



[haematologica]  
2004;89:1299-1305

## Hemochromatosis gene mutations and iron metabolism in celiac disease

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A B S T R A C T

**Background and Objectives.** Iron deficiency anemia is a common manifestation of celiac disease, which may be due to genetic and environmental factors. *HFE* mutations, frequent in Caucasian populations, can cause increased intestinal iron absorption and thus could protect against the development of iron deficiency. The aim of this study was to evaluate the prevalence of *HFE* mutations and their effect on iron metabolism in Italian celiac patients at diagnosis and after a gluten-free diet.

**Design and Methods.** *C282Y* and *H63D* mutations were assessed by polymerase chain reaction (PCR) and restriction enzyme digestion in 203 patients with celiac disease and in 206 controls. HLA alleles were determined by sequence-specific primers and PCR. Duodenal histology was graded using Marsh's classification, and iron parameters measured by standard techniques.

**Results.** The frequency of the *C282Y* mutation was similar in celiac patients and controls (0.034 vs. 0.031); comparable frequencies were detected also for the *H63D* allele (0.170 vs. 0.136 in celiac patients and controls, respectively). Neither of the two *HFE* mutations affected iron indices in celiac patients at diagnosis, whereas a significant inverse correlation was detected between hemoglobin or ferritin and severity of histological damage (Marsh 3C or 3B vs. 3A,  $p < 0.05$  for both parameters). After a gluten-free diet, a slight increase in hemoglobin levels was observed in *C282Y* carriers as compared to controls, but only in female patients ( $p = 0.044$ ).

**Interpretation and Conclusions.** In Italian patients with untreated celiac disease, *HFE* mutations do not constitute a protective factor against the development of iron deficiency, which seems to be mainly determined by the severity of the intestinal lesions.

**Key words:** celiac disease, *HFE* mutation, iron metabolism, HLA.

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Celiac disease is a disorder caused, in genetically susceptible subjects, by gluten (a protein fraction present in wheat, barley and rye). It is a frequent condition in both Europe and the USA, affecting about 1 in every 200/100 individuals.<sup>1</sup> Although the pathogenesis is not completely understood, intestinal T lymphocytes and cytokines play important roles in causing the small bowel mucosal damage which is characterized by flattening of villi, increased crypt depth and inflammatory infiltration.<sup>2</sup>

The presence of a genetic component in this disease is supported by several pieces of evidence, including the fact that first degree relatives of a patient with celiac disease have a 30-fold higher risk of developing disease than do the general population.<sup>3</sup> A specific HLA class II heterodimer, DQA1\*0501/ DQB1\*02 (DQ2), has been

detected with a significantly higher frequency among celiac patients,<sup>4,5</sup> being present in 80–90% of them as compared to about 12% of control subjects. Moreover, among DQ2 negative patients, the DQA1\*030/DQB1\*0302 heterodimer (DQ8) is present at a high frequency.<sup>6</sup> The presence of these two heterodimers seems to be important for the antigen presentation of gliadin peptides which, after deamidation by tissue transglutaminase (tTG),<sup>7,8</sup> are responsible for T-lymphocyte activation.<sup>9,10</sup> Since HLA class II loci are located on the short arm of chromosome 6 (6p), where there is an extensive linkage disequilibrium, it has been suggested that a haplotype, involving not only DQ but extending toward the telomere and thus including HLA class I, could be present. In fact, a B8-DR3-DQ2 haplotype has been reported in association with celiac disease.<sup>11</sup> The symptoms, age at

onset, prognosis, complications, as well as the duration and severity of mucosal damage of celiac disease differ greatly. Often the disease starts at weaning, when gluten is introduced into the diet, but more and more subjects are being diagnosed in adulthood<sup>2</sup> and iron deficiency anemia is one of the most frequent signs, being present in about 75% of cases.<sup>2</sup> Iron deficiency has been attributed to different factors including reduced absorption and intestinal losses.

