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Effects of *HFE* C282Y and H63D Polymorphisms and Polygenic Background on Iron Stores in a Large Community Sample of Twins

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The aim of this study was to assess and to compare the role of *HFE* polymorphisms and other genetic factors in variation in iron stores. Blood samples were obtained from 3,375 adult male and female twins (age range 29–82 years) recruited from the Australian Twin Registry. There were 1,233 complete pairs (562 monozygotic and 571 dizygotic twins). Serum iron, transferrin, transferrin saturation with iron, and ferritin were measured, and the HFE C282Y and H63D genotypes were determined. The frequency of the C282Y allele was .072, and that of the H63D allele was .141. Significant sources of variation in the indices of iron status included age, sex, age-sex interaction, body-mass index, and both the C282Y and H63D genotypes. The iron, transferrin, and saturation values of CC and CY subjects differed significantly, but the ferritin values did not. After correction for age and body-mass index, 23% and 31% of the variance in iron, 66% and 49% of the variance in transferrin, 33% and 47% of the variance in transferrin saturation, and 47% and 47% of the variance in ferritin could be explained by additive genetic factors, for men and women, respectively. *HFE* C282Y and H63D variation accounted for <5% of the corrected phenotypic variance, except for saturation (12% in women and 5% in men). We conclude that *HFE* CY and HD heterozygotes differ in iron status from the CC and HH homozygotes and that serum transferrin saturation is more affected than is serum ferritin. There are highly significant effects of other as-yet-unidentified genes on iron stores, in addition to *HFE* genotype.

Introduction

Iron is an essential element, mainly because of its role within oxygen-binding and respiratory proteins. However, it also gives rise to dangers such as generation of free radicals and consequent tissue damage. Regardless of its cause, high liver iron content leads to an increased risk of cirrhosis, hepatoma, porphyria cutanea tarda, and other liver diseases (Hsing et al. 1995; Stal 1995; Bonkovsky et al. 1996; Mandishona et al. 1998; Sampietro et al. 1999). Increased iron also favors infection (Brock 1994); in addition, it has been reported as a risk factor for atherosclerosis (Kiechl et al. 1997; Tuomainen et al. 1998), although this link is controversial (Danesh and Appleby 1999). Iron deposition in the pancreas may play a role in some cases of non–insulin-dependent diabetes (Kwan et al. 1998), although this, too, is controversial and is probably dependent on the clinical population studied. Iron deficiency, on the other hand, can proceed to iron-deficiency anemia and other adverse conditions, including poorer pregnancy outcomes and, possibly, impaired intellectual development in infancy (see Baynes 1994). Therefore, there are hazards associated with both insufficient and excessive iron content in the body. Iron levels within cells and extracellular fluids are strongly regulated through controls on absorption, storage, transport, and utilization.

Factors Influencing Iron Stores

Many factors are known to affect iron status, including dietary availability and the biological processes of intake, absorption, and loss. Since urinary excretion is slight and apparently is not regulated, control of absorption provides the main safeguard against both iron overload and iron depletion. Variation between individuals may be the result of combinations of—and interactions between—genetic and environmental differences. Dietary availability of iron (through choice of diet), iron absorption, absorption-diet interactions, and variation in iron loss (particularly in women) are all potentially subject to genetic influences. Little is currently known about the genetic factors that may influence iron stores in humans, except for hemochromatosis.

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Hemochromatosis

There has recently been substantial progress in determination of the molecular and cellular bases of iron metabolism, because of characterization of the gene mutation mainly responsible for hemochromatosis (Feder et al. 1996). This autosomal recessive condition with sex-dependent penetrance causes iron overload and iron deposition in the tissues, as a result of inappropriately high absorption of iron. The clinical consequences of this iron overload occur both more commonly and at an earlier age in men than in women (Powell et al. 1994); that the consequences of iron overload occur less commonly in women may generally be ascribed to the fact that women have physiological blood loss and lower iron stores.

At least 85% of cases of genetic hemochromatosis are believed to be the result of homozygosity for the C282Y mutation in the *HFE* gene at chromosome 6p. The C282Y form of the gene encodes a protein that does not bind β_2-microglobulin and that, therefore, is not presented on the cell surface, where the wild-type protein associates with transferrin receptors. This is believed to interfere with the regulation of iron absorption from the intestine (Zoller et al. 1999). The role, if any, of the slightly more-frequent H63D polymorphism in the same gene is unclear, but the findings of some reports (Aguilar Martinez et al. 1997; Sanchez et al. 1998; Brissot et al. 1999; Moirand et al. 1999) have suggested that compound heterozygotes (CY/HD) are occasionally affected by hemochromatosis. Current clinical and scientific questions relating to this disease have been summarized elsewhere (Bacon et al. 1999).

Polymorphic Variation in HFE

The dysfunctional C282Y form of the *HFE* gene is common among populations of European descent, with reported frequencies of 5%–15% (see Burt et al. 1998; Jouanolle et al. 1998; Lucotte 1998; Murphy et al. 1998; Nielsen et al. 1998; Merryweather-Clarke et al. 1999). These estimates are consistent with the findings of earlier studies (Edwards et al. 1988; Leggett et al. 1990), which detected homozygotes in unselected populations through identification of a phenotype of high serum ferritin and high transferrin saturation with iron.

There are uncertainties about the natural history of homozygosity for the Y allele and about how frequently—and at what age—it leads to clinically significant iron overload. To assess the desirability of population screening for hemochromatosis, it is important to undertake population-based studies of the prevalence and the effects of the Y allele. Presymptomatic but genetically affected subjects, or "obligate heterozygotes," that, in the past, were detected by screening of relatives of patients with hemochromatosis may differ from C282Y heterozygotes or from Y homozygotes in the general population in significant ways.

Since the C282Y polymorphism is both common and, at least in homozygous individuals, potentially harmful, it may persist through conferment of some selective advantage in the heterozygous state—most obviously, through a reduced risk of iron deficiency. Some reports of subjects ascertained, by means of haplotype studies within affected families, to be heterozygous for the hemochromatosis gene (Bulaj et al. 1996) and of subjects genotyped as being heterozygous for the *HFE* C282Y mutation (Burt et al. 1998; Datz et al. 1998; Moirand et al. 1999) have found a positive effect on iron stores and, by inference, a reduced risk of iron deficiency.

HFE *as a Contributor to Genetic Variation*

It is unlikely that *HFE* variation is the only genetic factor affecting iron stores; most quantitative characteristics with a genetic component appear to be affected by multiple genes. Moreover, variation in the penetrance and age at onset of symptoms of hemochromatosis may be caused by the additive or interactive effects of other genes. Similarly, some individuals may be more vulnerable to iron deficiency because of the actions of unidentified genes.

The aims of the present study were to determine the allele frequencies of C282Y and H63D in a large group of presumably healthy subjects and to compare iron stores in subjects with differing CY and HD genotypes. The study was performed on twin subjects, so that heritability—the sum of all gene effects—could be estimated and compared with the effects of *HFE* variation. This design allowed us to determine whether a search for further iron-storage genes is necessary.

We have assessed iron stores by measurement of iron, transferrin, and ferritin in blood serum and by calculation of transferrin saturation with iron. Data on a number of covariates have been included in the analysis, to determine the relative importance of *HFE* genotype, age, sex, and ethnicity with regard to iron stores within the Australian population.

