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The penetrance of hereditary hemochromatosis

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Since its original description as a rare disease of iron overload resulting in liver disease, diabetes mellitus, and bronzing of the skin ('bronze diabetes'), hereditary hemochromatosis has undergone several redefinitions leading to widely varying estimates of its prevalence. Over the last decade, the finding of a relatively high prevalence of the C282Y polymorphism of the *HFE* gene associated with hemochromatosis in Northern European populations suggested that the disease may be much more common than previously thought. However, several large population-based studies have now shown that the penetrance of the C282Y/C282Y genotype is very low, indicating that C282Y homozygosity is a necessary but not sufficient factor in causation of the disease. Studies are now focusing on other genetic and environmental factors, including alcohol, that may contribute to differential expression of C282Y homozygosity.

Key words: iron metabolism; hemochromatosis; HFE; C282Y; H63D.

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HISTORICAL PERSPECTIVE

In its original description as a 'classic triad' of cirrhosis of the liver, diabetes mellitus, and bronzing of the skin, hemochromatosis was understood to be rare.[1] Developments in the 1980s leading to new methods of defining and identifying the disease challenged this concept. During this time, the disease was linked to human leukocyte antigen A (HLA-A) and HLA-B. In addition, means of estimating the state of iron homeostasis through measurement of serum iron, iron-binding capacity, and serum ferritin became widely available. Screening for the disease based on elevated levels of serum iron, indicators of transferrin saturation (at thresholds >45–60%) and serum ferritin (>200 μg/l), led to much higher estimates of prevalence, with estimates of 2.4, 5.6, and 8.3 per thousand reported in surveys based on transferrin saturation levels.[2]

With the discovery of the *HFE* gene in 1996, the disease underwent further redefinition. The c.845G$>$A/c.845G$>$A (C282Y/C282Y) genotype occurred in more than 80% of Northern European patients previously diagnosed with hemochromatosis, and the c.845G$>$A/c.387C$>$G (C282Y/H63D) compound heterozygous genotype accounted for many of the rest. This raised the possibility of diagnosing the disease by screening for *HFE* polymorphisms.[3] The C282Y polymorphism was found to be highly prevalent in Caucasian populations, with an allele frequency of 10–15%, the C282Y homozygous genotype occurring in approximately five in 1000 persons. Thus, based on genetic diagnosis, prevalence of hemochromatosis was estimated to be 0.5%. Defined either by the genotype or even on the basis of an elevated transferrin saturation and increased serum ferritin, hemochromatosis was considered to be the most common autosomal recessive disorder in populations of Northern European origin.[4]

In the past few years, the understanding of the prevalence of hemochromatosis has undergone yet another revision. The editorial accompanying the issue when iron disorders, including hemochromatosis, were last reviewed in this journal in 2002, noted that 'hemochromatosis is now considered rare again', an observation based on several large studies involving *HFE* genotyping of non-clinically selected populations and the finding that most C282Y homozygotes had no symptoms of disease.[5]

In the intervening years, several additional studies of this type have yielded similar results, providing further evidence that the penetrance of the C282Y homozygous genotype is low.[6,7] In this article, we will briefly summarize the earlier studies and review the new evidence on penetrance of the C282Y genotype as well as current research on other genetic and environmental factors that may influence the expression of the C282Y/C282Y genotype. We also review what is known about the effects of other HFE genotypes on iron metabolism and their possible disease relationships. The review is limited to studies defining hereditary hemochromatosis with *HFE* polymorphisms. We do not discuss the penetrance of the less common forms of iron storage disease associated with other mutations or polymorphisms of iron-regulatory genes, such as ferroportin-1, hemojuvelin, hepcidin, transferrin receptor-2, or ceruloplasmin.[8]

THE PENETRANCE OF THE HOMOZYGOUS C282Y/C282Y GENOTYPE

Biochemical phenotype of C282Y homozygotes

Studies involving genotyping of large populations have consistently shown significantly higher mean transferrin saturation and serum ferritin levels among C282Y

Table 1. Transferrin saturation and serum ferritin levels by *HFE* genotype and sex in 32 820 Caucasians.

Genotype	Men		Women	
	Mean (95% CI) transferrin saturation (%)	Geometric mean (95% CI) serum ferritin (μg/l)	Mean (95% CI) transferrin saturation (%)	Geometric mean (95% CI) serum ferritin(μg/l)
C282Y/C282Y	65.3 (60.6, 70.0)	420 (308, 573)	47.1 (42.1, 52.1)	161 (114, 228)
C282Y/H63D	39.2 (37.7, 40.7)	185 (168, 204)	32.3 (30.8, 33.8)	71 (63, 79)
H63D/H63D	33.7 (32.6, 34.8)	138 (127, 151)	29.1 (28.0, 30.2)	60 (54, 66)
C282Y/wt	30.6 (30.1, 31.1)	118 (113, 123)	26.9 (26.4, 27.4)	57 (55, 59)
H63D/wt	29.3 (29.0, 29.6)	117 (114, 120)	24.8 (24.5, 25.1)	55 (53, 56)
wt/wt	26.7 (26.5, 26.9)	111 (109, 113)	22.8 (22.6, 23.0)	53 (52, 54)

Adapted from Waalen et al (2002, *Blood Cells, Molecules, and Diseases* 29: 418–432) with permission.

homozygotes compared to sex-matched subjects of other *HFE* genotypes (Table 1). As in wild-type subjects, mean levels of both serum iron indicators are higher in male homozygotes compared with female homozygotes. Although cut-off values have varied across studies, the majority of individual C282Y homozygotes in these surveys had elevated transferrin saturation and serum ferritin levels (Table 2). In most studies, elevated transferrin saturation is the more frequent finding.