During the last few years several genes involved in iron transport have been identified, including *HFE*, localized on 6p21.3 three megabases telomeric to the HLA class I locus, which codifies for an atypical MHC class I membrane protein.<sup>12</sup> Two major missense mutations have been described in *HFE*, a cysteine to tyrosine substitution at amino acid 282 (*C282Y*) and a histidine to aspartic acid substitution at amino acid 63 (*H63D*).<sup>12</sup> The functional role of the 282 amino acid substitution has been demonstrated in animal models, in which liver iron overload and increased intestinal iron absorption were detected.<sup>13</sup> Moreover, patients affected by genetic hemochromatosis type I are homozygous for the *C282Y* mutation in 60–100% of the cases,<sup>12,14,15</sup> whereas its allelic frequency can reach 10% in the general population.<sup>16</sup> The allelic frequency of the *H63D* mutation in the general population is up to 20%,<sup>16</sup> and in the homozygous state the mutation can contribute to increased transferrin saturation.<sup>17</sup>

The prevalence of both celiac disease and hemochromatosis is high in Caucasian populations, and thus the alleles predisposing to these diseases could be present in the same subject and influence the phenotype. The aim of this study was to evaluate the prevalence of *HFE* mutations in a population of Italian celiac patients, and to assess whether these mutations affect iron metabolism parameters at diagnosis and after a gluten-free diet.

## Design and Methods

### Subjects

This study included 203 adult celiac patients (53 males and 150 females) who attended the Gastroenterology Unit at the Department of Medical Sciences, IRCCS – Ospedale Maggiore, Milan, Italy. The diagnosis of celiac disease was based on the presence of symptoms and signs, positivity to one or two specific antibodies and compatible histological alterations, and had been established when the patients were  $\leq 2$  years old in 50 cases, between 3–18 years old in 32 cases and over  $\geq 19$  years old in 121 cases. All patients underwent regular follow-up visits (including blood tests and autoantibody assays), and the mean follow-up was  $13.5 \pm 10.3$  years (range 3–30). Iron parameters were measured both at diagnosis,

and in remission on a gluten-free diet. Patients were considered in remission when clinically asymptomatic, serologically negative and, whenever available, with normal histology. Histological reassessment was performed in about 60% of the patients. The control group included 206 adult subjects (62 males and 144 females), matched for sex and age, randomly selected from a large study performed on the population from the same geographical area for health surveillance purposes. The study was approved by the ethics committee of the IRCCS – Ospedale Maggiore, Milan, and all patients gave their informed consent to participation in the study.

### Serology

Serum immunoglobulins were determined by standard techniques in order to exclude IgA deficiency; IgA anti-gliadin antibodies (AGA) were analyzed with a commercial ELISA kit (OR-GenTec Diagnostika GmbH, Germany), whereas anti-endomysial IgA antibodies (EMA) were evaluated through an indirect immunofluorescence method on monkey esophagus (Biognost Endomysiale AK, Bios GmbH Labordiagnostik, Germany).

### Histology

At least four biopsies were taken from the distal duodenum of celiac patients using standard biopsy forceps during upper gastrointestinal tract endoscopy; samples were processed and stained with hematoxylin-eosin in accordance with standard techniques. Histological findings were described using the Marsh classification, modified by Rostami *et al.*<sup>18</sup> Celiac disease was only diagnosed if there were grade 3 alterations [grade 3 = villous atrophy moderate (A), subtotal (B) or total (C)].

### DNA analysis for *HFE* mutations

DNA was extracted from peripheral blood lymphocytes using the Wizard DNA purification kit (Promega, Milan, Italy) according to the manufacturer's instructions. PCR products, amplified using the primers described by Jazwinska *et al.*<sup>14</sup> and Jeffrey *et al.*,<sup>19</sup> were subsequently digested overnight with *Sna*B I or *Mbo* I to assess the presence of the *C282Y* and *H63D* mutations, respectively (Promega),<sup>14</sup> and the digestion products were separated on 2% agarose gels.

### HLA typing

Low resolution HLA class I genotyping and high resolution class II genotyping for the DRB1, DQA1 and DQB1 loci were performed by sequence-specific primers (SSP) and PCR using commercial kits according to manufacturers' instructions (Olerup, Uppsala, Sweden and Dynal, Oslo, Norway).

### Other assays

Hemoglobin (Hb) and serum iron concentrations were measured by standard methods, whereas serum ferritin levels were assessed by radio-immunoassay (liso-Phase; Lepetit, Milan, Italy). All measurements were performed on morning samples taken after an overnight fast.

