Evidence for an Association between Compound Heterozygosity for Germ Line Mutations in the Hemochromatosis (HFE) Gene and Increased Risk of Colorectal Cancer


Introduction

Iron is a pro-oxidant that can catalyze the formation of mutagenic hydroxyl radicals leading to DNA damage. Iron is unable to move freely across cellular membranes and has to be transported across bound to transferrin. The cell surface transferrin receptor (TFR) binds to transferrin allowing cellular uptake. One study has suggested that whereas the serine/glycine variant at codon 142, may alter the level of nucleotide 845G → A; cysteine → tyrosine) and H63D (exon 2; nucleotide 187C → G; histidine → aspartic acid). Most patients (60-100%) satisfying criteria for hemochromatosis are homozygous for the C282Y mutation; a few are compound heterozygotes (C282Y/wild type and H63D/wild type) or apparently C282Y/wild-type heterozygotes (9-12). Homozygosity for the H63D mutation confers only a relatively small risk of developing hemochromatosis, and there are no reported patients who carry the C282Y and H63D mutations on the same chromosome (11, 12). Although allele frequency varies widely, the two mutations are relatively common throughout Caucasian populations and may be regarded as polymorphisms. In Europe, the C282Y and H63D allele frequencies vary between 1% and 10% and 1% and 28%, respectively (11, 13). Given the high frequency of these alleles in Caucasians, the effect on the number of cancer cases may be large.

The C282Y mutation has been shown to prevent functional interaction between the HFE and the TFR proteins thereby increasing cellular iron uptake (1, 14). The interaction is not prevented by H63D, but the mutation causes a failure to decrease the affinity of the TFR protein for transferrin, resulting in increased cellular iron intake. As the receptor binds transferrin, polymorphisms in the TFR gene, such as the serine/glycine variant at codon 142, may alter the level of cellular iron uptake. One study has suggested that whereas there was no association for HFE and TFR independently, individuals heterozygous or homozygous for the C282Y mutation increased risk associated with colorectal cancer might be increased by compound heterozygosity for the HFE mutations in the small number of subjects studied. TFR gene polymorphism was not an independent risk factor and did not modify the disease risk associated with HFE mutation. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1460–3)
mutation and homozygous for the S142 allele had an increased risk of multiple myeloma, breast cancer, and colorectal cancer (15, 16). The lack of association between HFE gene mutations, when considered in isolation, and colorectal cancer has been supported by two other investigations (17, 18), although another study has found a modestly increased risk of colon cancer for carriers of any HFE mutation compared with subjects with no mutation (adjusted odds ratio [OR], 1.40; 95% confidence interval [95% CI], 1.07-1.87; ref. 19).

To determine whether there is an increased risk of colorectal cancer in individuals carrying a mutant HFE and TFR alleles, independently or as a combination, we established genotype frequencies of mutant alleles in a well-defined population with a family history of colorectal cancer and in a randomly selected control group. The use of cases with a family history increases the statistical power of the study compared with a set of unselected cases (20).

Materials and Methods

Patients and HFE Genotyping. The study involved a total of 327 unrelated individuals (191 females and 136 males) who had been diagnosed with colorectal cancer; were known to be negative for predisposing mismatch repair, APC and MYH germ line mutations; and had a family history of colon cancer with a minimum of two affected family members but no reported hemochromatosis. Cases were recruited from Regional Genetic Centres in the South of England and either the case or a relative was affected at <70 years of age. The cases in this study had an age range of 30 to 70 years. Controls comprised 322 individuals (168 females and 154 males) who were selected and recruited from households in the South of England and randomized by using a random number generator. Controls had no previous diagnosis of colorectal neoplasia or family history of disease, had not been investigated for colonic and/or rectal disease within 2 years before recruitment, and had an age range of 50 to 75 years. Both cases and controls were of Caucasian origin. Without taking into account the increased statistical power provided by the use of family history cases (20), a study of this size using a random selection of both cases and controls would have 80% power to detect a 4-fold increase in the frequency of a variant between controls (2%) and cases (8%). All individuals provided peripheral blood and DNA was extracted using standard protocols. PCR-RFLP analysis was used to screen for the HFE germ line mutations and for the TFR polymorphism, G142S as described previously (6, 15, 19). Primers for the H63D region were -AGACTGTTGGAGTACTTCACTCC-3′ and -ATGGTTAAGGCCCTGTTCGCTGTC-3′ and -AGACTGTTGGAGTACTTCACTCC-3′. All digests were conducted alongside control PCR products of known genotype. To ensure accuracy of the HFE genotyping, we also directly sequenced almost all the samples for C282Y and a random 20% of samples for H63D.

