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Morbidity and mortality in first-degree relatives of C282Y homozygous probands with clinically detected haemochromatosis compared with the general population: the HEmochromatosis FAmily Study (HEFAS)

E.M.G. Jacobs[1,2], J.C.M. Hendriks[3], J.J.M. Marx[1,4], C.Th.B.M. van Deursen[5], H.G. Kreeftenberg[6], R.A. de Vries[7], A.F.H. Stalenhoef[8], A.L.M. Verbeek[3], D.W. Swinkels[1*]

Departments of [1]Clinical Chemistry, [2]Haematology, [3]Epidemiology and Biostatistics, Radboud University Nijmegen Medical Centre, the Netherlands, [4]Eijkman Winkler Institute, University Medical Centre Utrecht, the Netherlands, [5]Department of Internal Medicine and Gastroenterology, Atrium Medical Centre, Heerlen/Brunssum, the Netherlands, [6]Department of Internal Medicine, University Medical Centre Groningen, the Netherlands, [7]Department of Hepato-Gastroenterology, Rijnstate Hospital, Arnhem, the Netherlands, [8]Department of Internal Medicine, Radboud University Nijmegen Medical Centre, the Netherlands, [*]corresponding author: tel.: +31 (0)24-361 89 57, fax: +31 (0)24-354 17 43, e-mail: d.swinkels@akc.umcn.nl

ABSTRACT

Background: Family screening has been suggested as a sophisticated model for the early detection of *HFE*-related hereditary haemochromatosis (HH). However, until now, controlled studies on the morbidity and mortality in families with HH are lacking.

Methods: Data on iron parameters, morbidity and mortality were collected from 224 Dutch C282Y-homozygous probands with clinically overt HH and 735 of their first-degree family members, all participating in the HEmochromatosis FAmily Study (HEFAS). These data were compared with results obtained from an age- and gender-matched normal population. HEFAS and controls filled in similar questionnaires on demographics, lifestyle factors, health, morbidity and mortality.

Results: A significantly higher proportion of the HEFAS first-degree family members reported to be diagnosed with haemochromatosis-related diseases: 45.7 *vs* 19.4% of the matched normal population (McNemar p<0.001). Mortality among siblings, children and parents in the HEFAS population was similar to that in the relatives of the matched controls.

Conclusion: In this study we show that morbidity among first-degree family members of C282Y-homozygous probands previously diagnosed with clinically proven HH is higher than that in an age- and gender-matched normal population. Further studies are needed to definitely connect these increased morbidity figures to increased prevalence of the C282Y mutated HFE-gene and elevated serum iron indices.

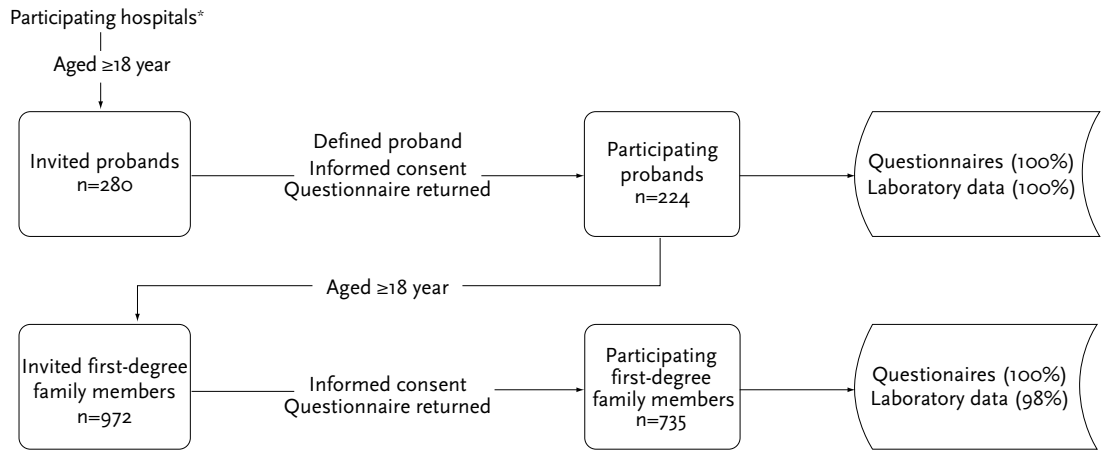
KEYWORDS

Family, hereditary haemochromatosis, *HFE*, morbidity, morality

INTRODUCTION

HFE-related hereditary iron overload is characterised by iron deposition in parenchymal organs.[1,2] Early detection and phlebotomy prevent tissue damage and result in long-term survival similar to that in the general population.[2-6] Of Northern European patients diagnosed with hereditary haemochromatosis (HH), 80% appear to be homozygous for the C282Y mutation in the *HFE* gene. The carrier frequency of this C282Y mutation in the general Caucasian population is estimated to be as high as one in every ten

Figure 1. *Flowchart of the invited and participating probands, the accompanying family members and their available data*



Probands were classified as 'defined proband' when symptoms consistent with hereditary haemochromatosis, C282Y homozygosity and iron overload were present, confirmed by either the plasma iron parameters, iron levels in a liver biopsy or the number of phlebotomies. Laboratory data: iron parameters (transferrin saturation, serum ferritin) and *HFE* genotyping. *Participating hospitals: Atrium Medical Centre, Heerlen/ Brunssum; Radboud University Nijmegen Medical Centre; Rijnstate Hospital Arnhem; University Medical Centre Groningen and University Medical Centre Utrecht.