### Data analysis and statistics

Carrier frequency was defined as the percentage of subjects who carried at least one *HFE* mutation, whereas allele frequency was calculated as the proportion of positive chromosomes divided by the total number of chromosomes analyzed. Carrier and allele frequencies were compared by  $\chi^2$  analysis with Yates' correction or Fisher's exact test when appropriate. Deviations of the frequencies of *HFE* mutated or wild type alleles from the Hardy-Weinberg equilibrium were tested with an exact test.<sup>20</sup> Differences between mean hemoglobin, serum iron and ferritin values were compared by the analysis of variance (ANOVA) test and Tukey's test for multiple comparisons. Given the small number of subjects who had more than one *HFE* mutation, these subjects were considered together with heterozygous individuals (for compound heterozygotes the *C282Y* mutation was considered). Statistical analysis was performed using SPSS statistical software (version 11) (Chicago, IL, USA).

## Results

### *HFE* mutations and HLA alleles in celiac patients and control subjects

Seventy-six out of the 203 celiac patients (37.4%) were carriers of one *HFE* mutation, this frequency not being significantly different from that observed in the control subjects (29.6%,  $p=0.116$ ) (Table 1). The frequency of *C282Y* mutation carriers was similar among celiac patients and controls (0.069 vs. 0.063), as was the allelic frequency (0.034 vs. 0.031). *H63D* carriership and allelic frequency were also similar in celiac patients and controls, (0.316 vs. 0.243 for carrier frequency and 0.170 vs. 0.136 for allelic frequency). No deviation from the Hardy-Weinberg equilibrium was observed for each genotype either when controls or celiac disease patients were considered, (for the *C282Y* mutation  $p=0.641$  and  $p=0.610$  and for the *H63D* mutation  $p=0.671$  and  $p=0.193$  in controls and celiac patients, respectively).

HLA-A1 and B8 alleles were detected in 14% and 19% of celiacs and in 8.5% and 7.7% of controls ( $p=0.012$ , and  $<0.001$ , respectively). DRB1\*03 was present in 124 celiac subjects and in 27 controls ( $p<0.001$ ), whereas DQA1\*0501 and DQB1\*02 alleles were detect-

**Table 1. Frequency of *HFE* mutations in 203 celiac patients and in 206 control subjects.**

<i>HFE</i> genotype	Celiac patients (203)		Control subjects (206)	
	No.	Frequency	No.	Frequency
282CC-63HH	127	0.626	145	0.704
282CY-63HH	12	0.059	11	0.053
282CY-63HD	2	0.010	2	0.010
282CC-63HD	57	0.281	42	0.204
282CC-63DD	5	0.020	6	0.029

  

<i>HFE</i> allele	Celiac patients' chromosomes (406)		Control subjects' chromosomes (412)	
	No.	Frequency	No.	Frequency
282Y	14	0.034	13	0.031
63D	69	0.170	56	0.136

*282C* and *63H* indicate the wild type alleles, whereas *282Y* and *63D* represent the mutated alleles. The allelic frequency has been calculated as the number of positive chromosomes divided by the total number of chromosomes.

ed in 88.7% and 96.5% of the celiacs vs. 46% and 37.8% of the controls ( $p<0.001$  in both cases). The combination of the DQA1\*0501 and DQB1\*02 alleles, which code for the DQ2 heterodimer, was present in 181 celiac patients but in only 19 controls (89.2% vs. 18.4%,  $p<0.001$ ). When HLA allelic frequencies were analyzed according to the presence of the *C282Y* and *H63D* mutations, no significant positive association could be observed.

### *HFE* mutations and iron parameters

To assess the possible role of *HFE* mutations on iron absorption in celiac patients, we analyzed hemoglobin, ferritin and serum iron levels in patients subdivided according to the presence of the *C282Y* and *H63D* mutations. No differences were observed in iron parameters in patients with different *HFE* genotypes, either at diagnosis or after a gluten-free diet (Tables 2-4). When the same analysis was performed only on female patients (150 cases, 75% of the celiac population analyzed), the presence of one or the other mutation did not influence iron parameters at diagnosis, but a difference in hemoglobin levels was observed after a gluten-free diet. In fact, female celiac patients heterozygous for the *C282Y* mutation showed a mean hemoglobin concentration higher than that observed in those without *HFE* mutations ( $p=0.044$ ) (Table 2). Iron parameters were also analyzed in the subgroup of celiac subjects diagnosed in adulthood ( $> 18$  years old, 121 patients), in particular considering that the possible protective effect of *HFE* on the development of iron deficiency may require a longer time to become

