Association of Mutations in the Hemochromatosis Gene With Shorter Life Expectancy

Lise Bathum, MD, PhD; Lene Christiansen, PhD; Hanne Nybo, MD; Karen Andersen Ranberg, MD; David Gaist, MD, PhD; Bernard Jeune, MD; Niels Erik Petersen, MD, PhD; James Vaupel, PhD; Kaare Christensen, MD, PhD

Background: To investigate whether the frequency of carriers of mutations in the *HFE* gene associated with hereditary hemochromatosis diminishes with age as an indication that *HFE* mutations are associated with increased mortality. It is of value in the debate concerning screening for hereditary hemochromatosis to determine the significance of heterozygosity.

Methods: Genotyping for mutations in exons 2 and 4 of the *HFE* gene using denaturing gradient gel electrophoresis in 1784 participants aged 45 to 100 years from 4 population-based studies: all 183 centenarians from the Danish Centenarian Study, 601 people aged 92 to 93 years from the Danish 1905 Cohort, 400 aged 70 to 94 years from the Longitudinal Study of Aging Danish Twins, and 600 aged 45 to 67 years from a study of middle-aged Danish twins.

Results: All participants (N = 1784) were screened for mutations in exon 4, and a trend toward fewer heterozygotes for the C282Y mutation—the mutation most often associated with hereditary hemochromatosis—was found. This was significant for the whole population ($P = .005$) and for women ($P = .004$) but not for men ($P = .26$). A group of 599 participants was screened for mutations in exon 2, and there was no variation in the distribution of mutations in exon 2 in the different age groups.

Conclusions: In a high–carrier frequency population like Denmark, mutations in *HFE* show an age-related reduction in the frequency of heterozygotes for C282Y, which suggests that carrier status is associated with shorter life expectancy.

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From the Department of Clinical Biochemistry, University Hospital, Odense, Denmark (Drs Bathum, Christiansen, and Petersen); The Danish Center for Demographic Research and Epidemiology, Institute of Public Health, University of Southern Denmark, Odense (Drs Nybo, Andersen Ranberg, Gaist, Jeune, Vaupel, and Christensen); the Max Planck Institute for Demographic Research, Rostock, Germany (Dr Vaupel); and the Terry Stanford Institute, Duke University, Durham, NC (Drs Vaupel and Christensen).

HEREDITARY hemachromatosis is the most common inherited disease in Europeans. It occurs in as many as 5 in every 1000 individuals of northern European heritage,[1] with 10% to 15% of the population being carriers of mutations in the *HFE* gene associated with hereditary hemochromatosis.

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Hereditary hemochromatosis is due to an abnormal absorption of iron from the intestine. As iron loading progresses, it leads to irreversible damage of many organs and tissues, resulting in hepatic fibrosis and cirrhosis, endocrine dysfunction, cardiomyopathy, or arthropathy.[2] The classic "bronzed diabetes" (skin pigmentation, diabetes mellitus, and cirrhosis) represents only a small fraction of affected individuals, usually those in whom the diagnosis has been undetected for many years. Hereditary hemochromatosis more often presents with nonspecific complaints, such as joint pain, fatigue, and abdominal pain.[3]

In 1996, the candidate gene for hereditary hemochromatosis, *HFE*, was identified.[4] This gene codes for a transmembrane protein that is presumed to be involved in the regulation of the intracellular iron level. Three common mutations have been found: C282Y, H63D, and S65C. Studies[1-8] in different countries have shown that approximately 80% to 100% of patients with clinically diagnosed hemochromatosis are homozygotes for the C282Y mutation, whereas the impact of H63D and S65C is more uncertain. It seems likely that the compound heterozygotes C282Y/H63D and C282Y/S65C are at increased risk of developing hemochromatosis.[5-8] The distribution of these mutations differs in different populations. The frequency of heterozygosity for C282Y is 9.6% in white people in the United States,[9] 17.3% in northern Ireland,[10] 13.2% in New Zealand,[11] 9.6% in northern Germany,[12] and 13.3% in Denmark.[13] This mutation is absent in populations of African, Asian, or Australian descent.[14]