persons.[7] Altogether, this would favour population screening to prevent disease-related morbidity. Recently, however, it was shown that not all C282Y-homozygous individuals develop symptoms of iron overload disease, debating the penetrance of the *HFE*-gene mutations.[8-11] Therefore, family screening has been suggested, since this has proven efficacy in the detection of latent homozygotes for frequent recessive mutations.[12] Nevertheless, until now, one important item in the World Health Organisation (WHO) guidelines for screening for disease, published in 1968, has remained unanswered for HH-related family screening: Is HH in these families an important health problem?[13] However, to date, to our knowledge there is no such a study that has extensively compared the morbidity and mortality in *HFE*-related HH families with the morbidity and family-related mortality of a general, apparently healthy, population, whereas these outcomes are required to legitimate further research on the implementation of family screening.

Therefore, the objective of the present study was to compare self-reported morbidity among first-degree family members (FDFM) of C282Y-homozygous probands previously diagnosed with clinically manifest HH, with data obtained from age- and gender-matched controls from a normal population. Furthermore the mortality rates among FDFM in these HH families, as reported by the HEFAS probands, were compared with the mortality among the FDFM of age- and gender-matched participants from the normal population. Notably, the study is observational and descriptive and not designed to explain the similarities and differences in outcomes of the morbidity and mortality rate for the two populations.

Data for the HH families were obtained from the HEmochromatosis FAmily Study (HEFAS), which was designed to collect clinical, biochemical, genetic and mortality data from Dutch C282Y-homozygous probands as well as from their first-degree relatives. All probands in the HEFAS had been previously diagnosed with symptomatic *HFE*-related HH. The controls were recruited from the Nijmegen Biomedical Study (NBS), a population-based survey conducted among 22,400 inhabitants of the Dutch city of Nijmegen in 2002-2003.[14]

STUDY POPULATION AND METHODS

HEFAS population

For this study, 280 probands diagnosed with symptomatic *HFE*-related HH from five different medical centres in the Netherlands were actively approached (*figure 1*). The local medical ethics committees of each of these centres approved the study protocol before the start of the study. A total of 224 probands participated. They provided the HEFAS with names and addresses of 972 first-degree relatives (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. Participants were included from May 2003 until August 2005.

Inclusion

Only subjects who gave written informed consent were included in the study. Probands had to be at least 18 years old and to have been clinically diagnosed with

C282Y-homozygous HH. The iron overload had to be confirmed by initial serum ferritin (SF) and transferrin saturation (TS) values exceeding the thresholds of SF ≥280 μg/l for men, SF >80 μg/l for women under the age of 50, SF ≥180 μg/l for women ≥50 years and TS >50% for both men and women. When either one or both pretreatment plasma iron parameters were unavailable, the presence of iron overload was alternatively confirmed by previously performed liver biopsy (grade 3 iron deposition according to Sindram) or by the number of phlebotomies required to normalise SF (males ≥22 phlebotomies = 5 g chelatable iron; females ≥13 phlebotomies = 3 g chelatable iron).[1,15]

Questionnaires

All participants were asked to fill in a questionnaire containing a large number of questions on demographics, lifestyle (smoking, use of alcohol, diet), health status, general medical history, morbidity, medical history for HH, implementation of family screening, legal, psychological and societal implications, and family structure including familial mortality.

Laboratory data

Data on the included probands and family members were extracted from the medical records of the participating hospitals. Information on iron parameters (TS and SF) and liver biopsy of the participants was obtained only at the time of diagnosis of HH or the time of screening for HH, whereas data on the number of phlebotomies were also collected at points in time after the initial investigations. When incomplete, the physician involved in the diagnosis and treatment of the participants was asked to provide the HEFAS team with these data. Finally, when the data remained deficient or the subjects declared that they had never been tested for HH, participants were offered counselling and blood testing by their general practitioner (GP).

Iron parameters for HEFAS were collected by several clinical laboratories. The TS and SF were quantified using validated, standardised, routine laboratory methods. The amount of iron in the liver biopsies was assessed semi-quantitatively.[15]

The Nijmegen Biomedical Study (NBS)

The Nijmegen Biomedical Study (NBS) is a population-based survey conducted among inhabitants of the city of Nijmegen in 2002-2003.[14] Nijmegen is a town in the eastern part of the Netherlands with 156,000 inhabitants, approximately 87% of Caucasian descent. The aim was to obtain a representative sample of the normal population in the Netherlands that could be used as a universal control population for a wide range of medical studies. Randomly selected, age- and gender-stratified inhabitants of Nijmegen (n=22,452) were taken from the population registry and received an invitation to fill in a postal questionnaire on lifestyle and medical and family history that was comparable with the HEFAS questionnaire. Approval to conduct the NBS was obtained from the Institutional Review Board of the Radboud University Nijmegen Medical Centre (RUNMC). The response to the questionnaire was 41.7% (n=9371). In addition, 69.1% of these responders donated 30 ml of blood each for DNA isolation, serum and plasma (n=6473). Analysis of the plasma iron parameters was performed in the Departments of Clinical Chemistry and Chemical Endocrinology of the RUNMC.

