clinical, personal and societal impact of hemochromatosis and iron overload in a multi-ethnic, primary care-based sample of 101,168 adults enrolled over a two-year period at four field centres in the United States and one in Canada. Participants, who were at least 25 years of age and able to give informed consent, were recruited from a health maintenance organization, diagnostic blood collection centres, and public and private primary care offices and ambulatory clinics associated with the field centres (20). Initial screening of participants included genotyping for the *HFE* C282Y and H63D alleles, measurement of serum iron and unsaturated iron binding capacity, calculated transferrin saturation (TfS) and SF levels (20).

Clinical examination

The study protocol was approved by the institutional review board of each field centre and informed written consent was obtained for screening and for a clinical examination (CE). The CE was offered to all *HFE* C282Y homozygotes and to all participants whose TfS and SF values exceeded study thresholds (TfS of more than 50% and SF more than 300 µg/L for

men; TfS more than 45% and SF more than 200 µg/L for women) (20). Participants were also informed of their genotype, TfS and SF results, and at that time were invited to return for the CE. The median length of time between initial screening and the CE was approximately eight months; participants generally received their results several months before the CE. Of 2265 participants invited for CEs, 1687 (75%) participated. Among C282Y homozygotes (n=333), the participation rate was 91% (n=302), of whom 94% (n=285) were non-Hispanic Caucasians. A control group of 642 participants who carried neither the C282Y nor H63D *HFE* alleles (ie, wild type), and had SF and TfS levels between the 25th and 75th percentiles of sex-specific distributions, also had CEs. Of these, 366 were non-Hispanic Caucasians. Control subjects were frequency-matched for age and sex to cases studied at each field centre. Three C282Y homozygotes lacked SF measurements and two controls reported a previous diagnosis of iron overload or hemochromatosis and were excluded from the analyses. Among the non-Hispanic Caucasian C282Y homozygotes, three study groups were designated: previously diagnosed participants, newly diagnosed participants with elevated SF (SF level of more than 300 µg/L for men or more than 200 µg/L for women) and newly diagnosed participants without elevated SF (Table 1). The group of previously diagnosed C282Y homozygotes included those who indicated that they had been diagnosed with hemochromatosis or iron overload before enrolling in the HEIRS study or had undergone therapeutic phlebotomy either before enrolling in the study or before returning for the CE.

The CE has been described in detail previously (20) and this included a participant-completed questionnaire addressing medical history and a focused physical examination of the heart, liver, spleen, skin and metacarpophalangeal (MP) joints. The medical history questionnaire and physical examination were designed to document the prevalence of the kinds of symptoms and clinical conditions that have been reported in previous studies to be associated with hemochromatosis and iron overload (1-5,13). Examiners were aware of each participant's genotype, TfS and SF results at the time of the examination. Participants also completed the Short Form 36 (SF-36) Health Survey (21). In total, 36 outcome variables (symptoms, clinical conditions and physical signs) were included in this evaluation. A morning fasting blood sample was obtained for confirmation of genotyping results for the C282Y and H63D alleles (6,22), repeat TfS and SF determinations (20,23) and measurements of serum glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), C-reactive protein (CRP) (Hitachi 911 analyzer, Roche Applied Science, USA), and serum insulin (DPC Immulite Analyzer, Diagnostic Products, USA). If the ALT concentrations were elevated, testing for hepatitis B surface antigen and hepatitis C antibody was performed (Vitros ECI, Ortho-Clinical Diagnostics Inc, USA).

Statistical methods

Binary outcomes were analyzed using logistic regression adjusted for sex, age and field centre, as appropriate. Some binary outcomes were positive too rarely to support reliable estimation and these were analyzed using Fisher's exact test for group effects without covariate adjustment. The SF-36 Health Survey was analyzed using analysis of covariance with covariate adjustment. The natural log transform was applied to fasting serum glucose, insulin, ALT, AST and GGT levels.

