Frequency of hemochromatosis gene (HFE) mutations in Russian healthy women and patients with estrogen-dependent cancers

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Abstract

Possible association between the C282Y and H63D mutations in the HFE gene and estrogen-dependent cancer risk was assessed. Genotyping was performed using PCR amplification followed by digestion of products with specific restrictases. In a population of 260 healthy women (permanent residents of the southwest European Russia), mutant allele frequencies at the C282Y and H63D sites were evaluated as 3.3 and 16.3%, respectively. In patients with breast, ovarian, and endometrial cancer, C282Y frequencies were also low (1.0, 1.3, and 3.8%, respectively), and no cancer risk associated with the C282Y mutation was found. Odds ratios for breast cancer risk associated with the H63D mutation increased significantly with age: 0.5 in women below 48 years old, 1.0 in a range of 48–57 years, and 4.4 in older women (P trend =0.002). The latter value was statistically significant (95% CI, 1.4–14.1), indicating that women bearing the H63D mutation may be at an increased breast cancer risk at an age above 57 years. Preliminary results obtained in patients with two other estrogen-dependent malignancies revealed the same tendency to OR increase with age in ovarian cancer patients (P trend =0.008), but no age-related OR differences in endometrial cancer patients.

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Keywords: HFE mutation; Iron metabolism; Oxidative stress; Cancer risk; Age

1. Introduction

Development of hemochromatosis, a multi-organ disease induced by a hereditary disorder of iron metabolism, is associated with two missense alterations in the HFE gene: a G to A transition at position 845 in exon 4, which results in a cysteine to tyrosine substitution at amino acid 282 (the G845A, or C282Y mutation), and a C to G change at position 187 in exon 2, resulting in a histidine to aspartic acid substitution at codon 63 (the C187G, or H63D mutation) [1]. It has been suggested that chronic iron overload, in addition to long-known hemochromatosis clinical manifestations (hepatic cirrhosis, cardiomyopathy, diabetes mellitus, and others), may predispose to cancer development—mainly due to oxidative stress created in cells and tissues by extra iron stores, and also because iron may be a nutritional requirement for cancer cells [2]. Studies on correlation between the HFE mutations and cancer risk gave contradictory results, some reporting a positive association [3–5], and others showing no association [6–8].

This correlation may be particularly pronounced in estrogen-dependent cancers, since these malignancies are associated with endogenous oxidative stress produced in target tissues by estrogen metabolites, and HFE-related iron overload may aggravate this stress [9]. To investigate this possibility, we determined HFE C282Y and H63D genotypes in patients with breast cancer (which is characterized by strong estrogen dependence [10]), and compared results with the HFE genotype distribution evaluated in healthy women. Also, we report preliminary evaluations of the HFE mutation frequencies in patients with two other estrogen-dependent cancers (ovarian and endometrial).
2. Materials and methods

2.1. Subjects

Patient groups were composed of women admitted to the Medical Radiological Research Center (MRRC) hospital between September 2001 and December 2004 with newly diagnosed breast, ovarian, or endometrial cancer (100, 40, and 53 cases, respectively). Patients with history of any other cancer were not included into the study. Blood samples from control subjects were collected in the same period. The control group included 260 women who had no any cancer or cancer history. Predominantly, they were volunteers recruited among employees of the MRRC and other organizations where authors’ acquaintances worked (158 subjects). In addition, the control group included 83 women examined in the course of routine medical examinations performed by MRRC physicians, and 19 women who presented to the MRRC outpatient clinics with breast or gynecological disorders. Both cases and controls, with rare exceptions, were residents of small towns (with 30–100 thousand inhabitants) in central European Russia to the southwest from Moscow (mainly, Kaluga Region).

Simultaneously with blood donation, each participant completed a questionnaire (under physician’s guidance), and provided her informed consent for DNA analyses. The questionnaire included self-reported data on age, height, weight, ethnicity, education, smoking habits, menstrual function, reproductive history, breast and gynecological disorders, chronic diseases, use of hormone preparations, and family history of cancer. However, in the course of interviewing, it became clear that cancer patients were much better informed on their health state than cancer-free women were. To avoid possible bias resulting from dealing with non-comparable between cases and controls data, the four latter characteristics were not included into analysis at this stage of the study.

The study was approved by the Medical Radiological Research Center Review Board.

