Original Paper

Human Heredity

Heritability of Serum Iron, Ferritin and Transferrin Saturation in a Genetically Isolated Population, the Erasmus Rucphen Family (ERF) Study

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Abstract
Background: Iron has been implicated in the pathogenesis of various disorders. Mutations in the HFE gene are associated with an increase in serum iron parameters. The aim of this study was to estimate the heritability in serum iron parameters explained by HFE. *Methods:* Ninety families (980 subjects) were included in the present analysis. Heritability estimation was conducted using the variance component method. The likelihood ratio test was used to compare models. Phenotypic and genetic correlations between serum iron parameters were calculated. *Results:* The heritability ($h^2 \pm$ SE) estimates were 0.23 ± 0.07 ($p < 0.0001$) for iron, 0.29 ± 0.09 ($p < 0.0001$) for ferritin and 0.28 ± 0.07 ($p < 0.0001$) for transferrin saturation while adjusting for age, age^2 and sex. The HFE genotypes explained between 2 to 6% of the sex and age-adjusted variance in serum iron, ferritin and transferrin saturation. There was a high genetic correlation between serum iron parameters, suggesting pleiotropy between these traits. *Conclusion:* A substantial proportion of the variance of iron, ferritin and transferrin saturation can be explained by additive genetic effects, independent of sex and age. The HFE genotypes explained a considerable proportion of serum iron parameters and may be an important factor in the complex iron network.

Introduction

Iron plays an important role in biochemical reactions [1, 2]. Low iron levels are associated with iron deficiency anaemia [2]. Increased iron stores in the body have been linked to several diseases including vascular disease(s) [3, 4], Parkinson's disease [5], diabetes mellitus [6], arthritis [7] and liver diseases [8].

Body iron status is not only influenced by genes but also by environmental factors like diet, iron intake, blood loss, pregnancy, alcohol intake and infections [9, 10]. In order to maintain iron levels within normal ranges and thus prevent the pathologic consequences of iron excess or iron deficiency, iron homeostasis has evolved as a complex trait and a tightly coordinated process of interplay between genes and environmental factors in order to maintain iron levels within the normal range. Several genes are known that are involved in iron regulation in humans including the hereditary hemochromatosis gene (HFE), the ferroportin1 gene (FPN1), the divalent metal transporter gene (DMT1), the hepcidin antimicrobial

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peptide (HAMP), the hemochromatosis type 2 gene (HFE2), and the transferin receptors gene (TFR) [11–16]. Mutations in these genes have been implicated in hereditary hemochromatosis, a disorder of iron metabolism, in which there is increased absorption and storage of iron in body tissues. The common and most important gene is HFE. The HFE gene has two main mutations, the C282Y and the H63D mutations. These two mutations are found in 85% of patients with hemochromatosis and are also associated with increased body iron concentrations in the general population [13]. HFE mutations are not fully penetrant and their contribution to the overall genetically determined variation in iron pool has not been clearly established.

Crawford et al. [17] studied the concordance of iron storage in siblings with genetic hemochromatosis. They found that siblings of similar sex accumulate similar amounts of liver iron. Their study demonstrated that iron storage in hemochromatosis is predominantly under genetic control. In a similar study, Whiting et al. [18] reported a concordance of iron indices in C282Y homozygote(s) and heterozygote(s) sibling pairs. There is only one twin study known on the heritability of iron parameters. Whitfield et al. [19] reported that 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, in men and women respectively, could be explained by additive genetic factors. However, this study did not address the question to what extend serum iron parameters are explained by known genes such as HFE. Lazarescu et al. [20] reported a residual heritability of 35% for serum ferritin in subjects with the C282Y genotype. Their study did not address other valuable iron parameters like serum iron and serum transferrin saturation, which are important for clinical diagnosis.

We estimated the proportion of serum iron, ferritin and transferrin saturation due to genetic factors and the proportion that could be explained by the HFE gene using a large family-based sample from a genetically isolated population in the Netherlands. Further, we estimated the genetic and phenotypic correlations between these traits.

Material and Methods

Study Population

This study was carried out within a family-based study of 3,000 inhabitants of a genetically isolated community in the Southwest of the Netherlands, the Erasmus Rucpen Family (ERF) study. The ERF study has been described in detail elsewhere [21]. Briefly, about 150 people who migrated to the area in the middle of the 18th century founded the study population. Since then there has been little emigration and immigration in the area and considerable population expansion. At present, there are about 20,000 inhabitants all living in 8 adjacent villages. The aim of the ERF study is to unravel the genetic determinants of complex diseases and the medical ethics committee of the Erasmus Medical Centre Rotterdam has approved this study. Informed consent and authorization to inspect and use medical records were obtained from all participants.