The effect of age on parameters of iron homeostasis in C282Y homozygotes compared with wild-type controls has been characterized in a study of 9174 subjects who were genotyped for *HFE* polymorphisms, including 23 C282Y homozygotes.[6]

Table 2. Biochemical penetrance of hemochromatosis: the frequency of elevated iron indices among C282Y homozygotes.

Study	Men			Women		
	n[a]	Elevated transferrin saturation[b] (%)	Elevated serum ferritin[c] (%)	*n*[a]	Elevated transferrin saturation[d]	Elevated serum ferritin[e]
Beutler et al[11,33]	73	75	76	78	40%	54%
Jackson et al[38]	29	86	34	40	45%	22%
Deugnier et al[12]	10	80	70	44	41%	33%
Andersen et al[6]	7	71	81	16	88%	63%
Chambers et al[36]	18	78	56	–[f]	–[f]	–[f]
Olynyk et al[9]	7	86	71	9	100%	33%
Adams et al[67]	5	60	60	11	55%	9%
Phatak et al[34]	4	75	100	8	100%	50%

[a] Sample sizes for some studies differ from those reported in table due to different selection criteria for measurement of serum iron indices and for ascertainment of symptoms.

[b] >50% except in studies by Deugnier et al (>55%) and Phatak et al (>45%).

[c] >250 μg/l except in studies by Jackson et al (>210 μg/l), Deugnier et al (>280 μg/l), Chambers et al (228 μg/l) and Phatak et al (200 μg/l).

[d] >50% except in study by Phatak et al (>45%).

[e] 200 μg/l except in studies by Jackson et al (>130 μg/l) and Deugnier et al (>130 μg/l).

[f] Study did not include women.

Subjects aged 25–85 years at baseline had been followed for up to 25 years as a part of the Copenhagen City Heart Study with determinations of serum transferrin saturation and ferritin levels made at up to four time points during follow-up. Among C282Y homozygotes, mean transferrin saturation levels increased from 50 to 70% in women aged 25–85 years, and from 70 to 80% in men aged 35–80 years. No increase in mean transferrin saturation occurred among wild-type controls matched with the C282Y homozygotes for age, sex, and alcohol consumption (Figure 1). Transferrin saturation levels were noted to have oscillated over time to a greater extent in C282Y homozygotes than in compound heterozygotes (C282Y/H63D) or wild-type controls. In women, ferritin levels increased with age in both C282Y homozygotes and wild-type