Subjects and Methods

The subjects participating in the present study were recruited through the Australian Twin Registry, initially for participation in questionnaire- and interview-based studies of alcohol use, alcohol dependence, and comorbid psychiatric conditions (Heath et al. 1997). The subjects who form the basis of this study subsequently volunteered for a study of genetic and genotype effects on health-related phenotypes. They gave informed con-

sent to be interviewed and, subsequently, to undergo blood collection and to allow use of their blood samples in the study. The studies were approved by appropriate ethics committees.

In all, 3,375 subjects provided blood samples from 1993–1996. At the time of blood collection, the subjects' height and weight were measured, and the subjects completed a brief questionnaire that included questions about their grandparents' countries of birth and ancestry. The time of blood collection was recorded, and the subjects reported the time of their last meal. All subjects were twins, but, in some cases, only one member of a twin pair provided blood. Consistent with the geographic distribution of the Australian population, most subjects who gave blood lived in either Adelaide, Brisbane, Melbourne, or Sydney or lived close enough to those cities to attend blood-collection sessions held there. A minority of blood samples were collected in more-remote sites; in these instances, blood was delivered to the laboratory within 24 h. Serum was separated and was stored at −70°C until it was analyzed, and DNA was extracted from EDTA or from heparin blood. Zygosity of all complete pairs was determined by typing of at least eight highly polymorphic microsatellite markers, the *HFE* genotypes, and the ABO, Rh, and MNS blood group systems.

Measurements of serum iron, transferrin, and ferritin were performed, by use of Roche methods, on a Hitachi 917 analyzer, and the percentage of iron saturation of transferrin was calculated as 100{[μmol iron per liter]/[(g tranferrin per liter) × 25]}. *HFE* genotypes for C282Y and H63D were determined by means of PCR, and allele-specific oligonucleotide hybridization (L.M.C. and E.C.J., personal communication), and those samples that were typed as YY by use of this method were confirmed with the use of modified primers, as described elsewhere (Jeffrey et al. 1999).

Complete results were obtained for 3,005 subjects, all phenotypic results were obtained for 3,118 subjects, and both *HFE* polymorphisms were typed for 3,176 subjects. For a further 254 subjects that were from monozygotic (MZ) twin pairs where only one of the pair underwent *HFE* genotyping, the genotype could be inferred from that of their twin. With the addition of this information, complete data were available for a total of 3,044 subjects, including 578 unpaired subjects (226 men and 352 women) and 1,233 pairs of twins (481 MZ female pairs, 181 MZ male pairs, 243 dizygotic [DZ] female pairs, 89 DZ male pairs, and 239 DZ male/female pairs).

Ancestry data provided by the subjects were used to test for differences in C282Y or H63D allele frequencies between ethnic groups. Given the nature of the population studied, the main available contrast was between subjects of English and Celtic ancestry. For each subject, information on the ancestry of four grandparents was provided, and the grandparents were divided into four groups (English, Celtic [from Ireland, Scotland, and Wales], Other European, and Non-European). Ancestry of the individual subjects could therefore be 100% attributable to one category, or it could be represented as a combination of categories—for example, 25% English and 75% Celtic, or 50% Celtic and 50% Other European. Multiple regression was used to test for the effects of the four ethnicity variables on the average number of Y and D alleles carried by each subject.

Initial investigation of the effects of known or suspected covariates was conducted by means of analysis of variance. This also generated means for each subgroup. These means and the size of the differences between subgroups were not affected by the twin nature of the sample. However, significance tests were biased in a nonconservative direction because of the sharing of genes within twin pairs. Statistical problems resulting either from missing data or from the use of twin subjects were overcome by means of maximum-likelihood methods. Models of sources of variation were fitted to values for the dependent variables (serum iron, transferrin, saturation, and ferritin) by use of the Mx 1.47c program, which also allows for modeling of the expected values of individuals in terms of such covariates as sex, age, and genotype.

The Mx program was used to test a succession of models of increasing complexity for their goodness of fit to the data. At each step, a covariate or gene effect was added to the model, and the increase in log likelihood was used in a χ^2 test for improvement of fit. At each step, the variance components and the twin-pair correlations corrected for covariates were estimated.

Models of genetic and environmental sources of variation were fitted to the data, with simultaneous correction for covariate effects. This was done both with and without incorporation of effects from the measured genotypes (*HFE* C282Y and H63D), and the relative importance of *HFE* polymorphisms and of other, unknown genes was estimated. Models were initially tested with additive genetic (A), nonshared environmental (E), and either dominant genetic (D) or shared environmental (C) sources of variation. The ACE or ADE models were then compared with models containing only A and E (AE models), and the AE models were compared with models containing either C and E or E alone. Sex-specific A and E components of variation were then compared with the common AE model (Zhu et al. 1999).

To confirm that the associations between genotype and phenotype were not the result of population stratification, contrasts within twin pairs (that share their ancestry) and between pairs (where stratification may be present) were tested as described elsewhere (Fulker et al. 1999). Finally, sources of covariation between

iron, transferrin, and ferritin were tested in a multivariate model incorporating both common and specific genetic and environmental sources of variation.

Results

HFE *Allele and Haplotype Frequencies*

Genotyping results, by sex, for the C282Y and H63D polymorphisms in the *HFE* gene are shown in table 1. These are the observed numbers for all subjects, without controlling for gene sharing within twin pairs. Allele and haplotype frequencies were also estimated by taking only one subject from each twin pair, with the addition of the subjects whose twins did not participate. This resulted in Y allele frequencies of .0669 ± .0064 and .0725 ± .0050 and in D allele frequencies of .1565 ± .0093 and .1351 ± .0065, in men and women, respectively (all estimates are ± standard error). The genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium. Maximum-likelihood estimation of the haplotype frequencies from the twin genotypes, by use of the program ASSOCIAT, showed that they did not differ significantly between men and women. The estimated frequencies were as follows: CH, .788; YH, .069; CD, .143; and DY, 0. The CY and HD loci were in significant linkage disequilibrium (women χ^2 = 19.62, men χ^2 = 11.37; both $P < .001$). Both the absence of subjects with CY/DD, YY/HD, or YY/DD and the zero estimate for the frequency of the DY haplotype are consistent with findings from previous reports indicating that Y and D alleles have never been found on the same chromosome.

The estimated effects of ethnic origin on the frequency of CY and HD are shown in table 2. The regression equations predicted that subjects who had 100% Celtic ancestry would have, on average, 0.180 Y alleles and 0.355 D alleles per person, resulting in allele frequencies of 9.0% and 17.8%, respectively. For subjects with no reported Celtic ancestry, the predicted allele frequencies were 6.0% for the Y allele and 13.6% for the D allele, and the effects of Celtic ancestry were marginally significant ($P < .05$ without correction for duplication within twin pairs) in each case.

Table 1

Observed Numbers of Twin Subjects, by *HFE* C282Y and H63D Genotypes

Sex of Subject and Genotype	Observed Number of Twin Subjects, by		
	HH	HD	DD
Male:			
CC	634	266	27
CY	106	17	0
YY	3	0	0
Female:			
CC	1,341	427	44
CY	251	45	0
YY	15	0	0

Note.—Numbers are not corrected for gene sharing within twin pairs.

Table 2

Relationship between Ancestry of Subjects and the Probability of Carrying a C282Y or H63D Allele

Ancestry of Subjects[a]	Probability of Carrying Allele	
	C282Y (b ± SE)[b]	H63D (b ± SE)[b]
(Constant)	.120 ± .017	.272 ± .024
English	.026 ± .022[c]	−.011 ± .030[c]
Celtic	.060 ± .026[d]	.083 ± .036[d]
Other European	−.019 ± .029[c]	−.066 ± .039[c]
Non-European	.007 ± .045[c]	.066 ± .061[c]

[a] As assessed from their reports on the ancestry of their grandparents.