Statistical Analysis. STATA statistical software version 8.0 (Stata Corp., College Station, TX) was used. The data were analyzed as a case-control study using single and multivariable logistic regression. The outcome variable was case or control, and the explanatory variables were genotypes or combinations of genotypes. Wald tests were used to compare a single characteristic of a variable with the baseline and the likelihood ratio test was used to test the significance of an overall variable. The study has been analyzed with adjustment for multiple post hoc testing using Bonferroni procedure to adjust for two post hoc tests (5% level of statistical significance attained at P = 0.025). Controlling for gender did not alter the results obtained from logistic regression and Wald tests, and this was not used in the results reported below.

Results

The frequencies of HFE gene mutations were established in 327 cases (191 females and 136 males) and 322 controls (168 females and 154 males). There was no significant difference in the distribution of males and females between cases and controls (x² = 2.55, P = 0.11). The allele frequencies of each HFE mutation were very similar in the cases and the controls (C282Y, 0.082 versus 0.073; H63D, 0.151 versus 0.138; P = 0.536 and 0.528, respectively, Fisher’s exact test; Table 1). The number of individuals heterozygous for the C282Y mutation was higher in the case population (50 of 327, 15.3%) than controls (39 of 322, 12.1%), although this difference was not significant (P = 0.255, Fisher’s exact test; Table 1). Heterozygosity for H63D occurred at a higher frequency in the case (83 of 327, 25.4%) than the control (73 of 322, 22.7%) populations (Table 1), although the difference was not significant (P = 0.463, Fisher’s exact test). The number of homozygotes for each of the two HFE mutations was very similar in cases and controls (2 and 4 and 8 and 8, respectively; Table 1); no compound homozygotes were identified. In both cases and controls, the HFE genotypes were in Hardy-Weinberg equilibrium (C282Y: cases, P > 0.5; controls, P > 0.3; and H63D: cases, and controls, P > 0.5).

The frequency of individuals with any HFE gene mutation was similar in cases (128 of 327, 39.1%) and controls (119 of 322, 37.0%). However, the frequency of the compound heterozygous HFE genotype (C282Y/wild type and H63D/wild type) was significantly higher in cases (15 of 327, 4.6%) than controls (5 of 322, 1.6%; P = 0.038, Fisher’s exact test; Table 2). We therefore identified three mutually exclusive groups of individuals: compound heterozygotes (both C282Y/wild type and H63D/wild type), “single mutation carriers” (C282Y/wild type or C282Y/H63D or H63D/wild type or H63D/H63D), and those who were wild type for both mutations (Table 2). Single variable logistic regression analysis showed that overall, the indicator variable for these three groups only reached borderline significance (P = 0.07). However, Wald Tests showed that single mutation carriers (individuals with mutation at C282 or H63 but not both) to be at no increased risk of colorectal cancer compared with wild-type subjects (OR, 1.01; 95% CI, 0.73-1.40; P = 0.95) and compound heterozygotes to be possibly at increased risk compared with individuals with a single mutation (OR, 3.03; CI, 1.06-8.61; P = 0.038). However, allowing for multiple post hoc testing using the Bonferroni correction for two post hoc tests (i.e., no mutation against a single mutation and compound mutation against single mutation), overall statistical significance at the 5% level would be attained only at P = 0.025.