Transformed variables were analyzed using multiple linear regression. Elevated CRP (more than 5.0 mg/L) was analyzed as a binary outcome. Primary analyses focused on the F test for effects among all study groups. A Bonferroni correction for multiple comparisons was applied to control the type 1 error rate at 0.05 for 36 signs and symptoms and, separately, for six laboratory tests. Pairwise comparisons among the study groups were made using estimated ORs or least-squares means. For signs and symptoms, a subset of outcomes for which results may be biased as a result of exclusion of controls with SF levels greater than the 75th percentile (246 µg/L for men and 121 µg/L for women) were specified; the outcomes that were designated as possibly subject to bias in comparisons between C282Y homozygotes and controls are indicated in Table 2.

RESULTS

Study population

The initial screening of participants included 63,550 women (62.8%) and 37,618 men (37.2%). The median age was 50 years (range 25 to 100 years). By self-identified race/ethnicity, 44% of the participants were non-Hispanic Caucasians. As previously reported (24), the frequency of homozygosity for the C282Y mutation in this group of non-Hispanic Caucasians was 4.4 per 1000 subjects; consistent with the findings of other screening studies (9,18,25-29). Of 285 non-Hispanic Caucasians homozygous for C282Y who attended the CE, 282 had complete data for analysis. There were 195 newly diagnosed homozygotes (122 [63%] women and 73 [37%] men) of median age 51 years (range 25 to 86 years). Among them, 131 (67%; 67 [51%] women and 64 [49%] men) had elevated SF (more than 300 µg/L in men, more than 200 µg/L in women) at the time of CE, and 64 (33%; 55 [86%] women and 9 [14%] men) had SF below these levels. Eighty-seven other participants with C282Y homozygosity reported they previously were told by a physician that they had hemochromatosis or iron overload, or had been treated by therapeutic phlebotomy, including 39 (45%) women and 48 (55%) men, of median age 56 years (range 25 to 83 years). The characteristics of the groups of C282Y homozygotes and control participants are shown in Table 1. Because the previously diagnosed group included participants who had undergone phlebotomy therapy before initial screening or had begun phlebotomy treatments before returning for CE, clinical manifestations were evaluated in this group without regard to SF levels. Control subjects (n=364) were comprised of 222 (61%) women and 142 (39%) men, of median age 56 years (range 25 to 92 years).

Symptoms, clinical conditions, physical signs and biochemical test results

Table 2 shows the proportions of participants who reported clinical symptoms or had abnormal findings on physical examination. After adjustment for imbalances in age and sex, statistically significant differences between C282Y homozygotes and controls were observed for the following five (of 36) outcomes: symptoms of chronic fatigue/weakness, unexplained weight loss, joint stiffness or pain, and findings on physical examination of swollen or tender MP joints and increased pigmentation. Although the observed prevalences of most other outcomes were higher in previously diagnosed C282Y homozygotes, and to a lesser extent, in newly diagnosed homozygotes with elevated SF than in controls, there were no statistically significant differences for these outcomes. There also were no

TABLE 1
Characteristics of non-Hispanic Caucasian C282Y homozygotes and control participants

Characteristics	C282Y homozygotes			
	Previously-diagnosed (n=87)	Newly diagnosed		Control participants (n=364)
		Ferritin elevated* (n=131)	Ferritin not elevated† (n=64)	
Age, years	57.4±13.5	51.7±13.6	45.7±10.3	56.3±14.1
Age range, years, n (%)				
24 to 44	14 (16)	40 (30)	27 (42)	75 (21)
45 to 64	47 (54)	65 (50)	36 (56)	180 (49)
65 and older	26 (30)	26 (20)	1 (2)	109 (30)
Women, n (%)	39 (45)	67 (51)	55 (86)	222 (61)
Transferrin saturation, %	63.4±25.9	75.6±20.4	51.0±27.1	28.5±9.1
Serum ferritin, µg/L‡	186.0 (58.0, 470.0)	616.0 (428.0, 911.0)	90.0 (32.0, 132.0)	84.0 (50.5, 138.0)