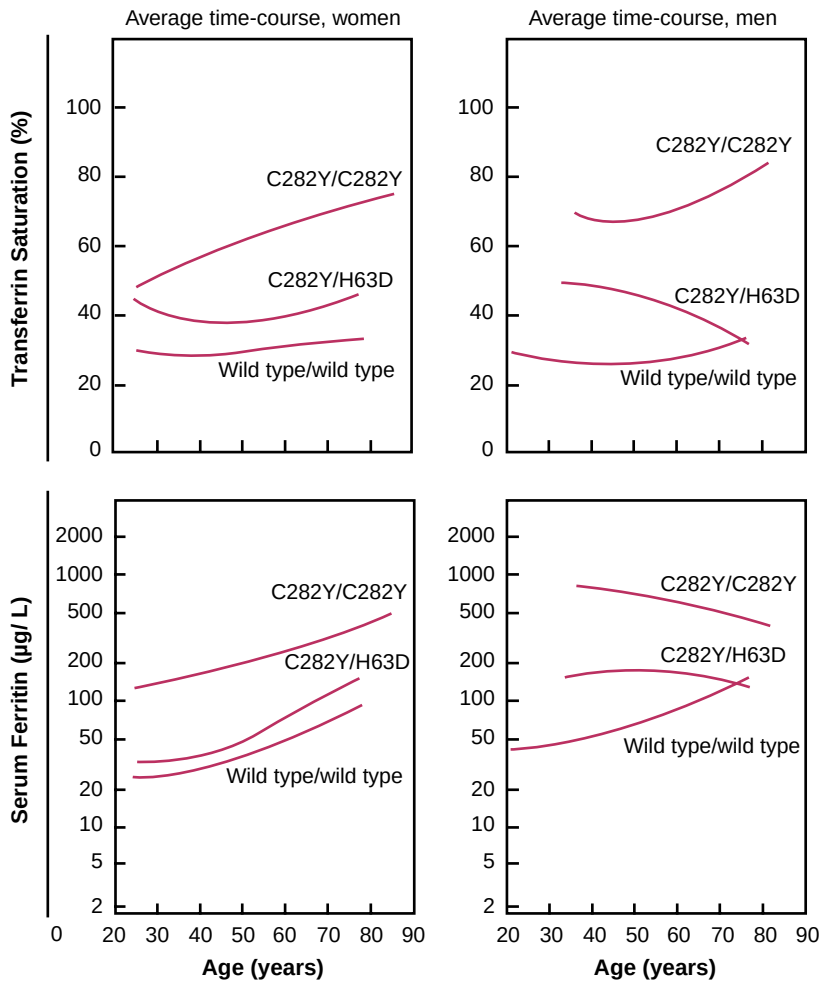

Figure 1. Levels of transferrin saturation and ferritin in selected genotypes over a follow-up period of up to 25 years, shown as a function of age at measurement. Twenty-three C282Y homozygotes were each matched for sex, age, and alcohol consumption, with two persons each of wild type/wild type and C282Y/H63D genotypes. Panels show local linear regression curves for women and men separately. From Andersen et al (2004, *Blood* 103: 2914–2919) with permission.

controls. In men, serum ferritin decreased with age in C282Y homozygotes while increasing in wild-type controls (Figure 1). This surprising result was attributed in part to very high serum ferritin values in several of the younger men. When changes were examined by time of follow up rather than age, a slight increase in serum ferritin over time was also observed in men. Reports by Olynyk et al. have also demonstrated a tendency for increases in transferrin saturation with marked variation in serum ferritin levels in C282Y homozygotes over time.[9,10]

Clinical phenotype of C282Y homozygotes

Description of the phenotype of the C282Y homozygous state in regard to symptoms are provided by studies identifying and clinically assessing relatively large cohorts of C282Y homozygotes. However, estimates of the penetrance of the C282Y/C282Y genotype can only be achieved by screening of populations not selected a priori for presence of symptoms or elevated iron levels. Although many such populations have been genotyped for *HFE* polymorphisms, relatively few studies have been of sufficient size to identify more than ten C282Y homozygotes. Because many of the symptoms associated with hemochromatosis and iron overload are non-specific and common, it is essential that the presence or absence of findings be established before the results of genotyping are known by either the patient or the examiner. The use of a comparison group is also essential in determining the burden of these diseases attributable to *HFE* polymorphisms. Control groups have been included in several of the most recent surveys (Table 3).

Symptoms

Overall, prevalence of non-specific symptoms varies appreciably between studies. The variability reflects the difficulty in ascertaining precise estimates of symptoms of a subjective nature such as fatigue and joint pain, and underscores the importance of ascertaining the prevalence of the same symptoms by the same methods in control groups.

Presence of symptoms in homozygotes does not appear to be related to levels of the serum iron, transferrin saturation, or ferritin in studies examining this relationship. In the Kaiser–Scripps study, we found no difference in the prevalence of symptoms among homozygotes with either elevated transferrin saturation or serum ferritin levels compared to those with normal levels.[11] Åsberg similarly reported that serum ferritin levels did not predict the presence of any of the conditions tested, except liver fibrosis.[7]

In addition, the studies of homozygotes identified from general populations have provided no evidence of an effect of age on expression of symptoms. In the Kaiser–Scripps study, homozygotes older than 55 years did not have significantly more symptoms than age-matched controls.[11] Additionally, in a study of younger female C282Y homozygotes, Deugnier et al found no significant difference in age between those who reported iron-overload-related symptoms and those who did not.[12]

General health

Attempts to assess the overall health status of homozygotes have shown no statistically significant differences between C282Y homozygotes and controls, whether defined in terms of 'limited health'[11] or 'very good or good health'.[7]

Table 3. Clinical penetrance of hemochromatosis. Clinical findings in C282Y homozygotes (and controls) identified from general populations.

Study	Source of population genotyped	*n*[a]	Mean age ± SD (y)	Included previously diagnosed subjects (*n*)	Fatigue	Dark skin	Joint pain	Elevated liver enzymes	Diabetes (%)
Åsberg et al[7]	Nord Trondelag (Norway) Health Study	297[b]	49 (median)	No	–[c]	NR	–[c]	NR	3
	Controls	64 224	NR		–[c]	NR	–[c]	NR	3
Beutler et al[11,33]	Health appraisal clinic in Southern California, USA	145	57 ± 14	Yes (25)[d]	26%	2%	43%	9% (AST)	8
	Controls	20 576			27%	7%	42%	4% (AST)	11
Deugnier et al[12]	Health appraisal clinic in France	54	Men: 25–40 y, Women: 35–50 y	No	38%	NR	18%	6% (ALT)	2
	Controls	9342			36%	NR	5%	5% (ALT)	1
Andersen et al[6]	Copenhagen City Heart Study in Denmark	23	56 ± 14	Yes (1)[e]	57%	0%	30%	4% (AST)	9
	Controls	6134	57 ± 15		41%	NR	28%	3% (AST)	4
Olynyk et al[9]	Busselton (Australia) Study	16	45 ± 13	Yes (4)	NR	38%	38%	NR	0
Phatak et al[34]	Primary care clinics in New York State, USA	12	57 ± 20	No[f]	NR	NR	50%	NR	0

SD, standard deviation; NR, not reported; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

[a] Sample sizes for some studies differ from those reported in Table 2 due to different selection criteria for measurement of serum iron indices and for ascertainment of symptoms.