We selected at random 171 cases and 184 control samples for TFR genotyping and found that the allelic frequencies for S142 and G142 were the same in both cases and controls (0.51 and 0.49; P = 0.940, Fisher’s exact test; Table 3). As allele frequencies are known to vary among ethnic groups (21),

Table 1. Frequency of HFE gene genotypes in study subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Case patients, % (n = 327)</th>
<th>Control subjects, % (n = 322)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282/C282</td>
<td>84.1 (275)</td>
<td>86.6 (279)</td>
</tr>
<tr>
<td>C282/Y282</td>
<td>15.3 (50)</td>
<td>12.1 (39)</td>
</tr>
<tr>
<td>Y282/Y282</td>
<td>0.6 (2)</td>
<td>1.2 (4)</td>
</tr>
<tr>
<td>H63/H63</td>
<td>72.2 (236)</td>
<td>74.8 (241)</td>
</tr>
<tr>
<td>H63/D63</td>
<td>25.4 (83)</td>
<td>22.7 (73)</td>
</tr>
<tr>
<td>D63/D63</td>
<td>2.4 (8)</td>
<td>2.5 (8)</td>
</tr>
<tr>
<td>Any HFE mutation</td>
<td>39.1 (128)</td>
<td>37.0 (119)</td>
</tr>
<tr>
<td>Allele frequency, Y282</td>
<td>0.083 (54/654)</td>
<td>0.073 (47/644)</td>
</tr>
<tr>
<td>Allele frequency, D63</td>
<td>0.151 (99/654)</td>
<td>0.138 (89/644)</td>
</tr>
</tbody>
</table>
these data provided good evidence of matching of the cases and controls used in this study. The frequencies of S142 heterozygotes and S142 and G142 homozygotes were also similar in cases and controls (Table 3). Statistical analysis failed to identify any interaction, as proposed by Beckman et al. (15), between TRF genotypes and either HFE mutation or compound HFE heterozygosity (Ps > 0.06).

Discussion

Recently, the presence of any germ line HFE mutation (C282Y or H63D) was shown associated with a modest but significant 1.4-fold increased risk (95% CI, 1.07-1.87) of colon cancer compared with those individuals with no HFE mutation; the level of increased risk was the same for those with or without a family history of colon cancer (19). Curiously, heterozygosity for either mutation conferred almost the same increase in colorectal cancer risk, although heterozygosity for the C282Y mutation has been reported to lead to more severe iron overload in carriers than the H63D mutation. The cases and controls in that study were made up of 43% African Americans and 57% Caucasian Americans, although the frequencies of the C282Y and H63D alleles in the two ethnic groups are very different (African Americans, 0.0067 and 0.0263; Caucasian Americans, 0.0507 and 0.1512, respectively; ref. 22). Despite the much lower mutant allele frequency in African Americans compared with those individuals with no HFE mutation; the level of increased risk was the same for those individuals with no family history of colon cancer (19).

Curiously, heterozygosity for either mutation conferred almost the same increase in colorectal cancer risk, although heterozygosity for the C282Y mutation has been reported to lead to more severe iron overload in carriers than the H63D mutation. The cases and controls in that study were made up of 43% African Americans and 57% Caucasian Americans, although the frequencies of the C282Y and H63D alleles in the two ethnic groups are very different (African Americans, 0.0067 and 0.0263; Caucasian Americans, 0.0507 and 0.1512, respectively; ref. 22). Despite the much lower mutant allele frequency in African Americans, those who did possess an HFE mutation were at significantly increased risk of colorectal cancer (OR, 2.1; 95% CI 1.1 to 3.9), whereas Caucasians with a similar genotype were not (OR, 1.2; 95% CI 0.8-1.6; ref. 19). By contrast, a study conducted in the Netherlands of postmenopausal women screened for C282Y mutations and followed for a period of 20 years showed no significant increase in colorectal cancer risk for women who were C282Y heterozygotes (23). Other studies have found no link between any HFE mutation and risk of colorectal cancer. For example, Beckman et al. (15) reported that genetic variants of HFE were not associated with increased risk of this disease or of multiple myeloma and breast cancer; two other studies found that heterozygosity for the C282Y mutation did not increase risk of colorectal cancer (17,18). Hence, it would seem that the higher overall statistically significant risk reported by Shaheen et al. (19) may be a consequence of inclusion of the African American subjects in their study. The reported increased risk of colorectal cancer in African Americans relative to Caucasians (24,25) may well relate to genetic factors other than the HFE C282Y and H63D mutations and/or environmental influences.