[b] b denotes the unstandardized regression coefficient from multiple regression of the number of Y or D alleles on the percentage of ancestry in each of the four ancestry groupings; SE = standard error.

[c] Not significant.

[d] $P < .05$.

Indices of Iron Stores

Frequency distributions of each variable (iron, transferrin, saturation, and ferritin) were assessed by use of normal probability plots, and ferritin results were log-transformed before further analysis. All references to ferritin are therefore log-transformed values, unless otherwise stated.

Initial assessments included tests for the effects of time of blood collection, age, sex, and obesity, without taking into account the twin structure of the cohort. Serum iron showed significant variation with regard to the time of day at which blood was collected (fig. 1), but it showed no significant effect of time since the last meal. Transferrin and ferritin showed no significant diurnal or fasting variation. Each of the four variables showed highly significant differences in means between men and women, and all but saturation showed changes with age in women. Among men, only transferrin differed significantly between the age groups. (Details of these tests of significance are not shown, since results of the more-appropriate model-fitting tests are described in the following paragraph.) Effects of age and sex on saturation and ferritin are shown in figure 2. Body-mass index (BMI) had highly significant effects on saturation and ferritin, respectively, in both men (R = −0.148 and 0.145) and women (R = −0.128 and 0.138); all effects are significant at $P < .001$. Obesity tended to increase ferritin, but it decreased transferrin saturation with iron. Division of the subjects into quintiles of BMI showed

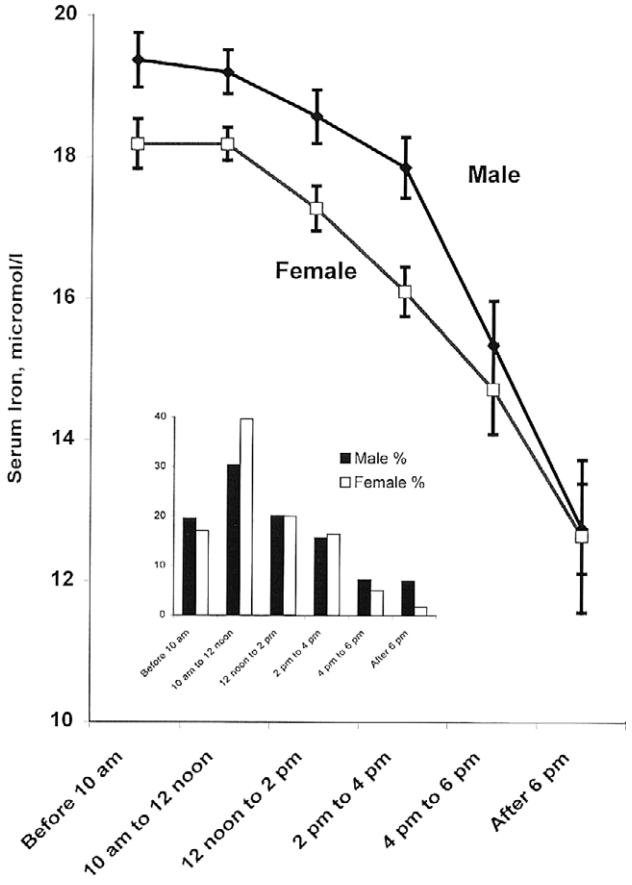

Figure 1 Effect of the time of blood collection on serum iron results in men and women in the present study. Points indicate means, and error bars show ± 1 SEM. Distributions of times of blood collection, for both men and women, are shown (*inset*).

that there was a continuous trend across all quintiles, rather than a major effect of an extremely low or high BMI (data not shown).

These effects were confirmed in the results of modeling with use of the Mx program. There was significant male/female heterogeneity in overall variances for iron, transferrin, and saturation ($\Delta\chi^2_1 = 37.3$, 52.9, and 23.2, respectively; all $P < .001$) but not (after the log-transformation) for ferritin ($\Delta\chi^2_1 = 1.2$; not statistically significant). The means for men and women differed significantly for all variables. In women, age had strong effects on transferrin and saturation and, to a lesser extent, on iron, but the effects of age were weaker in men. Addition of age^2—to allow for nonlinear effects of age—produced little improvement, except for transferrin, among men. BMI had significant effects on all four variables, but it should be remembered that these effects were different (opposite) for iron and ferritin (see preceding paragraph). Collection time had major effects on iron and, consequently, on saturation.

C282Y and H63D Genotypes and Iron Stores

Because of the sex differences in iron-store markers and allele frequencies and because of the difference in age effects between men and women, the effects of genotype on the dependent variables were initially assessed separately for men and women. These results for saturation and ferritin are shown in figure 3. In men, the C282Y genotype had significant effects on iron, transferrin, and saturation but not on ferritin. For iron and saturation, values increased across the groups CC→CY→YY, but the value for transferrin decreased. However, it should be noted that only one YY man was included in this analysis. In women, all four variables were significantly affected by the CY genotype. Again, iron, saturation, and ferritin increased—but transferrin decreased—across the groups CC→CY→YY.

For the H63D genotype, only iron and saturation showed significant effects in men. The mean values were lowest in HH subjects and were highest in DD subjects. Among women, only saturation showed a significant H63D effect. However, the effects of the C282Y and H63D alleles on iron-store markers could be confounded because of the linkage disequilibrium between them. Subjects carrying the D allele (the putative high–iron-storage form) were less likely to be carrying the Y allele.

These initial results were supported by Mx model fitting. After adjustment for the covariates described above, genotype effects were added. First, the effects of the C282Y polymorphism were tested under the assumption of additive effects (i.e., that the effect of two Y alleles would be twice as great as that of one allele). This produced highly significant results for all four variables (step 1; table 3). Next, the effect of the H63D polymorphism was tested under the same additive assumption; again, highly significant results were obtained for iron and saturation (step 2). A minor effect was found for transferrin, but none was found for ferritin. It should be noted that we found these H63D effects after accounting for the C282Y effect. In step 3, addition of H63D without C282Y produces a lesser (but still significant) change in the fit of the models for iron and saturation, and this difference in the HD effect size can be ascribed to the interaction of the C282Y and H63D gene frequencies.

In steps 1–3, additive allelic effects of *HFE* variation were assumed. Further models were tested that examined the effects of genotype and that allowed for the possibility of dominance (i.e., that the heterozygote value may be closer to one homozygote than to the other). Step 4 allows for a difference between the CC and CY genotypes. The change in χ^2 values shows that, for iron,