Data presented as mean ± SD, unless otherwise indicated. *Serum ferritin >200 µg/L for women, >300 µg/L for men; †Serum ferritin ≤200 µg/L for women, ≤300 µg/L for men; ‡Serum ferritin results presented as median (25th percentile, 75th percentile)

significant differences among the three groups of C282Y homozygotes for any symptom, sign or clinical condition. The only significant differences were between groups of C282Y homozygotes and controls (Table 3). In contrast, there were significant differences in serum ALT between groups of C282Y homozygotes (Table 4), with higher levels in previously diagnosed homozygotes and in newly diagnosed homozygotes with elevated SF than in newly diagnosed homozygotes with normal SF ($P<0.001$). However, the prevalences of self-reported liver disease, porphyria cutanea tarda and hepatomegaly on physical examination were not significantly different among groups of C282Y homozygotes and control subjects (Table 2). Of 76 participants with elevated ALT or AST, none tested positive for hepatitis B surface antigen and four tested positive for hepatitis C antibody, including one of 14 previously diagnosed C282Y homozygotes and three of 32 controls (data not shown). There was no significant difference among any of the groups in levels of fasting serum glucose, insulin, AST, GGT or prevalence of elevated CRP (Table 4).

Among symptoms with significantly different prevalences between C282Y homozygotes and controls, chronic fatigue/weakness was more prevalent both in previously diagnosed C282Y homozygotes and in newly diagnosed homozygotes with elevated SF. Self-reported unexplained weight loss was significantly more common among all groups of C282Y homozygotes than among controls, none of whom reported any unexplained weight loss. The SF-36 Health Survey score revealed no statistically significant differences among any of the groups (Table 2).

The prevalences of symptoms possibly related to heart disease, such as self-reported shortness of breath or ankle swelling, were not significantly different among any of the subgroups of C282Y homozygotes and control subjects (Table 2). There also was no significant difference in the prevalence of possible signs of heart disease such as frequent premature contractions, other rhythm disturbance or ankle edema determined by physical examination.

As shown in Table 2, self-reported joint stiffness was more common among C282Y homozygotes than in controls, and the prevalence was highest for newly diagnosed C282Y homozygotes with elevated SF, although a history of arthritis

TABLE 2

Proportions of C282Y homozygotes and control participants with symptoms and signs

Symptoms and clinical conditions	C282Y homozygotes			Control participants (n=364)	Nominal P ‡
	Previously diagnosed (n=87)	Newly diagnosed			
		Ferritin elevated*(n=131)	Ferritin not elevated† (n=64)		
General manifestations					
Chronic fatigue/weakness in the last 12 months§	51.7	39.7	34.4	23.6	<0.001
Unexplained weight loss in the last 12 months §	5.8	6.9	3.1	0.0	<0.001
Health Survey score (mean)§	60.3	70.1	66.8	69.5	0.066
Cardiac symptoms					
Swelling of feet or ankles in the last 12 months§	41.4	37.4	32.8	34.1	0.70
Shortness of breath when walking quickly or uphill	69.0	62.6	65.6	61.3	0.62
Shortness of breath when walking on level ground	27.6	12.2	20.3	12.1	0.033
Shortness of breath while resting in a chair	6.9	3.8	6.3	2.5	0.61
Shortness of breath in the last 12 months	41.4	29.8	31.3	23.6	0.10
Heart failure or weak heart, ever	9.2	1.5	3.1	5.2	0.33
Abnormal heart rhythm, heart beat or action arrhythmia, ever	23.0	15.3	18.8	21.2	0.74
Other heart disease or heart attack, ever	10.3	8.4	7.8	11.0	0.41
Diabetes					
Diabetes, ever	16.1	9.9	10.9	8.5	0.57
Liver disease					
Abdominal swelling or fluid in last 12 months§	16.1	11.5	20.3	8.8	0.42
Unexplained abdominal pain or discomfort in the last 12 months§	23.0	16.8	28.1	11.3	0.037
Liver disease, ever§	16.1	9.2	7.8	6.0	0.094
Porphyria cutanea tarda, ever§	4.6	0.8	1.6	0.3	0.038
Arthritis					
Joint stiffness/pain/ache in last 12 months§	73.6	74.1	70.3	55.5	<0.001
Arthritis, ever§	57.5	35.1	29.7	38.7	0.084
Neurological symptoms					
Unexplained confusion or memory loss in last 12 months	27.6	16.8	18.8	9.1	0.078
Hypogonadism					
Trouble having an erection or loss of sexual drive in last 12 months (men only)	56.3¶	44.4**	44.4††	36.6‡‡	0.023
Other endocrine					
Thyroid disease, ever	16.1	15.3	21.9	19.8	0.93
Skin changes					
Change in skin colour in last 12 months§	12.6	10.7	12.5	3.0	0.0015
Bone disease					
Osteoporosis, ever	8.1	3.8	7.8	7.1	0.51
Other					
Cancer§	10.3	16.8	14.1	14.3	0.048
Signs on physical examination					
Heart arrhythmia: bradycardia	0.0	0.0	0.0	0.0§§	1.00
Heart arrhythmia: tachycardia	1.2	0.0	0.0	0.0§§	0.23
Frequent premature contractions (>1 ectopic beat/min)	6.9	3.1	3.1	1.7§§	0.049
Other abnormal rhythm	3.5	0.0	0.0	3.3§§	0.058
Heart murmur	8.1	6.1	7.8	10.2§§	0.51
Edema§	11.5	7.6	6.3	15.5§§	0.73
Hepatomegaly§	10.3	9.2	4.7	3.3§§	0.068
Splenomegaly§	2.3	0.0	1.6	0.6§§	0.14
Metacarpophalangeal joints swollen or tender§	21.8	13.7	3.1	5.0§§	<0.001
Increased pigmentation on sun-exposed or unexposed areas§	21.8	9.9	7.8	4.4§§	<0.001
Blistering, ulcers, scarring of sun-exposed skin§	3.5	2.3	0.0	2.5§§	0.64
Hypertrichosis (excess hair growth in sun-exposed areas)§	0.0	1.5	1.6	0.3§§	0.17