The importance of hereditary hemochromatosis is based on its prevalence, its remarkably diverse clinical spectrum, and

SUBJECTS, MATERIALS, AND METHODS

We conducted 4 major surveys and collected biological material from 1995-1999: the Danish 1905 Cohort, all Danes born in 1905 (1632 DNA samples)[23]; the Longitudinal Study of Aging Danish Twins, all Danish twins 70 years and older (2265 DNA samples)[24]; a study of middle-aged Danish twins, a random sample of twin pairs born between 1931 and 1952 (4171 DNA samples)[25]; and the Danish Centenarian Study, all persons living in Denmark who celebrated their 100th birthday between April 1, 1995, and May 31, 1996, and centenarians from the island of Funen, who participated in the pilot study in 1994 (183 DNA samples).[22,26] These studies comprised a home-based 2-hour multidimensional interview and sampling of DNA by means of a finger prick or a cheek swab, except in the Danish Centenarian Study, in which full blood samples were collected. We randomly selected DNA samples from 600 individuals in the middle-aged twins study and from 400 in the Longitudinal Study of Aging Danish Twins, but only 1 participant from each twin pair was included. From the Danish 1905 Cohort, we selected 300 men and 301 women. From the Danish Centenarian Study, DNA samples from all participants were included. This selection scheme aimed at getting precise estimates at middle ages and among the oldest old.

DNA samples were isolated from cheek swabs and blood spots using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Exons 2 and 4 were screened using denaturing gradient gel electrophoresis[27] after previous amplification using an external set of primers. Exons 2 and 4 were analyzed in 599 samples (200 each from the Longitudinal Study of Aging Danish Twins and middle-aged twins cohort and 199 from the Danish 1905 Cohort) and only exon 4 was analyzed in the remaining samples. In case of abnormal patterns, the relevant exons were sequenced using a Thermo Sequenase Fluorescent–labeled primer cycle sequencing kit (Amersham, Pharmacia Biotech Inc, Piscataway, NJ) and Alf Express (Amersham, Pharmacia Biotech AB, Uppsala, Sweden).

Hardy-Weinberg equilibrium was tested using the χ^2 test. The 95% confidence intervals for the proportion of heterozygotes for C282Y in an age group were calculated using the normal distribution. The χ^2 test for trend was used for comparing the frequencies among the different age groups, and the uncorrected χ^2 test was used for comparing proportions.[28] EpiCalc 2000 (version 1.02; Gilmann and Myatt, Brixton, England) was used for statistical analysis.

the fact that early treatment (ie, venesection) is effective in preventing its clinical manifestations. The clinical diagnosis of hereditary hemochromatosis is established much more rarely than expected, and strong association between C282Y homozygosity and hereditary hemochromatosis makes it tempting to suggest population-based genetic screening for this mutation.[1,15] However, several issues need to be clarified before the conditions for screening are fulfilled. What proportion of individuals with hereditary hemochromatosis on a molecular level will develop serious clinical manifestations, and what is the impact on heterozygotes? Examinations of the frequency of C282Y homozygotes in healthy populations and in other diseases have shown that the clinical penetrance of this mutation is low. Some studies[16,17] have identified individuals who are homozygous for C282Y but do not fulfill the clinical and biochemical criteria for hemochromatosis.

Heterozygotes for mutations in *HFE* have significantly higher serum iron and transferrin saturation[11] and a lower frequency of iron deficiency anemia.[9] Furthermore, new studies strongly suggest a relation between the storage of iron and cardiovascular diseases. Two studies[18,19] have shown that heterozygosity for mutations in *HFE* confers a significant increase in risk for cardiovascular events in men and women. However, the evidence for adverse effects of being a carrier is still sparse.

Iron is a potent promotor of generation of free radicals—strong reactive ions that react with cell membranes and cell organelles. Free radicals are believed to play a role in the development of cancer and cardiovascular diseases and in aging, although there is no good experimental evidence.[20,21] *HFE* mutations thus represent a group of common mutations that may affect the aging processes, morbidity, and mortality possibly through the accumulation of iron. Except for apolipoprotein E,[22] no longevity-associated genes have been firmly established, and the *HFE* mutations are of particular interest because of the potential for prevention of harmful effects through venesection.