Family Structure

The (first) 980 participants considered in the present study came from 90 families and included 369 parent-offspring pairs, 561 sibling pairs, 41 half-sibling pairs, 872 avuncular pairs, 32 grandparent-grandchild pairs, 52 half avuncular pairs, 2,258 first cousins pairs, 50 half first cousin pairs.

Phenotypic Measurements

Participants were invited for a battery of clinical examinations at the research centre. Each participant provided blood samples and further information was obtained during an interview at the research centre and after participants returned a take-home questionnaire. Fasting blood samples were drawn by venepuncture between 7:00 and 10:00 a.m. Serum samples were obtained from whole blood and plasma samples were obtained from blood collected in EDTA.

The serum ferritin was measured by a solid-phase, two-site chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, Cirrus). Total serum iron was measured by colorimetric assay with the use of ascorbate/FerroZine reagents from Roche on an Aeroset from Abbott. The unsaturated iron binding capacity (UIBC) was measured by adding a known quantity of Fe(III) to the serum sample, reducing it with ascorbate to Fe(II) and measuring it with FerroZine as described above (Roche reagents on an Aeroset). The total iron binding capacity (TIBC) was calculated as: Fe + UIBC = TIBC. Serum transferrin saturation (%) was calculated as follows: (serum iron/TIBC) × 100%. Genotyping of the HFE C282Y and H63D variants were done as described elsewhere [22].

Statistical Analyses

The heritability of serum iron parameters was estimated using the variance component method as implemented in the program SOLAR [23]. The heritability is calculated by partitioning the phenotypic variance (Vp) of the serum iron parameter into components that include the additive effect of genes or polygenic variance (Vg), the variance due to measured environmental risk factors (Ve) such as age, sex and other covariates, and the residual or non-shared environmental variance (Vr). The heritability is defined as $h^2 = Vg/(Vp - Vr)$. We accounted for the pedigree structure by using the kinship matrix as a covariate. The kinship matrix captures the proportion of genes shared in common between all pairs of individuals in the family, based on their relationship (sibling(s) pairs share 50% of their genes in common, grandparent-grandchild pairs share 25% of their genes in common, unrelated spouses share no gene in common). The likelihood ratio test was used to test for significance. Two times the difference between the log-likelihoods of the two models has a chi-square dis-

tribution with degree of freedoms equal to the difference in the number of covariates in the models that are being compared.

The association between the traits and HFE polymorphisms was tested by including the genotypes as covariates; the genotypes were coded as number of 'Y' (C282Y) and 'D' alleles (H63D). The proportion of variance explained by inclusion of the genotypes was estimated as (Ve′ – Ve)/(Vp – Ve), where Ve is variance explained by sex, age and age^2 and Ve′ is the variance explained by including the above covariates and the genotype. The total significance of the inclusion of HFE genotypes was tested using Likelihood Ratio Test on 2 degrees of freedom.

We further investigated whether significant associations between serum iron parameters could be explained by common genetic influences, shared environmental factors or unmeasured non-genetic factors using bivariate analysis. We partitioned the phenotypic correlation between a pair of quantitative traits into their additive genetic effect and random environmental effect taking into account the kinship correlation.

The significance of the phenotypic, genetic and environmental correlations were evaluated using the likelihood ratio test by comparing the model in which the parameter is set to zero to the model in which the parameter is estimated. The inbreeding coefficient was estimated using the PEDIG package [24]. In all analyses age, age^2, sex and inbreeding coefficient were included as covariates in the model. Serum iron parameters exhibiting a skewed distribution were normalized prior to data analysis.

Results

The characteristics of the study population are shown in table 1. In total of 980 subjects were included in the analysis and consisted of a large number of relative pairs, including 894 first-degree relative pairs, 652 second-degree relative pairs and 2,360 third degree relative pairs. The mean serum iron, ferritin and transferrin saturation for men and women are presented in table 1. Our reference range for iron was 10–25 μmol/l and 15–50% for transferrin saturation. The reference ranges for ferritin are not uniform, due to less optimal standardization. Therefore reported ferritin values vary greatly between laboratories. Our reference values for ferritin were 15–280 μg/l for men, 6–80 μg/l for premenopausal women and 15–190 μg/l for postmenopausal women.

