Penetrance of 845G→A (C282Y) *HFE* hereditary haemochromatosis mutation in the USA

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Summary

Background There has been much interest in screening populations for disease-associated mutations. A favoured candidate has been the *HFE* gene, mutations of which are the most common cause of haemochromatosis in the European population. About five people in 1000 are homozygotes for the 845G→A mutation, but little is known of how many have mutation-caused clinical manifestations.

Methods We screened 41 038 individuals attending a health appraisal clinic in the USA for the 845G→A and 187C→G *HFE* mutations, and analysed laboratory data and data on signs and symptoms of haemochromatosis as elicited by questionnaire.

Findings The most common symptoms of haemochromatosis, including poor general health, diabetes, arthropathies, arrhythmias, impotence, and skin pigmentation were no more prevalent among the 152 identified homozygotes than among the controls. The age distribution of homozygotes and compound heterozygotes did not differ significantly from that of controls: there was no measurable loss of such individuals from the population during ageing. However, there was a significantly increased prevalence of a history of hepatitis or "liver trouble" among homozygotes and in the proportion of homozygotes with increased concentrations of serum aspartate aminotransferase and collagen IV; these changes were not related to iron burden or to age. Only one of the 152 homozygotes had signs and symptoms that would suggest a diagnosis of haemochromatosis.

Interpretation The normal age distribution of people with the haemochromatosis genotype, and the lack of symptoms in patients of all ages, indicate that the penetrance of hereditary haemochromatosis is much lower than generally thought. The clinical penetrance of a disorder is an essential consideration in screening for genetic disease; disorders with low penetrance are more expensive candidates for screening than disorders with high penetrance. Our best estimate is that less than 1% of homozygotes develop frank clinical haemochromatosis.

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Introduction

The revolution in molecular biology has made possible the ready determination of a person's genotype at any given locus. This opportunity has engendered some enthusiasm for the screening of populations for mutations associated with disease or disease susceptibility, particularly diseases that are readily treated or prevented. Three factors must be taken into account in deciding how appropriate and cost-effective such screening is: prevalence, effectiveness of intervention, and penetrance. For example, Tay-Sachs disease is frequent in the Ashkenazi Jewish population and the penetrance (proportion of affected individuals who show the mutation phenotype) is high; screening is readily justified in populations where termination of the pregnancy is an option.[1] However, the case for screening for mutations of genes such as *BRCA1* is much less clear because of the low or uncertain penetrance of mutations and the drastic nature of some of the preventative measures that have been suggested.[2]

Hereditary haemochromatosis is an iron-storage disease whose phenotype is characterised by biochemical changes reflecting abnormal iron homoeostasis (eg, changes in transferrin saturation and serum ferritin concentrations) and total-body iron burden; signs and symptoms (eg, darkening of the skin, arrhythmias, loss of body hair, or impotence in men); pathological changes in organs (eg, hepatic fibrosis, abnormal liver function tests, or raised blood-sugar levels); and death. The *HFE* gene was cloned in 1996,[3] and homozygosity for the 845G→A (C282Y) mutation has been found in more than 80% of patients diagnosed with iron overload due to hereditary haemochromatosis.[3–6] Compound heterozygotes for the C282Y and 187C→G (H63D) mutations are also over-represented in the haemochromatosis population.[7,8] This disease has been regarded as one of the most common genetic disorders of northern Europeans[9,10]—about five per 1000 population are homozygous for the C282Y mutation.

Because the homozygous genotype for haemochromatosis is common, and because iron overload is readily treated by phlebotomy, the mutation has been regarded as ideally suited for population screening. Even before detection of homozygotes and compound heterozygotes by DNA analysis was possible, screening was thought to be cost-effective.[11] With the availability of DNA-based detection of the haemochromatosis genotypes, the case for screening has seemed even more compelling.[12] However, although most patients with European ancestry who have been diagnosed clinically with haemochromatosis have either the C282Y/C282Y or the C282Y/H63D genotypes, there is scant and conflicting information about the number of people in the general population with this genotype who have clinical manifestations caused by the mutation and not merely associated with it.[13] In reality, practising physicians rarely encounter patients with the "bronzed diabetes" that is the classical hallmark of the severe, advanced form of the disease.[14] None of the previous studies of the penetrance of clinical manifestations of

haemochromatosis compared patients with an adequate control group to make it possible to assess which symptoms were due to haemochromatosis. To address this question, we assessed more than 41 000 individuals attending a health appraisal clinic in the USA for haemochromatosis mutation status, signs and symptoms of haemochromatosis, and biochemical abnormalities suggestive of the disorder.

Methods

Kaiser-Permanente is a large system of integrated medical care with about 8 000 000 members in several states of the USA. The current studies were carried out in San Diego, CA, where 500 000 individuals, representing about a third of the population of the metropolitan area, are members of the Kaiser Permanente medical care programme. There is no barrier to entry into the programme for patients who join as a part of an employee group or who have MediCare. In any 4-year period, 81% of adult members older than 26 years attend the Health Appraisal Clinic in which these investigations were done.

All participating individuals gave informed consent for these studies, which were approved by the institutional review boards of Kaiser Permanente and the Scripps Research Institute. All completed a detailed health questionnaire before their assessment, and were interviewed and examined by a physician or a physician's assistant. Blood from all patients was analysed for full blood count, serum cholesterol concentration, more than 3-h fasting serum glucose concentrations, serum iron concentrations, iron binding capacity, and serum ferritin concentration. Serum aspartate aminotransferase concentrations were measured in all C282Y homozygotes and on all participants during part of January and September, 2000, to provide control values.