In view of these apparent contradictions, we have evaluated whether individuals with a family history of colorectal cancer tend to carry mutant HFE alleles, independently or in combination. When HFE mutations were considered separately, there was only a small, nonsignificant increased risk of colorectal cancer; in this respect therefore, our data support the earlier, negative findings (15,17,18). Our study also considered the colorectal cancer risk to an individual carrying both C282Y and H63D compound heterozygous mutations. The data presented showed that there was evidence of a possible increased risk of colorectal cancer (3.03-fold) only when the two different HFE mutations were present in the same individual.

The study of Shaheen et al. did not show an increased frequency of HFE compound heterozygotes in colorectal cancer cases (1.1%) compared with controls (1.3%; ref. 19). The disparity between this result and our own findings of an association between HFE compound heterozygosity and colorectal cancer risk may relate to differences in case and control selection between the two studies. In support of our findings, an increased frequency of compound heterozygotes has been reported in randomly selected sets of Swedish Caucasian patients diagnosed with either multiple myeloma (2.2%), breast cancer (3.6%), or colorectal cancer (2.9%) compared with controls (1.4%; ref. 15). Although HFE compound heterozygote mutants are relatively uncommon and hence detection of true associations may not be straightforward, the consistency between the Swedish study and our own work suggests that this genotype may truly be associated with increased risk of colorectal cancer.

We found no evidence to suggest that subjects homozygous for either HFE mutation were at increased risk of disease, although the numbers reported in this study and by others (15,19) were very small. It has been shown that C282Y/C282Y homozygotes have the highest iron load (6-9) and if the association between HFE variants and colorectal cancer is mediated directly through iron excess, these individuals might be expected to have the highest risk of colorectal cancer. Owing to the very low frequency of this genotype, it is not possible to exclude an association with colorectal cancer.

An earlier investigation identified a 7.7-fold increase in colorectal cancer risk in HFE Y282 homozygotes and C282Y/H63D heterozygotes who were also homozygous for TFR S142 suggesting that an interaction between HFE and TFR increased risk for neoplastic disease (15). Using a similar number of case and control samples to this earlier study, we have found no evidence to support the earlier observation; our data indicate that homozygosity for the S142 does not modify the influence of the C282Y mutation on colorectal cancer risk.

In summary, we have found evidence to suggest that individuals with a single HFE C282Y or H63D mutation are unlikely predisposed to develop colorectal cancer and that TFR genotype in combination with C282Y mutation does not influence the likelihood of disease. In contrast, compound heterozygosity for the HFE mutations might increase the risk of colorectal cancer.

Table 2. Frequency of single and compound HFE gene mutations among cases and controls

<table>
<thead>
<tr>
<th>Variable*</th>
<th>Case patients, % (n = 327)</th>
<th>Control subjects, % (n = 322)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>60.9 (199)</td>
<td>63.0 (203)</td>
<td></td>
</tr>
<tr>
<td>Single mutation</td>
<td>34.6 (113)</td>
<td>35.4 (114)</td>
<td>1.01 (0.73-1.40)</td>
</tr>
<tr>
<td>Compound mutation</td>
<td>4.5 (15)</td>
<td>1.6 (5)</td>
<td>3.06 (1.09-8.38)</td>
</tr>
</tbody>
</table>

*Singles mutation, C282Y or H63D as homozygotes or heterozygotes. Compound mutation, all were heterozygous for C282Y/wild type and H63D/wild type.

Table 3. Frequency of TRF polymorphisms in study subjects

| Genotype | Case patients, % (n = 171) | Control subjects, % (n = 194) |
|----------|-----------------------------|-------------------------------|-------------|
| S142/S142 | 23.0 (40)                  | 25.0 (47)                     |             |
| S142/G142 | 55.0 (94)                  | 50.0 (92)                     |             |
| G142/G142 | 22.0 (37)                  | 25.0 (45)                     |             |

| Allele frequency, S142 | 0.51 (174/342) | 0.51 (186/368) |
| Allele frequency, G142 | 0.49 (168/342) | 0.49 (182/368) |

Acknowledgments

We thank the patients, their doctors, and other staff from UK Genetics Departments.

References

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