Figure 1 shows the mean serum iron, ferritin and transferrin saturation by HFE C282Y genotype. Serum iron, ferritin and transferrin saturation were significantly higher (p < 0.001) in women homozygous for the C282Y mutation compared to wild type homozygotes. Although subjects heterozygous for the C282Y mutation had higher serum iron indices compared to wild type homozygotes, this did not reach statistical significance.

Table 2 shows the heritability estimates (h^2 ± standard error) for serum iron, serum ferritin and transferrin

Table 1. Characteristics of the study population

Men, %	40
Age, years	53.52 ± 0.47
Serum iron, μmol/l	
Men	20.96 ± 0.33
Women	18.82 ± 0.26
Serum ferritin, ng/ml	
Men	223.71 ± 8.84
Women	99.34 ± 4.28
Serum transferrin saturation, %	
Men	35.30 ± 0.59
Women	30.78 ± 0.45
Inbreeding coefficient	0.006 ± 0.00004

Figures are mean ± standard error or percentage.

saturation. When HFE C282Y and H63D genotypes were considered in the model, the heritability estimates were 0.20 ± 0.07 (p < 0.0001) for iron, 0.22 ± 0.07 (p < 0.0001) for ferritin and 0.23 ± 0.07 (p < 0.0001) for transferrin saturation. The effects of HFE C282Y and H63D genotypes was highly significant (p < 0.001 for all three traits). The effect of the 282Y allele was 1.49 + 0.54 on serum iron level, 80.29 + 11.23 on ferritin and 4.34 + 0.98 on transferrin saturation. The effect of the 63D allele was somewhat lower: 1.49 + 0.44, 16.22 + 9.11 and 2.81 + 0.77 for iron, ferritin and transferrin saturation, respectively. The proportion of variance explained by HFE gene mutations was 2.1% for serum iron level, 5.6% for ferritin and 3.5% for transferrin saturation.

Table 3 shows the phenotypic (P) and genetic (G) correlations between serum iron, serum ferritin and transferrin saturation. As expected, there was a high and significant phenotypic and genotypic correlation between serum iron and serum ferritin.

The genetic correlation estimates vary little between models with and without the HFE genotypes included (data not shown). This suggests that HFE mutations equally affect serum iron, ferritin and transferrin saturation levels or that there are other, even more important pleiotropic genes.

To test the effect of inbreeding on the results presented here, we computed the mean of serum iron parameters by level of inbreeding. We categorised subjects into three categories; no inbreeding (0) for subjects with no inbreeding or inbreeding coefficient = 0, low inbreeding (1) for subjects with inbreeding coefficient ≤ to the mean inbreeding coefficient and high inbreeding coefficient (2) for subjects with inbreeding coefficient > mean inbreed-
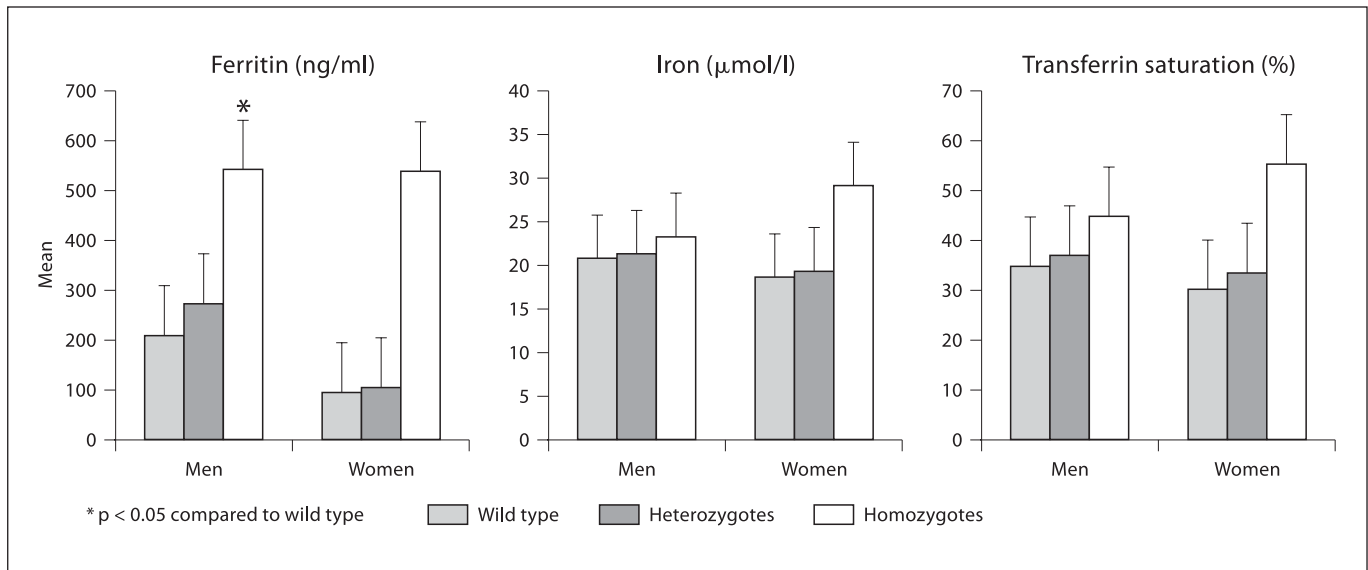
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Fig. 1. Mean (SE) level of serum iron indices by C282Y genotype.