Mutation analysis was done as described elsewhere.[15] Concentration of plasma type IV collagen—a surrogate of hepatic fibrosis[16]—was measured by a two-step sandwich enzyme immunoassay. The capture antibody was a mouse monoclonal antibody against human type IV collagen (clone IV-4H12, ICN Biomedicals, Aurora, OH, USA), and the antibody used for detection was a rabbit polyclonal antibody (ICN Biomedicals). A horseradish-peroxidase-conjugated goat IgG fraction to rabbit IgG (ICN Biomedicals) was added, and colour was developed with ABTS (2,2′-azino-bis-[3-ethylbenzthiazoline-6-sulphonic acid]; Sigma, St Louis, MO, USA).

Comparisons of symptoms and laboratory findings between wildtype (wt/wt) controls and either homozygotes (C282Y/C282Y) or compound heterozygotes (C282Y/H63D) were made by means of standard binomial tests for differences in proportions, and Mann-Whitney *U* statistics for quantitative data. In this regard, the accrued sample sizes were sufficient to detect differences of 0·16 or greater in the frequencies of individual symptoms between the wildtypes and homozygotes, or of 0·07 or greater between the wild types and compound heterozygotes, with a power exceeding 0·9, by standard two-sided binomial tests at α=0·05. Logistic regression was also used for assessing the associations between symptoms and genotypes, controlling for sex and age.

Reported p values are nominal, and have not been corrected for multiple comparisons. The age distributions of homozygotes and compound heterozygotes were compared with that of wildtype controls by quantile-quantile plots. Such plots compare the quantiles (or percentiles) of one distribution with the corresponding quantiles of a second distribution.[17] They are useful in assessing whether two distributions are similar. If the two datasets are identically distributed, the points in this plot cluster along the straight diagonal line through the origin with slope 1 (ie, the line y=x).

Role of the funding source

The funding sources had no role in the writing of the report or the decision to publish the results.

Results

The present analysis was undertaken after 41 038 individuals had been screened and 152 homozygotes for the C282Y mutation and 616 compound heterozygotes for the C282Y and H63D mutations had been identified. The demographic composition of the population is summarised in table 1. Of the 152 homozygotes, 45 had previously been diagnosed with haemochromatosis. In most of these individuals, the disorder was detected during an earlier visit to the Health Appraisal Clinic at a time when attendees were screened for haemochromatosis by determining transferrin saturations. Although health records were available for these patients, only the 17 patients for whom we had detailed questionnaire data from before diagnosis were included in the assessment of symptoms, since individuals who believed that

Race and sex	Number of participants	Mean (SD) age (years)	Number with college education*	Number with less than high school education
White				
Men	15 113 (38·3%)	58·7 (13·7)	7541 (49·9%)	575 (3·8%)
Women	15 305 (38·8%)	58·0 (13·9)	5801 (37·9%)	612 (4·0%)
Hispanic				
Men	1776 (4·5%)	50·5 (13·9)	554 (31·2%)	316 (17·8%)
Women	2273 (5·8%)	48·4 (13·9)	566 (24·9%)	489 (21·5%)
Black				
Men	712 (1·8%)	51·5 (12·5)	259 (36·4%)	26 (3·6%)
Women	750 (1·9%)	50·2 (12·8)	272 (36·3%)	33 (4·4%)
Asian				
Men	736 (1·9%)	50·2 (12·8)	388 (52·7%)	41 (5·6%)
Women	1038 (2·6%)	49·9 (12·8)	566 (54·5%)	81 (7·8%)
Other/mixed†				
Men	814 (2·0%)	51·5 (13·9)	313 (38·5%)	45 (5·5%)
Women	939 (2·4%)	48·8 (14·5)	282 (30·0%)	65 (6·9%)

For whole of USA, census for 2000 gives following percentages for same ethnic groups: white (non-Hispanic) 62·5%; Hispanic 12·5%; African American 12·3%; Asian 3·6%. *4-year college degree or higher. †American Indian, Pacific Islander, mixed race, or unknown.