[b] Genotyping was carried out only in subjects found to have elevated serum transferrin saturation and ferritin levels. This number therefore presumably represents only the 50–66% most affected subjects.

[c] Several different questions related to specific aspects of this symptom were included in the study. No single measure is available for comparison with other studies.

[d] Results were similar when previously diagnosed homozygotes were removed from the analysis.[11]

[e] Diagnosed by elevated serum ferritin levels and was asymptomatic.

[f] Clinical evaluation was performed after determination of *HFE* genotype.

Diabetes mellitus

Diabetes, a diagnosis based on relatively standard criteria, is consistently shown to occur at a low rate among C282Y homozygotes across studies. In the four studies with a control group, differences in prevalence between homozygotes and controls were not statistically significant.[6,7,11,12] It is notable that this is remarkably different from the classical 1935 description of the disease by Sheldon[1], in which he averred that 78% of the reported patients had diabetes. This discrepancy re-emphasizes the difference between the classical clinical disease and the disease as defined by biochemical or genetic markers alone.

Joint involvement

The prevalence of joint pain, a more subjective clinical finding, was more variable across studies and occurred at a relatively high prevalence ($>30\%$) in most (Table 3). In two studies with controls, however, joint pain occurred at an equally high rate among C282Y homozygotes and controls[6,11]; in another, involving a younger population, distal arthralgias were reported more frequently by homozygotes among both men (20 versus 4%, $P<0.05$) and women (17.5 versus 6%, $P<0.03$).[12] Of the 17 questions about joint pain included in the study by Åsberg et al, statistically significant differences were found for only two, and only among numerous specific subgroups, making the results difficult to interpret given the problem with multiple comparisons.[7]

Despite the lack of evidence that arthritis occurs at a higher rate among C282Y homozygotes, its relationship with iron overload continues to be of interest, given the high prevalence of arthritis in the juvenile form of hemochromatosis associated with mutations of the gene encoding hemojuvelin on chromosome 1q.[13]

Fatigue

Prevalence of fatigue exhibited even greater differences between studies, likely due in part to variability in defining this symptom. In all three studies with controls, however, the prevalence of fatigue was not significantly different in C282Y homozygotes than in controls.[6,11,12] In the study by Deugnier et al, a significantly greater prevalence of fatigue was reported among male C282Y homozygotes but not in females homozygotes compared to sex-specific controls.[12] However, there were only ten males in this study, seven of whom complained of fatigue; fatigue was actually less common in homozygous women than in controls. The results were not statistically significant when corrected for multiple comparisons. The results from the Kaiser–Scripps study comparing symptoms in men and women separately showed no sex-specific differences in fatigue.[11]

Skin pigmentation

Darkening of skin, another subjective finding, was reported at a high rate (6/16, 38%) in only one uncontrolled study in which both patients and examiners were aware of the diagnosis.[9] In the Kaiser–Scripps study, increased skin pigmentation was reported at a higher rate in controls.[11]

Hepatic dysfunction

Effects on the liver have also been variably defined between studies. In the Kaiser–Scripps investigation, elevated aspartate aminotransferase (AST), increased levels of plasma collagen IV (a surrogate for hepatic fibrosis), and self-reported 'liver problems' were the only conditions found to occur at a significantly higher rate among C282Y homozygotes compared with controls.[11] Likewise, Åsberg et al reported significantly high levels of alanine aminotransferase (ALT) in homozygotes.[7] Other studies, however, did not find elevated AST[6] or ALT.[12]

Pathologic findings on liver biopsy of clinically unselected C282Y homozygotes have been reported in only two studies. Both reported relatively high prevalence of cirrhosis (3[7] and 9%[9]), fibrosis (8[7] and 27%[9]), and elevated hepatic iron (96[7] and 73%[9]), but comparisons to controls were not possible.

Studies of homozygotes identified from clinical populations of patients previously diagnosed with hemochromatosis based on a variety of criteria, often not specified, also report prevalences of cirrhosis and fibrosis that are much higher than in homozygotes identified from population studies.[14–16] Serum ferritin has been identified as a significant predictive factor, with cirrhosis being found only among homozygotes with serum ferritin levels >1000 μg/l in studies controlling for alcohol consumption.[15]

In a study of previously undiagnosed C282Y homozygotes identified from genotyping of relatives of clinically affected C282Y homozygotes, a very high rate of cirrhosis was reported, occurring in 14 of 113 (12%) of men and two of 101 (2%) of women.[14] The results are difficult to reconcile with the lower rates reported by other studies of unselected homozygotes, but may in part reflect the somewhat subjective pathologic criteria for diagnosis of cirrhosis or may result from other familial factors that influence expression of the genotype.

