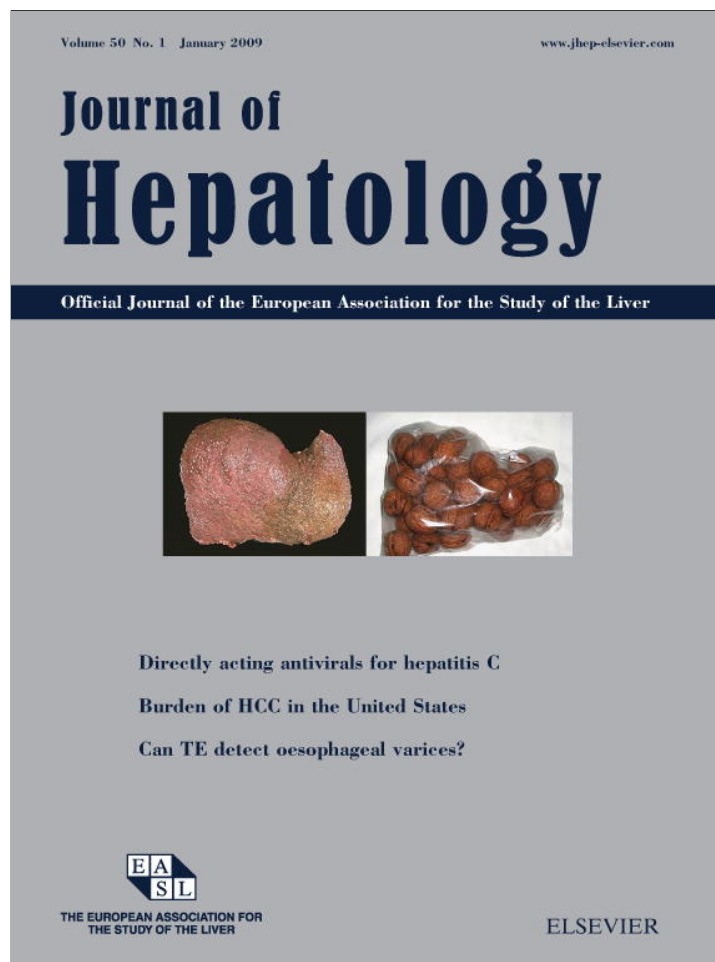




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Severity of iron overload of proband determines serum ferritin levels in families with HFE-related hemochromatosis: The HEmochromatosis FAmily Study[☆]

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Background/Aims: In families of patients with clinically detected hereditary hemochromatosis (HH) early screening has been suggested to prevent morbidity and mortality. Here, we aim to identify determinants for iron overload in first-degree family members of C282Y homozygous probands with clinically detected HH.

Methods: Data on HFE-genotype, iron parameters, demographics, lifestyle factors and health, were collected from 224 Dutch C282Y homozygous patients with clinically diagnosed HH and 735 of their first-degree family members (FDFM), all participating in the HEmochromatosis FAmily Study (HEFAS).

Results: The best predictive multivariable model forecasted 45% of variation of the serum ferritin levels. In this model severity of iron overload in the proband significantly predicted serum ferritin levels in FDFM. Other significant determinants in this model consisted of C282Y homozygosity, compound heterozygosity, age at testing for serum ferritin and supplemental iron intake, whereas a low body mass index showed a protective effect.

Conclusions: This study provides a model to assess the risk of development of iron overload for relatives of probands with HH. These results might be instrumental in the development of an optimal strategy for future family screening programs. © 2008 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Keywords: Hereditary hemochromatosis; HFE; Iron; Ferritin; Family; Screening

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Abbreviations: HH, hereditary hemochromatosis; FDFM, first-degree family members; HEFAS, hemochromatosis family study; TS, transferrin saturation; SF, serum ferritin; OR, odds ratio; BMI, body mass index; AUC, area under the curve; CI, confidence interval; WT, wild type.

1. Introduction

HFE-related hereditary hemochromatosis (HH) is an autosomal recessive disease characterized by a progressive deposition of iron in joints, pancreas, liver, heart and other vital organs, that might ultimately result in arthralgia, diabetes mellitus, liver cirrhosis, cardiac failure, rhythm disorders (reviewed in [1–3]). Organ failure and early death can be prevented through removing the accumulated iron by phlebotomy before irreversible organ damage occurs [4].

Because the penetrance of the C282Y homozygous HFE-genotype is low [3,5–9], the incremental benefits of population screening programs are likely to be small. Alternatively, targeted screening in high risk groups such as family members of clinically detected C282Y homozygous probands is an attractive strategy [10–13].

Identification of the determinants of disease development in the relatives of HH patients will contribute to the cost-effectiveness of these family screening programs. So far, searches for additional gene mutations that might identify those individuals have been largely fruitless [14,15] (and reviewed in [2,3]). The most robust determinant for disease development appeared to be the levels of serum iron indices, especially of serum ferritin [16,17].

Accordingly, we analysed the data from first-degree family members (FDFM) of clinically diagnosed C282Y homozygous probands, recruited in the Dutch Hemochromatosis Family Study (HEFAS) [18] for determinants of body iron stores.

2. Study population and methods

A detailed description of the HEFAS study has previously been reported [18].

2.1. HEmochromatosis FAmily Study (HEFAS) population

Only subjects who gave written informed consent were included in the study. Proband had to be at least 18 years old and clinically diagnosed with C282Y homozygous HH. The iron overload had to be confirmed by initial transferrin saturation (TS) and serum ferritin (SF) concentrations exceeding the reference value thresholds; TS > 50% for both men and women, SF $\geq$ 280 μ g/L for men, SF > 80 μ g/L for women under the age of 50, and SF $\geq$ 180 μ g/L for women $\geq$ 50 years, or corresponding values for SF depending on the reference values of the laboratories. When either one or both pre-treatment serum iron parameters were not available, the presence of iron overload was alternatively confirmed by previously performed liver biopsy (grade 3 iron deposition according to Sindram [19], in 6 of the 224 probands) or by the number of phlebotomies required to normalize SF (males $\geq$ 22 phlebotomies = 5 g chelatable iron; females $\geq$ 13 phlebotomies = 3 g chelatable iron; in 6 of the 224 probands) [20]. A total of 224 probands participated. They provided the HEFAS team with names and addresses of 972 FDFM defined in this study as biological parents, full siblings, and biological children, 18 years of age and older, of whom 735 met the inclusion criteria. Of these FDFM 45.7% reported to be diagnosed with hemochromatosis-related diseases [18]. Participants were recruited from May 2003 until August 2005.