2.2. Detection of HFE mutations

Blood samples were collected into EDTA-containing tubes and stored at −80 °C until DNA isolation. Isolated DNA was stored at −20 °C until analysis. Genotypes at the G845A (C282Y) and C187G (H63D) sites were determined by a PCR-based RFLP (restriction fragment length polymorphism) technique (generally, as described in [12]). Primers used to amplify a 389-bp fragment containing the G to A transition at position 845 in exon 4 of the HFE gene (the C282Y mutation) were 5'-TGCAAGGGTAAACAGATCC (forward) and 5'-CTCAGGCACCTCCTCACC (reverse). A primer pair for amplification of a 208-bp fragment encompassing the C to G transversion at nucleotide 187 (the H63D mutation) was 5'-ACATGGT- TAAAGGGCTGTTGCA and 5'-GCCGACATCGGGGTTGAATT. The fragments were amplified in 25-μL reactions containing 0.625 U of HotStarTaq polymerase (Qiagen, Valencia, USA), 0.15 mM dNTPs, 0.2 μM primers, and 50 ng of genomic DNA. Thermal cycling was carried out as follows: 15-min denaturing at 95 °C, 30 amplification cycles (30 s at 94 °C, 1 min at 60 °C, and 30 s at 72 °C), and 10-min extension at 72 °C. At the first stages of the study, C282Y and H63D amplifications were performed separately; later, we combined both reactions in one tube, since conditions for both fragments were similar and the difference in fragment length was enough to distinguish between them clearly. Amplified fragments were digested for 4 h at 37 °C with either Rsal or BclI for detection of the C282Y or H63D mutations, respectively (both enzymes were purchased from Takara Biomedicals, Shiga, Japan), in 10-μL reaction mixtures containing 0.4 μL of PCR products and 0.5 U of the respective enzyme. After Rsal digestion, a sequence containing G at the G845A site was cut into fragments of 249 and 140 bp, whereas an A-containing sequence resulted in 249- and 111-bp fragments. BclI digestion produced 138- and 70-bp fragments in a sequence containing C at the C187G site, whereas a G-containing product, which did not carry restriction sites for this enzyme, remained intact at 208 bp in length. PCR products and digested fragments were run on 8% polyacrylamide gels, stained with ethidium bromide, and visualized by UV. Genotyping was performed blindly relative to the case or control status of the blood sample donor.

2.3. Statistical analysis

Numerical variables were compared between study groups using the two-sided t-test. The two-sided exact Fisher’s test was used for comparing proportions. The Hardy–Weinberg equilibrium was assessed by the χ² goodness-of-fit test. The χ² criterion was also used in heterogeneity tests in 2 × K contingency tables. In comparisons of genotype proportions, contingency tables were generated for the C282Y and H63D mutations separately. Since homozygous HFE mutations (both at the C282Y and H63D sites) were rare, they were combined with heterozygotes. Significance of a trend in proportions was evaluated by the χ² test for trend. Evaluations of cancer risk associated with the HFE mutations were based on odds ratios (OR) derived from 2 × 2 tables (wild type/wild type genotypes were regarded as a low-risk category). Logit limits were used to evaluate 95% confidence intervals (CI). A group-matching approach was applied for control of confounding: the study population was stratified into subgroups, within which cases and controls were balanced against putative confounding variables. To analyze OR estimates in a series of resulting 2 × 2 tables, the expected number of cases carrying a high-risk genotype was calculated for each table, based on the Mantel–Haenszel common OR. Then, a variant of the χ² goodness-of-fit statistic for logistic models was computed to assess homogeneity of ORs in the series. To test for a trend in ORs, the single degree of freedom χ² for trend was used. In the choice of statistical approaches, we followed recommendations given in [13].

3. Results

The primary attention in this study was focused on breast cancer, and the main analysis refers to the breast cancer group. Also, we show data obtained in ovarian and endometrial cancer patients. These data are preliminary, because of the insufficient group sizes; yet, they may be of interest for comparing genotyping results between various types of estrogen-dependent malignancies.

Characteristics of the study groups, including selected cancer risk factors and demographic features, are shown in Table 1.

The mean variables that were significantly different between controls and breast cancer cases included age (and, consequently, the proportion of postmenopausal women), age at menopause, and BMI. All these indices were higher in patients than in controls. Demographic characteristics (ethnicity, education level, smoking habits, and parity) were similar in the study groups, indicating that even if any bias associated with selection of the control group (the “healthy volunteer effect”) was introduced into the study, it was not essential.