Life expectancy in C282Y homozygotes

Because hemochromatosis is considered to be a late-onset disease, the seriousness of the clinical phenotype can also be assessed by the degree to which homozygotes may disappear from the population as it ages. Several studies have addressed this question by comparing the frequency of the C282Y/C282Y genotype in very elderly populations with the frequency in younger populations. No significant differences were found in the frequency of homozygotes among elderly populations, including centenarians, in France[17] and Finland[18] and subjects 85 years and older in the Netherlands[19] compared with younger groups.

To further determine whether C282Y homozygotes are lost from the population with age, we performed a meta-analysis of five large population screening studies determining the frequency of *HFE* genotypes for subjects of an age range of at least 30–79 years. Frequency of C282Y homozygotes was determined for each age group in intervals of 10 years for each study. Frequencies by age group were then weighted by sample size and combined to yield a summary frequency. The results of the meta-analysis showed no statistically significant differences in homozygote frequency by age for either men or women (Tables 4 and 5).

Hardy–Weinberg equilibrium

Determining whether there is a selective loss of homozygotes in a population can also be assessed by examining adherence to the Hardy–Weinberg equilibrium.

Table 4. Number of C282Y homozygotes by age group in studies of general populations: men.

Study	Age (years)					
	20–29	30–39	40–49	50–59	60–69	70+
Beutler et al[11,33]	3/368 (0.82%)	5/1207 (0.41%)	19/2859 (0.66%)	17/4169 (0.41%)	17/4016 (0.42%)	10/3618 (0.28%)
Ellervik et al[68]	0/200 (0%)	1/490 (0.20%)	1/564 (0.18%)	3/904 (0.33%)	1/989 (0.10%)	1/949 (0.11%)
Olynyk et al[9]	0/132 (0%)	1/275 (0.36%)	2/313 (0.64%)	3/286 (1.05%)	0/294 (0%)	1/220 (0.45%)
Phatak et al[34]	1/330 (0.33%)	0/330 (0%)	0/330 (0%)	1/330 (0.33%)	2/330 (0.60%)	0/330 (0%)
Steinberg et al[69]	0/103 (0%)	1/96 (1.0%)	0/96 (0%)	0/74 (0%)	1/150 (0.67%)	2/200 (1.0%)
Weighted summary[a]	0.55%	0.37%	0.50%	0.41%	0.36%	0.27%

[a] $P = 0.4808$ (chi square with 5 degrees of freedom).

The Hardy–Weinberg equilibrium describes the distribution of alleles in a population with the equation $p^2 + 2pq + q^2$, where p and q represent the frequency of respective alleles, in this case C282Y and wild type. Departure from the Hardy–Weinberg equilibrium—i.e. the occurrence of a smaller number of C282Y homozygotes than that predicted from the allelic frequency—would suggest that C282Y homozygotes are differentially lost from the population, as would occur in the case of decreased survival or non-participation in a screening study due to illness.

Because few individual studies have adequate sample sizes to address the question, we performed a meta-analysis of all studies reporting frequency of *HFE* genotypes among European populations. Studies were identified through searching

Table 5. Number of C282Y homozygotes by age group in studies of general populations: women.

Study	Age (years)					
	20–29	30–39	40–49	50–59	60–69	70+
Beutler et al[11,33]	1/479 (0.21%)	6/1384 (0.43%)	11/3076 (0.36%)	23/4051 (0.57%)	18/4116 (0.44%)	15/3477 (0.43%)
Ellervik et al[68]	1/243 (0.41%)	1/523 (0.19%)	4/622 (0.64%)	2/995 (0.20%)	5/1300 (0.38%)	3/1395 (0.22%)
Olynyk et al[9]	0/120 (0%)	2/220 (0.91%)	4/284 (1.41%)	2/294 (0.68%)	0/319 (0%)	1/254 (0.39%)
Phatak et al[34]	1/419 (0.24%)	1/419 (0.24%)	1/419 (0.24%)	1/419 (0.24%)	1/419 (0.24%)	3/627 (0.48%)
Steinberg et al[69]	0/143 (0%)	1/188 (0.53%)	0/140 (0%)	0/148 (0%)	1/131 (0.76%)	0/346 (0%)
Weighted summary[a]	0.23%	0.41%	0.45%	0.47%	0.40%	0.38%

[a] $P = 0.8091$ (chi square with 5 degrees of freedom).

Medline and other databases using 'iron', '*HFE*', 'C282Y', or 'hemochromatosis' as search words. An advantage of this meta-analysis is that it is not likely to have been affected by publication bias, i.e. the bias toward publishing studies with positive results, a problem inherent to many meta-analyses, because the primary purpose of most of the studies included in this meta-analysis was simply to report the frequency of the C282Y polymorphism in a defined population. Thus, the meta-analysis used data reported in the studies to answer a question not addressed by most of the studies individually.

The frequency of the C282Y polymorphism, number of C282Y homozygotes, and total number of subjects genotyped were abstracted or requested from the authors when not available in the published report. Studies included those in which subjects were drawn from: (1) the general population; (2) elderly populations; (3) selected healthy populations (i.e. those in which subjects were blood donors or employee groups likely to be healthier than the general population); and (4) control groups for studies of *HFE* gene polymorphism frequencies in subjects with a specific disease. Reports of C282Y frequencies among groups of subjects selected for a specific disease were not included in the analysis. Meta-analysis was limited to studies having an expected number of C282Y homozygotes of ≥ 1. Studies excluded by this criterion were largely from populations with very low frequency of C282Y allele or studies with small sample sizes of less than 300 subjects.