2.2. Questionnaires

All participants were asked to fill out a questionnaire containing a large number of questions on demographics, lifestyle (smoking, alcohol intake, meat consumption), health status, general medical history, medication (i.e. use of iron supplements now or in the past), morbidity, medical history for HH, and family structure.

2.3. Laboratory data

Data on the included probands and family members were extracted from medical records of the participating hospitals or acquired from the physicians involved in diagnosis and treatment of the patients. Information on iron parameters (TS and SF) and liver biopsy of the participants was obtained at the time of diagnosis or screening for HH, whereas data on HFE-genotype and especially on the number of phlebotomies were also collected at points in time after the initial investigations. When incomplete, participants were offered counselling and blood testing by their general practitioner. Iron parameters for HEFAS were collected by several clinical laboratories. The TS and SF were quantified using validated routine laboratory methods. HFE-genetic test results were obtained from routinely used genetic tests.

2.4. Statistical methods

In this study we aimed at distinguishing FDFM of probands with clinically detected HFE-related HH, at risk for iron accumulation from those not at risk. For this purpose, elevated TS was defined as TS above 50% and elevated SF as SF above the gender and calendar time-specific local upper laboratory reference values. In some cases where the SF reference values were not available, the 67-percentile of all reference values was used. Furthermore, different reference values for premenopausal and postmenopausal women were taken into account when provided by the laboratories.

In the following data analyses the probands were excluded. Univariable logistic regression was used to study the ability of environmental, life habits and genotype variables to discriminate FDFM with elevated serum iron parameters from FDFM with non-elevated iron parameters, for each variable separately. The dependent variables were elevated TS and elevated SF, respectively. The crude odds ratios (OR) with 95% confidence intervals (CI) are presented.

Multivariable logistic regression with stepwise selection procedures was used to identify variables that contributed independently to the risk of elevated iron parameters, either in addition to genotype or in addition to family-degree. In this way genotype models and family-degree models were studied. Again, the dependent variables were elevated TS and elevated SF, respectively.

Putative iron accumulation determining variables used in the selection procedure consisted of gender, age at testing, body mass index (BMI), iron supplements (now or in the past, yes/no), alcohol use (>2 units a day), meat consumption (>200 g a day) and familial iron severity. Familial iron severity was defined as the value of TS of the proband in case elevated TS was studied and the value of SF of the proband divided by the upper reference value in case of elevated SF. The adjusted odds ratios with 95% confidence intervals of the final model are presented. The total R^2 is presented to indicate the total percentage explained variance in the outcome and the area under the curve (AUC) of the receiver operating characteristic curve is presented as measure of predictive discrimination.

The fit of model is visualized in a figure that shows the estimated and observed iron overload. The results of the final genotype model are also used to estimate the probability of elevated ferritin levels (with 95%CI) of C282Y homozygous family members by gender, age, BMI, use of iron supplements and familial iron severity. Note that this estimates the penetrance (of “elevated ferritin values”), including modifying factors.

All statistical analyses were performed using the SAS package version 8.2.

3. Results

3.1. Characteristics of the study population

The family members of the HEFAS population comprised 224 probands, 428 siblings, 241 children and 66 parents (Table 1). The percentage of male participants was slightly higher in the group of probands, 62.5% ($n = 140$), but lower among the sibling group, 46.3% ($n = 0198$) and children 40.7% ($n = 98$). The percentage of male participants of the parents was even lower (33.3%, $n = 22$), reflecting the higher age of survival of the women. The ages of the participants varied from median 56 years for probands, 54 years for siblings, 32 years for children, to 70 years for parents, respectively.

Table 1 also presents the genotype characteristics of the HEFAS population. One hundred percent of the probands were C282Y homozygous (by definition), compared to 29.9% ($n = 110$) of the siblings, 5.7% ($n = 11$) of the children and 2.2% ($n = 1$) of the parents, whereas C282Y heterozygosity was determined in 39.7%

($n = 146$), 78.1% ($n = 150$) and 78.3% ($n = 36$) of the siblings, children and parents, respectively.

The mean TS found for the probands was 86.8% (Quartile (Q)1–Q3; 74.0–96.3%), which was significantly higher than the mean TS of the other family groups, $p < 0.0001$. The same is true for the TS > 50%. The probands also revealed significantly higher values of absolute SF compared to the other FDFM, $p < 0.0001$. Of note is that not in all probands both the TS value and the SF value were elevated. In that case the excess of iron was confirmed either by liver biopsy or by the number of phlebotomies required to normalize serum ferritin levels.

In the C282Y homozygous HEFAS population TS > 50% was found in 93.2% ($n = 192$) of the probands, 86% ($n = 84$) of the siblings, 56% ($n = 5$) of the children and 100% ($n = 1$) of the parents. For an elevated SF these percentages amounted to 86.3% ($n = 183$) of the probands, 80% ($n = 84$) of the siblings, 22% ($n = 2$) of the children and 100% ($n = 1$) of the parents.