*Serum ferritin > 200 µg/L for women, >300 µg/L for men. [†]Serum ferritin ≤200 µg/L for women, ≤300 µg/L for men. [‡]P value for the group effect from logistic regression or Fisher's exact test. Nominal P values are compared with 0.00139 (0.05/36) to assess significance with a 5% type-1 error rate; [§]Outcomes possibly subject to bias in comparisons between C282Y homozygotes and controls; [¶]Denominator = 48; ^{**}Denominator = 63; ^{††}Denominator = 9; ^{‡‡}Denominator = 142; ^{§§}Denominator = 362

was not reported more frequently by C282Y homozygotes than control subjects. On physical examination, previously diagnosed and newly diagnosed C282Y homozygotes with elevated SF, but not newly diagnosed C282Y homozygotes

with normal SF, had swollen MP joints more often than in controls (Table 3).

The prevalences of self-reported impotence in men, unexplained confusion or memory loss, and a change in skin colour

were not significantly different across subgroups of C282Y homozygotes. In contrast, there was a significantly higher prevalence of increased pigmentation detected on physical examination in previously diagnosed and newly diagnosed C282Y homozygotes with increased SF than in controls (Table 3). There was no significant difference between C282Y homozygotes and controls in the prevalence on physical examination of other skin changes such as blistering or hypertrichosis (Table 2).

The prevalences of self-reported diabetes mellitus, thyroid disease, osteoporosis and cancer were similar in C282Y homozygotes and controls (Table 2).

DISCUSSION

Our results indicate that most symptoms and clinical manifestations associated with hemochromatosis were not statistically significantly more common among C282Y homozygotes identified by screening than among controls in this primary care population. In particular, the prevalences of certain serious clinical manifestations of hemochromatosis, such as symptoms or signs related to liver disease or heart disease, were not significantly higher in C282Y homozygotes than in controls after correction for multiple comparisons. The clinical consequences of advanced iron overload have been documented in previous studies (30), particularly in patients identified on the basis of symptoms or signs of organ damage. In contrast, participants in the large multicentre HEIRS Study were enrolled without regard to any clinical conditions. Our findings are consistent with the results of several single-centre and regional population- and primary care-based studies (15-18). A recent study of first-degree relatives of patients with known C282Y hemochromatosis also found a low prevalence of clinical manifestations in family members who were C282Y homozygotes or C282Y/H63D compound heterozygotes, despite evidence of iron overload in most cases (31). However, a recent meta-analysis (32), based mainly on case-control studies, found an increased risk of liver disease among C282Y homozygotes compared to wild type/wild type control subjects. In that study, hemochromatosis genotypes were not associated with an increased risk of diabetes mellitus, heart disease, arthritis, stroke or cancer in the overall analysis, although there was an increased risk of diabetes mellitus in C282Y homozygotes compared with controls among subjects of northern European descent.