Table 1: **Demographic characteristics of participants**

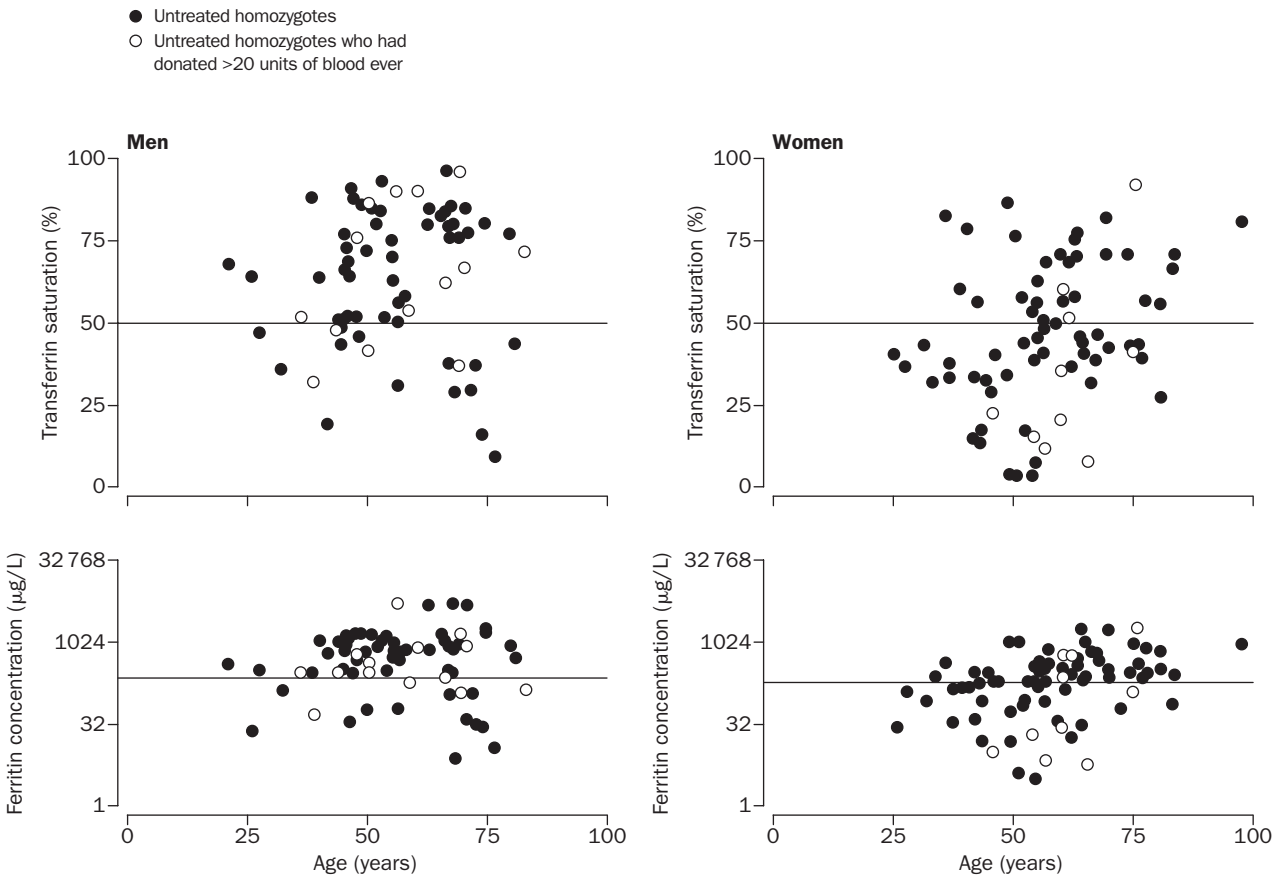

Figure 1: **Transferrin saturation and serum ferritin concentrations of C282Y/C282Y homozygotes**
Horizontal lines=50% transferrin saturation, and ferritin concentrations of 200 μg/L for women and 250 μg/L for men.

they had haemochromatosis might have responded differently from those who were unaware of their condition. Transferrin saturation and ferritin concentration were reported only on the homozygotes and compound heterozygotes for whom data were available before phlebotomy therapy had been initiated. Some of the compound heterozygotes were excluded because of a previous diagnosis of haemochromatosis or lost questionnaires, but 607 of the 616 were included.

The individuals homozygous for the C282Y mutation were almost entirely white (149 patients, including nine who were of Hispanic ancestry and one of white/Hispanic ancestry), with one patient each of mixed white/Asian and white/Native American and Native American ancestry. Their mean age was 56·9 years (SD 14·0). Among the compound heterozygotes (mean age 57·3 years [13·9]) only five did not have some white or Hispanic ancestry. The 22 394 white and Hispanic participants with the wildtype genotype, on whom questionnaire data were available, served as controls (mean age 57·0 years [14·2]).

Serum ferritin and transferrin saturation were all calculated from pretreatment values and are shown in figure 1 and table 2. Among the homozygotes for the C282Y mutation, the pretreatment serum transferrin saturation was greater than 50% in 55 of 73 (75%) men and 31 of 78 (40%) women. Serum ferritin concentrations were greater than 250 μg/L in 55 of 72 (76%) homozygous men and more than 200 μg/L in 43 of 79 (54%) homozygous women. These percentages increased to 76%, 41%, 77%, and 56%, respectively, after frequent blood donors were excluded. These results are similar to those documented at an earlier point in this study when about 10 000 individuals had been examined.[18]

The symptoms and laboratory findings of the participants were compared by use of the criteria summarised in the panel.[19–21] No symptoms were significantly more common in patients with

Sex and genotype	Mean (95% CI) transferrin saturation (%)	Geometric mean (95% CI) serum ferritin concentration (μg/L)
Men		
wt/wt	26·69 (26·53–26·85 [n=12 601])	118 (116–120 [n=12 193])
C282Y/wt	30·63 (30·12–31·14 [n=1603])	122 (117–127 [n=1555])
C282Y/H63D	39·59 (38·12–41·06 [n=300])	191 (171–212 [n=301])
C282Y/C282Y	64·08 (59·27–68·89 [n=73])	395 (287–545 [n=72])
Women		
wt/wt	22·66 (22·51–22·81 [n=13 674])	52 (51–53 [n=13 206])
C282Y/wt	26·77 26·30–27·24 [n=1690])	56 (54–59 [n=1637])
C282Y/H63D	32·05 (30·63–33·47 [n=305])	70 (63–78 [n=301])
C282Y/C282Y	46·45 (41·76–51·14 [n=79])	159 (114–222 [n=79])

wt=wild-type.