Statistical methods

In order to compare the data from HEFAS with those of the general population, a one-to-one age- and gender-matched sample was randomly drawn from the 9371 participants in the NBS. The cut off values at 65% of the scales of general mental health, physical functioning, vitality[16] and fatigue[17] were used for further evaluation.

Haemochromatosis-related medication use was calculated by counting the use of (1) analgesics, (2) antirheumatic drugs and (3) cardiovascular medication (i.e. use of at least one of the following: antihypertensive drugs, cardiovascular drugs and diuretics), for each person resulting in a score that ranged from 0-3. Similarly, the number of haemochromatosis-related diseases was calculated by counting the presence of (1) diabetes mellitus, (2) liver disease, (3) rheumatism, (4) fatigue (score ≥8) and (5) cardiovascular disease, for each person resulting in a score that ranged from of 0-5. Haemochromatosis-related medication use (yes, no) and haemochromatosis-related morbidity (yes, no), were used for further evaluation.

We compared HEFAS and NBS with regard to i) the percentage of elevated iron parameters using local reference values for each of the participating laboratories, and ii) the absolute values of iron parameters using data obtained in only one single laboratory, that of the RUNMC (ca. 25%). The rationale for choosing this laboratory is that the sera of all participants in the NBS were analysed at this location. Prior to the analysis, both the actual iron parameters and the body mass index (BMI) were transformed logarithmically to improve skewness. Differences in the means of the logarithmically transformed data between the HEFAS and the age- and gender-matched sample from the NBS were tested for statistical significance using the t-test for paired data. The back-transformed mean differences with the 95% confidence intervals (95% CI) are presented. Differences in single proportions between the HEFAS probands and the age- and gender-matched sample from the NBS were tested for statistical significance using McNemar's test. The percentage differences between the HEFAS and the NBS samples were calculated together with the 95% CI that takes into account the matched pair design. Because p values and the corresponding confidence intervals are then univocally

related, it is not necessary to present both; therefore, only the differences with the corresponding confidence intervals are presented here. As this is a descriptive study, no corrections for multiple comparisons were performed.

The mortality within HEFAS families, as reported by the probands, was compared with the mortality in the families of the matched NBS participants. Differences in mortality between the HEFAS and the matched NBS sample were tested for statistical significance using Fisher's exact test, separately among parents, siblings and children.

A two-tailed p value <0.05 was considered statistically significant. Analyses were performed using SAS version 8.2.

RESULTS

Study population

Of the 280 probands, 224 (80.0%) filled in the questionnaires and the informed consent forms (*figure 1*). These 224 probands provided names and addresses of 972 FDFM, ≥18 years of age, of whom 735 (75.6%) were included. Of these 735 relatives, 155 reported to have been diagnosed with HH in the past. *Figure 1* shows that 100% of the included probands gave permission for analysis of their laboratory results, whereas 17 (2%) family members did not approve retrieval of laboratory data from their records or agree to additional withdrawal of blood for laboratory tests if data were missing.

Table 1 shows the size and structure of the families of the included HEFAS probands. Twenty-four (10.7%) of the 224 probands who entered the study had more than five participating siblings, whereas 78 (34.8%) had no participating siblings. Four probands had more than five children included in the study, whereas 105 probands had no participating children. In total, this study involved 224 probands, 428 siblings, 241 children and 66 parents.

Demographics and lifestyle

Table 2 shows the results of the self-reported demographics and lifestyle characteristics of the FDFM and the matched NBS participants. The median age at participation was 48 years (range: 18-97 years), and 56.7% of the participants were women. Because of the matched design these values are identical in both studies.

Table 1. *Size and structure of the families of the HEmochromatosis FAmily Study (HEFAS) probands*

		Siblings						
		0	1	2	3	4	≥5	*Total*
Children:	0	45	17	15	9	10	9	*105*
	1	13	9	6	7	3	6	*44*
	2	13	7	7	8	4	7	*46*
	3	6	3	0	5	3	2	*19*
	4	1	1	1	2	1	0	*6*
	≥5	0	1	1	0	2	0	*4*
Total		*78*	*38*	*30*	*31*	*23*	*24*	*224*
Both parents		5	3	3	1	3	3	*18*
Father or mother		3	4	9	6	2	6	*30*
No parents		70	31	18	24	18	15	*176*

Table 2. *Characteristics of the first-degree family members of the HEFAS probands and of the age- and gender-matched NBS participants*

	HEFAS		NBS		HEFAS - NBS	
	Total	Median (range)/n (%)	Total	Median (range)/n (%)	Total*	Difference#(%) (95% CI)
Demographics:						
• Age at participation (years)	735	48 (18-97)	735	48 (18-97)	735	n.a.
• Men	735	318 (43.3)	735	318 (43.3)	735	n.a.
• Education (≥secondary)	689	198 (28.7)	732	285 (38.9)	686	-9.9 (-14.5; -5.3)
• Household (single with or without children)	723	136 (18.8)	734	230 (31.3)	722	-12.3 (-16.4; -8.3)
• Paid job (≥32 hrs/week)	342	185 (54.1)	458	208 (45.4)	290	2.8 (- 4.8; 10.3)
Lifestyle:						
• Alcohol (>2 units/day)	628	163 (26.0)	702	234 (33.3)	602	-8.3 (-13.2; -3.4)
• Smoking (ever)	727	463 (63.7)	733	460 (62.8)	725	0.8 (- 0.4; 5.7)
Blood loss:						
• Blood donation (never)	705	560 (79.4)	727	544 (74.8)	698	4.3 (0.0; 8.7)
• ♀Menarche (≤12 years)	403	128 (31.8)	404	127 (31.4)	391	-0.7 (-7.4; 5.9)
• ♀Pregnancies (>3)	417	79 (18.9)	417	51 (12.2)	417	6.7 (2.0; 11.5)