Table 1
Characteristics of the HEFAS population by family-degree

	Probands		Siblings		Children		Parents	
	Total	Median (Q1–Q3)/n (%)	Total	Median (Q1–Q3)/n (%)	Total	Median (Q1–Q3)/n (%)	Total	Median (Q1–Q3)/n (%)
	224		428		241		66	
Demographics								
Male	224	140 (62.5)	428	198 (46.3)	241	98 (40.7)	66	22 (33.3)
Age at participation (yr)	224	56 (48–63)	428	54 (47–62)	241	32 (26–38)	66	70 (63–78)
Education ($\geq$ secondary)	212	59 (27.8)	395	105 (26.6)	233	86 (36.9)	61	7 (11.5)
Paid job ($\geq$ 32 h/week)	78	44 (56.4)	178	87 (48.9)	160	97 (60.6)	4	1 (25.0)
Body mass index (kg/m ²)	215	25.9 (23.5–27.9)	417	25.4 (23.3–27.8)	238	23.4 (21.6–26.5)	62	26.2 (24.0–29.1)
Lifestyle								
Smoking (ever)	220	168 (76.4)	421	289 (68.7)	241	129 (53.5)	65	45 (69.2)
Alcohol >2 units/day	189	44 (23.3)	374	103 (27.5)	204	55 (27.0)	50	5 (10.0)
Iron supplements (yes)	218	14 (6.4)	416	52 (12.5)	240	26 (10.8)	62	9 (14.5)
Meat consumption (>200 g/d)	214	85 (39.7)	398	129 (32.4)	230	95 (41.3)	59	11 (18.6)
Blood donation (never)	212	152 (71.7)	409	310 (75.8)	239	200 (83.7)	57	50 (87.7)
Menarche ($\leq$ 12 yr)**	80	5 (6.3)	223	30 (13.5)	141	18 (12.8)	39	6 (15.4)
Pregnancies (>3)**	67	23 (34.3)	196	97 (49.5)	84	33 (39.3)	41	33 (80.5)
Genotype	224		368		192		46	
C282Y/C282Y		224 (100)		110 (29.9)		11 (5.7)		1 (2.2)
C282Y/H63D		0		19 (5.2)		27 (14.1)		8 (17.4)
C282Y/WT*		0		146 (39.7)		150 (78.1)		36 (78.3)
WT/WT†		0		93 (25.3)		4 (2.1)		1 (2.2)
Iron parameters‡								
TS (%)	206	86.8 (74.0–96.3)	361	36.7 (27.5–59.6)	189	36.4 (26.3–49.1)	49	36.2 (27.7–46.6)
TS > 50%	206	192 (93.2)	361	119 (33.0)	189	47 (24.9)	49	10 (20.4)
SF (μ mol/L)	212	1031 (389–2025)	366	155 (62–383)	191	70 (33–160)	48	113 (74–235)
SF above normal (μ mol/L)§	212	183 (86.3)	366	133 (36.4)	191	32 (16.8)	48	13 (27.1)

HEFAS, HEMochromatosis FAmily Study; Q1–Q3, inter quartile range; n , number; **, only women; *, C282Y/WT includes C282Y/WT and C282Y/unknown; †, WT/WT includes H63D/H63D; H63D/WT, WT/WT; education ($\geq$ secondary), education consisted of at least secondary school; WT, wild type; ‡, at time of being tested for hereditary hemochromatosis; §, serum ferritin above the local upper reference value; TS, transferrin saturation; SF, serum ferritin; SF above normal, SF above the gender- and calendar time-specific local laboratory upper reference values. In case reference values were not available, the 67th percentile of all references ranges was used.

3.2. Determinants of elevated serum iron indices

Table 2 demonstrates the ability of the candidate predisposing iron storage factors to discriminate between FDFM with and without elevated serum iron indices. As expected being C282Y homozygous was correlated with a significantly raised risk of both elevated TS and elevated SF compared to being wild type/wild type (WT/WT) (OR 80.3, 95%CI 36.8–175 and OR 22.5, 95%CI 12.2–41.4, respectively). Similarly, being compound heterozygous (C282Y/H63D) increased the risk of elevated TS and elevated SF with OR 4.84 (95%CI 1.96–12.0) and OR 4.02 (95%CI 1.84–8.80), respectively. Compared to the other FDFM, the probands showed the highest probability of elevated TS (OR 53.5, 95%CI 22.1–129.1) and elevated SF (OR 17.0, 95%CI 8.05–35.9), which is likely to be due to the higher prevalence of C282Y homozygosity among probands. Male gender also significantly increased the risk of elevated TS (OR 2.04, 95%CI 1.54–2.70), and of elevated SF (OR 1.91, 95%CI 1.45–2.54). The age at testing was only identified as a significant risk factor of an elevated SF, as was having an abnormal BMI and higher meat consumption. Alcohol consumption (>2 units/day) and blood donation showed no influence on the iron parameters in this univariable analysis. The intake of iron supplements lowered the risk of elevated TS (OR 0.59,

95%CI 0.37–0.95), whereas women who had >3 pregnancies, had a higher risk of elevated TS (OR 1.51, 95%CI 0.96–2.36) and of elevated SF (OR 1.59; 95%CI 1.01–2.48). Note that these crude odds are inconsistent with a biological system and therefore are likely to be confounded by other variables. In the next paragraph multivariable analysis is performed to obtain adjusted or unbiased estimated odds ratios.

3.3. Predictive models for elevated iron parameters

3.3.1. Genotype model

We next sought to identify the variables that contributed independently to the risk of elevated iron parameters in addition to the combination of genotype, age, gender and familial iron severity (defined as the TS or the SF value relative to the reference of the proband). Table 3 shows the adjusted odd ratios of this so called “genotype model” for the prediction of elevated TS and of elevated SF.