Of the 158 studies identified, 46 (29%) having an expected number of C282Y homozygotes of ≥ 1, as predicted by the Hardy–Weinberg equilibrium, were included. Allelic frequency of the C282Y polymorphism ranged from 0.032 to 0.109. Linear regression of the observed/expected number of C282Y homozygotes weighted by sample size of the 46 studies yielded a regression line with a slope (95% confidence interval) of 1.178 (1.172, 1.184), suggesting a small excess of C282Y homozygotes

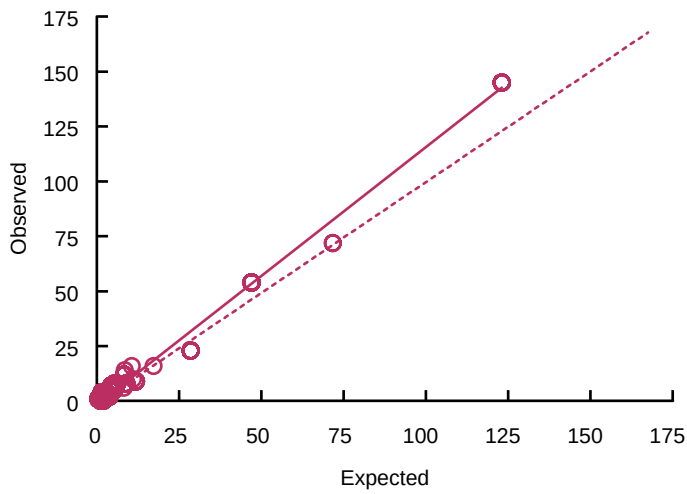

Figure 2. Linear regression of observed number of C282Y homozygotes by expected number from Hardy–Weinberg equilibrium. Analysis included 46 studies with an expected number of C282Y homozygotes of one or greater. Cases were weighted by *n* of the study sample. Linear regression: slope (95% confidence interval) = 1.175 (1.170, 1.180). Dotted line represents slope of 1.0 (observed = expected) for reference.

(Figure 2). Had selection bias excluded ill patients with hemochromatosis, the opposite result would have been expected.

Admixture of inbreeding subpopulations with different frequencies of a mutation or polymorphism can cause a departure from the Hardy–Weinberg equilibrium that results in increased homozygote frequency in the overall population. To test whether the excess of C282Y homozygotes in the Kaiser–Scripps study of US Whites may have resulted from this phenomenon, known as the Wahlund effect, we examined the Hardy–Weinberg equilibrium separately in Whites reporting Northern European and Southern European ancestry. As expected, the allelic frequency of C282Y was higher in Whites of Northern European ancestry compared to those with Southern European ancestry (6.9 versus 2.7%). In both subpopulations, however, the observed number of C282Y homozygotes was greater than the expected (106 observed versus 91 expected in Northern Europeans and six observed versus three expected in Southern Europeans), suggesting that the admixture was not responsible for a major part of the small excess observed in the white population overall. However, admixtures of smaller, unrecognized subpopulations may have contributed.

Factors influencing the penetrance of the C282Y/C282Y genotype

The growing evidence that the C282Y homozygous genotype alone does not result in symptomatic disease has led to the search for other factors that influence penetrance. These factors fall into three broad categories: genetic, epigenetic, and environmental. Relatively few studies have been published to date examining these factors. Most involve comparison of 'expressing' homozygotes with 'non-expressing' homozygotes. The studies have limited power due to the relatively small numbers of subjects in either category.

Genetic factors

Polymorphisms of numerous candidate genes involved in iron homeostasis have been studied for associations with elevated transferrin saturation, serum ferritin, or both among C282Y homozygotes. In the Kaiser–Scripps study of multiple candidate genes, including other genes with mutations associated with iron-storage disease such as transferrin, transferrin receptor-2, ferroportin, ceruloplasmin and hepcidin, none were found to occur at a significantly different frequency between homozygotes with high and low transferrin saturation and ferritin levels.[20] However, in one pedigree co-inheritance of the heterozygous state for an *HFE* and a mutation of the hepcidin gene (*HAMP*) manifested more iron storage than subjects with only one of the two mutations.[21] This could easily have been a coincidence, but another group examining a larger cohort of 392 C282Y homozygotes found a higher mean transferrin saturation level among the five subjects carrying *HAMP* mutations, suggesting that this gene may indeed influence expression.[22] Nine additional C282Y homozygotes from the latter cohort have been found to be heterozygous for missense mutations of the gene associated with juvenile hemochromatosis (*HJV*). These homozygotes have significantly higher mean serum ferritin levels, implicating the *HJV* gene as another possible modifier of expression.[23]