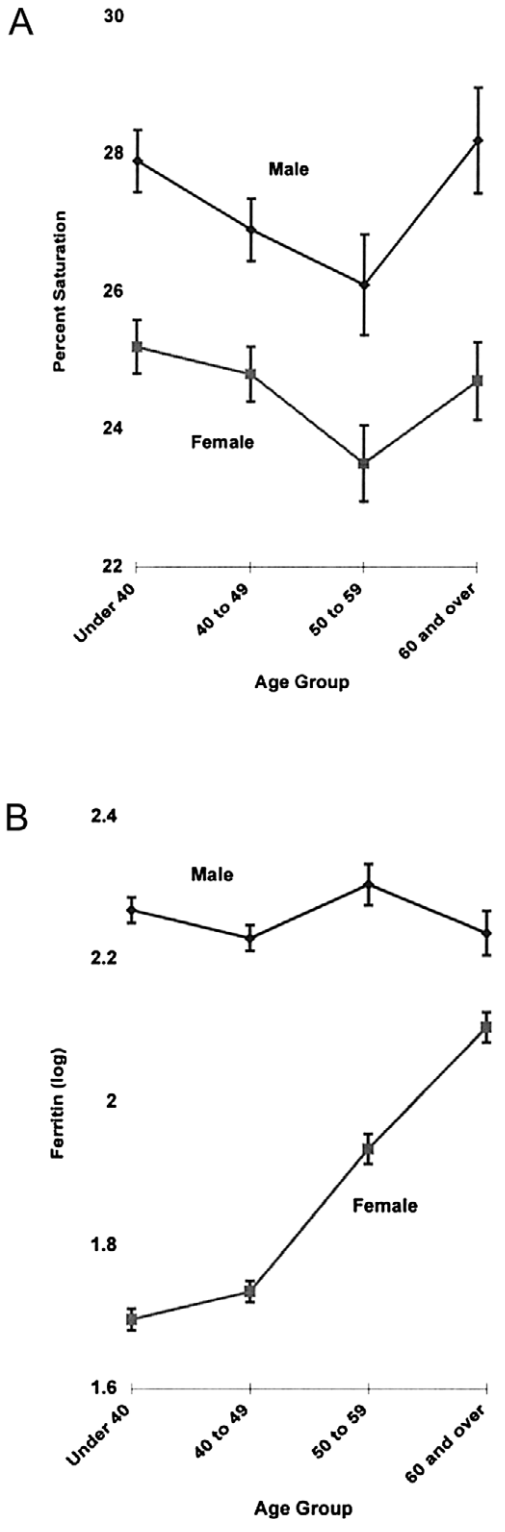

Figure 2 Effects of age group and sex on (*A*) saturation and (*B*) ferritin. Points indicate means, and error bars show ±1 SEM. Mean values are adjusted for collection time and for BMI. Sex had highly significant effects on both variables, and age affected ferritin in women.

transferrin, and saturation—but not for ferritin—there is a significant difference between the CC homozygote and the CY heterozygote. In step 5, allowing for a difference between CY and YY produces significant improvement in fit for all four variables, consistent with the results shown in figure 3. Comparison of this two-stage process, which allows for unequal effects of the first and second Y allele (dominance), with use of the model in which equal effects (additivity) are assumed, shows that the χ^2 change between the dominance-effects model at step 5 and at baseline is much greater for every variable than is the χ^2 change between the additive-effects model at step 1 and at baseline.

Similarly, after adjustment for the differences in the mean values associated with variation in the C282Y genotype, steps 6 and 7 (see table 3) test for differences between HH and HD and, then, test for additional effects of the DD genotype. HD heterozygotes showed significant differences, compared with HH homozygotes, for iron and saturation but not for transferrin or ferritin (step 6), whereas the DD genotype showed minor effects for iron, transferrin, and saturation but, again, none for ferritin (step 7). For this polymorphism, the model permitting dominance effects of two D alleles showed no significant improvement over the additive model (the sum of χ_1^2 values for steps 6 and 7 is not appreciably greater than the χ_1^2 value for step 2). Steps 8 and 9 are similar to step 3, in that C282Y effects are not included. Again, the HH/HD differences were more detectable when CY effects were included (in a comparison of χ_1^2 for step 8 vs. that for step 6), and the effects of DD were slight (step 9 vs. step 7).

Are HFE *Effects Due to Population Stratification?*

Results of the Fulker test, which assessed population stratification as a possible cause of some of the genotype/iron-stores associations, are shown in table 4. Since this technique makes use of complete DZ pairs only, the number of subjects is reduced; for a constant effect size, this analysis will have less power to detect allelic effects than will the full Mx model. Nevertheless, the genetic associations that were found to be significant in the model fitting (see preceding section, and table 3) were confirmed as resulting from pleiotropic allelic effects rather than from population stratification. This is tested in model 5 (presented in table 4), in which dropping within-pair differences produces a significant deterioration for iron (for C282Y and H63D), transferrin (for C282Y only), and saturation (for C282Y and H63D). For ferritin, for which the effect size is smaller than that for the other analytes, the reduction in sample size obtained by using only complete DZ pairs renders the association no longer significant.

Genetic and Environmental Sources of Variation in Iron-Store Markers

At each step of the model-fitting process described above, the within-pair correlations, by zygosity, were estimated after adjustment for the covariates and genotypic effects in the model. Details of these correlations at two important stages are given in table 5: first, after adjustment for the covariates but before introduction of the measured genotypes and, second, after inclusion of the CY and HD genotypes. Models of genetic and environmental sources of variation were fitted to these data by use of Mx.

In all cases, models containing either unshared environmental effects only (E) or unshared and shared environmental effects (CE) were strongly rejected. Models containing unshared and shared environmental effects together with additive genetic effects (ACE) showed no significant improvement when compared with models containing only unshared environmental and additive genetic effects (AE). Allowing different A and E components of variance for men and women (A_mE_m/A_fE_f) showed highly significant improvements, compared with the common AE model, for all four variables. No further improvement in fit was gained by relaxation of the constraint on perfect correlation between additive genetic effects in men and women; from this, we can conclude that there is no evidence for nonscalar sex limitation of gene effects on these variables (Neale and Cardon 1992).