HEFAS = HEmochromatosis FAmily Study, encompassing probands with clinically overt *HFE*-related haemochromatosis and their first-degree family members; NBS = Nijmegen Biomedical Study, consisting of a representative sample of the Dutch population; CI = confidence interval, using the matched pair design; n.a. = not applicable, because the first-degree family members of the HEFAS and the NBS participants are matched one-to-one by age and gender. *Number of matched pairs with valid data; #the increase from HEFAS to NBS, using the matched pair design.

Table 3. *General health, medication, morbidity and iron parameters in the first-degree family members of the HEFAS probands and of the age- and gender-matched NBS participants*

	HEFAS		NBS		HEFAS - NBS	
	Total	Median (range)/n (%)	Total	Median (range)/n (%)	Total*	Difference#(%) (95% CI)
Body mass index (kg/m²)	717	24.9 (15.2-60.6)	718	24.4 (16.9-62.4)	701	1.7 (0.1; 2.4)
General health:						
• Exercise (≤1 hr/week)	415	109 (26.3)	412	151 (36.7)	250	-4.4 (-12.0; -3.2)
• Health (>2)‡	722	204 (28.3)	733	162 (22.1)	720	6.5 (2.2; 10.9)
• General mental health last 4 weeks (≤23)§	684	339 (49.6)	697	461 (51.8)	650	-1.7 (-7.1; 3.7)
• Physical functioning at this moment(≤23)##	656	108 (16.5)	686	72 (11.5)	617	6.0 (2.5; 9.5)
• Vitality last 4 weeks (≤17)¥¥	680	376 (55.3)	701	325 (46.4)	649	9.1 (3.7; 14.4)
Medication used (yes):						
• Analgesics	627	321 (51.2)	691	285 (41.2)	593	9.8 (4.1; 15.4)
• Antihypertensive drugs	654	146 (22.3)	690	94 (13.6)	617	8.8 (4.9; 12.6)
• Antirheumatic drugs	601	63 (10.5)	673	35 (5.2)	556	5.9 (2.9; 9.0)
• Cardiovascular drugs	614	70 (11.4)	681	50 (7.3)	574	4.4 (1.2; 7.5)
• Diuretics	606	73 (12.0)	683	61 (8.9)	572	3.2 (0.1; 6.2)
• Folic acid	583	67 (11.5)	655	61 (9.3)	531	2.4 (-1.0; 5.8)
• Lipid-lowering drugs	614	57 (9.3)	682	48 (7.0)	576	3.0 (0.1; 5.8)
• Iron supplements	718	87 (12.1)	674	141 (20.9)	659	-9.0 (-12.7; -5.2)
• Tranquillizers	618	148 (24.0)	696	150 (21.6)	590	3.0 (-1.5; 7.6)
• (Multi)vitamin preparations	613	221 (36.0)	675	199 (29.5)	570	6.0 (0.5; 11.5)
• Vitamin B complex	593	139 (23.4)	668	124 (18.6)	542	5.5 (1.0; 10.2)
• Vitamin C complex	601	197 (32.8)	670	174 (26.0)	556	7.9 (2.7; 13.1)
Haemochromatosis-related medication (analgesics, antirheumatic drugs and cardiovascular medication)	*677*	*421 (62.2)*	*708*	*348 (49.2)*	*652*	*13.3 (8.2; 18.4)*
Morbidity¥:						
• Anaemia	620	99 (16.0)	674	90 (13.4)	575	3.0 (-1.1; 7.0)
• Cancer	621	35 (5.6)	683	48 (7.0)	584	-1.4 (-4.1; 1.4)
• Cardiovascular disease	620	65 (10.5)	685	28 (4.1)	582	5.5 (2.7; 8.3)
• Cerebrovascular accident	604	17 (2.8)	675	9 (1.3)	561	1.4 (-0.1; 3.0)
• Diabetes mellitus	620	25 (4.0)	677	31 (4.6)	574	0.4 (-1.8; 2.5)
• Fatigue (≥18)**	688	90 (13.1)	683	54 (7.9)	643	5.9 (2.7; 9.1)
• Hypercholesterolaemia	623	97 (15.6)	684	80 (11.7)	582	4.5 (0.8; 8.1)
• Hypertension	648	184 (28.4)	689	138 (20.0)	609	7.9 (3.5; 12.3)
• Infertility	604	22 (3.6)	669	28 (4.2)	557	-0.5 (-3.0; 1.9)
• Liver disease	611	31 (5.1)	669	17 (2.5)	563	3.2 (1.0; 5.4)
• Osteoporosis	612	47 (7.7)	677	25 (3.7)	570	4.2 (1.8; 6.6)
• Rheumatism	638	199 (32.2)	678	41 (6.0)	594	24.6 (20.6; 28.6)
• Surgery	722	499 (69.1)	728	482 (66.2)	715	2.6 (-2.2; 7.2)
• Thyroid disease	610	28 (4.6)	671	30 (4.5)	565	0.0 (-2.4; 2.4)
Haemochromatosis-related diseases (diabetes mellitus, liver disease, rheumatism, fatigue and cardiovascular disease)	*652*	*298 (45.7)*	*675*	*131 (19.4)*	*599*	*25.7 (20.9; 30.5)*
Iron parameters†:						
• Serum transferrin saturation >50%	599	176 (29.4)	494	21 (4.2)	403	25.3 (20.5; 30.1)
• Serum ferritin above normal (µmol/l) ‡‡	487	198 (40.7)	409	106 (21.2)	333	16.5 (9.7; 23.3)
• Serum transferrin saturation (%)§§	207	38.4 (3.2-107.3)	135	29.5 (4.8-97.7)	135	37.1 (23.4; 52.5)
• Serum ferritin (µmol/l)§§	207	119.0 (4.0-2308)	137	93.9 (6.6-4737)	137	32.4 (7.4; 63.1)