We observed significant differences between groups of C282Y homozygotes and controls for five of 36 outcomes:

TABLE 3
Associations between study groups and medical conditions related to hemochromatosis

Symptoms and signs	OR (95% CI)
General	
Chronic fatigue/weakness last 12 months.	
Previously diagnosed	2.8 (1.34 to 5.95)
Newly diagnosed with elevated serum ferritin	2.0 (1.07 to 3.75)
Newly diagnosed with normal serum ferritin	1.6 (0.66 to 3.68)
Controls	1.0
Arthritis	
Joint stiffness/pain/ache in last 12 months.	
Previously diagnosed	1.9 (0.85 to 4.29)
Newly diagnosed with elevated serum ferritin	2.7 (1.38 to 5.30)
Newly diagnosed with normal serum ferritin	2.3 (0.98 to 5.63)
Controls	1.0
Signs on physical examination	
Metacarpophalangeal joints swollen or tender	
Previously diagnosed	4.2 (1.37 to 13.03)
Newly diagnosed with elevated serum ferritin	3.3 (1.17 to 9.49)
Newly diagnosed with normal serum ferritin	1.0 (0.11 to 8.75)
Controls	1.0
Increased pigmentation on sun-exposed or unexposed areas	
Previously diagnosed versus controls	4.7 (1.50 to 15.06)
Newly diagnosed with elevated serum ferritin	3.7 (1.10 to 12.16)
Newly diagnosed with normal serum ferritin	4.6 (0.87 to 24.25)
Controls	1.0

For signs and symptoms with a significant overall group effect by the Bonferroni multiple comparisons procedure, pairwise comparisons significant at the nominal level ($\alpha=0.05$) are shown

symptoms of chronic fatigue/weakness, weight loss, joint stiffness or pain, and findings on physical examination of swollen or tender MP joints, and increased pigmentation. However, only C282Y homozygotes with elevated SF levels showed significant differences from controls for all five outcomes. Newly diagnosed homozygotes with normal SF (86% females) did not show significant differences from controls for most of these symptoms and conditions (Table 3). The prevalences of these symptoms and signs did not differ statistically among previously diagnosed C282Y homozygotes and newly diagnosed homozygotes, nor between newly diagnosed C282Y homozygotes with elevated SF and newly diagnosed homozygotes without SF elevation.

Several factors may have favoured increased or decreased prevalences of outcomes. It is possible that C282Y homozygotes identified by screening in a primary care population represent a relatively healthy subgroup of all C282Y homozygotes. A

TABLE 4
Results of serum biochemical tests in C282Y homozygotes and control participants

Test*	C282Y homozygotes				Nominal P [§]
	Previously diagnosed (n=87)	Newly diagnosed		Control participants (n=364)	
		Serum ferritin elevated [†] (n=131)	Serum ferritin not elevated [‡] (n=64)		
Fasting glucose, (mmol/L)	5.61 (4.94, 6.22)	5.22 (4.83, 5.61)	5.05 (4.80, 5.52)	5.16 (4.88, 5.66)	0.062
Fasting insulin, (pmol/L)	76 (49, 97)	56 (35, 83)	56 (42, 76)	56 (42, 80)	0.20
Alanine aminotransferase (U/L)	20.0 (15.0, 30.0)	22.0 (16.0, 33.0)	16.0 (11.0, 20.0)	19.0 (14.5, 25.0)	0.0047
Aspartate aminotransferase (U/L))	22.0 (18.0, 29.0)	22.0 (19.0, 30.0)	19.0 (16.0, 21.5)	21.0 (17.0, 25.0)	0.040
Gamma-glutamyltransferase (U/L)	25.0 (17.0, 37.0)	23.0 (15.0, 34.0)	16.5 (12.0, 29.5)	19.0 (13.0, 31.0)	0.070
C-reactive protein >5.0 mg/L, n (%)	27 (31)	41 (31)	25 (39)	106 (29)	0.62