The probability of having an elevated TS was significantly increased by C282Y homozygosity and compound heterozygosity compared to WT/WT (OR 59.9, 95%CI 21.2–169 and OR 4.89, 95%CI 1.67–14.3, respectively). Interestingly, a BMI > 30 kg/m² independently diminished the risk of TS elevation. Both the *R*² and the AUC of this genotype model are relatively large,

Table 2

The crude odds ratios with 95% confidence intervals of elevated transferrin saturation and serum ferritin values among first-degree family members using univariable logistic regression

	Elevated transferrin saturation Odds ratio (95%CI)	Elevated ferritin concentration Odds ratio (95%CI)
Genotype		
C282Y/C282Y	80.3 (36.8–175)	22.5 (12.2–41.4)
C282Y/H63D	4.84 (1.96–12.0)	4.02 (1.84–8.80)
C282Y/WT*	1.73 (0.82–3.66)	0.81 (0.43–1.51)
WT/WT†	1.00 (reference)	1.00 (reference)
Family-degree		
Probands	53.5 (22.1–129)	17.0 (8.05–35.9)
Siblings	1.92 (0.93–3.98)	1.54 (0.79–3.00)
Children	1.29 (0.60–2.79)	0.54 (0.26–1.14)
Parents	1.00 (reference)	1.00 (reference)
General characteristics		
Male	2.04 (1.54–2.70)	1.91 (1.45–2.54)
Age at testing (yr)	1.00 (1.00–1.02)	1.03 (1.02–1.04)
Education (≥secondary school)	0.95 (0.69–1.31)	1.10 (0.80–1.51)
Paid job (≥32 h/week)	1.00 (0.65–1.53)	1.04 (0.68–1.60)
BMI (kg/m ²)	0.99 (0.95–1.02)	1.08 (1.04–1.12)
Alcohol >2 units/day	0.76 (0.54–1.01)	0.80 (0.59–1.12)
Iron supplements (yes)	0.59 (0.37–0.95)	0.85 (0.54–1.33)
Meat consumption (>200 gr/d)	0.85 (0.63–1.14)	1.51 (1.12–2.04)
Blood donation (never)	0.79 (0.56–1.10)	1.11 (0.79–1.56)
Menarche (≤12 yr)**	0.83 (0.44–1.56)	1.12 (0.60–2.10)
Pregnancies (>3)**	1.51 (0.96–2.36)	1.59 (1.01–2.48)

*, C282Y/WT includes C282Y/WT and C282Y/unknown; †, WT/WT includes H63D/H63D, H63D/WT, WT/WT; **, only women; WT, wild type; BMI, body mass index; elevated TS, TS > 50%; elevated SF, SF above the gender- and calendar time-specific local laboratory upper reference values. In case reference values were not available, the 67th percentile of all references ranges was used.

Table 3

The adjusted odds ratios of the genotype model of elevated transferrin saturation and serum ferritin values, among first-degree family members using multivariable logistic regression

	Elevated transferrin saturation OR _{adj} (95%CI) [^]	Elevated ferritin concentration OR _{adj} (95%CI) [^]
Genotype		
C282Y/C282Y	59.9 (21.2–169)	20.9 (9.74–44.7)
C282Y/H63D	4.89 (1.67–14.3)	5.30 (2.26–12.5)
C282Y/WT*	1.75 (0.72–4.27)	0.99 (0.50–1.93)
WT/WT [†]	1.00 (reference)	1.00 (reference)
Gender		
Male	1.66 (0.08–1.66)	1.34 (0.82–2.17)
Female	1.00 (reference)	1.00 (reference)
Familial iron severity	1.00 (0.99–1.02)	1.06 (1.02–1.10)
Age at testing (yr)	1.00 (0.98–1.01)	1.03 (1.01–1.05)
BMI (kg/m ²)		
<20	0.42 (0.11–1.53)	0.25 (0.07–0.94)
20–25	1.00 (reference)	1.00 (reference)
25–30	0.66 (0.36–1.02)	1.45 (0.87–2.42)
>30	0.21 (0.07–0.62)	1.12 (0.52–2.41)
Iron supplements	n.s.	
Yes	–	2.16 (1.10–4.24)
No	–	1.00 (reference)
Alcohol		n.s.
>2 units/day	0.58 (0.32–1.05)	–
≤2 units/day	1.00 (reference)	–

Selection was performed when genotype, age, gender and severity were already included. Of the other putative candidates for iron loading, e.g. BMI, use of iron supplements, alcohol >2 units a day and meat consumption, only those that significantly contribute to elevated serum iron indices are shown; age at testing, age at which serum iron indices were measured; n.s., these variables were not selected; OR_{adj}, odd ratio adjusted for the other variables in the model; *, C282Y/WT includes C282Y/WT and C282Y/unknown; †, WT/WT includes H63D/H63D, H63D/WT, WT/WT; ^, R^2 and area under the curve are $\pm 45\%$ and 83% for these both two respective models.

WT, wild type; familial iron severity, the value of transferrin saturation of the proband in case elevated transferrin saturation was studied and the value of serum ferritin of the proband divided by the reference value in case of elevated serum ferritin; elevated TS, TS > 50%; elevated SF, SF above the gender- and calendar time-specific local laboratory upper reference values. In case reference values were not available, the 67th percentile of all references ranges was used.

i.e. in total 44.6% (R^2) of the variance in elevated TS could be explained by the selected variables and the discriminatory power was 83.2% (AUC).

The risk of an elevated SF was also significantly increased by being C282Y/C282Y or C282Y/H63D compared to WT/WT (OR = 20.9, 95%CI: 9.74–44.7 and OR = 5.30, 95%CI 2.26–12.5, respectively). Furthermore, the familial iron severity was predictive for an elevated SF (OR 1.06, 95%CI 1.02–1.10), as was the age of testing (OR 1.03, 95%CI 1.01–1.05). In this genotype model a BMI < 20 kg/m² was protective for the risk of elevated SF, whereas the intake of iron supplements was associated with increased risk. Noteworthy is that gender was not statistically significant in this multivariate logistic regression analysis. This may be indicative for the idea that gender is not an independently risk factor of an elevated SF that is based on age and gender specific reference values. The R^2 and AUC were similar to those of the genotype model of elevated TS, 45.3% and 84.7%, respectively.

