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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.**

***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.

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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. Probands 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 $\geqslant$ 280 μg/L for men, SF > 80 μg/L for women under the age of 50, and SF $\geqslant$ 180 μg/L for women $\geqslant$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 $\geqslant$22 phlebotomies = 5 g chelatable iron; females $\geqslant$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.