Table 2 presents genotype distributions at the HFE C282Y and H63D loci, as well as C282Y and H63D allele frequencies, in healthy women and cancer patients. The genotypes were in the Hardy–Weinberg equilibrium in each group (all P values exceeded 0.6). The only C282Y homozygote for all 453 women genotyped for this mutation was found in a 61-year-old endometrial cancer patient with the wild-type H63D genotype. Compound C282Y/H63D
Table 1
Characteristics of study subjects (controls, healthy women; BC, OC, and EC patients, women with breast, ovarian, and endometrial cancer, respectively)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 260)</th>
<th>BC patients (n = 100)</th>
<th>P value</th>
<th>OC patients (n = 40)</th>
<th>EC patients (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>13.8 ± 1.4 (10–18)</td>
<td>13.9 ± 1.7 (11–19)</td>
<td>&gt;0.10</td>
<td>13.7 ± 1.4 (12–18)</td>
<td>13.6 ± 1.5 (10–17)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.2 ± 5.3 (16.9–46.6)</td>
<td>28.8 ± 5.5 (19.1–47.7)</td>
<td>0.01</td>
<td>27.2 ± 6.3 (18.8–47.0)</td>
<td>32.2 ± 6.7 (20.3–52.3)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>28 (10.8)</td>
<td>11 (11.0)</td>
<td>&gt;0.10</td>
<td>3 (7.5)</td>
<td>3 (5.7)</td>
</tr>
<tr>
<td>East Slavic, n (%)</td>
<td>250 (96.2)</td>
<td>96 (96.0)</td>
<td>&gt;0.10</td>
<td>36 (90.0)</td>
<td>51 (96.2)</td>
</tr>
<tr>
<td>Higher school graduates, n (%)</td>
<td>99 (38.1)</td>
<td>35 (35.0)</td>
<td>&gt;0.10</td>
<td>10 (25.0)</td>
<td>18 (34.0)</td>
</tr>
</tbody>
</table>

P values are given for comparisons between BC patients and controls.

- a Mean ± SD (range).
- b Data were missing for 28 control subjects, 3 BC patients, 1 OC patient, and 1 EC patient.
- c Including women with amenorrhea period up to 6 months.
- d Data were missing for 2 control subjects, and 1 OC patient.
- e Data were missing for 2 control subjects, and 1 BC patient.
- f East Slavics were predominantly Russians (over 90% in each group), Ukrainians, and Belarusians.
- g As opposed to subjects with secondary and elementary education (these two categories were combined, since in total there were only 8 subjects with elementary education); data were missing for 20 control subjects, and 1 OC patient.

heterozygotes were rare: four in the control group, one in each of the breast and endometrial cancer groups, and none in the ovarian cancer group. Differences from the control values in the genotype distributions, as well as in the mutant allele frequencies, were statistically insignificant in each patient group.

Age, BMI, age at menarche, and age at menopause are known to influence breast cancer risk [10], and, presumably, distribution of each of these variables in a population may be associated with the HFE genotype; thus, these characteristics may represent classic confounding factors. The case and control samples were not balanced in respect of most of these factors. Since obesity is more frequent in older persons, and since the mean ages at menarche and at menopause are, in general, dependent on the calendar date of birth, the differences in these factors could ensue, at least partly, from the difference in age distributions. Thus, we had to deal with correlated confounding factors. To control for them, we chose a “group-matching” approach (or stratified analysis), because logistic regression, though a more powerful statistical tool, may be error-prone in cases of correlated regression variables (see pp. 233–236 in [13]). However, stratified analysis was only applied to H63D data, as the low population frequency of the C282Y mutation did not allow reasonable stratification.

To take an initial look at possible association of the above-mentioned factors with the H63D genotype, we dichotomized the control group into carriers of a H63D mutation (homo- and heterozygotes combined) and wild-type homozygotes (Table 3). Of all the putative confounders compared between the resulting subgroups, only the age differences were statistically significant, indicating that age was the most potent confounder. This impression was further indirectly supported by results of tests for heterogeneity in proportions of the mutant genotypes in breast cancer patients and healthy controls stratified in various ways: by menopausal status, age, BMI, or other factors (χ² tests in 2 × 4 tables). All these tests gave negative results (data not shown), with the exception of age stratification: percentages of the mutant genotypes in cases and controls older and younger than 54 years (the median age in the breast cancer group) appeared heterogeneous (P = 0.02).