Table 2. Heritability (h^2) estimates of serum iron, ferritin and transferrin saturation

	Serum iron	Serum ferritin	Serum transferrin saturation
Covariates			
Age + sex + age^2	0.23 ± 0.07 (p < 0.0001)	0.29 ± 0.09 (p < 0.0001)	0.28 ± 0.07 (p < 0.0001)
Age + sex + age^2 + HFE genotypes	0.20 ± 0.07 (p < 0.0001)	0.22 ± 0.07 (p < 0.0001)	0.23 ± 0.07 (p < 0.0001)

Figures are proportion of variance ± standard error.

Table 3. Phenotypic and genetic correlations between serum iron, ferritin and transferrin saturation

Serum iron and serum ferritin	P = 0.25, p < 0.001	G = 0.40, p < 0.001
Serum ferritin and serum transferrin saturation	P = 0.40, p < 0.001	G = 0.73, p < 0.001

P = Phenotypic correlation; G = genetic correlation.

ing coefficient. Figure 2 shows the mean iron indices by level of inbreeding. There was a significant difference in mean serum ferritin by level of inbreeding, predicting a recessive model. We observed no significant difference in the mean of serum iron and serum transferrin saturation by level of inbreeding.

Discussion

We have studied the contribution of genetic and environmental factors on serum iron indices. We found that a significant proportion of serum iron (23%), serum ferritin (29%) and transferrin saturation (28%) could be attributed to genetic factors. A large proportion of serum iron parameters is determined by environmental factors. We also found that serum iron indices are significantly

correlated, indicating that they share common genetic and environmental factors.

Compared to the twin study of Whitfield et al. [19], our heritability estimates are somewhat lower. This can be explained by the differences in study populations. We have studied large families from a genetic isolate and we have conditioned on the pedigree structure and accounted for relationship between individuals in our analysis. Heritability estimates from twin studies are based on differences in trait correlations between monozygotic and dizygotic twin pairs and are based on the assumption that environmental influences are the same for both twin pairs whereas differences between dizygotic twin pairs may be attributable to both genetic and environmental factors. Another explanation for the small differences in heritability estimates between these two studies can be the difference in the population's genetic make up as they studied an Australian population comprising subjects of Celtic origin. Celts are known to have a higher frequency of the HFE C282Y mutation and therefore increased iron stores. It is also possible that different methods of measurements of serum iron indices can lead to subtle differences in heritability estimates. We have calculated serum transferrin saturation using TIBC, whereas Whitfield et al. [19] used transferrin. Although some articles suggest that the relation between transferrin and TIBC is not fixed, others found that when each test is measured precisely and without bias, the ratio of TIBC (in μmol/l) to transferrin (in g/l) is indeed close to the expected value of 25.0 [25, 26]. Also, recent studies found high significant correlations between the TIBC and the serum transferrin values, even without this proposed calibration [27].

Our results in this genetic isolate are comparable to our previous report in another Dutch population [22] where we found that the HFE genotypes explained less than 5% of the variability in serum ferritin and about 6% variability in serum transferrin saturation.

The major strength of our study is its population structure. We did not only use related individuals but also included spouses and relatives not living in the same household. This has the advantage of reducing confounding by shared environment leading to more accurate estimates of the genetic effect.