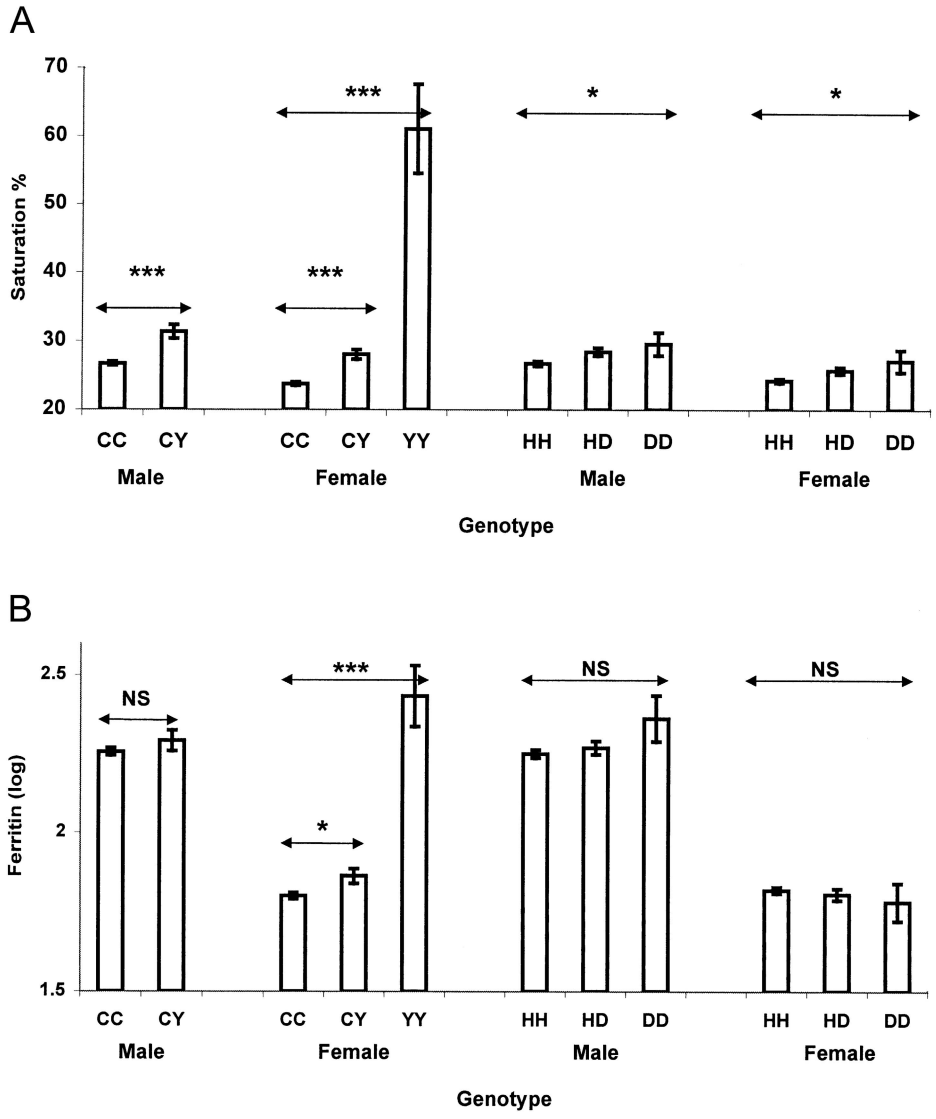

Figure 3 Effects of *HFE* C282Y and H63D genotypes on (*A*) saturation and (*B*) ferritin. Mean values are adjusted for sex, age (in women), BMI, and collection time. Columns indicate means; error bars show ±1 SEM. $^{***}P < .001$; $^{*}P < .05$. NS = not significant.

Table 3

Tests for Effects of *HFE* Genotype on Indices of Iron Stores, Including Data from Both Male and Female Subjects

Step	Model[b]	Compared Model[c]	$\Delta\chi_1^{2a}$ in Iron	Transferrin	Saturation	Ferritin
1	Y only	Baseline	39.2*	64.5*	115*	9.4**
2	Y + D	1	25.3*	6.5***	36.1*	.7[d]
3	D only	Baseline	18.0*	2.4[d]	21.2*	.2[d]
4	CY	Baseline	15.9*	39.4*	45.8*	1.3[d]
5	CY + YY	4	47.2*	35.1*	144*	26.5*
6	CY + YY + HD	5	22.2*	1.4[d]	27.3*	.0[d]
7	CY + YY + HD + DD	6	4.3***	6.9**	9.9**	2.0[d]
8	HD only	Baseline	16.6*	.2[d]	16.3*	.0[d]
9	HD + DD	8	2.7[d]	3.9***	5.2***	1.6[d]

[a] Maximum-likelihood estimates created by means of the computer program Mx.

[b] The improvements in fit, as genotypic information is added, are tested for each variable. Y is the additive effect of C282Y mutations, and D is the additive effect of H63D mutations. CY, YY, HD, and DD are genotypic effects and allow for unequal (dominance) effects of the heterozygous and homozygous states.

[c] The baseline model allows for effects of age, sex, BMI, and collection time.

[d] Not significant.

* $P < .001$.

** $P < .01$.

*** $P < .05$.

Models allowing for unshared environmental effects together with both additive and dominance genetic effects (ADE) gave no improvement in fit, compared with the AE models, except possibly for saturation, before allowing for effects of measured *HFE* genotypes. For the data allowing for covariate effects alone, the ADE/AE change in χ^2 was almost significant ($\Delta\chi_1^2 = 3.52$; $P = .06$). On the basis of this saturation ADE model, before the *HFE* variation was omitted, almost one-third (32%) of the variance was estimated to be the result of dominance effects, and this dropped sharply (to 14%, not significantly different from zero) after allowance was made for the effects of *HFE* C282Y and H63D variation. This is consistent with the dominance effects of the CY genotype, as shown in table 3.

Sex-dependent heritability estimations on the A_mE_m/A_fE_f models, before allowing for measured genotypes (i.e., with C282Y and H63D effects included among the additive genetic effects), were 23% and 31% for iron, 66% and 49% for transferrin, 33% and 47% for saturation, and 47% and 47% for saturation, for men and women, respectively. Although the *HFE* polymorphisms had significant effects on the phenotypic variables, the background genetic effects were substantially greater in each case. Proportions of variance caused by environmental, non-*HFE* genetic, and *HFE* effects on iron, transferrin, saturation, and ferritin are shown in figure 4.

Sources of Covariation between Markers of Iron Stores

There were highly significant—and moderately strong—correlations between results for transferrin and ferritin (men −0.358, women −0.413; both $P < .001$). Iron was weakly correlated with transferrin in men (0.121; $P < .001$) but not in women (0.024; $P = .274$) and with ferritin in both sexes (men 0.070, $P = .024$; women 0.228, $P < .001$). To determine whether these correlations were caused by genetic factors, environmental factors, or both, a Cholesky model (Neale and Cardon 1992) of the sources of residual polygenic genetic and environmental variation in iron, transferrin, and ferritin was also computed. Values of path coefficients were similar, with or without adjustment for *HFE* effects in the means model. Genes with a primary influence on iron had only minor effects on ferritin and transferrin, whereas most of the polygenic variation for transferrin was specific to transferrin. The negative relationship between ferritin and transferrin is modulated through both genes and specific environment.

Discussion

The main aim of the present study was to assess the effects of the *HFE* C282Y and H63D polymorphisms on iron stores in the general population and to compare their magnitude with other genetic effects through use of a twin-study design. The results lead to three main conclusions about *HFE* polymorphisms and iron stores: (1) both C282Y and H63D affect aspects of iron stores or iron metabolism, even when they are present in the heterozygous state; (2) *HFE* effects on serum ferritin are minor, compared with effects on serum iron and on the saturation of serum transferrin with iron; and (3) other

Table 4

Fulker Test for Within-Pair Associations between Iron-Related Phenotypes and the *HFE* C282Y and H63D Polymorphisms in Complete DZ Twin Pairs Only

	Model 2[a]	Model 3[b]	Model 4[c]	Model 5[c,d]
Description	Drop ADs	ab + aw	Drop ab	Drop aw
Compared model	Full	Full	3	4
Δdf	1	1	1	1
Iron:				
$\Delta\chi^2$ CY	7.83*	1.35	2.63	6.55*
$\Delta\chi^2$ HD	12.1*	.33	6.36*	6.07**
Transferrin:				
$\Delta\chi^2$ CY	21.8*	.17	15.3*	6.64*
$\Delta\chi^2$ HD	1.11	.00	.65	.46
Saturation:				
$\Delta\chi^2$ CY	29.5*	.74	15.1*	15.1*
$\Delta\chi^2$ HD	17.2*	.25	9.26*	8.16*
Ferritin:				
$\Delta\chi^2$ CY	2.13	.94	.33	2.74
$\Delta\chi^2$ HD	.07	.10	.10	.00

[a] Tests for the deterioration in the fit of the model brought about by constraining allelic deviations to zero across all individuals. ADs = allelic deviations.

[b] Partitions ADs into those occurring between (ab) and within (aw) sib pairs.

[c] ADs partitioned in model 3 are set to zero. See footnote b for definition of "ab."

[d] ADs partitioned in model 3 are set to zero. Significant aw (see footnote b for definition) demonstrates that ADs are not solely the result of population stratification.

* $P < .01$.

unknown genes have effects on iron stores that are considerably greater than those of *HFE*.