**Table 2. Hemoglobin levels (Hb, expressed as g/L), at diagnosis and on a gluten-free diet, in celiac patients subdivided according to HFE genotype.**

	Hb at diagnosis		Hb on gluten-free diet	
	No.	Mean ( $\pm$ SD)	No.	Mean ( $\pm$ SD)
<b>All CD patients</b>				
282CC-63HH	127	116.7 ( $\pm$ 18)	127	134.2 ( $\pm$ 12)
282CC-63HD/DD	62	118.2 ( $\pm$ 20)	62	138.1 ( $\pm$ 14)
282CY-63HH/HD	14	110.4 ( $\pm$ 25)	14	138.6 ( $\pm$ 7)
<b>Female CD patients</b>				
282CC-63HH	101	112.9 ( $\pm$ 16)	101	130.8 ( $\pm$ 10)
282CC-63HD/DD	37	113.3 ( $\pm$ 18)	37	132.2 ( $\pm$ 12)
282CY-63HH/HD	12	110.1 ( $\pm$ 22)	12	136.9* ( $\pm$ 6)

\* $p=0.044$  vs. female CD patients with the 282CC/63HH genotype.**Table 4. Serum iron levels (expressed as  $\mu$ mol/L), at diagnosis and on a gluten-free diet, in celiac patients subdivided according to HFE genotype.**

	Serum iron at diagnosis		Serum iron on gluten-free diet	
	No.	Mean ( $\pm$ SD)	No.	Mean ( $\pm$ SD)
<b>All CD patients</b>				
282CC-63HH	84	8.91 ( $\pm$ 4.8)	104	15.8 ( $\pm$ 3.2)
282CC-63HD/DD	37	7.44 ( $\pm$ 3.7)	48	15.4 ( $\pm$ 2.8)
282CY-63HH/HD	10	6.88 ( $\pm$ 3.5)	14	15.5 ( $\pm$ 2.9)
<b>Female CD patients</b>				
282CC-63HH	55	8.22 ( $\pm$ 3.9)	72	15.2 ( $\pm$ 3.1)
282CC-63HD/DD	24	7.33 ( $\pm$ 3.6)	33	15.0 ( $\pm$ 2.7)
282CY-63HH/HD	8	6.76 ( $\pm$ 3.5)	12	15.0 ( $\pm$ 2.7)

evident. In this group 9 subjects carried the C282Y mutation, whereas the H63D substitution was detected in 39 patients. No significant difference was detected in hemoglobin, ferritin or serum iron levels in subjects with or without HFE mutations (hemoglobin:  $108.3\pm 27$ ,  $118.6\pm 21$  and  $117.5\pm 19$  g/L; ferritin:  $19.6\pm 16.3$ ,  $21.5\pm 22$  and  $20.4\pm 19$   $\mu$ g/L; serum iron  $7.22\pm 3.5$ ,  $7.55\pm 3.8$  and  $8.99\pm 5$   $\mu$ mol/L in C282Y and H63D carriers and wild type celiacs, respectively). After a gluten-free diet higher hemoglobin levels were still detected in female C282Y carriers than in female celiac patients with a wild type genotype ( $136.7\pm 6$  vs.  $129.3\pm 11$  g/L,  $p=0.04$ ). The presence of the C282Y or H63D mutation did not affect the hemoglobin level in the control subjects, since the mean levels were  $143.2\pm 16$  and  $141\pm 17$  g/L respectively, as compared to  $142.3\pm 13$  g/L observed in individuals without HFE mutations (data available for 152 subjects). Unfortunately, in this population, ferritin values were available

**Table 3. Ferritin levels (expressed as  $\mu$ g/L), at diagnosis and on a gluten-free diet, in celiac patients subdivided according to HFE genotype.**

	Ferritin at diagnosis		Ferritin on gluten-free diet	
	No.	Mean ( $\pm$ SD)	No.	Mean ( $\pm$ SD)
<b>All CD patients</b>				
282CC-63HH	104	19.8 ( $\pm$ 31)	127	72.1 ( $\pm$ 65)
282CC-63HD/DD	55	16.6 ( $\pm$ 23)	62	95.6 ( $\pm$ 80)
282CY-63HH/HD	14	17.1 ( $\pm$ 10)	14	51.8 ( $\pm$ 42)
<b>Female CD patients</b>				
282CC-63HH	92	19.7 ( $\pm$ 29)	101	51.9 ( $\pm$ 52)
282CC-63HD/DD	30	16.6 ( $\pm$ 27)	37	51.6 ( $\pm$ 37)
282CY-63HH/HD	12	17.1 ( $\pm$ 16)	12	51.6 ( $\pm$ 42)