Table 2: **Transferrin saturations and serum ferritin concentrations in participants with different genotypes**

Symptoms and laboratory findings most commonly encountered in patients with hereditary haemochromatosis

Symptom	Statements or questions
General health	My health (1) allows full activity or (2) limits activity to some degree
Chronic fatigue	I believe I am more tired or have less energy compared to other people my age
	I currently have severe fatigue, extreme tiredness, or exhaustion
Joint symptoms	I currently have pain or stiffness in my joints on most days
	I have been diagnosed with (1) osteoarthritis or (2) other forms of arthritis
Skin darkening	I have darkening of the skin
Abdominal pain	In the last year I have had recurrent abdominal pain
Impotence	I currently have (1) problems getting an erection; (2) problem with impotence or maintaining an erection during sex; or (3) I am taking Viagra
Depression	I often (1) feel like crying; (2) feel hopeless or down in the dumps; (3) have problems with depression; or (4) feel suicidal
Weight loss	During the last year I have had distinct weight loss
Body-hair loss	I have loss of body hair other than scalp
Arrhythmias	Do you get episodes of fast heart beats or skipped beats?
Diabetes	Non-fasting glucose concentration >6·7 mmol/L*
	I have been diagnosed by a doctor with borderline diabetes
	Are you diabetic?
Liver disorders	I have been diagnosed by a doctor with (1) liver trouble or (2) hepatitis
Increased AST	AST >40 U/L*

Phrases represent exact wording of questionnaire answered by all but 15 patients. Phrasing was very similar in prediagnosis questionnaire completed by 15 patients diagnosed before current study was undertaken. When more than one question or criterion is listed, affirmative answer to any question was regarded as positive response in that category. History of liver disease and increased aspartate aminotransferase (AST) concentration were considered separately because AST values were only available in a small subset of controls. *As assessed by laboratory analysis.

haemochromatosis genotypes than in controls (figure 2). After controlling for age and sex, homozygotes were 2·1 (95% CI 1·1–4·0) and 2·1 (0·9–5·1) times more likely to report liver problems or to have increased aspartate aminotransferase concentrations, respectively, than were wild-type controls. Darkening of the skin was less common in homozygotes than in controls. Homozygotes were only 19·0% (0·5–0·8) as likely to report this symptom as were wild-type controls. Symptoms and laboratory findings were also stratified by sex, serum ferritin concentration, transferrin saturation, and age (table 3). Even the subsets of men, older patients, or patients with higher iron measurements showed no increases in any symptoms or laboratory findings, except for a history of liver disease and aspartate amino-transferase concentrations, compared with controls.

Figure 3 shows the plasma collagen IV concentrations of 124 homozygotes and 117 controls matched for age and sex and ethnic origin (a few control samples were lost for technical reasons). The mean and median collagen IV concentrations in the 25 homozygotes who had been on treatment at the time the plasma was obtained were somewhat higher than those of the 99 who were untreated, but not significantly so, and the results have been pooled in the analysis. There were five obvious outliers among the 117 controls; the mean plasma collagen IV concentrations of the 112 other controls was 120·7 μg/L (SD 33·7). The upper limit of normal was therefore regarded as 187 μg/L. 13 of 117 (11·1%) normal controls exceeded this value, compared with 32 of 124 (25·8%) homozygotes (p=0·005, Fisher's exact test). The difference between the median plasma collagen IV concentration in controls (117·3 μg/L) and homozygotes (138·8 μg/L) was also significant (p=0·001, Mann-Whitney *U* test). Plasma collagen IV concentrations showed a slight increase with increasing age in homozygotes (1·76 μg/L per year [SD 0·85]) and in controls (0·05 μg/L per year [0·55]), but this difference was not significant. There was no significant correlation between plasma collagen IV and ferritin concentrations.

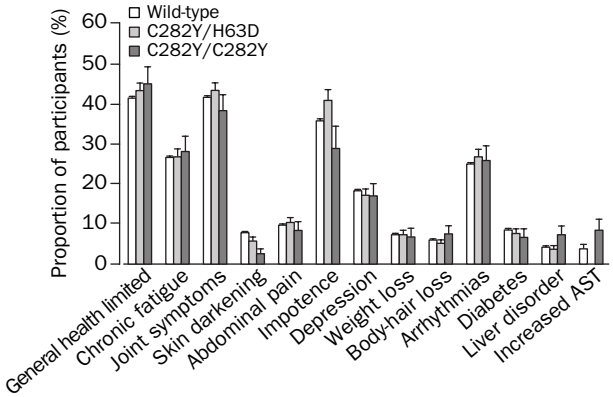

Figure 2: **Frequency of symptoms in individuals of homozygous hereditary haemochromatosis genotype (C282Y/C282Y), of compound heterozygous genotype (C282Y/H63D), and without any *HFE* mutations (wild-type)**
Error bars=SE.

Since haemochromatosis presents as a clinical syndrome, we assessed the frequency of multiple signs and symptoms of the disorder, choosing those manifestations that have been most frequently associated with the full-blown disease (ie, general health limited to some degree, chronic fatigue, joint symptoms, skin darkening, impotence, diabetes, arrythmias, and aspartate aminotransferase concentation >40 U/L; figure 4). The number of symptoms reported by C282Y individuals and controls did not differ significantly.