HEFAS = HEmochromatosis FAmily Study, encompassing probands with clinically overt *HFE*-related haemochromatosis and their first-degree family members; NBS = Nijmegen Biomedical Study, consisting of a representative sample of the Dutch population; CI = confidence interval, using the matched pair design. *Number of matched pairs with valid data; #increase from HEFAS to NBS, using the matched pair design, ‡1: feeling good to 5: feeling bad; §5: bad mental health to 30: good mental health, using the SF-36 health survey score,[16] ##10: bad physical functioning to 30: good physical functioning, using the SF-36 health survey score,[16] ¥¥4: low vitality to 24: high vitality, using the SF-36 health survey score;[16] ¥self-reported diagnosis of morbidity made by a physician; **4: fatigue absent to 24: fatigue present, using the shortened fatigue questionnaire score;[17] †at time of being tested for hereditary haemochromatosis; ‡‡serum ferritin above the local upper reference value; §§only participants tested in the Radboud University Nijmegen Medical Centre.

The number of participants with at least secondary education was significantly lower in the FDFM of the HEFAS population compared with the matched NBS participants (HEFAS% minus NBS%: -9.9%) while the percentage of participants with paid jobs was similar for both populations. The HEFAS FDFM reported a significantly lower alcohol intake compared with the NBS controls (>2 units alcohol/day, HEFAS%-NBS%: -8.3%). Yet, the smoking behaviour of both groups was similar.

General health, medication, morbidity and iron parameters

Table 3 summarises the general health, medication, morbidity and iron parameters of the FDFM in the HEFAS population and the age- and gender-matched NBS participants. The median BMI of the HEFAS FDFM was slightly but significantly higher than that of the population-based controls of the NBS (HEFAS%-NBS%: 1.7%, 95% CI 0.1-2.4%). The HEFAS FDFM reported significantly more hours of exercise during the week, they also felt better (health) but had a lower level of physical functioning and vitality.

Significantly more FDFM of the HEFAS population were on antihypertensive drugs (HEFAS%-NBS%: 8.8%) analgesics ((HEFAS%-NBS%: 9.8%), antirheumatic drugs (HEFAS%-NBS%: 5.9%) and cardiovascular drugs (HEFAS%-NBS%: 4.4.%). Iron supplements were less frequently taken by the HEFAS FDFM, than by the matched NBS participants (HEFAS%-NBS%: -9.0%).

Cardiovascular disease, hypercholesterolaemia and hypertension were reported significantly more frequently by the FDFM of the HEFAS population than by the participants in the control population (*table 3*). Fatigue (HEFAS%-NBS%: 5.9%), liver disease (HEFAS%-NBS%: 3.2%), osteoporosis (HEFAS%-NBS%: 4.2%) and especially rheumatism (HEFAS%-NBS%: 24.6%) were also diagnosed significantly more frequently among the FDFM of the HEFAS population. In contrast, diabetes mellitus and infertility were diagnosed with similar frequencies in both populations (*table 3*). The iron parameters TS and SF were both significantly more often elevated in the FDFM of the HEFAS probands compared with the matched NBS participants, with a difference between HEFAS and NBS for TS of 25.3% and for SF of 16.5% (*table 3*). Similarly, the relative differences in the absolute values of TS and SF between the FDFM of the HEFAS and the matched NBS participants were 37.1 and 32.4%, respectively, using only the samples measured in the RUNMC.

Figure 2 shows both the amount of haemochromatosis-related medication use and number of diseases of the FDFM of the HEFAS population and the age- and gender-matched NBS participants. A significantly higher percentage of FDFM used haemochromatosis-related

Figure 2. *The amount of haemochromatosis-related medication use and the number of haemochromatosis-related diseases in the first-degree family members of the HEFAS probands (black) and of the age- and gender-matched NBS participants (grey)*

