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

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Abstract

Background The C282Y mutation in the gene for haemochromatosis (HFE) has been associated with various diseases at middle age. However, recent studies indicate that penetrance of the C282Y mutation is low. We explored the association between the C282Y mutation, iron metabolism, and morbidity and mortality in participants of the Leiden 85-plus.

Study design A cross-sectional comparison and prospective follow-up was conducted in two unselected cohorts of 661 and 552 subjects. All subjects were aged 85 years and over. We determined the prevalence of C282Y homozygous and heterozygous subjects, and the association between the C282Y mutation and iron metabolism, all-cause and specific causes of death.

Results Prevalence of C282Y homozygosity in both cohorts was 0.2% (1/661 and 1/552, respectively) and of C282Y heterozygosity was 12.4% (82/661) and 11.4% (63/552), respectively. These estimates coincide exactly with reported estimates in younger age groups. Median ferritin level was 97 μg L\(^{-1}\) (IQR 39–162) for heterozygous carriers and 89 μg L\(^{-1}\) (IQR 41–157) for noncarriers (\(P = 0.66\)). The serum ferritin concentration for one C282Y homozygous subject, a woman aged 86 years at the time of enrollment in 1986, was 392 μg L\(^{-1}\).

Cardiovascular morbidity was comparable between the C282Y heterozygous subjects and the noncarriers in both study cohorts. All-cause and cardiovascular mortality of carriers of the C282Y mutation was similar to that in noncarriers.

Conclusions We found two C282Y homozygous subjects, illustrating that homozygosity can be compatible with survival in very old ages. C282Y heterozygosity was not associated with history of cardiovascular disease morbidity, all cause mortality, cardiovascular mortality, or biochemical phenotype of haemochromatosis at old age.

Keywords Elderly, genetics, haemochromatosis, single nucleotide polymorphism

Introduction

Haemochromatosis is a disorder of iron metabolism in which excess iron absorption leads to deposition of iron in multiple organs, resulting in hepatic cirrhosis, diabetes, cardiomyopathy, hypogonadism, and early death [1]. In 1996, the gene for haemochromatosis (HFE) was identified and two missense mutations were reported: C282Y and H63D [2]. The estimated population frequency for C282Y in Caucasian subjects ranges between 0.1% and 1.0% for homozygosity and between 10% and 15% for heterozygosity [3,4]. In most studies, more than 90% of typical haemochromatosis patients are homozygous for the C282Y mutation [5]. Moreover, C282Y heterozygosity has been associated with increased risk of cardiovascular disease [6,7] and cardiovascular mortality [8].
Recent evidence indicates that penetrance of the C282Y mutation is rather low. Screening of 41,038 Americans revealed 152 homozygotes, of whom only one had signs and symptoms that would suggest a diagnosis of haemochromatosis [9]. A similarly low penetrance was found in large-scale population screens of 10,556 Welsh blood donors [10] and 9,890 Danes [11]. Moreover, heterozygous C282Y subjects do not generally develop haemochromatosis [12] and several studies have not found an association between C282Y heterozygosity and coronary artery disease [13–15].

We explored the association between the C282Y mutation, iron metabolism, and morbidity and mortality in participants of the Leiden 85-plus Study, a population-based, longitudinal cohort study of the oldest old. First, we investigated whether in old age the prevalence of homozygous C282Y subjects was nil and that of heterozygous carriers depleted. Second, we examined the distribution of cardiovascular morbidity between heterozygous C282Y carriers and noncarriers. Third, we prospectively studied all-cause and cardiovascular mortality risk according to genotype.

Methods
The Leiden 85-plus Study consists of two separate cohorts. Subjects of the first cohort were enrolled between 1 December 1986 and 1 March 1988. Subjects of the second cohort were enrolled between 1 September 1997 and 1 September 1999. The Medical Ethics Committee of the Leiden University Medical Center approved the study protocols of both cohorts and informed consent was obtained from all participants.

First cohort
A detailed description of the first cohort has been presented elsewhere [16]. In short, on 1 December 1986 the total number of inhabitants aged 85 years and over in Leiden, the Netherlands, was 1,258. Between December 1986 and March 1989, 977 subjects (78%) were included in the first cohort, 221 subjects (18%) died before they could be enrolled, while 60 subjects (5%) refused to participate. At baseline, data on past and present morbidity were obtained by medical history taking. We have previously shown that this procedure yields valid estimates [17]. During a separate home visit a venous blood sample was drawn. For the present genetic study sufficient cell material was available from 666 subjects. Subjects were all followed up for mortality until 1 October 1996. Primary causes of death were obtained from the Dutch Central Bureau of Statistics and categorized for cardiovascular disease (ICD-9 codes 390–519), ischaemic heart disease (ICD-9 codes 410–414), cerebrovascular disease (ICD-9 codes 430–438) and all-causes (ICD-9 codes 000–999).