The age distributions of screened individuals with the C282Y/C282Y and C282Y/H63D genotype and the controls were compared by means of a quantile-quantile plot (figure 5). There was close agreement between the age distribution of those with mutant genotypes and the wild-type controls. Although the slight displacement of male homozygotes towards younger ages was not significant with the 73 individuals examined, we expect that it would be significant in a larger number of

	All	Men	Women	High ferritin concentration*	Transferrin saturation >50%	Age >55 years
General health limited to some degree						
Controls	9261/22 347 (41·4%)	4206/10 889 (38·6%)	5055/11 458 (44·1%)	812/1855 (43·8%)	88/232 (37·9%)	5654/12 270 (46·1%)
C282Y/H63D	255/594 (42·9%)	127/292 (43·5%)	128/301 (42·5%)	65/137 (47·4%)	32/68 (47·1%)	153/325 (47·1%)
C282Y/C282Y	52/124 (41·9%)	21/56 (37·5%)	31/68 (45·6%)	39/80 (48·8%)	33/69 (47·8%)	33/60 (55·0%)
Fatigue						
Controls	5923/22 347 (26·5%)	2286/10 889 (21·0%)	3637/11 458 (31·7%)	492/1855 (26·5%)	58/232 (25·0%)	2485/12 270 (20·3%)
C282Y/H63D	157/594 (26·4%)	62/292 (21·2%)	95/301 (31·6%)	35/137 (25·5%)	14/68 (20·6%)	67/325 (20·6%)
C282Y/C282Y	34/124 (27·4%)	12/56 (21·4%)	22/68 (32·4%)	25/80 (31·3%)	20/69 (29·0%)	19/60 (31·7%)
Arthropathy						
Controls	9299/22 347 (41·6%)	3975/10 889 (36·5%)	5324/11 458 (46·5%)	770/1855 (41·5%)	80/232 (34·5%)	6300/12 270 (51·3%)
C282Y/H63D	255/594 (42·9%)	112/292 (38·4%)	143/301 (47·5%)	60/137 (43·8%)	25/68 (36·8%)	165/325 (50·8%)
C282Y/C282Y	45/124 (36·3%)	16/56 (28·6%)	29/68 (42·6%)	36/80 (45·0%)	28/69 (40·6%)	31/60 (51·7%)
Darkening of skin						
Controls	1701/22 347 (7·6%)	521/10 889 (4·8%)	1180/11 458 (10·3%)	118/1855 (6·4%)	16/232 (6·9%)	943/12 270 (7·7%)
C282Y/H63D	30/594 (5·1%)	10/292 (3·4%)	20/301 (6·6%)	6/137 (4·4%)	1/68 (1·5%)	18/325 (5·5%)
C282Y/C282Y	2/124 (1·6%)	2/56 (3·6%)	0/68	2/80 (2·5%)	2/69 (2·9%)	2/60 (3·3%)
Abdominal pain						
Controls	2144/22 347 (9·6%)	719/10 889 (6·6%)	1425/11 458 (12·4%)	141/1855 (7·6%)	16/232 (6·9%)	1073/12 270 (8·7%)
C282Y/H63D	60/594 (10·1%)	18/292 (6·2%)	42/301 (14·0%)	9/137 (6·6%)	6/68 (8·8%)	26/325 (8·0%)
C282Y/C282Y	10/124 (8·1%)	4/56 (7·1%)	6/68 (8·8%)	7/80 (8·8%)	4/69 (5·8%)	5/60 (8·3%)
Impotence						
Controls	3904/10 889 (35·9%)	3904/10 889 (35·9%)	..	508/1381 (36·8%)	54/163 (33·1%)	3224/6157 (52·4%)
C282Y/H63D	118/292 (40·4%)	118/292 (40·4%)	..	44/101 (43·6%)	15/46 (32·6%)	96/164 (58·5%)
C282Y/C282Y	15/56 (26·8%)	15/56 (26·8%)	..	12/44 (27·3%)	14/44 (31·8%)	12/24 (50·0%)
Depression						
Controls	4066/22 347 (18·2%)	1228/10 889 (11·3%)	2838/11 458 (24·8%)	262/1855 (14·1%)	34/232 (14·7%)	1756/12 270 (14·3%)
C282Y/H63D	100/594 (16·8%)	32/292 (11·0%)	68/301 (22·6%)	14/137 (10·2%)	14/68 (20·6%)	46/325 (14·2%)
C282Y/C282Y	22/124 (17·7%)	5/56 (8·9%)	17/68 (25·0%)	15/80 (18·8%)	9/69 (13·0%)	9/60 (15·0%)
Weight loss						
Controls	1638/22 347 (7·3%)	774/10 889 (7·1%)	864/11 458 (7·5%)	155/1855 (8·4%)	18/232 (7·8%)	888/12 270 (7·2%)
C282Y/H63D	42/594 (7·1%)	17/292 (5·8%)	25/301 (8·3%)	10/137 (7·3%)	8/68 (11·8%)	23/325 (7·1%)
C282Y/C282Y	8/124 (6·5%)	2/56 (2·0%)	6/68 (8·8%)	6/80 (7·5%)	4/69 (5·8%)	3/60 (5·0%)
Hair loss						
Controls	1324/22 347 (5·9%)	381/10 889 (3·5%)	943/11 458 (8·2%)	111/1855 (6·0%)	7/232 (3·0%)	1064/12 270 (8·7%)
C282Y/H63D	29/594 (4·9%)	127/292 (1·0%)	26/301 (8·6%)	3/137 (2·2%)	2/68 (2·9%)	24/325 (7·4%)
C282Y/C282Y	9/124 (7·3%)	3/56 (5·4%)	6/68 (8·8%)	5/80 (6·3%)	6/69 (8·7%)	8/60 (13·3%)
Arrhythmias						
Controls	5599/22 347 (25·1%)	1869/10 889 (17·2%)	3730/11 458 (32·6%)	375/1855 (20·2%)	47/232 (20·3%)	3143/12 270 (25·6%)
C282Y/H63D	156/594 (26·3%)	53/292 (18·2%)	103/301 (34·2%)	33/137 (24·1%)	16/68 (23·5%)	88/325 (27·1%)
C282Y/C282Y	31/124 (25·0%)	8/56 (14·3%)	23/68 (33·8%)	19/80 (23·8%)	16/69 (23·2%)	15/60 (25·0%)
Diabetes						
Controls	1888/22 347 (8·4%)	1045/10 889 (9·6%)	843/11 458 (7·4%)	304/1855 (16·4%)	25/232 (10·8%)	1356/12 270 (11·1%)
C282Y/H63D	44/594 (7·4%)	29/292 (9·9%)	15/301 (5·0%)	12/137 (8·8%)	2/68 (2·9%)	32/325 (9·8%)
C282Y/C282Y	7/124 (5·6%)	1/56 (1·8%)	6/68 (8·8%)	5/80 (6·3%)	3/69 (4·3%)	5/60 (8·3%)
Liver problem or hepatitis						
Controls	926/22 347 (4·1%)	534/10 889 (4·9%)	392/11 458 (3·4%)	113/1855 (6·1%)	16/232 (6·9%)	12/12 270 (3·7%)
C282Y/H63D	22/594 (3·7%)	11/292 (3·8%)	11/301 (3·7%)	6/137 (4·4%)	4/68 (5·9%)	12/325 (3·7%)
C282Y/C282Y	10/124 (8·1%)	4/56 (7·1%)	6/68 (8·8%)	4/80 (5·0%)	5/69 (7·2%)	4/60 (6·7%)
AST >40 U/L						
Controls	13/344 (3·8%)	8/173 (4·6%)	5/171 (2·9%)	5/38 (13·2%)	1/3 (33·3%)	7/224 (3·1%)
C282Y/H63D	0/8	0/4	0/4	0/3	0/1	0/5
C282Y/C282Y	10/122 (8·2%)	4/54 (7·4%)	6/68 (8·8%)	7/78 (9·0%)	5/68 (7·4%)	6/60 (10·0%)