3.3.2. Family-degree model

We also investigated the factors that contributed to the prediction of iron overload in addition to the combination of family-degree, age, gender and family iron severity. Table 4 shows the adjusted odd ratios of this “family-degree model” for the prediction of elevated TS and of elevated SF. The variables to predict elevated TS in this family-degree model only reached the level of borderline significance resulting in a low fit and a moderate discriminatory power ($R^2 = 4.0\%$, AUC = 61.7%). The factors predicting an elevated risk of increased SF were comparable to the factors mentioned in Table 3, though their influence appeared to be less clear: familial iron severity (OR 1.04, 95%CI 1.01–1.08) and age at testing (OR 1.02, 95%CI 1.003–1.05). The percentage explained variance and the discriminatory power were only slightly better than in the family-degree model of elevated TS ($R^2 = 15.3\%$, AUC = 70.3%).

Taken together, the most important findings are (i) the severity of the iron overload in the proband contrib-

Table 4
The adjusted odds ratios of the family-degree model of elevated TS and SF values, among first-degree family members using multivariable logistic regression

	Elevated transferrin saturation OR _{adj} (95%CI) ^Δ	Elevated ferritin concentration OR _{adj} (95%CI) ^Δ
Family-degree		
Siblings	1.82 (0.67–4.98)	2.16 (0.84–5.55)
Children	1.80 (0.51–6.29)	0.76 (0.21–2.70)
Parents	1.00 (reference)	1.00 (reference)
Gender		
Male	1.51 (0.98–2.33)	1.27 (0.82–1.98)
Female	1.00 (reference)	1.00 (reference)
Familial iron severity	1.00 (0.99–1.01)	1.04 (1.01–1.08)
Age at testing (yrs)	1.01 (0.99–1.03)	1.02 (1.003–1.05)
Meat consumption	n.s.	
>200 g per day	–	1.61 (1.01–2.56)
≤200 g per day	–	1.00 (reference)
Alcohol		n.s.
>2 units/day	0.65 (0.41–1.04)	–
≤2 units/day	1.00 (reference)	–

Selection was performed when family-degree, age, gender and familial iron severity were already included. Of the other putative candidates for iron loading, e.g. BMI, use of iron supplements, alcohol >2 units a day and meat consumption, only those that significantly contribute to elevated serum iron indices are shown; age at testing, age at which serum iron indices were measured; n.s., these variables are not selected; ^Δ, R^2 is 4% and 15% and the area under the curve is 62% and 70% for the two models for the prediction of TS and ferritin, respectively.

OR_{adj}, Odds Ratio adjusted for the other variables in the model; familial iron severity, the value of TS of the proband in case elevated TS was studied and the value of SF of the proband divided by the reference value in case of elevated SF; elevated TS, TS > 50%; elevated SF, SF above the gender- and calendar time-specific local laboratory upper reference values. In case reference values were not available, the 67th percentile of all references ranges was used.

uted to the prediction of iron overload in his/her relatives and (ii) the genotype models outperform by far the family-degree models using both the percentage explained variance and the discriminatory power.

3.4. Observed and predicted probability of elevated serum ferritin levels

We next compared the *predicted* SF levels, as obtained by this genotype model, with the *observed* elevated SF levels. This is visualized in Fig. 1. The observed elevated SF (in panels A) and the expected probability of elevated SF (in panels B) are shown for the C282Y/C282Y genotype (upper panels) and the C282Y/H63D genotype (lower panels) as a function of familial iron severity and age of testing. The figure shows that black spots in the A panels, representing FDMD with elevated SF, correspond very closely with the larger bubbles in the B panels, representing FDMD with higher predicted probability of elevated SF. This indicates the large discriminatory power of our model. The black spots between the open spots in the A panels may indicate that in addition to age and familial severity alone also other factors contribute to the elevated SF level. Similarly, large bubbles between small bubbles in the B panels point to the influence of other unknown variables that next to age and familial severity contribute to the prediction of an elevated SF.

3.5. Tabulated risk calculation to develop iron overload

Finally the risk for a C282Y homozygous FDFM to develop iron overload is presented in Table 5 (males) and Table 6 (females) by age, BMI, use of iron supplements and familial iron severity. For instance a 50 yr old male, with a BMI of 25 kg/m², not using iron supplements, with a related proband presenting with an SF level of four times the reference value, has an estimated risk for iron overload of 0.80 (95%CI 0.69–0.87).

4. Discussion

The potentially positive effects of family screening for HH to prevent iron accumulation and its related morbidity and mortality in individuals at risk for HH calls for a thorough investigation of the usefulness of family screening for the early detection of HH in the light of the discussion on the penetrance of the HFE-gene mutation [10–13]. Recently, we demonstrated a strongly increased HH related morbidity in family members of probands with clinically detected HH compared to an age and gender matched healthy population [18]. In the search for co-factors that contribute to iron overload, we showed that the risk of elevated body iron stores is strongly related to the genotype of the FDFM, e.g. C282Y homozygosity and compound