Data are presented as median (25th percentile, 75th percentile) unless otherwise indicated. *Laboratory reference ranges: fasting glucose: 3.3–6.38 mmol/L; fasting insulin: 0–139 pmol/L; alanine aminotransferase: 0–31 U/L (women), 0–40 U/L (men); aspartate aminotransferase: 0–31 U/L (women), 0–37 U/L (men); gamma-glutamyltransferase: 7–33 U/L (women), 11–51 U/L (men); C-reactive protein: 0–5.0 mg/L. [†]Serum ferritin >200 µg/L for women, >300 µg/L for men; [‡]Serum ferritin ≤200 µg/L for women, ≤300 µg/L for men; [§]P-value for the study group effect. Nominal P-values are compared with 0.0083 (0.05/6) to assess significance with a 5% type 1 error rate

potential bias possibly favouring increased prevalence of morbidity among C282Y homozygotes is that, by design, the selection criteria for control participants required SF levels between the 25th and 75th percentiles to exclude participants with increased or decreased iron stores. Thus, some control subjects having liver disease or inflammatory disorders and an associated increase in SF levels (33) were excluded by this criterion. In addition, capture of the medical history and physical examination data was not masked to genotype information, and this may have led to some overprobing of anticipated findings in C282Y homozygotes or underprobing in controls. However, a lack of strong bias is suggested by the fact that only a few outcomes were significantly more common in homozygotes despite previous knowledge of genotype by participants and examiners.

The higher prevalence of self-reported chronic fatigue and unexplained weight loss in C282Y homozygotes than in controls in the current study is consistent with previous reports of symptoms in patients with hemochromatosis (1-4,13,28,34). Fatigue is commonly reported by patients diagnosed with hemochromatosis, especially in those having serum ferritin levels greater than 1000 µg/L (35). Weight loss in hemochromatosis patients has been attributed to diabetes mellitus, other endocrinopathy, cirrhosis of the liver or other complications of advanced iron overload typical of such patients. However, weight loss is infrequently reported by persons diagnosed to have hemochromatosis in screening programs, presumably because most of them do not have severe iron overload (13). The relatively low prevalence of weight loss reports among C282Y homozygotes in the current study is consistent with the low prevalence of severe iron overload observed in homozygotes in the HEIRS Study (24). Similarly, there was no significant difference between C282Y homozygotes and controls in the prevalence of most other self-reported symptoms commonly associated with hemochromatosis, including diabetes mellitus. The lack of association between *HFE* mutations and diabetes is consistent with the results of some (16,24,36,37), but not all (32,38) previous studies.

The greater prevalence of self-reported joint stiffness/pain and swollen or tender MP joints on physical examination in C282Y homozygotes than in controls in the current study may represent clinical expression of the arthropathy of hemochromatosis (1-4). Although we did not find a significantly higher prevalence of self-reported arthritis in C282Y homozygotes than in controls, arthritis is one of the most common clinical conditions in hemochromatosis patients and often is the first to develop (39-42). In the current study, the prevalence of joint stiffness or pain in newly diagnosed C282Y homozygotes with normal SF was similar to that in previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated SF (Table 2). This raises the possibility that arthralgia may be an early manifestation of hemochromatosis that precedes the development of iron overload (42).

Increased pigmentation in patients with hemochromatosis can be manifested as either a bronze or slate grey appearance (1,2,4,13), although its presence may be subject to interobserver variability. In the current study, increased pigmentation on physical examination was observed more often in previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated SF (but not in newly diagnosed homozygotes with normal SF) than in control participants, consistent with a relationship between this finding and the severity of iron overload.