These data suggested that the difference in age distributions between breast cancer patients and healthy controls should be the factor of major concern to further analysis. Since there were many more controls than cases, we stratified both groups by the age tertiles in breast cancer patients. Table 4 shows that this stratification yielded acceptable matching of cases and controls within each of the three age strata for each of the putative confounders.

Table 2
HFE genotype distribution in healthy women (controls) and patients with breast, ovarian, and endometrial cancer (BC, OC, and EC, respectively)

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 260)</th>
<th>BC (N = 100)</th>
<th>OC (N = 40)</th>
<th>EC (N = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H63D genotypes, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild/wild</td>
<td>243 (93.5)</td>
<td>98 (98.0)</td>
<td>39 (97.5)</td>
<td>50 (94.3)</td>
</tr>
<tr>
<td>Wild/C282Y</td>
<td>17 (6.5)</td>
<td>2 (2.0)</td>
<td>1 (2.5)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td>C282Y/C282Y</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (1.9)</td>
</tr>
<tr>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.11</td>
<td>0.48</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>C282Y allele, %</td>
<td>3.3 ± 0.8</td>
<td>1.0 ± 0.7</td>
<td>1.3 ± 1.2</td>
<td>3.8 ± 1.9</td>
</tr>
<tr>
<td>p&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.09</td>
<td>0.32</td>
<td>0.79</td>
<td></td>
</tr>
<tr>
<td>H63D genotypes, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild/wild</td>
<td>180 (69.2)</td>
<td>67 (67.7)</td>
<td>30 (75.0)</td>
<td>41 (77.4)</td>
</tr>
<tr>
<td>Wild/H63D</td>
<td>75 (28.8)</td>
<td>30 (30.3)</td>
<td>9 (22.5)</td>
<td>10 (18.9)</td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>5 (1.9)</td>
<td>2 (2.0)</td>
<td>1 (2.5)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td>p&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.78</td>
<td>0.58</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>H63D allele, %</td>
<td>16.3 ± 1.6</td>
<td>17.2 ± 2.7</td>
<td>13.8 ± 3.9</td>
<td>13.2 ± 3.3</td>
</tr>
<tr>
<td>p&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.79</td>
<td>0.56</td>
<td>0.42</td>
<td></td>
</tr>
</tbody>
</table>

- a For one patient, H63D genotyping was not successful.
- b Exact Fisher’s test for the proportions of subjects bearing at least one mutant allele (orthogonal comparisons with controls).
- c Exact Fisher’s test for the mutant allele proportions (orthogonal comparisons with controls).
Table 3
Selected characteristics of the control group by H63D genotype (Wild, wild-type homozygotes; Mutant, carriers of at least one H63D allele)

<table>
<thead>
<tr>
<th>Agea, years</th>
<th>Wild (n = 180)</th>
<th>Mutant (n = 80)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at menarchea</td>
<td>45.7±10.7</td>
<td>43.0±9.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Postmenopausal, N (%)</td>
<td>13.8±1.4</td>
<td>13.8±1.3</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>BMIa, kg/m²</td>
<td>50 (27.8)</td>
<td>18 (22.5)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Age at menopausea</td>
<td>48.3±4.7</td>
<td>47.8±2.6</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>BMIa, kg/m²</td>
<td>27.3±5.4</td>
<td>26.9±5.2</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

a Mean±S.D.

Comparison of proportions of mutant H63D genotypes between controls and breast cancer cases within each of the matched age groups is presented in Table 5. In healthy women, the mutant frequency tended to decrease with age; however, neither the differences between the age groups (homogeneity test in a 2 × 3 contingency table) nor the trend of the decrease were statistically significant. Data in the three age groups of breast cancer patients were inhomogeneous at a borderline significance; moreover, the trend of an age-related increase in the mutant frequency (that is, an opposite tendency, as compared with controls) was significant at P=0.018. Odds ratios shown in Table 5 were calculated in each age stratum separately (e.g., cases aged 25–47 years were compared with controls aged 25–47 years, and so on). The stratum-specific OR values were significantly heterogeneous, indicating that a summary measure of relative risk would be misleading. The trend to OR increase with age was also significant. In the two first age strata, the ORs did not differ from 1, within their respective confidence intervals; however, an estimate in the oldest age group (OR=4.4) appeared statistically significant.