In this study, we genotyped 1784 individuals aged 45 to 100 years from population-based studies for mutations in *HFE* to investigate whether the frequency of heterozygotes for mutations in *HFE* diminishes by age. If so, this would indicate that heterozygotes for the mutations in *HFE* have increased mortality rates starting at midlife.

RESULTS

A total of 599 samples were screened for mutations in exon 2. The H63D and S65C mutations were found with the same frequency across the age groups (**Table 1**). Exon 4, the clinically most important, was screened in all samples. The C282Y carrier frequencies and 95% confidence intervals are given in **Table 2**. The overall distribution was in Hardy-Weinberg equilibrium ($P=.4$). All samples showing variant band patterns were sequenced (2 samples), and the following mutations were found in exon 4: C917T (this participant was also heterozygous for the H63D mutation in exon 2) and T1105C. The reduction in heterozygotes for C282Y is significant in the population as a whole ($P=.005$) and in women ($P=.004$) but not in men ($P=.26$). The frequency of heterozygotes for C282Y in the youngest age group was 12.4% in men and 19.5% in women, but this difference was not significant ($P=.09$).

Table 1. Frequency of Mutations in Exons 2 and 4 in 599 Individuals Screened in Exons 2 and 4 of the *HFE* Gene*

	Age, y					
Allele	**45-54**	**55-64**	**65-74**	**75-84**	**85-94**	**Total**
Wt/Wt	48 (60.0)	53 (59.6)	61 (60.4)	71 (67.7)	141 (62.9)	**374** (62.4)
Wt/H63D	14 (17.5)	19 (21.3)	26 (25.7)	23 (21.9)	52 (23.2)	**134** (22.4)
H63D/H63D	0	2 (2.2)	1 (1.0)	1 (1.0)	4 (1.8)	**8** (1.3)
Wt/S65C	2 (2.5)	4 (4.5)	4 (4.0)	1 (1.0)	5 (2.2)	**16** (2.7)
Wt/C282Y	16 (20.0)	7 (7.9)	8 (7.9)	9 (8.6)	20 (8.9)	**60** (10.0)
H63D/C282Y	0	3 (3.4)	1 (1.0)	0	1 (0.4)	**5** (0.8)
H63D/S65C	0	1 (1.1)	0	0	1 (0.4)	**2** (0.3)
Total	**80**	**89**	**101**	**105**	**224**	**599**

*Data are given as number (percentage). The normal, nonmutated allele is denoted wildtype (WT); the mutated alleles are denoted H63D, S65C, and C282Y.

Table 2. C282Y Carrier Frequency (and 95% Confidence Intervals [CIs]) in the Different Age Groups in Men, Women, and the Total Population

	Age, y					
	45-54	**55-64**	**65-74**	**75-84**	**85-94**	**100**
Men						
No.	161	91	111	83	325	46
Frequency (95% CI)	0.124 (0.073-0.175)	0.088 (0.03-0.146)	0.081 (0.03-0.132)	0.108 (0.041-0.175)	0.071 (0.043-0.099)	0.13 (0.033-0.227)
Women						
No.	200	85	91	115	339	137
Frequency (95% CI)	0.195 (0.14-0.25)	0.176 (0.095-0.257)	0.099 (0.038-0.16)	0.096 (0.042-0.15)	0.094 (0.063-0.125)	0.139 (0.081-0.197)
Total						
No.	**361**	**176**	**202**	**198**	**664**	**183**
Frequency (95% CI)	0.163 (0.125-0.201)	0.131 (0.081-0.181)	0.089 (0.05-0.128)	0.101 (0.059-0.143)	0.083 (0.062-0.104)	0.137 (0.087-0.187)

COMMENT

In this study, we investigated the frequency of carriers of 3 common *HFE* gene mutations—C282Y, H63D, and S65C—in different age groups. No apparent difference was found for H63D and S65C (Table 1). However, the results indicate an age-related reduction in the carrier frequency of the hemochromatosis-related C282Y mutation (Table 2). These findings are consistent with the hypothesis that there may be a survival difference from middle age onward, especially for women, among carriers and noncarriers of the C282Y allele. The age-related reduction is only minor after age 65 years—a trend that has also been shown for obesity.[29] Our data thus imply that there is selection against carriers of C282Y and that this manifests before age 65 years.