We found that about 20 to 30% of serum iron indices can be attributed to genetic factors. We also observed a high genetic correlation between serum iron, ferritin and transferrin saturation suggesting that these traits are likely regulated by the same genes. It has been reported that iron regulates ferritin production [28].
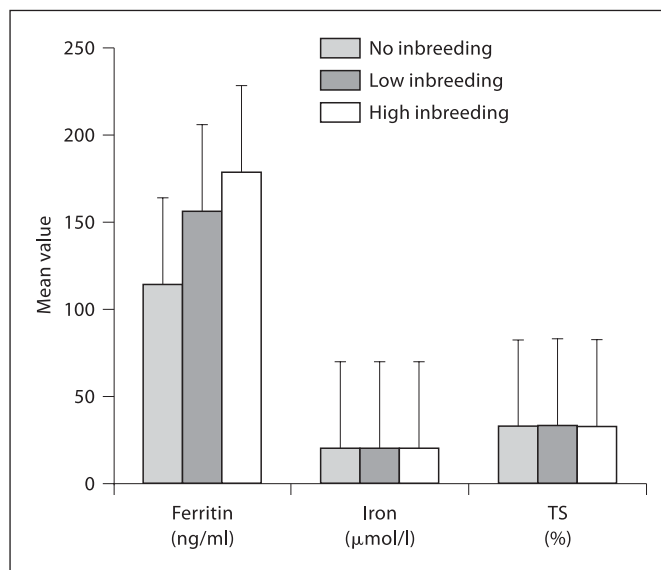

Fig. 2. Mean (SE) iron parameters by degree of inbreeding.

The HFE C282Y and H63D genotypes could explain 2 to 6% of the sex and age-adjusted variation in iron indices. This is in agreement with the study of Crawford et al. [14] who found a heritability of 3.5% for serum ferritin in sibs with hemochromatosis. The proportions themselves (2 to 6 %) may seem small at the first glance. However, for the iron levels and transferrin saturation these effects are comparable to those exhibited by such important factor as sex (2.6% for iron and 3.7% for transferrin saturation). Moreover, in the context of genetics of complex traits, an effect size in the magnitude of 5% is high. For example, a gene that is consistently implicated in human complex genetics is the APOE gene. The e2/e3/e4 polymorphism of this gene explains about 3 to 5% of the variation of total cholesterol level [29].

The observation of a 2 to 6% influence of the HFE on a complex trait such as iron points to a significant role of HFE in the complex iron network. This network according to the current knowledge involves numerous other proteins connecting several pathways [30–32]. Also the effect estimate for the C282Y and H63D mutations is in agreement with the observation that heterozygosity for the C282Y mutation in the HFE gene shows increased iron parameters compared to wild type and this mutation is associated with significantly higher iron loading compared to the H63D [22].

Without being the devil's advocate, these results bring back HFE to the central role it had been given just after

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its discovery in 1996 but that appears to have faded away since (i) the clinical penetrance of homozygosity for the C282Y mutation was shown to be low in 2001 [33], and (ii) other proteins of significant importance in iron metabolism were identified the last few years.

The control of iron homeostasis involves numerous genetic and environmental factors that are part of a wide network of metabolic pathways. This regulation appears to take place at both the systemic and cellular level with the intestine, bone marrow, macrophages and hepatocytes as the main regulatory sites and cell types [31]. In recent years, identification and characterization of new transporters, scavengers and regulatory proteins have considerably increased our understanding of iron homeostasis and its link with other pathways, such as innate immunity and signal transduction that have roles in a myriad of biological activities [31, 32]. The newly identified key players of iron metabolism among others include HFE, transferrin receptor 2 (TfR2), ferroportin and hemojuvelin, as well as the iron regulatory protein hepcidin [30]. The latter protein is a small peptide synthesized in the liver in response to the total burden of iron, but also to non-iron stimuli such as inflammation and oxidative stress.

Although the exact role of HFE is not known yet, the available data do suggest that HFE/TfR1 in parallel to TfR2 is implicated in the iron sensing pathway of hepatocytes that controls hepcidin synthesis (reviewed in [34]). But also HFE in macrophages might influence hepatic hepcidin expression [35].

Altogether, these proteins, signals and regulatory mechanism orchestrate expression of iron genes that involve both genetic and environmental modulation of transcription, translation and post-translational modifications. Thus it is likely that there is a multifactorial influence on serum iron parameters including a multigenetic rather than an oligogenetic effect.

The effect of inbreeding in our study population was significant for serum ferritin only, predicting a recessive mode of inheritance.

Future studies aiming at identifying genes influencing serum iron and ferritin levels in this population are necessary and can be done using a quantitative trait loci (QTL) method.

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