Allele and Haplotype Frequencies

The C282Y and H63D gene frequencies found among the subjects were consistent with findings from recent reports from Europe, and there was some evidence of association between the Y allele and Celtic ancestry. In a review of geographic variation in *HFE* gene frequencies (Lucotte 1998), the highest frequency of the Y allele was found among populations in the more-northern parts of Europe. Despite some difficulties with the definition of Celtic populations, high frequencies have been reported from Ireland, Scotland, Wales, and Brittany. In surveys concentrating on Ireland, Murphy et al. (1998) found a Y allele frequency of .099, and Ryan et al. (1998) reported a frequency of .140.

Our results also show some association between Celtic ancestry and the H63D allele, although this has a more widespread occurrence than does C282Y. In Europe, reported frequencies of the D allele include .11 in Germany (Nielsen et al. 1998), .13 in Denmark (Steffensen et al. 1998), .14 in Ireland (Murphy et al. 1998), and .22 in Spain (Sanchez et al. 1998). Other reported frequencies of the D allele are .14 in New Zealand (Burt et al. 1998) and .16 among whites in America (McDonnell et al. 1999). These are all similar to our frequency of .14.

Effects of HFE *Variation*

The most-prominent effect of *HFE* genotype was the major effect of homozygosity for the C282Y allele. As expected, YY women had much higher values for iron, saturation, and ferritin and had lower results for transferrin than did either CC or CY subjects (fig. 3). The only YY man for whom quantitative results were available also had results consistent with high iron stores. This large effect of the second Y allele shows the quantitative dominance of the C allele and is consistent with the recessive inheritance of the clinical condition of hemochromatosis. This finding was apparent in the major improvement in fit for all variables when an effect of YY (additional to that of CY) was introduced into the model of sources of variation. Comparison of ADE and AE models of sources of variation, before adjustment for *HFE*, also suggests a dominant genetic component in the variation in iron and saturation (although purely additive models are not rejected). These estimated dominant genetic components are reduced after the effects of CY and HD are omitted. Thus, the results of variance-component analysis are consistent with the fixed-effect modeling of genotypic contribution to expected values (means; fig. 3) and with clinical experience of the inheritance of hemochromatosis.

Compound heterozygotes with CY/HD have been reported by some studies as presenting with clinical hemochromatosis. For this reason, the results for CY/HD

Table 5

Maximum-Likelihood Estimates of Twin Correlations, before and after Correction for the Effects of *HFE* Genotypes in the Model for the Means

	Maximum-Likelihood Estimates of Twin Correlations for				
	MZ		DZ		
Variable and Correction	Both Female	Both Male	Both Female	Both Male	Male and Female
Iron:					
Covariates only	.317	.222	.100	.149	.130
With CY and HD	.275	.203	.120	.138	.131
Transferrin:					
Covariates only	.481	.665	.311	.350	.245
With CY and HD	.453	.658	.307	.371	.224
Saturation:					
Covariates only	.488	.329	.106	.222	.170
With CY and HD	.389	.296	.138	.219	.142
Ferritin:					
Covariates only	.463	.501	.300	.098	.170
With CY and HD	.452	.498	.314	.091	.176

Note.—All correlations are adjusted for effects of the covariates sex, age, BMI, and collection time.

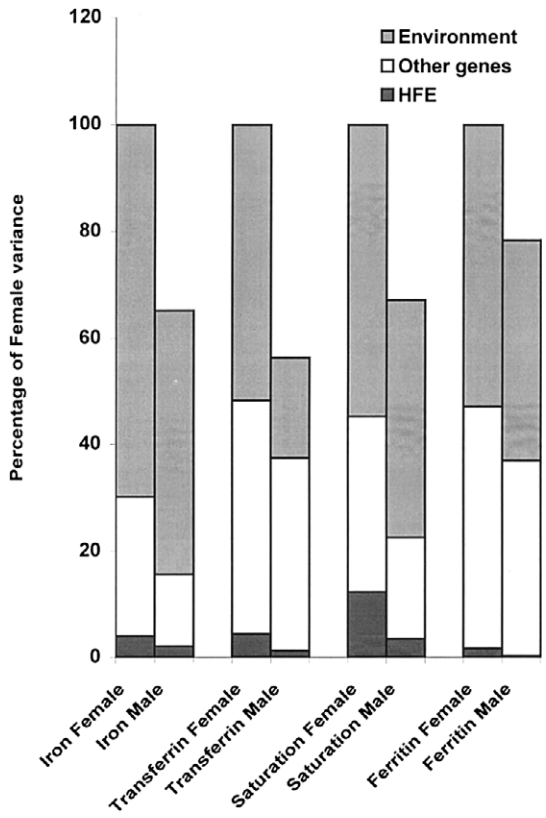

Figure 4 Proportions of variance ascribable to *HFE* gene variation, the additive effects of other genes, and the nonshared environmental effects in men and women. The total variance in women has been standardized to 100 for each variable.

subjects were examined for evidence of additive or synergistic effects of the two *HFE* polymorphisms. In women, the effects were approximately additive for both saturation and ferritin. In men, the effects were nonadditive, with ~10% increases in both saturation and ferritin being associated with CY or HD alone (compared with CC/HH subjects) and with increases of 40%–45% above this baseline occurring in the compound heterozygotes. However, there was no evidence that the combination of Y and D alleles could produce iron accumulation similar to that found in YY subjects. For comparison, the saturation and ferritin results in YY/HH subjects were 240%–390% of those in CC/HH subjects. Elsewhere in Australia, Olynyk et al. (1999) have reported that compound heterozygotes (those carrying both the C282Y and the H63D mutations) had higher transferrin saturation than either heterozygotes with the C282Y mutation alone or subjects with neither mutation; ferritin values were also greater in the compound heterozygotes, but only among the men. These findings suggest that compound heterozygotes are unlikely to be affected, unless other genetic or dietary risks are substantial. This is consistent with the findings from clinical reports; compound heterozygotes will be approximately twice as common as YY homozygotes, but, in studies that have genotyped patients diagnosed before this genotyping became available, they comprise only a small percentage of subjects with apparent hereditary hemochromatosis.

Any finding of association between a genotype with a frequency that varies among populations and a phenotype that may vary for a number of reasons raises the question of whether there is a true cause-and-effect relationship between the allele and the phenotype or whether some form of population stratification is causing the apparent association. Specifically, an association between high iron stores and the Y allele could be the result of this allele being more common among Irish Australians, with some unrelated genetic or nongenetic factor causing higher iron stores in this group. Apart from considerations of biological plausibility, these possibilities can be distinguished statistically in studies that use related subjects, such as siblings (in this case, the DZ twin pairs). Application of this test involves testing for differences in genotype and phenotype within pairs of siblings, and the results (table 4) confirmed that not only CY but, also, HD has genuine effects on serum iron and transferrin saturation. The absence of significant results for ferritin is because restriction of the analysis to complete DZ pairs has reduced the power to detect the comparatively small effect.

The genotype-phenotype associations found in this study are similar to those reported in other community-based studies. In New Zealand, Burt et al. (1998) found increased iron and transferrin saturation in both C282Y and H63D heterozygotes. Mean ferritin values were also slightly higher than those in the CC/HH group, but this did not reach statistical significance. In C282Y heterozygotes, Datz et al. (1998) reported increased iron, saturation, and, interestingly, hemoglobin. H63D was not typed. Their subjects were all women (age range 18–40 years) who were recruited from health-care staff in Austria. These studies and the present study show that *HFE* polymorphisms affect iron stores even among heterozygotes but that ferritin is less affected than is transferrin saturation.

HFE *Variation and Selection*

As mentioned above, the persistence of at least the C282Y mutation, with its clear disease association in homozygous men, suggests some advantage for heterozygotes. The most-obvious possibility is protection against iron deficiency, which not only puts the adult individual (particularly women) at risk but which also has adverse effects on pregnancy outcome.