The cardiac manifestations of hemochromatosis often resolve after treatment to remove excess iron by therapeutic phlebotomy, and this may account for our observation that the prevalence of cardiac symptoms among previously diagnosed C282Y homozygotes was not significantly increased in comparison with controls. Hepatic fibrosis, and occasionally cirrhosis, can also be reversed by iron removal (43), but cirrhosis in most cases is irreversible and is associated with an increased risk of primary hepatocellular carcinoma. We previously reported that male C282Y homozygotes in the HEIRS Study were more likely to report a history of liver disease at the time of the initial screening examination than were participants without *HFE* mutations (24). A recent longitudinal study from Australia also found that male C282Y homozygotes with a serum ferritin level higher than 1000 µg/L were more likely to report a history of liver disease than men without *HFE* mutations (35). Although we did not find a significantly increased prevalence of self-reported liver disease in C282Y homozygotes relative to controls in the current study, this could have been an effect of the selection of controls for the clinical examination or differential recall bias between the time of initial screening and the subsequent clinical examination. Hepatic fibrosis and cirrhosis in hemochromatosis patients can also be silent, and a recent report documents comparable prevalences of fibrosis and cirrhosis on liver biopsy in apparently healthy Australian C282Y homozygotes identified either by screening during health checks (19% fibrosis and 12% cirrhosis in males) or because there was a family history of hemochromatosis (23% and 6%, respectively) (40). In a Norwegian population-screening study, similar but slightly lower prevalences of fibrosis (11%) and cirrhosis (4%) were found in male C282Y homozygotes undergoing biopsy (44). In the current study, ALT levels were higher in previously diagnosed C282Y homozygotes and newly diagnosed homozygotes with elevated SF than in newly diagnosed homozygotes with normal SF. We previously reported that, of 11 C282Y homozygotes with elevated SF (497 µg/L to 5200 µg/L) in the HEIRS Study who underwent clinical liver biopsy, fibrosis was present in eight cases, including one with cirrhosis (45). Thus, some C282Y homozygotes had previously undiagnosed liver damage that was revealed by biopsy.

A possible interpretation of the relative lack of an increased prevalence of symptoms and signs among C282Y homozygotes in the current study may be that most homozygotes do not develop sufficient iron overload to cause symptoms and clinical manifestations (15-18), even though the majority have evidence of excess iron accumulation (46). Previous studies of hemochromatosis patients (47) in referral practice settings have shown that the risk of hepatic cirrhosis in C282Y homozygotes begins to increase when SF levels rise above 1000 µg/L, but this SF level was exceeded in only 29 of 131 newly diagnosed C282Y homozygotes with elevated SF in the current study (24). Thus, the prevalence of disease manifestations in C282Y homozygotes identified by screening in primary care is likely to be much less than in C282Y homozygotes receiving care in referral settings, such as practices specializing in the care of patients with liver disease (48). This was pointed out previously by Edwards et al (49), who found that patients with hemochromatosis diagnosed as a result of clinical symptoms tended to have more advanced iron overload and more severe disease manifestations than asymptomatic patients diagnosed by screening.

It has been estimated that 10% to 33% of C282Y homozygotes may eventually develop hemochromatosis-associated

morbidity (29). The results of the current study indicate that C282Y homozygotes identified by screening for hemochromatosis or iron overload in primary care do not have a significantly increased prevalence of most symptoms and clinical conditions typically associated with hemochromatosis at the time of screening. The traditional approach is to screen for hemochromatosis in patients with classic manifestations such as hepatic and cardiac complications, diabetes, gonadal dysfunction and arthritis. Our results suggest that using this traditional approach, a majority of C282Y homozygotes may remain undetected. Whether, or the rate at which, such individuals will develop clinical symptoms related to iron overload in the future is unknown (50). However, a longitudinal study (40) has shown that in the absence of therapeutic phlebotomy, a progressive increase in SF occurs in a majority of C282Y homozygotes. Three other longitudinal studies have assessed morbidity. One population-based study (51) of 23 C282Y homozygotes found no evidence of hemochromatosis-associated liver disease after 25 years, although three of these subjects died before they could be examined. In contrast, a similar study (52) of 10 C282Y homozygotes over a 17-year study period documented cirrhosis or fibrosis in three of six subjects who had liver biopsies. In the recent Australian study (35), iron overload-related disease was documented in 28.4% of 203 male C282Y homozygotes followed for an average of 12 years. Prospective longitudinal studies are needed to assess iron accumulation in C282Y homozygotes with initially normal SF levels and to evaluate treatment outcomes in patients undergoing therapeutic phlebotomy (29).

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