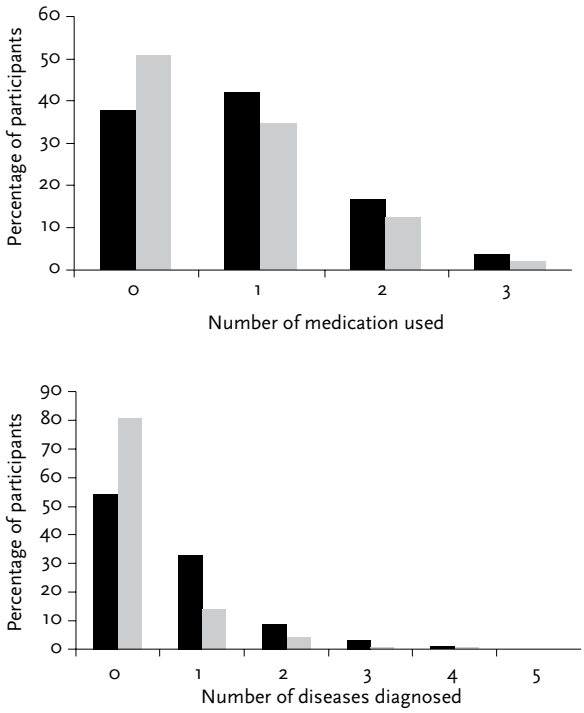


Table 4. *Mortality among first-degree family members of both HEFAS probands and age- and gender-matched NBS participants*

	HEFAS			NBS			
	Families	n	Deceased n (%)	Families	n	Deceased n (%)	P value*
Parents	224	427	299 (70.0)	224	421	310 (73.6)	0.25
Siblings	224	709	93 (13.1)	224	752	99 (13.2)	1.00
Children	224	414	8 (1.9)	224	372	5 (1.3)	0.59

Families = number of families reported by the HEFAS probands or the age- and gender-matched NBS participants; n = number of family members reported by the proband or the age- and gender-matched NBS participant, respectively. *P value for difference in proportion between the HEFAS and the NBS group, using Fisher's exact test.

medication, compared with the NBS participants, i.e. a difference between HEFAS and NBS of 13.3%. Similarly, a significantly higher percentage of FDFM reported to be diagnosed with one or more disease, i.e. a difference between HEFAS and NBS of 25.7%.

Mortality

All 224 HEFAS probands provided data on the mortality of their first-degree relatives. The probands provided information on 427 parents, of whom 70.0% (n=299) had died by the end of our study (*table 4*). These mortality figures did not differ significantly from the reported 73.6% (n=310) deceased parents of the 224 age- and gender-matched NBS participants (p=.025). Similarly, the mortality among the siblings and children of the HEFAS families did not differ significantly from that of the NBS families.

DISCUSSION

Family screening can be a sophisticated model for screening of HH. However, to date, to the best of our knowledge controlled studies on morbidity and mortality in families with HH are lacking. Indeed, the present study reveals more haemochromatosis-related diseases in the HEFAS population compared with the general population. In contrast, the mortality in the HEFAS population was not significantly higher than in the normal population.

Earlier studies have already described fatigue, weakness and arthropathy as being related to *HFE* gene mutations, whereas diabetes mellitus, abnormal liver function tests, impotence, hypothyroidism, cardiomyopathy and hepatocellular carcinoma were mentioned as some of the more specific, organ-related problems leading to increased morbidity and mortality.[1,2-5] If HH were diagnosed and treated in time, tissue damage could be prevented and a long-term survival similar to that in the general population could be achieved.[2-6] Nevertheless, recent studies claim that although some iron-overloaded patients with homozygosity for the C282Y mutation in the *HFE* gene have a high and probably preventable morbidity, even more subjects with this genotype had no symptoms at all.[8-11] Moreover, studies performed in several European countries could not detect significant differences in the prevalence of untreated homozygotes among elderly populations compared with younger groups.[18-21] This cast doubt on the adequacy of presymptomatic population screening. Thus, family screening was suggested as it was thought to increase the chances to find both C282Y homozygosity (theoretically present in 25% of the siblings) and an elevated penetrance of iron overload due to the sharing of iron metabolism modifying genes or environmental factors with the clinically expressing proband. Indeed, focusing on FDFM of C282Y-homozygous patients with clinically overt HH has been shown to produce a significant yield of C282Y-homozygous individuals with high penetrance of iron accumulation, but with an unknown increase of morbidity compared with the normal population.[22-24] McCune *et al.* recently reported that despite the presence of elevated iron parameters, the morbidity among C282Y-homozygous relatives of probands identified by screening a group of blood donors was similar to that of C282Y-homozygous relatives of probands presenting as patients.[25] Assuming that the C282Y homozygous blood donors had less morbidity than the probands of identical genotype presenting clinically, this cast doubt on the contribution of the higher penetrance of iron overload within HFE-mutated families and therefore the significance of family screening. In the present study, however, we demonstrated that first-degree relatives of patients with clinically overt HFE-related HH do have a higher morbidity in comparison with the general population. Admittedly, this study was not designed to clarify the factor that is responsible for the observed morbidity differences. It is evident, however, that HEFAS relatives have a higher possibility of being homozygous and heterozygous for the C282Y mutation compared with the normal population. These differences in genetic predisposition are likely to be the cause of the elevated serum iron indices of the HEFAS relatives and the higher incidence of HH-related symptoms. To analyse this further we evaluated the relation between HH-related symptoms and TS, and observed a significant relation between rheumatism and TS%, and a nonsignificant correlation between 'cardiovascular disease' and TS%. Thus, additional studies are warranted to definitely attribute the morbidity differences to *HFE* genotype and iron parameters.