From our model, we can estimate the probability of

each genotype being iron deficient, which we define as falling below a threshold of 15% for saturation and, for ferritin, 20 mg/liter. For a woman of age 40 years, we predict that 18% of normal CC/HH women will fall below the saturation threshold, whereas only 8% of CY women and none of the YY women will have such a decrease. There is also some protective effect of the H63D polymorphism, since 12% of HD women and 9% of DD women are predicted to be deficient. Protection against low ferritin is less dramatic; 21% of normal women are predicted to have ferritin concentrations <20 mg/liter, whereas 18% of CY women and 1% of YY women will have such concentrations; HD women show no protection (21%), but only 16% of DD women have low ferritin.

A similar calculation can be performed to assess the probability of iron overload, estimated for men of age 50 years; as with the calculations assessing women, a BMI of 25 and a midmorning collection time are assumed for the calculations assessing men. The following probabilities of exceeding a saturation value of 50% were estimated: CC/HH, 2%; CY, 7%; YY, 99%; HD, 4%; and DD, 6%. For a ferritin value >500, the probabilities were CC/HH, 15%; CY, 18%; YY, 73%; HD, 15%; and DD, 20%.

Translating these values into selective advantage can only be speculative, but it will be seen that *HFE* C282Y and H63D genotypes have substantial influence on the chance of low transferrin saturation in women and that the C282Ygenotype has such an influence on the chance of high transferrin saturation in men. The effects on ferritin are smaller, but each polymorphism may affect iron overload in men. However, bearing in mind that, in our sample, there was only one YY man for whom we had quantitative data, we must be very cautious about the estimates of iron-overloaded men predicted from our model.

It is likely that, throughout most of the past 15 centuries, these polymorphisms have had effects on survival and reproductive fitness. Whether the sex-associated risks and advantages are now in equilibrium is unknown. The effects will vary according to the chance of iron deficiency or overload in the two sexes and in the population in question, and this might account for geographic differences in gene frequency. If the C282Y mutation arose in a single founder within the last 60–70 generations (Ajioka et al. 1997), then this implies an increase of ~20% per generation. Even if the age of the mutation is underestimated because of drastically reduced recombination in the major-histocompatibility-complex region, it seems likely that there has still been a very substantial selective advantage for the Y allele.

Which Aspect of Iron Metabolism Is Most Affected?

One surprising finding was that the effects of C282Y variation on iron, transferrin, and saturation were greater than the effect on ferritin, at least for the heterozygotes. This raises the question of which is the best index both of body iron stores and the risks of deficiency or overload. There is evidence that serum ferritin is well correlated with body iron reserves (Skikne et al. 1990). Although it may also be increased as an acute-phase protein during infection or inflammation, these would be uncommon among subjects in the present study. Serum iron, on the other hand, is not regarded as a good measure of iron stores, although the saturation calculated from the combination of iron and transferrin (which increases in iron deficiency and decreases in iron overload) may be better. Part of the problem with use of serum iron as a measure of iron status is its within-person variation, and the correction for time of blood collection would have partly compensated for this.

Although the details of how *HFE* variation affects iron absorption and iron stores are not fully worked out, it seems that the primary effect of the Y mutation is to change the interactions between transferrin, the transferrin receptor, and the *HFE* gene product (Salter-Cid et al. 1999). Effects on iron-transport proteins in the intestine (Zoller et al. 1999) and, ultimately, on body iron stores and ferritin, are consequences of this. From this perspective, the fact that *HFE* variation accounts for a greater proportion of variance in transferrin saturation than in ferritin is not so surprising, although it does imply that there are other mechanisms that can keep down ferritin levels in CY heterozygotes, even when the availability of iron bound to transferrin in the circulation and at the cell surface is relatively high.

Perspective on HFE *Mutations*

Although the C282Y variant of *HFE* has major effects on iron stores in the homozygous state and produces a potentially fatal disease in the long term, and although both the C282Y and H63D polymorphisms lead to highly significant variation in indices of iron reserves within the general population, they are not the only genes affecting iron status. The remaining differences between MZ and DZ correlations, after allowing for *HFE* effects (table 5), show that other genes account for ~45% of the phenotypic variation in serum ferritin and for 30% of the variation in transferrin saturation.

The possibility of achieving this perspective is one of the strengths of combining twin or family studies with measurement of specific genotypes, and, in this case, it shows that there are other genes to be found. Such genes may or may not affect the penetrance or age at onset of hemochromatosis, and they may or may not be linked

with the *HFE* locus. There are likely to be a number of genes with individually small and additive effects. Location of such genes may be achieved through linkage studies with further polymorphic markers in the chromosome 6 major histocompatibility complex/*HFE* region, or it may require a genomewide search. The S65C polymorphism in *HFE* (Mura et al. 1999) may influence iron status, but its low frequency (~.02) suggests that it can have only minor effects on population variation.

Finally, we may consider the effects of genes affecting iron stores on diseases other than hemochromatosis or iron-deficiency anemia. It is known that high hepatic iron stores are associated with many types of liver disease, from viral hepatitis to alcoholic cirrhosis to hepatocellular carcinoma, although the direction of causal relationships is not yet clear (George et al. 1999). Recent studies (Roest et al. 1999; Tuomainen et al. 1999) have strengthened the proposed association between iron and cardiovascular disease. Given the differences in iron stores produced by the known forms of *HFE* variation and, even more strongly, by other unknown genes, this may be a route to understanding genetic differences in susceptibility to a range of more-common diseases.

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Electronic-Database Information

URLs for data in this article are as follows:

Lab of Statistical Genetics at Rockefeller University, ftp://linkage.rockefeller.edu/software/utilities/ (for ASSOCIAT, version 2.33)

Mx: Statistical Modeling, 4th ed., http://views.vcu.edu/mx/index.html

References

Aguilar Martinez P, Biron C, Blanc F, Masmejean C, Jeanjean P, Michel H, Schved JF (1997) Compound heterozygotes for hemochromatosis gene mutations: may they help to understand the pathophysiology of the disease? Blood Cells Mol Dis 23:269–276

Ajioka RS, Jorde LB, Gruen JR, Yu P, Dimitrova D, Barrow J, Radisky E, et al (1997) Haplotype analysis of hemochromatosis: evaluation of different linkage-disequilibrium approaches and evolution of disease chromosomes. Am J Hum Genet 60:1439–1447

Bacon BR, Powell LW, Adams PC, Kresina TF, Hoofnagle JH (1999) Molecular medicine and hemochromatosis: at the crossroads. Gastroenterology 116:193–207

Baynes RD (1994) Iron deficiency. In: Brock JH, Halliday JW, Pippard MJ, Powell LW (eds) Iron metabolism in health and disease. WB Saunders, London, pp 189–225

Bonkovsky HL, Banner BF, Lambrecht RW, Rubin RB (1996) Iron in liver diseases other than hemochromatosis. Semin Liver Dis 16:65–82

Brissot P, Moirand R, Jouanolle AM, Guyader D, Le Gall JY, Deugnier Y, David V (1999) A genotypic study of 217 unrelated probands diagnosed as "genetic hemochromatosis" on "classical" phenotypic criteria. J Hepatol 30:588–593

Brock JH (1994) Iron in infection, immunity, inflammation and neoplasia. In: Brock JH, Halliday JW, Pippard MJ, Powell LW (eds) Iron metabolism in health and disease. WB Saunders, London, pp 353–389