Second cohort
A detailed description of the second cohort has been presented elsewhere [18]. In short, between 1 September 1997 and 1 September 1999 all inhabitants of Leiden were approached for participation in the study in the month after their 85th birthday. During this 2-year enrollment period 705 inhabitants of Leiden reached the age of 85 years. Of those, 599 subjects (85%) were included in the second cohort, 92 subjects (13%) refused to participate, while 14 subjects (2%) died before they could be enrolled. All subjects were visited at their place of residence to obtain information on past and present morbidity. Medical history information was obtained from each subjects’ general practitioner. From all participants a venous blood sample was drawn. For the present study sufficient cell material was available from 561 subjects.

Assessment of HFE genotype and iron stores
An 89-bp fragment containing the HFE mutation was amplified using the primers gataaaccttgctgtaacctccct (forward) and gataacgactgagcgttcct (reversed). The accumulation of allele-specific PCR products was monitored using molecular beacons (TFT-gccgagaataacctgTccaggtgcgtcgg-DABCYL and FAM-gccgagataacccAgcaggtgcgtcgg-DABCYL for detection of wild-type and mutant alleles, respectively) and an ABI 3700 real time PCR machine (Applied Biosystems, Foster City, CA, USA). A randomly chosen 10% of the samples was reamplified and digested with RsaI. All previous genotyping results were confirmed. For five subjects in the first cohort and nine subjects in the second cohort HFE genotypes could not be determined because of technical reasons.

Serum ferritin levels, a measure of body iron stores, and C-reactive protein (CRP) were determined for all subjects. Serum ferritin levels were excluded. The reference value for CRP was 10 mg L$^{-1}$.

Statistical analysis
Distributions of dichotomous variables between genotypes were compared by the Chi-squared test and continuous variables were compared by the Mann–Whitney U-test. Mortality risks and corresponding 95% confidence intervals (95%CI) were estimated with Cox’s proportional hazard model.

Results
The prevalence of C282Y homozygosity in the two cohorts was 0.2% (1/661; 95%CI 0.006–0.8) and 0.2% (1/552; 95%CI 0.006–1.0), respectively. The prevalence of C282Y heterozygosity was 12.4% (82/661; 95%CI 9.9–14.9) in the first cohort and 11.4% (63/552; 95%CI 8.8–14.1) in the second cohort. Our prevalence estimates of homozygous
and heterozygous subjects in both cohorts coincide exactly with reported estimates in younger age groups [3,4].

We measured ferritin in all subjects of the first cohort and the median level was 97 mg L\(^{-1}\) (IQR 39–162) among the heterozygous carriers and 89 mg L\(^{-1}\) (IQR 44–157) among the noncarriers (\(P = 0.66\)). The C282Y homozygous carrier, at the time of enrollment in 1986 an 86-year-old woman without a history of cardiovascular disease, had a serum ferritin of 392 mg L\(^{-1}\). She survived until the end of the follow up to an age of 96 years.

Morbidity from cardiovascular disease was comparable between the group of C282Y heterozygous subjects and the noncarriers in both study cohorts (Table 1). During the 10-year follow-up period of the first cohort, 588 deaths occurred (89·2% of the original sample, two subjects were lost to follow up). The all-cause mortality of carriers of the C282Y mutation as compared to the noncarriers was similar (RR = 1·1, 95% CI = 0·9–1·4; Table 2). Mortality risks for specific causes of death were also not different for the heterozygous C282Y carriers as compared to the noncarriers (Table 2).

Discussion

We found no differential survival nor increase in mortality and morbidity in carriers of the C282Y mutation in the hereditary haemochromatosis gene (HFE). At age 85 years the prevalence of homozygous and heterozygous C282Y subjects was equivalent to reported prevalences in the general population. The distribution of cardiovascular disease between the heterozygous carriers of the C282Y mutation compared with the noncarriers of the same age was identical. During the prospective follow up, no difference in all-cause and cardiovascular mortality was found between the heterozygous subjects and the noncarriers.

### Homozygous subjects

In each of the two 85-year-old populations we found one C282Y homozygous subject, resulting in a point prevalence of 0.2%. These findings are in line with a report by Willis et al. [19], who found no depletion of C282Y homozygous subjects in an elderly population, and also coincides with estimates in younger age groups, as recently reported by Beutler et al. [9]. Hence homozygosity can be compatible with survival in very old ages. A likely explanation is that other genes or environmental factors are necessary for the HFE gene to come to expression [5]. Such a complex genetic background explains why there are only few patients with the clinical picture of haemochromatosis when compared with the prevalence of C282Y homozygous carriers in the general population.