A remarkable finding in this study is the discrepancy between the higher morbidity and similar mortality among the FDFM of the HEFAS probands compared with the matched NBS population. Several explanations can be given. First of all, HEFAS family members as well as their general practitioners may be more aware of the symptoms typical for HH, leading to an advantage in diagnosis and treatment.[23] Secondly, the age of the C282Y homozygous siblings (mean 54 years, interquartile range Q1-Q3 47-62 years) might be too low for *HFE*-related mortality and the study might also comprise too few C282Y homozygous parents to influence the mortality differences between both parental populations. Next to this, other confounding factors that were not measured may have influenced the comparative mortality. It has, for instance, been suggested that C282Y polymorphism may protect against several infectious agents, either by the synthesis of a dysfunctional HFE protein as target receptor for infectious agents, by lowering the iron levels inside macrophages and so inducing resistance to ferrophilic micro-organisms, or

by altering immunological processes, all leading to an advantage in survival.[3,26-29] More recent investigations have demonstrated that non-transferrin-bound iron in the sera of homozygotes and even heterozygotes for the C282Y mutation promoted the adhesion of monocytes to endothelial cells, which may be another advantage of immune defence.[30] Furthermore, the *HFE* gene mutations may provide a survival advantage by ameliorating the iron deficiency seen in another common *HLA*-defined condition, such as coeliac disease.[31] Meanwhile, however, questions on the survival advantage of *HFE* polymorphism remain.

It should be noted that our study includes a self-reporting questionnaire. Therefore, to diminish a potential registration bias, the questionnaires for both HEFAS and NBS participants were identical on the questions evaluated in the present study in that participants were asked to report diseases as diagnosed by their physicians and the fatigue and general health questions were scored by validated questionnaires.

Taken together, this study demonstrates that the morbidity among first-degree relatives of probands with clinically overt *HFE*-related HH is higher than in the normal population. These findings challenge us to definitely link these morbidity figures to haemochromatosis in future studies.

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REFERENCES

1. Adams P, Brissot P, Powell LW. EASL International Consensus Conference on Haemochromatosis. J Hepatol 2000;33:485-504.
2. McDonnell SM, Preston BL, Jewell SA, et al. A survey of 2,851 patients with hemochromatosis: symptoms and response to treatment. Am J Med 1999;106:619-24.
3. Powell LW, Dixon JL, Ramm et al. Screening for hemochromatosis in asymptomatic subjects with or without a family history. Arch Intern Med 2006;166:294-301.
4. Milman N, Pedersen P, Steig T, et al. Clinically overt hereditary hemochromatosis in Denmark 1948-1985: epidemiology, factors of significance for long-term survival, and causes of death in 179 patients. Ann Hematol 2001;80:737-44.
5. Niederau C, Fischer R, Purschel A, et al. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-19.
6. Bomford A, Williams R. Long term results of venesection therapy in idiopathic haemochromatosis. Q J Med 1976;45:611-23.
7. Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 2001;154:193-206.
8. Beutler E, Felitti VJ, Koziol JA, et al. Penetrance of 845G--> A (C282Y) HFE hereditary haemochromatosis mutation in the USA. Lancet 2002;359:211-8.
9. Andersen RV, Tybjaerg-Hansen A, et al. Hemochromatosis mutations in the general population: iron overload progression rate. Blood 2004;103:2914-9.
10. Asberg A, Hveem K, Thorstensen K et al. Screening for hemochromatosis: high prevalence and low morbidity in an unselected population of 65,238 persons. Scand J Gastroenterol 2001;36:1108-15.
11. Olynyk JK, Cullen DJ, Aquilia S, et al. A population-based study of the clinical expression of the hemochromatosis gene. N Engl J Med 1999;341:718-24.
12. Krawczak M, Cooper DN, Schmidtke J. Estimating the efficacy and efficiency of cascade genetic screening. Am J Hum Genet 2001;69:361-70.
13. Wilson JMG, Jungner G. Principles and practice of screening for disease. Geneva: WHO 1968.
14. Hoogendoorn EH, Hermus AR, de Vegt F, et al. Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex. Clin Chem 2006;52:104-11.
15. Sindram JW, Marx JJ. Localisation of iron in the hepatic acini and in bile duct epithelium as a tool for estimation of liver iron overload. Ann N Y Acad Sci 1988; 526:361-2.
16. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.
17. Alberts M, Vercoulen JHMM, Bleijenberg G. Assessment of fatigue - the practical utility of the subjective feeling of fatigue in research and clinical practice. Assessment in Behavioral Medicine. Brunner-Routledge, 2001, pp 301-27.
18. Coppin H, Bensaid M, Fruchon S, et al. Longevity and carrying the C282Y mutation for haemochromatosis on the HFE gene: case control study of 492 French centenarians. BMJ 2003;327:132-3.
19. Piippo K, Louhija J, Tilvis R, et al. You may live to the age of more than 100 years even if you are homozygous for a haemochromatosis gene mutation. Eur J Clin Invest 2003;33:830-31.

20. Van Aken MO, De Craen AJ, Gussekloo et al. No increase in mortality and morbidity among carriers of the C282Y mutation of the hereditary haemochromatosis gene in the oldest old: the Leiden 85-plus study. Eur J Clin Invest 2002;32:750-4.

21. Willis G, Wimperis JZ, Smith KC, et al. Haemochromatosis gene C282Y homozygotes in an elderly male population. Lancet 1999; 354:221-2.