For comparison, Table 5 shows similar data for ovarian and endometrial cancer patients. As noted above, the numbers of patients with these malignancies were insufficient for reliable OR evaluations (currently, we continue collecting DNA samples for the analysis). In ovarian cancer patients, these preliminary results indicated the same tendency as was found in the breast cancer group: cancer risk in H63D carriers increased with age. No such tendency was visible in endometrial cancer patients.

Table 4
Stratification of the control (Co) and breast cancer (BC) groups by age (values closest to the age tertiles in cases were used as cut-off points)

<table>
<thead>
<tr>
<th>Agea, years</th>
<th>Co (n=167)</th>
<th>BC (n=32)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aged 25–47 years</td>
<td>38.8±6.1</td>
<td>39.7±5.7</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Age at menarchea</td>
<td>13.9±1.4</td>
<td>13.4±1.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>6 (3.6)</td>
<td>4 (12.5)</td>
<td>&gt;0.10</td>
</tr>
<tr>
<td>Age at menopausea</td>
<td>42.8±5.5</td>
<td>43.8±2.1</td>
<td>&gt;0.10</td>
</tr>
</tbody>
</table>

Nulliparousa | 13 (7.8) | 1 (3.1) | >0.10 |
| BMIa, kg/m² | 26.0±5.1 | 26.2±5.1 | >0.10 |
| Slavica | 160 (95.8) | 32 (100.0) | >0.10 |
| Smokers | 72 (44.0) | 8 (25.0) | >0.10 |
| Higher school graduates | 63 (42.0) | 12 (37.5) | >0.10 |

a Mean±S.D.

b 0.05 < P < 0.06.

c n (%).

4. Discussion

Prevalence of the C282Y mutation in various ethnic populations has been reported in numerous studies (reviewed, for example, in [11]). Published data show an overall decrease of the mutant frequency from northwest Europe both to the south and east: from as high as 0.142 in Ireland [14] to zero in northern Greece [15], and Japan [16]. As for Slavic populations, values reported for the C282Y allele frequency were 0.031 in a sample of 871 Poles [17] and 0.037 in a sample of 150 Russians residing in Siberia [18]. An estimate of 0.033 obtained in this study is virtually consistent with the cited results.

Reported frequency of the mutant H63D allele also shows pronounced ethnic dependence, but, compared with that of the C282Y, the rate is relatively high in Europe (around 0.160 in Spain [19] and northern Italy [20], 0.125 in Sicily [20], and 0.090 in Greece [15]), whereas in Asian populations the mutation is rare (below 0.01 in Japan [16]). The estimations reported for Polish and Russian populations are 0.162 and 0.133, respectively [17,18]. In frames of statistical errors, they are quite similar to our estimate (0.163).

Evaluation of the population frequencies of the HFE mutations made in this study was based on genotyping 260 women residing mostly in Kaluga Region and adjacent areas in southwest European Russia. This sample was 96.2% East Slavics (predominantly Russians, plus Ukrainians and Belorussians). As compared with data reported by the Russian Federal Service of State Statistics in 2002 [21], this percentage coincided with 96.3% in Kaluga Region, and was fairly close to 93.8% in the Central Federal District. Since published data did not show sex-related difference in the HFE genotype distributions [22,23], we believe that our sample was representative enough to provide an evaluation of the C282Y and H63D mutation frequencies in healthy population of central European Russia.

Carriers of the mutant HFE alleles are prone to accumulating extra iron in body tissues. The correlation between mutant genotype and iron overload is more distinct for C282Y than for...
H63D [24–27]; yet, data on impaired iron metabolism in H63D homo- and heterozygotes have also been published [28,29]. Results of a large epidemiological study indicated increased cancer risk in subjects with elevated body iron level [2]. Stevens et al. [30] further hypothesized that the association between the HFE mutations and cancer risk may be particularly pronounced for breast cancer. The latter is known to be aggravated by extra iron stores. In particular, it was shown that redox cycling of estrogen metabolites released Fe$^{2+}$ ions from ferritin, which resulted in generating additional free radicals [9]. Following the same arguments, an increased risk associated with the mutant HFE can be assumed for other oxidative stress-related malignancies, in particular, ovarian and endometrial cancers, both of which are estrogen-dependent [31,32].