**Table 5. Distribution of celiac patients, subdivided according to HFE genotype and hemoglobin levels at diagnosis.**

HFE genotype	Males (53)		Females (150)	
	<120 g/L	>120 g/L	<110 g/L	>110 g/L
	No. (%)	No. (%)	No. (%)	No. (%)
282CC-63HH	11 (47.8)	17 (56.7)	45 (65.2)	54 (66.7)
282CC-63HD/DD	10 (43.5)	13 (43.3)	17 (24.6)	22 (27.1)
282CY-63HH/HD	2 (8.7)	0	7 (10.2)	5 (6.2)

for only a few subjects, and no meaningful comparison could be performed. Even when data were analyzed according to the presence or absence of iron deficiency anemia, the distribution of the different HFE genotypes did not differ among the celiac patients (Table 5).

#### Severity of histological damage and iron parameters in celiac patients

Since the severity of the intestinal damage affects the number of differentiated enterocytes which can take up dietary iron, celiac patients were subdivided according to the degree of histological damage (categorized according to the Marsh classification) at diagnosis. As reported in Table 6, the severity of the damage affected both hemoglobin and ferritin levels, but no difference was observed in serum iron concentrations among the three different classes. In detail, hemoglobin levels were significantly lower in Marsh

class 3C and 3B than in class 3A ( $p < 0.001$  and  $p = 0.018$ , respectively); likewise serum ferritin concentration was significantly lower in both class 3B and 3C than in class 3A ( $p = 0.014$  and  $p < 0.001$ , respectively). When both Marsh class and HFE mutations were taken into account, similar results for hemoglobin and ferritin levels were obtained in celiac patients without mutations and in H63D carriers. As regards C282Y carriers, almost all the patients were in class C, thus no comparison among all the classes could be performed. However, when all class C patients with different HFE genotypes were compared, no differences were observed in the serum indices (hemoglobin:  $101.1 \pm 21.6$ ,  $111.1 \pm 14.9$  and  $112.1 \pm 16.5$  g/L; ferritin:  $9.5 \pm 9.7$ ,  $12.6 \pm 11.1$  and  $11.1 \pm 17.8$   $\mu$ g/L in C282Y and H63D carriers and wild type celiac patients, respectively). Similar results were obtained when only adult patients were considered, with a significant decrease in hemoglobin and serum ferritin levels in subjects with more severe lesions (hemoglobin:  $131.6 \pm 16.4$ ,  $124.3 \pm 18.3$  and  $109.7 \pm 18.5$  g/L in patients with class 3A, B and C lesions, respectively,  $p < 0.001$  3C vs. 3A,  $p = 0.003$  3C vs. 3B; ferritin  $58.1 \pm 42$ ,  $30.6 \pm 28$  and  $10.2 \pm 9$   $\mu$ g/L in patients with 3A, B and C lesions, respectively,  $p = 0.017$  3C vs. 3A,  $p < 0.05$  3C vs. 3B and 3B vs. 3A). Even in this subgroup of patients the presence of HFE mutations did not affect the hemoglobin or ferritin levels of celiac patients with a grade 3C lesion (data not shown).

## Discussion

Iron deficiency anemia is one of the most frequent signs of celiac disease,<sup>2</sup> being present in about 75% of patients at diagnosis. Its pathogenesis is probably multifactorial, since blood loss, insufficient iron intake and decreased absorptive surface may determine this iron deficiency. Only recently, genes coding for proteins directly involved in intestinal iron absorption (such as *DMT1*) have been investigated, demonstrating the presence of a normal regulation of their expression in celiac patients as compared to in control subjects.<sup>21</sup> However, another gene which may play an important role in iron metabolism is *HFE*,<sup>12</sup> since mutations in its sequence have been associated with increased intestinal iron uptake.<sup>13,22,23</sup> In 2002, Butterworth *et al.*<sup>24</sup> observed a higher proportion of C282Y carriers in celiac patients from England than in the control group, a fact which was associated with higher hemoglobin levels at diagnosis. These findings prompted the authors to suggest that the development of iron deficiency in celiac patients may be prevented by the presence of the C282Y mutation even in the heterozygous state. Moreover, they suggested that there is a specific haplotype, including the C282Y mutation and DQB1\*02 in the celiac