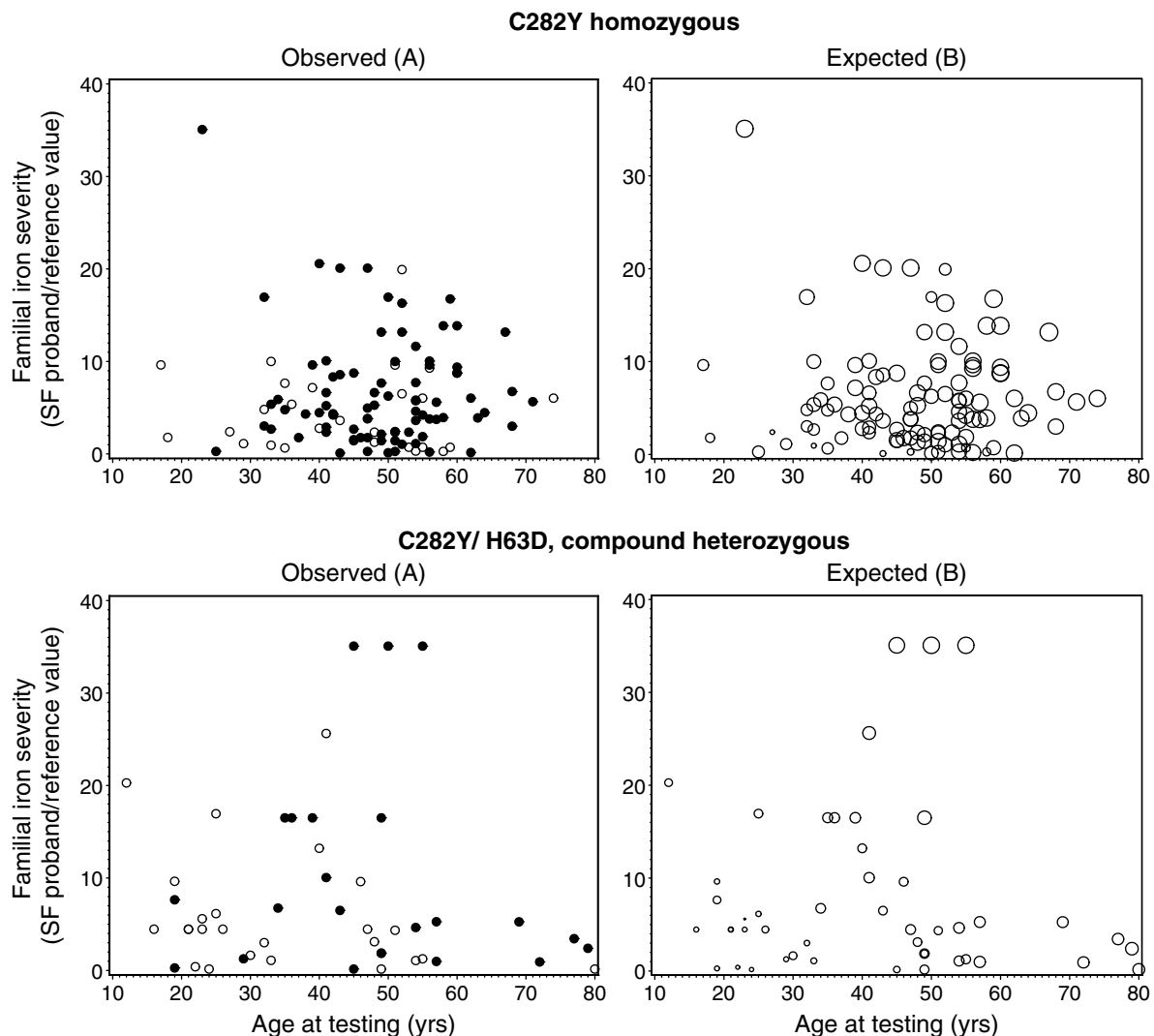


Fig. 1. The observed (A) and predicted probability (B) of elevated serum ferritin concentration (SF) by severity of iron overload in the family and by age of first-degree family members with the homozygous genotype (upper panel) or with compound heterozygous genotype (lower panel). The black spots in panel A indicate family members with elevated SF and the size of the bubbles in panel B indicate the predicted probability of elevated SF, using the final multivariable logistic regression model. Severity of iron overload in the family was defined as the value of SF of the proband divided by the upper reference value of the local laboratory.

heterozygosity, and much less to family-degree. In addition to these factors, an older age at testing for ferritin levels, a more severe iron overload in the related proband at presentation and the use of iron supplements all contributed significantly to the prediction of iron overload in the FDFM. In contrast, a low BMI protected against iron accumulation, whereas the family-degree itself (sibling, parents, child) provided no additional information.

Our finding on HFE-genotype as the strongest predictor for the accumulation of iron, corroborates previous reports [21–24]. In addition to that we are the first to confirm a positive relationship between the severity of iron overload found for the proband and that detected in the relatives. These results suggest the presence of fac-

tors that modify the biochemical penetrance of the HFE-genotype, such as co-inherited genes or family related environmental factors. Previously, Whiting et al. observed a concordance of iron indices in 35 homozygote and 35 heterozygote sibling pairs in hemochromatosis families, suggesting the existence of these familial modification factors [25]. Two other studies, however, do not corroborate our findings. Mura et al. could not demonstrate a familial predisposition for iron overload, as they found no significant correlation between SF or TS between small series of sex matched homozygous sibling pairs ($n = 18$) in which one patient exhibited total body iron overload and the other was clinically diagnosed as HH [26]. McCune et al. also looked at a familial predisposition of HFE-gene expres-

Table 5

The estimated probability (95%CI) of developing iron overload in C282Y homozygous FDFM males by age, BMI, use of iron supplements and familial severity using multivariable logistic regression