The observed reduction in C282Y carrier frequency persisted until age 95 years; however, the frequency in the centenarian group is the same as that in the youngest group for both men and women. There are several possible explanations for this. Although 183 centenarians is a large sample, including most of the centenarians in Denmark who reached age 100 years in the study year, it is not large enough to give a reliable carrier frequency. This can be seen in the broad 95% confidence intervals in Table 2; therefore, it may be a chance finding. Another possible explanation is that heterozygosity for C282Y has a higher mortality rate in the younger groups but becomes beneficial in the oldest old (antagonistic pleiotropy). Furthermore, the fact that some become octogenarians or nonagenarians with a "bad" gene could be balanced by a good composition of other relevant genes or lifestyle, making it more likely that they become centenarians.

There are several limitations to our study. The major limitation is the inability to study directly the effect of the C282Y allele on survival. The results suggest that there is a survival difference, but more specific data on the relationship of the C282Y allele to mortality rates are needed before such a conclusion can be made because the design used here is vulnerable to migration and association to twin status. However, it seems unlikely that migration or twin status should be associated with *HFE* mutation status. Another limitation is that our study does not give an idea of the mechanisms behind this survival difference. It is tempting to suggest that the difference is owing to accumulation of iron, but this should be further investigated. It is well known that heterozygotes for mutations in *HFE* have a higher iron content, but it has until now been assumed that this was without significant influence on morbidity and mortality rates. Our finding that heterozygotes for C282Y may have reduced life expectancy raises the question of whether the elevated iron stores affect mortality and morbidity rates, although some other effect of C282Y is possible.

It is well known that there is a low incidence of myocardial infarction in menstruating women. The finding that heterozygosity for mutations in *HFE* confers a significant increase in risk for cardiovascular events in men and wom-

en[18] is compatible with the hypothesis that iron plays an important role in ischemic injury. In that case, iron depletion could have a large protective effect.[19] However, if the accumulation of iron is in fact the mechanism behind this apparently increased mortality rate among heterozygotes for C282Y, then our study would probably underestimate the increased mortality rate because several individuals without this mutation may nonetheless have higher-than-normal stored iron levels owing to other known or unidentified iron-loading mutations.

Several studies have demonstrated a familial aggregation of premature myocardial infarction.[30] Some of this can be explained by hypertension or hyperlipidemia; however, in a large proportion of high-risk families, no aggregation of the known risk factors is seen. It is possible that this familial aggregation is due to a gene that favors iron absorption.[31] A relevant question then is whether the increased mortality can be prevented by deliberate iron depletion by regular blood donation, by recommending a diet with a low iron content, or by warning against taking vitamins that contain iron.

The finding of 3 homozygotes for C282Y (2 aged 93 years and 1 aged 70 years) is not surprising because earlier studies have shown that the penetrance of hereditary hemochromatosis in homozygotes for C282Y is low—probably less than 50%. According to the Hardy-Weinberg law, we should have found 6 participants homozygous for C282Y, with an overall allele frequency of 6%.

This study shows an age-related reduction in the carrier frequency of C282Y in the *HFE* gene, suggesting that carriers have a shorter life expectancy. Our study does not shed light on which mechanisms are behind this shorter life expectancy or whether this increased mortality can be prevented by, for instance, venesection. Future research needs to elucidate this before a decision concerning population-based screening for mutations in *HFE* is made.

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Corresponding author and reprints: Lise Bathum, MD, PhD, Department of Clinical Biochemistry, Odense University Hospital, Sdr. Blvd 29, DK-5000 Odense C, Denmark (e-mail: Lise.Bathum@ouh.fyns-amt.dk).

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