AST=aspartate aminotransferase. Controls were 22 394 participants who identified their ethnic origin as white, Hispanic, or white and Hispanic, and whose genotype was wild-type. Numerator of each proportion is the number of patients with a positive response or finding as defined in the panel. Denominator is the number of people for whom the data were available. *>200 μg/L for women, and >250 μg/L for men.

Table 3: **Effect of sex, ferritin concentration, transferrin saturation, and age on findings in homozygotes and compound heterozygotes for *HFE* mutations**

individuals, since severe haemochromatosis is sometimes life-threatening. Calculation of the Hardy-Weinberg equilibrium of individuals who identified themselves as "white only" revealed that the gene frequency for the C282Y allele in this group of 30 672 individuals was 0·0622, predicting a homozygote frequency $3{\cdot}872\times10^{-3}$. The actual homozygote frequency was $4{\cdot}60\times10^{-3}$ in women and $4{\cdot}46\times10^{-3}$ in men, indicating no selective loss of homozygotes from the population. The slightly higher than predicted prevalence of homozygotes might be due to the greater tendency for individuals from the same geographic area to mate, but similar results were obtained when the analysis was limited to people of northern European ancestry.

Discussion

We found that the symptoms commonly associated with haemochromatosis were not significantly more prevalent among homozygotes or compound heterozygotes than among controls. This finding was true even among subsets of older patients or of patients with raised ferritin concentrations. There was no significant aggregation of symptoms associated with hereditary

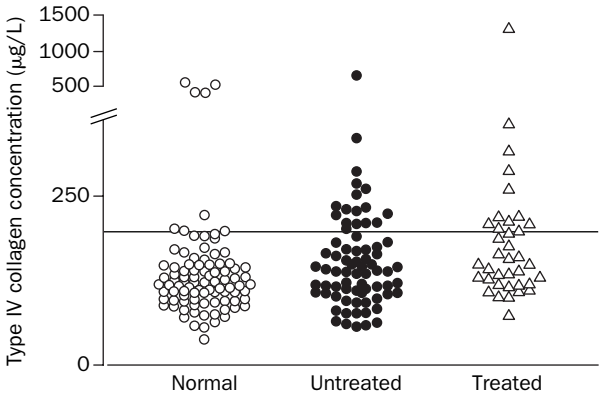

Figure 3: **Plasma collagen IV concentrations of 124 homozygotes for the C282Y *HFE* mutation and 117 normal controls**

"Untreated" represents homozygotes whose plasma collagen IV concentrations were measured before therapy had started; "treated" represents homozygotes on whom at least one phlebotomy had been done when plasma was obtained for collagen IV assay. Horizontal line=upper limit of normal, derived from control population.

haemochromatosis in C282Y homozygotes, even when stratified by age or biochemical measurements of iron load. Only a history of hepatitis or other liver disorders was significantly more frequent in homozygotes for the C282Y mutation than in wild-type controls. No such effect was seen in the compound heterozygotes.

By contrast with ourselves, some have assumed that virtually all patients with the C282Y/C282Y genotype develop symptoms of the disease at some point. For example, Edwards and colleagues[22] wrote: "Although the time required to become iron loaded is variable, it is clear that most homozygotes will eventually become symptomatic"; a meta-analysis of data from seven studies concluded that clinical manifestations were present in 50% of male and 44% of female homozygotes;[23] and, although readily acknowledging the need for further studies, an expert panel[20] held that 95% of patients over 45 years of age and homozygous for the C282Y mutation have significant morbidity from haemochromatosis.