Age (yr)*	BMI (kg/m ²)	Use of iron supplements									
		Yes					No				
		Familial iron severity					Familial iron severity				
		1	2	3	4	5	1	2	3	4	5
30	<20	0.45	0.46	0.48	0.49	0.50	0.27	0.29	0.30	0.31	0.32
		(0.15–0.78)	(0.16–0.79)	(0.17–0.80)	(0.18–0.81)	(0.19–0.82)	(0.09–0.59)	(0.10–0.60)	(0.10–0.62)	(0.11–0.63)	(0.11–0.64)
	20–30	0.79	0.80	0.81	0.82	0.83	0.64	0.65	0.66	0.68	0.69
		(0.60–0.90)	(0.61–0.91)	(0.62–0.91)	(0.64–0.92)	(0.65–0.92)	(0.48–0.77)	(0.50–0.78)	(0.52–0.78)	(0.53–0.79)	(0.54–0.80)
	>30	0.78	0.79	0.80	0.81	0.82	0.62	0.64	0.65	0.66	0.68
		(0.53–0.92)	(0.55–0.92)	(0.56–0.92)	(0.58–0.93)	(0.59–0.93)	(0.40–0.81)	(0.41–0.81)	(0.43–0.82)	(0.44–0.83)	(0.46–0.84)
40	<20	0.52	0.54	0.55	0.57	0.58	0.34	0.35	0.37	0.38	0.39
		(0.20–0.83)	(0.21–0.84)	(0.22–0.84)	(0.23–0.85)	(0.24–0.86)	(0.12–0.66)	(0.67–0.72)	(0.13–0.68)	(0.14–0.70)	(0.15–0.71)
	20–30	0.84	0.84	0.85	0.86	0.87	0.71	0.72	0.73	0.74	0.75
		(0.67–0.93)	(0.69–0.93)	(0.71–0.93)	(0.71–0.94)	(0.72–0.94)	(0.57–0.81)	(0.59–0.82)	(0.60–0.83)	(0.62–0.83)	(0.63–0.84)
	>30	0.83	0.84	0.84	0.85	0.86	0.69	0.70	0.72	0.73	0.74
		(0.62–0.93)	(0.63–0.94)	(0.64–0.94)	(0.66–0.94)	(0.67–0.94)	(0.48–0.84)	(0.50–0.85)	(0.51–0.86)	(0.53–0.87)	(0.54–0.88)
50	<20	0.60	0.61	0.63	0.64	0.65	0.41	0.43	0.44	0.46	0.47
		(0.25–0.87)	(0.27–0.87)	(0.28–0.89)	(0.29–0.89)	(0.30–0.89)	(0.16–0.73)	(0.17–0.74)	(0.17–0.75)	(0.18–0.76)	(0.19–0.77)
	20–30	0.87	0.88	0.89	0.89	0.90	0.77	0.78	0.79	0.80	0.80
		(0.74–0.95)	(0.75–0.95)	(0.76–0.95)	(0.77–0.95)	(0.78–0.96)	(0.65–0.85)	(0.66–0.86)	(0.68–0.87)	(0.69–0.87)	(0.70–0.88)
	>30	0.87	0.87	0.88	0.89	0.90	0.75	0.76	0.78	0.79	0.79
		(0.69–0.95)	(0.70–0.95)	(0.71–0.96)	(0.72–0.96)	(0.74–0.96)	(0.56–0.88)	(0.58–0.89)	(0.59–0.89)	(0.61–0.90)	(0.62–0.90)
60	<20	0.67	0.68	0.70	0.71	0.72	0.49	0.50	0.52	0.53	0.55
		(0.31–0.90)	(0.33–0.91)	(0.34–0.91)	(0.35–0.92)	(0.37–0.92)	(0.20–0.79)	(0.20–0.80)	(0.22–0.80)	(0.23–0.81)	(0.24–0.82)
	20–30	0.90	0.91	0.91	0.92	0.92	0.82	0.82	0.83	0.84	0.85
		(0.79–0.96)	(0.80–0.96)	(0.81–0.96)	(0.82–0.97)	(0.83–0.97)	(0.71–0.89)	(0.72–0.90)	(0.73–0.90)	(0.75–0.91)	(0.76–0.91)
	>30	0.90	0.90	0.91	0.91	0.92	0.81	0.82	0.82	0.83	0.84
		(0.75–0.96)	(0.76–0.97)	(0.77–0.97)	(0.78–0.97)	(0.79–0.97)	(0.63–0.91)	(0.65–0.91)	(0.66–0.92)	(0.67–0.92)	(0.69–0.93)

Iron overload is defined by a serum ferritin level above the gender- and calendar time-specific local laboratory upper reference values. In casereference values were not available, the 67th percentile of all references ranges was used. FDFM, first-degree family members; *, age at time the serum ferritin levels was measured; familial iron severity, the level of serum ferritin of the proband divided by the reference value; a “2” implicates a serum ferritin level that is two times the upper reference value of the laboratory; BMI, body mass index.

sion. They compared the amount of iron overload of C282Y homozygous relatives of 60 clinically affected C282Y homozygous index cases with the amount of iron overload found in C282Y homozygous relatives of 59 C282Y homozygous blood donors detected by genetic screening [27]. After correcting for HFE-genotype, life style and gender, the multivariable analyses showed that, in contrast to our findings, being related to a clinically affected proband was no longer a significant risk factor for iron overload.

In a study that is similar to ours, Lazarescu et al. [28] report for 53 hemochromatosis pedigrees (each consisting of at least two untreated C282Y homozygotes, and comprising a total of 296 individuals) that by multivariate analyses increased ferritin levels in C282Y homozygotes were significantly associated with male sex, increasing age and absence of a non-expressor in the family. These findings corroborate our results in that next to C282Y genotype we also found increasing age as a predictor for ferritin levels. The fact that in our study gender did not add any predictive value for iron overload in contrast to findings in literature, is inherent to our use of gender and menopause specific SF reference value. In addition, the potentially protective effect for iron overload by a low BMI (<20 kg/m²) might be attributed to the increased num-

ber of individuals in this group with severe diseases reducing iron stores.

We found that a high BMI (>30 kg/m²) independently reduces the risk of an elevated TS in FDFM of clinically detected C282Y homozygotes. These results confirm those obtained by Laine et al. who reported that the phenotypic expression of C282Y homozygous women, measured by TS, depended on their BMI, as 82% of the women with a BMI > 27 kg/m² were non-expressing hemochromatosis patients (TS < 45%) [29]. A biological explanation might be found in the increased circulating hepcidin levels in obese patients, through both hepcidin production by the adipose tissue [30], and the (low grade) inflammatory condition of the patients which is likely to increase liver hepcidin synthesis. As hepcidin decreases the intestinal iron uptake and the reticuloendothelial system iron release, these increased hepcidin levels are predicted to result in lower circulating iron levels (reviewed in [31]). For severely obese C282Y homozygotes one could therefore postulate that the increased hepcidin expression in the adipose tissue as well as an intact hepatic inflammatory induction of hepcidin, compensates for the innately low hepcidin expression in the liver of C282Y homozygotes [32], protecting these patients from iron overload. We

Table 6

The estimated probability (95%CI) of developing iron overload in C282Y homozygous FDFM females by age, BMI, use of iron supplements and familial severity using multivariable logistic regression