22. Bulaj ZJ, Ajioka RS, Phillips JD, et al. Disease-related conditions in relatives of patients with hemochromatosis. N Engl J Med 2000;343:1529-35.

23. Adams PC, Kertesz AE, Valberg LS. Screening for hemochromatosis in children of homozygotes: prevalence and cost-effectiveness. Hepatology 1995; 22:1720-7.

24. Gleeson F, Ryan E, Barrett S, et al. Clinical expression of haemochromatosis in Irish C282Y homozygotes identified through family screening. Eur J Gastroenterol Hepatol 2004;16:859-63.

25. McCune CA, Ravine D, Carter K, et al. Iron loading and morbidity among relatives of HFE C282Y homozygotes identified either by population genetic testing or presenting as patients. Gut 2006;55:554-62.

26. Rochette J, Pointon JJ, Fisher CA, Perera G, et al. Multicentric origin of hemochromatosis gene (HFE) mutations. Am J Hum Genet 1999;64:1056-62.

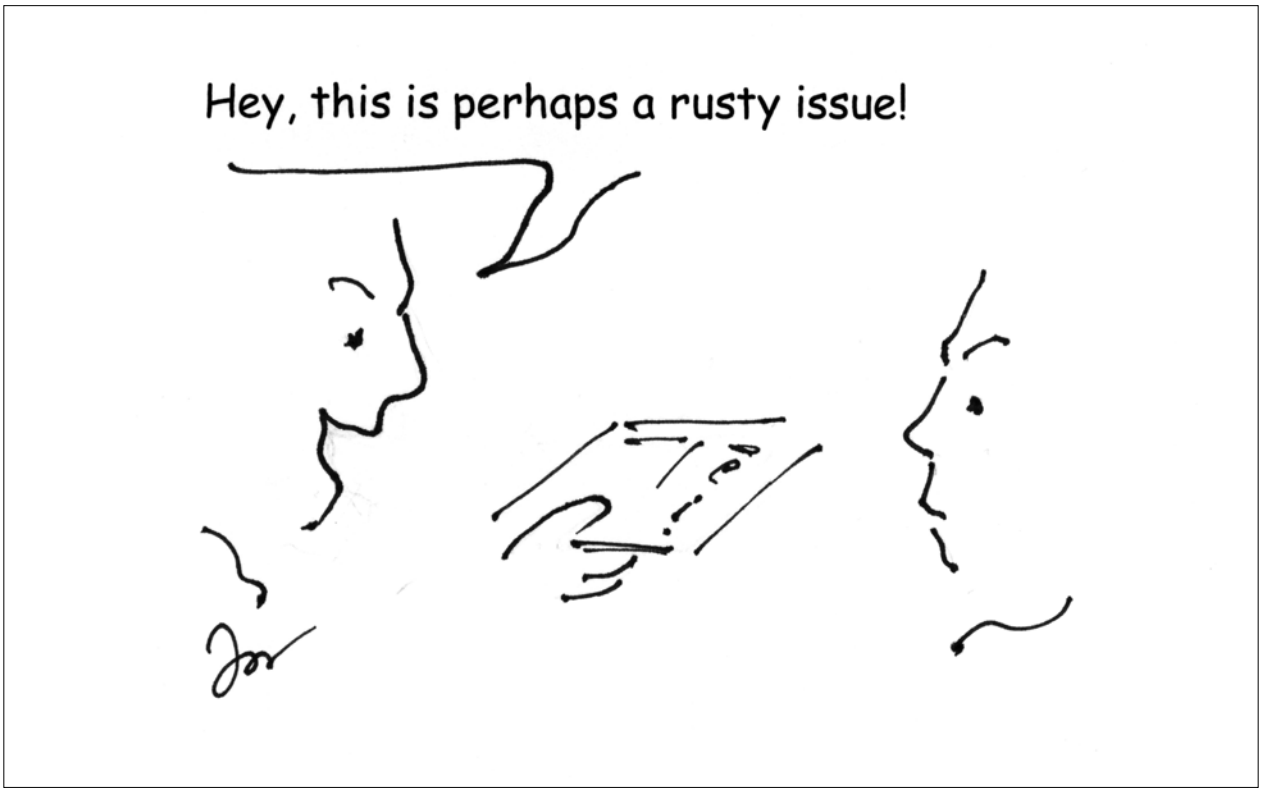
27. Moalem S, Percy ME, Kruck TP, et al. Epidemic pathogenic selection: an explanation for hereditary hemochromatosis? Med Hypotheses 2002;59:325-9.

28. De Almeida SF, Carvalho IF, Cardoso CS, et al. HFE crosstalks with the MHC class I antigen presentation pathway. Blood 2005;106:971-7.

29. Cardoso CS, de Sousa M. HFE, the MHC and hemochromatosis: paradigm for an extended function for MHC class I. Tissue Antigens 2003;61:263-75.

30. Kartikasari AE, Georgiou NA, Visseren FL, et al. Endothelial activation and induction of monocyte adhesion by nontransferrin-bound iron present in human sera. FASEB J 2006;20:353-5.

31. Butterworth JR, Cooper BT, Rosenberg WM et al. The role of hemochromatosis susceptibility gene mutations in protecting against iron deficiency in celiac disease. Gastroenterology 2002;123:444-9.



Jacobs, et al. Morbidity in families with haemochromatosis.