Results indicating a possible role of genes involved in oxidative stress control in ovarian or endometrial cancer development have been published [32,33].

We could not find either studies addressing ovarian and endometrial cancers in relation to HFE mutations or reports on H63D distribution in breast cancer patients. However, an increased breast cancer risk associated with the mutant C282Y allele has recently been reported [5]. In our study, no significant differences in the C282Y frequencies were found between breast cancer patients and healthy women (the mutant frequency was even somewhat lower in cases than in controls; Table 2). In effect, this is not contradictory to the cited report, as statistical power of our study was obviously insufficient to detect effects of the C282Y, a mutation characterized by a relatively low population frequency. The mutant C282Y frequencies preliminary evaluated in smaller groups of ovarian and endometrial cancer patients also did not differ significantly from the control value. It should be noted that all comparisons related to the C282Y frequencies were made between the case and control groups that were not matched for confounding factors.

For the H63D mutation, due to its higher population frequency, it was possible to apply stratified analysis and perform more accurate comparisons between breast cancer cases and controls. Stratification by age allowed obtaining three strata such that, within each stratum, breast cancer cases, and controls were matched not only for age but also for other putative confounders (Table 4). It appeared that ORs for breast cancer were significantly inhomogeneous in the three strata: they increased from 0.5 in women aged below 48 years through 1.0 in women aged 48 to 57 years to 4.4 in older women (Table 5). The latter value was statistically significant, indicating that carriers of the H63D mutation aged above 57 years were at increased risk of developing breast cancer. The smooth positive trend in stratum-specific ORs (which is generally regarded as an indication of causality of an association) gave additional support to this conclusion.

These OR values are not adjusted for alcohol consumption (unfortunately, a question on alcohol consumption was not included into our questionnaire); however, this factor is not likely to be a source of serious bias, since reported relative risk for breast cancer in moderate drinkers vs. nondrinkers (1.41 [34]) is essentially lower than OR of 4.4 found in this study for elderly carriers of the H63D allele.

A similar pattern of genotype distributions in the same age strata was observed in ovarian cancer patients (the age-related trend was also significant) but not in women with endometrial cancer. It would be preliminary to discuss this difference between breast and ovarian cancer, on the one hand, and endometrial cancer, on the other; probably, it may be attributable to an important dissimilarity of endometrium from breast and ovarian tissue (periodical tissue loss during menstruation).

It is not of surprise that H63D-associated breast cancer risk was revealed only in elder women. Iron overload increases with age, and its deleterious effects may become more apparent in the elderly. In women, age-related changes in iron accumulation may be even more pronounced because menopause transition puts an end to significant iron loss that occurs in women of reproductive age due to menstruation and pregnancies.

We would like to add some conjectural comments on why the H63D frequency in breast cancer patients increased with age (with a significant trend), whereas in healthy controls the pattern tended to be opposite (though neither the differences between the age strata nor the trend were significant). Health effects of HFE mutations are ambiguous. From the first perspective, they predispose to diseases associated with iron
overload [12,35]; conversely, HFE mutation carriers may be protected against diseases connected with iron deficiency [11], and references herein). One might assume that, whereas the former (adverse) effect of the mutant genotype is more pronounced in older subjects, at younger age the latter (favorable) health effect prevails, especially in women. An OR value of 0.5 obtained in the youngest age strata (25–47 years) was not reliable statistically; yet, some health-protecting role (not necessarily specific for breast cancer) of HFE mutations at a young age seems plausible. Similar data were reported for colon cancer risk in subjects bearing HFE mutations: OR values in age ranges of <50, 50–69, and ≥70 years were 0.59, 1.29, and 1.90, respectively (only the latter value was statistically significant) [4]. In published works, we did not find straightforward data either confirming or contradicting this assumption. Among indirectly relevant studies, we found, on the one hand, data on a shortened life expectancy in HFE heterozygotes [36]; on the other hand, data showing no under-representation of HFE mutations in women aged 85 years and older [23], and a report on a trend for an increased H63D frequency in centenarian women [37]. No significant differences were found in prevalence of HFE mutations in a large sample stratified by the age groups 12–19, 20–39, 40–59, and ≥60 years [22]. Evidently, not only a much larger than ours but a specially designed study is required to verify the above assumption.

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