Haptoglobin, a protein that influences iron metabolism by binding hemoglobin and promoting its excretion, has been one focus of study. Haptoglobin type 2-2 (Hp2-2)

has been associated with increased transferrin saturation and increased serum ferritin levels in healthy men[24] and in subjects homozygous for the C282Y *HFE* polymorphism.[25] However, these results could not be confirmed.[26,27]

Iron deposition and loading can also occur as a result of impaired mitochondrial function. Variants in mitochondrial DNA (mtDNA) have been associated with a variety of diseases; mtDNA variant at position 16 189 involving a T→C substitution has been identified as a risk factor for diabetes mellitus, among other diseases. The frequency of the 16 189 variant was reportedly greater among 292 C282Y homozygotes with elevated transferrin saturation and serum ferritin levels than in C282Y homozygotes with normal serum iron indices[28]; however, we were not able to confirm these results in another group of patients.[29]

Environmental factors

Alcohol consumption is associated with increased serum iron and ferritin levels as well as levels of iron in the liver.[2,30] Scotet et al studied 378 C282Y homozygous patients who were treated at a blood center in western Brittany and who had initial transferrin saturation levels of $>45\%$. Significantly higher levels of serum ferritin and serum iron were found among the 33 subjects who had 'excessive alcohol consumption' defined as >60 g/day[31], but clinical symptoms did not occur at a higher rate in these subjects. The association of alcohol intake with cirrhosis in homozygotes is striking. In a study of 206 C282Y homozygotes identified from a clinical population previously diagnosed with hemochromatosis and having undergone liver biopsy, Fletcher et al found a ninefold increase in cirrhosis among homozygotes who drank >60 g alcohol per day (66%) compared with those who drank less (7%).[32]

C282Y HETEROZYGOTES

Effects of the compound heterozygous C282Y/H63D genotype

Compound heterozygotes having the C282Y/H63D genotype account for approximately 5–10% of patients diagnosed with hemochromatosis and subsequently genotyped for *HFE* polymorphisms.[3] Mean values of both transferrin saturation and serum ferritin have been consistently found to be elevated among compound heterozygotes (Table 1). The percentage of compound heterozygotes having elevated transferrin saturation and serum ferritin levels, however, is much lower than the percentage of C282Y homozygotes having elevated values.[9,33,34] It has been calculated that with respect to being diagnosed as 'hemochromatosis' prior to cloning of the *HFE* the penetrance of the compound heterozygous state gene is only about 1% of that of the homozygous state.[35] Given that the penetrance of the homozygous state is now recognized to be very low, it is clear that compound heterozygotes with clinical disease must be scarce indeed. Levels of transferrin saturation and serum ferritin increase modestly with age in compound heterozygotes at a rate similar to that in wild-type controls.[6] As with C282Y homozygotes, subjects with the C282Y/H63D genotype identified from population-based studies have not been found to have a higher rate of iron-overload-related symptoms compared to wild-type controls.[11,33]

Effects of the heterozygous C282Y/wt genotype: biochemical phenotype of heterozygotes

One of the most consistent observations among studies involving genotyping of large populations is that carriers of either common *HFE* polymorphism have increased mean transferrin saturation and serum ferritin levels compared to wild-type controls. The results from the Kaiser–Scripps survey of subjects attending a health appraisal clinic demonstrate the dose–response-like effect of the polymorphisms on these parameters of iron homeostasis (Table 1). Among both men and women, subjects with the C282Y/C282Y genotype have the highest mean transferrin saturation, followed in decreasing order by subjects with the C282Y/H63D, H63D/H63D, C282Y/wt, H63D/wt and wt/wt genotypes. C282Y homozygotes also have the highest mean serum ferritin levels, with subjects with other *HFE* genotypes having lower values in a trend similar to, but less distinct than, that seen with transferrin saturation. Results from numerous other population-based studies involving genotyping of a large number of subjects have been very similar in regard to mean levels of transferrin saturation and serum ferritin as well as trends by genotype.[34,36–40]

Selective advantage of *HFE* mutations?

The fact that the *HFE* gene polymorphisms have reached a very high prevalence, in the case of C282Y over a relatively short period of time, suggests that the carriers of these polymorphisms may have a selective advantage. Because these polymorphisms are associated with accumulation of iron, one obvious advantage would be resistance to iron deficiency and, as a result, iron-deficiency anemia. Examining these parameters in 30 000 white subjects, we found that carriers of *HFE* polymorphisms had higher mean hemoglobin levels and lower prevalence of iron depletion—not of iron-deficiency anemia—compared with wild-type controls.[41] These results are consistent with several other smaller studies that have shown no protection from iron-deficiency anemia among *HFE* carriers.[38,42,43] A direct comparison of the hemoglobin distributions in C282Y polymorphism carriers and wild-type controls in our study revealed a shift toward higher hemoglobin values in the middle (non-anemic) hemoglobin distribution among C282Y carriers, contributing to an increase in mean hemoglobin without affecting the lower (anemic) part of the distribution.[41]

Alternatively, it has been suggested that the C282Y polymorphism may be associated with protection from some infectious agents. Two mechanisms have been proposed. Rochette et al[44] rejected the concept that this pressure was the prevention of iron deficiency, averring that this would have reached fixation of the polymorphism in groups with a high prevalence of iron-deficiency anemia, and speculated instead that the HFE protein may act as a receptor for an infectious agent, as has been proposed for the CFTR protein in cystic fibrosis. In this instance, protection from infection would result from the fact that the C282Y mutant protein does not reach the cell surface. Moalem et al have proposed that the relative iron deficiency in macrophages occurring in association with the polymorphism could provide protection against bacteria that multiply mainly in iron-rich macrophages, including strains of *Chlamydia*, *Coxiella*, *Francisella*, *Mycobacterium*, *Salmonella*, and *Yersinia*.[45] Models based on these theories have yet to be tested in animal or epidemiological studies.