Bulaj ZJ, Griffen LM, Jorde LB, Edwards CQ, Kushner JP (1996) Clinical and biochemical abnormalities in people heterozygous for hemochromatosis. N Engl J Med 335:1799–1805

Burt MJ, George PM, Upton JD, Collett JA, Frampton CMA, Chapman TM, Walmsley TA, et al (1998) The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 43:830–836

Danesh J, Appleby P (1999) Coronary heart disease and iron status: meta-analyses of prospective studies. Circulation 99: 852–854

Datz C, Haas T, Rinner H, Sandhofer F, Patsch W, Paulweber B (1998) Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation, and hemoglobin in young women: a protective role against iron deficiency? Clin Chem 44: 2429–2432

Edwards CQ, Griffin LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP (1988) Prevalence of hemochromatosis using 11,065 presumably healthy blood donors. N Engl J Med 318:1355–1362

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, et al (1996) A novel MHC class 1–like gene is mutated in patients with hereditary hemochromatosis. Nat Genet 14:249–251

Fulker DW, Cherny SS, Sham PC, Hewitt JK (1999) Combined linkage and association sib-pair analysis for quantitative traits. Am J Hum Genet 64:259–267

George DK, Powell LW, Losowsky MS (1999) The haemochromatosis gene: a co-factor for chronic liver diseases? J Gastroenterol Hepatol 14:745–749

Heath AC, Bucholz KK, Madden PAF, Dinwiddie SH, Slutske WS, Statham DJ, Dunne MP, et al (1997) Genetic and environmental contributions to DSM-IIIR alcohol dependence risk in a national twin sample: consistency of findings in women and men. Psychol Med 27:1381–1396

Hsing AW, McLaughlin JK, Olsen JH, Mellemkjar L, Wacholder S, Fraumeni JF (1995) Cancer risk following primary hemochromatosis: a population-based cohort study in Denmark. Int J Cancer 60:160–162

Jeffrey GP, Chakrabarti S, Hegele RA, Adams PC (1999) Polymorphism in intron 4 of *HFE* may cause overestimation of C282Y homozygote prevalence in haemochromatosis. Nat Genet 22:325–326

Jouanolle AM, Fergelot P, Raoul ML, Gandon G, Roussey M,

Deugnier Y, Feingold J, et al (1998) Prevalence of the C282Y mutation in Brittany: penetrance of genetic hemochromatosis? Ann Genet 41:195–198

Kiechl S, Willeit J, Egger G, Poewe W, Oberhollenzer F (1997) Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study. Circulation 96: 3300–3307

Kwan T, Leber B, Ahuja S, Carter R, Gerstein HC (1998) Patients with type 2 diabetes have a high frequency of the C282Y mutation of the hemochromatosis gene. Clin Invest Med 21:251–257

Leggett BA, Halliday JW, Brown NN, Powell LW (1990) Prevalence of hemochromatosis amongst asymptomatic Australians. Br J Haematol 74:525–530

Lucotte G (1998) Celtic origin of the C282Y mutation of hemochromatosis. Blood Cells Mol Dis 24:433–438

Mandishona E, MacPhail AP, Gordeuk VR, Kedda MA, Paterson AC, Rouault TA, Kew MC (1998) Dietary iron overload as a risk factor for hepatocellular carcinoma in Black Africans. Hepatology 27:1563–1566

McDonnell SM, Hover A, Gloe D, Ou CY, Cogswell ME, Grummer-Strawn L (1999) Population-based screening for hemochromatosis using phenotypic and DNA testing among employees of health maintenance organizations in Springfield, Missouri. Am J Med 107:30–37

Merryweather-Clarke AT, Simonsen H, Shearman JD, Pointon JJ, Norgaard-Pedersen B, Robson KJ (1999) A retrospective anonymous pilot study in screening newborns for *HFE* mutations in Scandinavian populations. Hum Mutat 13:154–159

Moirand R, Jouanolle A-M, Brissot P, Le Gall J-Y, David V, Deugnier Y (1999) Phenotypic expression of *HFE* mutations: a French study of 1110 unrelated iron-overloaded patients and relatives. Gastroenterology 116:372–377

Mura C, Raguenes O, Ferec C (1999) *HFE* mutations in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. Blood 93:2502–2505

Murphy S, Curran MD, McDougall N, Callender ME, O'Brien CJ, Middleton D (1998) High incidence of the Cys 282 Tyr mutation in the *HFE* gene in the Irish population—implications for haemochromatosis. Tissue Antigens 52: 484–488

Neale MC, Cardon LR (1992) Methodology for genetic studies of twins and families. Kluwer, Dordrecht, The Netherlands

Nielsen P, Carpinteiro S, Fischer R, Cabeda JM, Porto G, Gabbe EE (1998) Prevalence of the C282Y and H63D mutations in the *HFE* gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany. Br J Haematol 103:842–845

Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW (1999) A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 341: 718–724

Powell LW, Jazwinska E, Halliday JW (1994) Primary iron overload. In: Brock JH, Halliday JW, Pippard MJ, Powell LW (eds) Iron metabolism in health and disease. WB Saunders, London, pp 227–270

Roest M, van der Schouw YT, de Valk B, Marx JJ, Tempelman MJ, de Groot PG, Sixma JJ, et al (1999) Heterozygosity for a hereditary hemochromatosis gene is associated with cardiovascular death in women. Circulation 100:1268–1273

Ryan E, O'Keane C, Crowe J (1998) Hemochromatosis in Ireland and *HFE*. Blood Cells Mol Dis 24:428–432

Salter-Cid L, Brunmark A, Li Y, Leturcq D, Peterson PA, Jackson MR, Yang Y (1999) Transferrin receptor is negatively modulated by the hemochromatosis protein *HFE:* implications for cellular iron homeostasis. Proc Natl Acad Sci USA 96:5434–5439

Sampietro M, Fiorelli G, Fargion S (1999) Iron overload in porphyria cutanea tarda. Haematologica 84:248–253

Sanchez M, Bruguera M, Bosch J, Rodes J, Ballesta F, Oliva R (1998) Prevalence of Cys282Tyr and His63Asp *HFE* gene mutations in Spanish patients with hereditary hemochromatosis and controls. J Hepatol 29:725–728

Skikne BS, Flowers CH, Cook JD (1990) Serum transferrin receptor: a quantitative measure of tissue iron deficiency. Blood 75:1870–1876

Stal P (1995) Iron as a hepatotoxin. Dig Dis 13:205–222

Steffensen R, Varming K, Jersild C (1998) Determination of gene frequencies for two common haemochromatosis mutations in the Danish population by a novel polymerase chain reaction with sequence-specific primers. Tissue Antigens 52:230–235

Tuomainen TP, Kontula K, Nyyssonen K, Lakka TA, Helio T, Salonen JT (1999) Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation: a prospective cohort study in men in eastern Finland. Circulation 100:1274–1279

Tuomainen TP, Punnonen K, Nyyssonen K, Salonen JT (1998) Association between body iron stores and the risk of acute myocardial infarction in men. Circulation 97:1461–1466

Zhu G, Duffy DL, Eldridge A, Grace M, Mayne C, O'Gorman L, Aitken JF, et al (1999) A major quantitative-trait locus for mole density is linked to the familial melanoma gene CDKN2A: a maximum-likelihood combined linkage and association analysis in twins and their sibs. Am J Hum Genet 65:483–492

Zoller H, Pietrangelo A, Vogel W, Weiss G (1999) Duodenal metal-transporter (DMT-1, NRAMP-2) expression in patients with hereditary haemochromatosis. Lancet 353:2120–2123