Age (yr)*	BMI (kg/m ²)	Use of iron supplements									
		Yes					No				
		Familial iron severity					Familial iron severity				
		1	2	3	4	5	1	2	3	4	5
30	<20	0.37	0.38	0.40	0.41	0.42	0.22	0.23	0.24	0.25	0.26
		(0.13–0.70)	(0.13–0.71)	(0.14–0.72)	(0.15–0.74)	(0.16–0.75)	(0.07–0.50)	(0.07–0.51)	(0.08–0.53)	(0.08–0.54)	(0.09–0.55)
	20–30	0.73	0.74	0.75	0.76	0.77	0.56	0.57	0.59	0.60	0.62
		(0.54–0.86)	(0.56–0.87)	(0.57–0.87)	(0.59–0.88)	(0.60–0.89)	(0.41–0.70)	(0.42–0.71)	(0.44–0.72)	(0.45–0.73)	(0.47–0.74)
	>30	0.72	0.73	0.74	0.75	0.76	0.54	0.56	0.57	0.59	0.60
		(0.48–0.88)	(0.50–0.88)	(0.51–0.89)	(0.53–0.89)	(0.54–0.90)	(0.33–0.74)	(0.35–0.75)	(0.36–0.76)	(0.37–0.77)	(0.39–0.78)
40	<20	0.44	0.46	0.47	0.49	0.50	0.27	0.28	0.30	0.31	0.32
		(0.17–0.76)	(0.18–0.77)	(0.19–0.78)	(0.19–0.79)	(0.20–0.80)	(0.09–0.57)	(0.10–0.59)	(0.11–0.60)	(0.11–0.61)	(0.12–0.62)
	20–30	0.79	0.80	0.81	0.82	0.82	0.63	0.65	0.66	0.67	0.69
		(0.63–0.89)	(0.64–0.90)	(0.65–0.90)	(0.67–0.91)	(0.68–0.91)	(0.50–0.75)	(0.51–0.76)	(0.53–0.77)	(0.54–0.78)	(0.56–0.79)
	>30	0.89	0.90	0.90	0.91	0.91	0.75	0.76	0.77	0.78	0.79
		(0.57–0.90)	(0.58–0.91)	(0.60–0.91)	(0.61–0.92)	(0.62–0.92)	(0.41–0.79)	(0.43–0.80)	(0.44–0.81)	(0.46–0.82)	(0.47–0.83)
50	<20	0.52	0.53	0.55	0.56	0.58	0.34	0.35	0.36	0.38	0.39
		(0.21–0.81)	(0.23–0.82)	(0.24–0.83)	(0.25–0.84)	(0.26–0.84)	(0.12–0.65)	(0.13–0.66)	(0.14–0.67)	(0.15–0.68)	(0.15–0.69)
	20–30	0.83	0.84	0.85	0.86	0.86	0.70	0.71	0.73	0.74	0.75
		(0.70–0.92)	(0.71–0.92)	(0.72–0.93)	(0.73–0.93)	(0.74–0.93)	(0.57–0.81)	(0.59–0.81)	(0.61–0.82)	(0.62–0.83)	(0.64–0.84)
	>30	0.83	0.83	0.84	0.85	0.86	0.69	0.70	0.71	0.73	0.74
		(0.64–0.93)	(0.66–0.93)	(0.67–0.93)	(0.68–0.94)	(0.69–0.94)	(0.49–0.84)	(0.51–0.84)	(0.52–0.85)	(0.54–0.86)	(0.55–0.86)
60	<20	0.60	0.61	0.62	0.64	0.65	0.41	0.42	0.44	0.45	0.47
		(0.27–0.86)	(0.28–0.86)	(0.29–0.87)	(0.30–0.88)	(0.32–0.88)	(0.16–0.72)	(0.17–0.73)	(0.18–0.74)	(0.19–0.75)	(0.20–0.76)
	20–30	0.87	0.88	0.89	0.89	0.90	0.76	0.77	0.78	0.79	0.80
		(0.75–0.94)	(0.76–0.94)	(0.77–0.95)	(0.78–0.95)	(0.79–0.95)	(0.64–0.85)	(0.65–0.86)	(0.67–0.87)	(0.68–0.87)	(0.70–0.88)
	>30	0.87	0.87	0.88	0.89	0.89	0.75	0.76	0.77	0.78	0.79
		(0.71–0.95)	(0.72–0.95)	(0.73–0.95)	(0.74–0.95)	(0.75–0.96)	(0.57–0.88)	(0.58–0.88)	(0.60–0.89)	(0.61–0.89)	(0.62–0.90)

Iron overload is defined by a serum ferritin level above the gender- and calendar time-specific local laboratory upper reference values. In case reference values were not available, the 67th percentile of all references ranges was used. FDFM, first-degree family members; *, age at time the serum ferritin levels was measured; familial iron severity, the level of serum ferritin of the proband divided by the reference value, a “3” implicates a serum ferritin level that is three times the upper reference value of the laboratory; BMI, body mass index.

found, however, no independent contribution of an elevated BMI to decreased ferritin levels. It is conceivable that the lower intestinal uptake of iron by elevated hepcidin levels is compensated by the increase in ferritin levels through (i) induction of ferritin synthesis by inflammatory cytokines, (ii) hepcidin-induced iron trapping in the RES and (iii) increased ferritin release in the circulation through lysis of hepatocytes damaged by steatosis [33]. Taken together, the relevance of obesity as a predictor for the iron overload in C282Y homozygous relatives of clinically affected C282Y homozygotes remains unclear. Therefore, to accurately assess the role of BMI in the biochemical penetrance of HFE-aberrations in HH-families assessment of liver iron overload by either MRI or liver biopsy is recommended.

Our results underline the relevance of family screening for HFE-related hemochromatosis by showing a strong relationship between HFE-genotype and iron overload. Further studies are needed to assess the association of these elevated iron indices with the development of iron-overload-related disease in these families of clinically expressing probands. We showed that HFE-genotype, age at testing for HH, severity of iron

overload within the family, BMI and the intake of iron supplements are the most important predictive factors for developing iron overload in FDFM of clinically diagnosed C282Y homozygous probands. These results might be instrumental in the development of an optimal strategy for future family screening programs.

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