Studies of disease risk in heterozygotes

Because *HFE* polymorphisms are known to affect iron metabolism, it has been suggested that heterozygosity may also increase the risk of disease. Most studies on the relationship of the C282Y heterozygous genotype to disease have been case–control studies comparing the frequency of heterozygotes among subjects with and without a specific disease. Diseases studied have included diabetes[46], arthritis[47–49], cardiovascular disease[50–56], cancer[57–60], and liver disease.[61–63] The studies have not consistently supported the C282Y heterozygous genotype as a risk factor for disease.

Life expectancy of C282Y heterozygotes

Analysis of all available data also does not support a decreased life expectancy among C282Y heterozygotes. In a study by Bathum et al, a significant trend for a decrease of C282Y heterozygotes in older age groups compared to subjects aged 45–54 years was found overall and in women, but not among men.[64] In addition, among the oldest (>100 years) there was no decrease in frequency. In other studies of elderly populations, no decrease in frequency or general health status of C282Y heterozygotes has been reported.[18,19] Similarly, in a study of 1000 men and women ages 85 years and older, none of the ten possible *HFE* genotypes was over- or under-represented compared with the expected frequency calculated from the Hardy–Weinberg equilibrium equation.[65] Analysis of the Kaiser–Scripps data involving 3145 white C282Y heterozygotes has also shown no decrease by age.[66]

SUMMARY

The C282Y polymorphism of the *HFE* gene was discovered to occur at a high frequency in patients previously diagnosed with hemochromatosis based on symptoms, iron overload, or both. Approximately 80% of these patients were homozygotes. Genotyping of non-clinical populations have shown the frequency of C282Y homozygosity to be approximately 0.5% in Northern European populations. Several large population-based studies have shown that while the majority C282Y homozygotes, particularly men, have elevated levels of transferrin saturation and serum ferritin, prevalence of symptoms associated with iron overload are no greater than in age- and sex-matched controls. Meta-analysis of studies involving genotyping of general populations also shows no evidence of a decrease in life expectancy among C282Y homozygotes. Thus, although in a few patients C282Y homozygosity may produce symptoms, these patients represent a small minority of C282Y homozygotes detected in general population surveys.

Identification of environmental and genetic factors that may influence the expression of the C282Y genotype has been limited to date. Alcohol intake of more than 60 g/day is associated with higher levels of transferrin saturation and serum ferritin in C282Y homozygotes. Of the many genes investigated as possible modifiers, polymorphisms in the hepcidin gene and the *HJV* gene associated with juvenile hemochromatosis are the only ones found to date to be associated with significantly higher levels of iron in small subsets of clinically identified C282Y homozygotes. C282Y heterozygosity is not associated with increased risk of disease and, due to its high prevalence, is hypothesized to confer a survival advantage, the basis for which has not been identified.

Practice points

- controlled studies of persons with the C282Y/C282Y genotype of the *HFE* hemochromatosis gene identified from screening of large non-clinical populations have shown that:
  - most C282Y homozygotes have increased levels of transferrin saturation (i.e. values of 50% or greater) and serum ferritin ($\geq$200–250 ng/ml)
  - prevalence of symptoms associated with iron overload—including diabetes, arthritis/arthralgias, fatigue, and skin pigmentation—is not greater in C282Y homozygotes compared with sex- and age-matched controls; thus, although in a few patients C282Y homozygosity may produce symptoms, these findings are not significantly increased in general population surveys
  - life expectancy is not significantly decreased among C282Y homozygotes
- prevalence of fibrosis and cirrhosis may be increased in C282Y homozygotes having serum ferritin levels of $>$1000 ng/ml
- alcohol intake of $>$60 g/day is associated with higher levels of transferrin saturation and serum ferritin among C282Y homozygotes
- few genetic factors influencing the expression of the biochemical or clinical hemochromatosis phenotype in C282Y homozygotes have been identified; polymorphisms of the hepcidin gene (*HAMP*) and the *HJV* gene associated with juvenile hemochromatosis may play a role
- transferrin saturation and serum ferritin levels may also be increased in individuals with the heterozygous genotypes C282Y/H63D or C282Y/wt; however, there is no evidence of increased disease risk among subjects with these genotypes

Research agenda

- demonstration of the low penetrance of the C282Y/C282Y genotype in subjects identified in controlled, population-based studies underscores the need to further define the influence of other genes and environmental factors (including alcohol) on expression of iron overload and clinically significant disease that occurs in a small subset of these subjects

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