Two studies attempted to estimate the prevalence of symptoms in homozygotes for the C282Y mutation. Olynyk and colleagues[24] reported that "eight of the 16 homozygous subjects had clinical findings that were

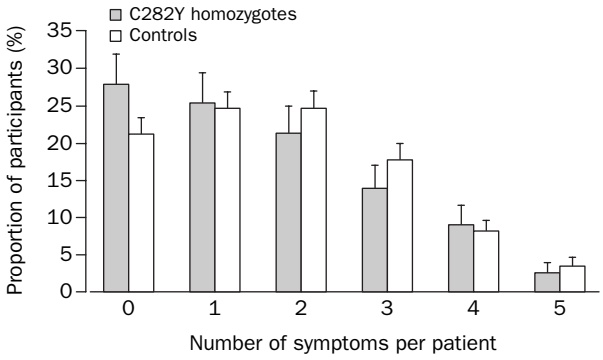

Figure 4: **Frequency of multiple symptoms or signs of haemochromatosis in C282Y homozygotes and controls**

Symptom categories were: general health limited to some degree, chronic fatigue, joint symptoms, skin darkening, impotence, diabetes, arrythmias, and aspartate aminotransferase concentration >40 U/L. Included are 112 C282Y homozygotes and 344 white (including Hispanic) controls on whom data were available on all categories.

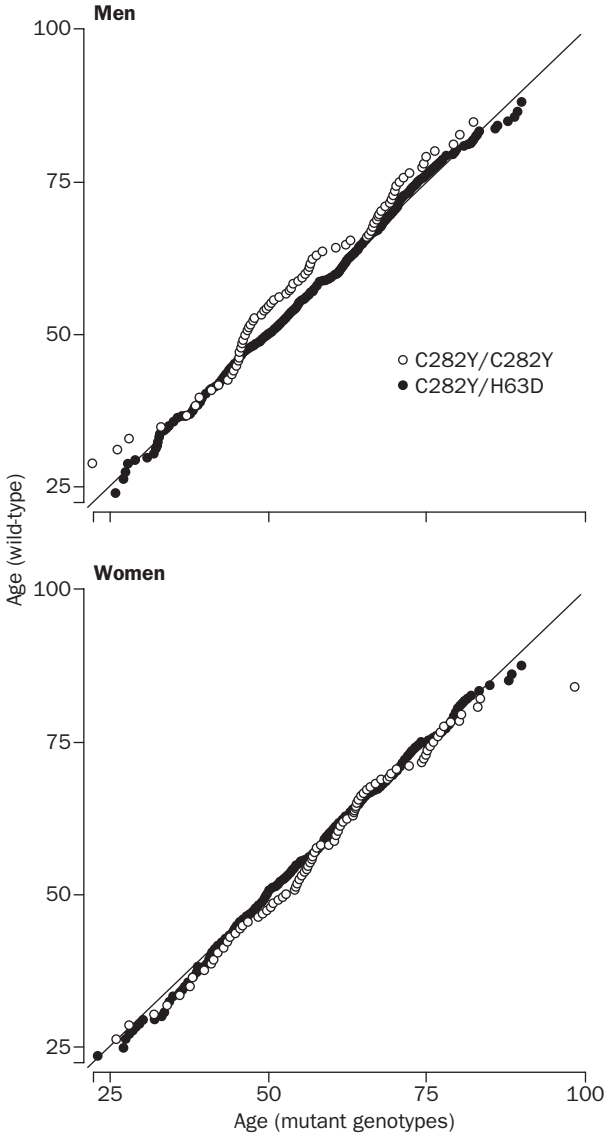

Figure 5: **Quantile-quantile plots of sample age distributions of C282Y homozygotes and compound heterozygotes versus wild-type**

Plot displays individual quantiles (percentiles) of one sample *vs* corresponding quantiles of second sample. Approximates to the straight diagonal line shown if samples follow same distribution.

consistent with the presence of hereditary haemochromatosis, such as hepatomegaly, skin pigmentation, and arthritis". Of key importance is the fact that the prevalence of these findings in matched controls was not reported. Thus, arthritis was reported in six (38%) of 16 patients and implicitly attributed to the iron-storage disease. This figure is similar to the 35·6% of individuals with joint symptoms found in our study, but 42·8% of our wild-type controls reported the same symptoms. Bulaj and colleagues[25] reported that 52% of men older than 52 years, and 16% of women older than 50 years with the homozygous genotype had at least one "disease-related condition". However, their data are also difficult to interpret because of the lack of any control group. Moreover, most of the patients had been enrolled in the study because they had relatives with haemochromatosis. Even if the frequency of symptoms in this population were higher than that in the general population, mutations in other genes are likely

to be important in determining whether clinical expression will occur, and the presence of such mutations is much more probable in relatives of patients who have been recruited because of clinical expression of the disease.

By contrast with publications that attribute high penetrance to the homozygous state, Willis and colleagues[26] found four men (0·67%) who were homozygous for the C282Y mutation among 600 men aged 70–97 years. This frequency was higher than the 0·44% predicted from the normal population, implying, in agreement with our observations, that people homozygous for the C282Y mutation were not removed from the population by early death. None of the four homozygotes had signs and symptoms of haemochromatosis. In another study,[27] only five of 190 biopsy samples from patients with cirrhosis were from homozygotes for the C282Y mutation; a penetrance for cirrhosis of only 2·5% was calculated on the basis of the number of homozygotes in the overall population.