### Table 1 Characteristics of the first and second Leiden 85-plus Study cohorts according to the hereditary haemochromatosis gene

<table>
<thead>
<tr>
<th>General characteristics</th>
<th>HFE heterozygous (n = 82)</th>
<th>Noncarriers (n = 578)</th>
<th>P-value</th>
<th>HFE heterozygous (n = 63)</th>
<th>Noncarriers (n = 488)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)*</td>
<td>89·9 (87·7–91·7)</td>
<td>89·1 (87·5–91·6)</td>
<td>0·53</td>
<td>85</td>
<td>85</td>
<td>0·07</td>
</tr>
<tr>
<td>Women (n)</td>
<td>64 (78%)</td>
<td>409 (70·8%)</td>
<td>0·18</td>
<td>48 (76%)</td>
<td>316 (65%)</td>
<td>0·07</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction (n)</td>
<td>6 (8%)</td>
<td>46 (9%)</td>
<td>0·07</td>
<td>6 (10%)</td>
<td>51 (10%)</td>
<td>0·81</td>
</tr>
<tr>
<td>Stroke (n)</td>
<td>2 (3%)</td>
<td>14 (3%)</td>
<td>0·98</td>
<td>5 (8%)</td>
<td>51 (10%)</td>
<td>0·55</td>
</tr>
<tr>
<td>Diabetes mellitus (n)</td>
<td>10 (14%)</td>
<td>62 (12%)</td>
<td>0·65</td>
<td>11 (18%)</td>
<td>77 (16%)</td>
<td>0·76</td>
</tr>
<tr>
<td>Hypertension (n)</td>
<td>15 (21%)</td>
<td>119 (23%)</td>
<td>0·67</td>
<td>18 (29%)</td>
<td>130 (27%)</td>
<td>0·76</td>
</tr>
</tbody>
</table>

*Variable is presented as median and interquartile range.

HFE, hereditary haemochromatosis gene.

### Table 2 All-cause and cause-specific 10-year mortality risks for subjects aged 85 years and over with the haemochromatosis heterozygous genotype as compared to noncarriers

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Deaths (n) (%)</th>
<th>Crude mortality risk for HFE-heterozygous subjects (95% CI)</th>
<th>Adjusted mortality risk for HFE-heterozygous subjects (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-cause</td>
<td>588 (89·2%)</td>
<td>1·1 (0·9–1·4)</td>
<td>1·1 (0·9–1·4)</td>
</tr>
<tr>
<td>Cardiovascular diseases (total)</td>
<td>246 (37·3%)</td>
<td>1·0 (0·7–1·5)</td>
<td>1·0 (0·7–1·5)</td>
</tr>
<tr>
<td>Ischaemic heart disease</td>
<td>62 (9·4%)</td>
<td>0·5 (0·2–1·4)</td>
<td>0·5 (0·2–1·4)</td>
</tr>
<tr>
<td>Other circulatory disease</td>
<td>102 (15·4%)</td>
<td>1·3 (0·8–2·3)</td>
<td>1·3 (0·8–2·3)</td>
</tr>
</tbody>
</table>

*Adjusted for age and gender.

HFE, hereditary haemochromatosis gene.
Heterozygous subjects

The ferritin concentration in the group of heterozygous HFE carriers was not different from the noncarriers. This finding seems to contradict the results of Bulaj et al. [12] who found that HFE heterozygous subjects had higher serum ferritin concentrations. However, the subjects in that study were family members of homozygous subjects with clinical expression of haemochromatosis. It is now clear that penetrance of the disease in homozygous subjects is very low. This means that it is possible that the subjects in the study by Bulaj et al. might have been different in their iron metabolism as a result of the selection pedigree of the haemochromatosis patients, resulting in high ferritin concentrations.

We also assessed whether heterozygosity of the C282Y mutation was associated with increased mortality by two different analytical strategies. First, the prevalence of heterozygosity for the C282Y mutation was not decreased in the two independent cohorts of subjects aged 85 years and over compared with reported prevalences in the general population [3,4]. Second, we showed that heterozygosity for the C282Y mutation did not predict all-cause or cardiovascular mortality over a 10-year follow-up period. These analyses indicate that heterozygosity for the C282Y mutation does not affect mortality in the general population. Our results are within the range of other published studies. Lao et al. [20] found that heterozygous C282Y carriers were significantly more prevalent in Italian nonagenarians and centenarians compared with young control subjects. Bathum et al. [21] found the opposite; they demonstrated an age-related reduction in the frequency of heterozygotes for C282Y in Denmark.

Some studies have shown an association between C282Y heterozygosity and cardiovascular disease [6,7] and cardiovascular death [8], while other studies have reported no association [12–14]. Most of the studies were carried out in 50–70-year-old men and women. There are two possible explanations for the apparent discrepancy between increased morbidity and mortality risk at age 50–70 years and no increased risk at a very old age. First, because a number of studies have shown no association and the effect of size in the positive studies was modest, there might be no important relation between the C282Y mutation and morbidity and mortality (i.e. type-1 error). Second, it could be that heterozygous subjects who do not develop cardiovascular disease at middle age have a genetic make-up protecting against the effects of the C282Y mutation.

Conclusion

We found two C282Y homozygous subjects, illustrating that heterozygosity can be compatible with survival in very old ages. C282Y heterozygosity was not associated with history of cardiovascular disease morbidity, all-cause mortality, cardiovascular mortality, or the biochemical phenotype of haemochromatosis at old age.

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References