Hepatic fibrosis has sometimes been detected on liver biopsy of symptom-free homozygotes. We did not feel justified in doing biopsies on our patients, and therefore used surrogates to assess the probability of liver disease. According to one large study,[28] only haemochromatosis patients with serum ferritin concentrations of more than 1000 μg/L and with raised aspartate aminotransferase concentrations develop severe fibrosis of the liver. In our study, only three of the 119 homozygotes for whom both aspartate aminotransferase and ferritin measurements were available fulfilled these criteria. One of these patients, an alcoholic, also had diabetes, heart failure, and possibly darkening of the skin. He is the only one of the 152 homozygotes who would be regarded by most clinicians as having fully manifest clinical haemochromatosis. However, even the frequency of one in 152 is a somewhat upward-biased estimate of the prevalence of fully manifest haemochromatosis; the frequency of these clinical findings in alcoholic controls is unknown, since aspartate aminotransferase concentrations were measured in only a small subset of individuals with the wildtype genotype.

Plasma collagen IV concentrations are an excellent surrogate for even mild hepatic fibrosis in patients with haemochromatosis.[16] The 25·8% of patients with raised collagen IV concentrations is similar to the 19% (three of 16, or four of 16 [25%] including one patient with alcoholism and cirrhosis) of patients that Olynyk and colleagues[24] found to have hepatic fibrosis by biopsy. Only two of our 32 homozygotes with increased collagen IV concentrations had an increased serum aspartate aminotransferase concentration. One was the patient alluded to above with a greatly increased ferritin concentration of 4555 μg/L, and the other had a serum ferritin concentration only modestly raised at 334 μg/L. Untreated homozygotes having increased collagen IV concentrations had a geometric mean ferritin concentration of 297 μg/L, whereas those with normal collagen IV concentrations averaged 189 μg/L.

Patients are sometimes assumed to have died of haemochromatosis without the diagnosis ever being made. Although some such deaths undoubtedly occur, the accumulating epidemiological evidence suggests that they do not happen as commonly as proposed. Necropsy studies have also failed to confirm this view,[29,30] and the prevalence of homozygotes in the elderly population seems to be similar to that in the young in the present study and in those of others.[26] If, in fact, patients with the homozygous state died prematurely, the prevalence of such patients should progressively decrease in the population, and there should be fewer homozygotes than predicted by the Hardy-Weinberg equilibrium. We saw neither of these predictions.

The decision about the appropriateness of genetic screening depends on the prevalence in the population of the genotype being detected, the extent to which the disease can be modified by intervention, and the proportion of patients with the genotype who are clinically affected. The latter consideration is particularly important, and the increase in genotyping of patients with genetic diseases has revealed that patients with the same genotypes often differ markedly with respect to disease expression.[31] For example, some patients with sickle-cell disease can survive in good health into their 70s,[32] and only about half of patients with the most common 1226G/1226G Gaucher's disease genotype are thought to come to medical notice.[33]

Hereditary haemochromatosis fits the prevalence and success of intervention criteria for genetic screening, but what about the penetrance? The only clinical abnormalities we found in screened patients who were homozygous for the C282Y *HFE* mutation were minor increases in liver-function tests, and a history of liver disease in less than 10% of the patients. Since the prevalence of these manifestations did not increase with age, they are probably not progressive or only very slightly so. Moreover, our inability to detect any decrease in the prevalence of the homozygous or compound heterozygous state with increasing age and the good fit of the data to the Hardy-Weinberg equilibrium strongly indicates that the liver abnormalities detected had little or no effect on survival.

Only one of the 152 homozygotes fitted the criteria usually applied for the clinical diagnosis of haemochromatosis. A similarly low penetrance was suggested by the necropsy studies of cirrhotic patients reported by Willis and colleagues.[27]

Severe haemochromatosis is a potentially fatal disease that responds well to phlebotomy. Physicians have an obligation to detect this disorder and to treat it, since logically, early diagnosis and treatment make it a preventable disease. Increased iron stores due to haemochromatosis-associated mutations are far more common than had been believed many years ago, and the high prevalence of haemochromatosis mutations was confirmed after cloning of the *HFE* gene. However, in the enthusiasm over this accumulating information, there has been a tendency to equate genotype with phenotype and to regard every homozygote as a patient with haemochromatosis. The results of our study suggest that screening for hereditary haemochromatosis, either by genetic testing or by measuring transferrin saturation, will be more costly per clinical case treated than had been realised. Mutations of the *HFE* gene are a necessary but not sufficient cause for fully developed haemochromatosis. What other mutations or environmental factors are required for the penetrance of haemochromatosis in patients with *HFE* mutations remain to be determined. These factors could differ from population to population, and penetrance could be higher in some populations than others. This possibility needs to be established in a controlled study in which morbidity and mortality of individuals with the wild-type genotype is taken into account.

The same principles that apply to screening for haemochromatosis must also be applied to other genetic disorders for which screening is contemplated.

Frequency of the genotype and treatability of the disorder is not enough. A high proportion of genotypically affected individuals must also manifest the disease phenotype. Controlled studies will always be essential to distinguish symptoms associated with a given genotype from those actually caused by it.

Contributors

Ernest Beutler designed the study, analysed the data, wrote the paper, and obtained funding. Vincent Felitti organised the acquisition of clinical data and critically assessed the paper. James Koziol and Ngoc J Ho designed and maintained the database and analysed the data. Terri Gelbart developed the laboratory methodology and supervised the mutation analysis and collagen IV analysis.

Conflict of interest statement

None declared.

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