**Table 6. Hemoglobin (Hb), ferritin and serum iron levels, in celiac patients subdivided according to the severity of histological damage at diagnosis. Hemoglobin is expressed as g/L, ferritin as  $\mu$ g/L, serum iron as  $\mu$ mol/L.**

Grade of histological lesion (Marsh)	Hb		Ferritin		Serum iron	
	No.	Mean ( $\pm$ SD)	No.	Mean ( $\pm$ SD)	No.	Mean ( $\pm$ SD)
3A	47	129.6 ( $\pm$ 16)	39	58.3 ( $\pm$ 41)	33	9.54 ( $\pm$ 6.8)
3B	31	119.3 ( $\pm$ 17) <sup>^</sup>	28	26.3 ( $\pm$ 27) <sup>^</sup>	23	7.07 ( $\pm$ 4.6)
3C	125	110.7 ( $\pm$ 17) <sup>^o</sup>	106	9.8 ( $\pm$ 10) <sup>ts</sup>	75	8.13 ( $\pm$ 4.1)

<sup>^</sup> $p = 0.018$  vs. Marsh 3A; <sup>\*</sup> $p < 0.001$  vs. Marsh 3A; <sup>^</sup> $p = 0.014$  vs. Marsh 3A; <sup>t</sup> $p < 0.001$  vs. Marsh 3A; <sup>o</sup> $p = 0.021$  vs. Marsh 3B; <sup>ts</sup> $p = 0.015$  vs. Marsh 3B.

ac population,<sup>24</sup> although this hypothesis was not supported by family studies.

We did not find statistically significant differences in the prevalence of either the C282Y or H63D mutation between Italian celiac patients and controls. The genotypic frequency of the C282Y mutation was lower than that reported by Butterworth *et al.*,<sup>24</sup> both in celiac patients and in controls (6.9% and 6.3% in our cohort as compared to 17% and 9%, in Butterworth's study). This finding confirms the idea that C282Y is mainly a Celtic gene and that there is a North-South gradient in its prevalence.<sup>12,14-16</sup> In fact, Jackson *et al.*<sup>25</sup> and Chambers *et al.*<sup>26</sup> observed a higher C282Y genotypic frequency in two large series of blood donors from South Wales and England respectively, than those reported in this study. In contrast, the HFE mutation frequencies observed in our cohorts are very similar to that observed in a large series of blood donors from the same geographical area.<sup>27</sup> The frequencies of the HLA alleles were significantly different in the celiac population than in the controls; in particular, HLA-A1, B8, DRB1\*03, DQA1\*0501, DQB1\*02 were significantly more prevalent in celiac patients, as also previously reported by several studies.<sup>4-6,11,24</sup> These alleles are part of the extended haplotype described in celiac disease,<sup>11</sup> which may include further genes involved in the development of the disease, such as *TNFA* and *MICA*.<sup>28,29</sup> In the two cohorts examined here, although a higher frequency of the A3 and B7 alleles was observed among C282Y carriers, the association between the C282Y mutation and the classical A3-B7 haplotype<sup>30,31</sup> did not reach the level of significance, probably because of the small number of HFE carriers in our populations.

The mechanisms involved in the regulation of iron

absorption in response to body iron stores have been recently further clarified, and the *stores' regulator* identified and named hepcidin.<sup>32-34</sup> Hepcidin is a peptide hormone synthesized in the liver and secreted as 20-25 amino acid peptides. Studies in genetically modified mice have demonstrated that hepcidin knockout animals develop severe iron overload,<sup>32</sup> whereas transgenic ones show iron deficiency.<sup>35</sup> An inverse relationship has been detected between hepatic hepcidin mRNA levels and duodenal expression of apical and basolateral iron transporters<sup>36</sup> and, more recently, functional studies have demonstrated the ability of hepcidin to decrease intestinal iron uptake both in wild type and *HFE* knockout mice,<sup>37</sup> probably through a direct interaction with enterocytes, as suggested by *in vitro* studies.<sup>38</sup> The *HFE* mutations do, however, still play an important role in intestinal iron absorption and could, in theory, affect iron metabolism also in celiac subjects. Studies performed before the identification of *HFE* gene on patients affected by genetic hemochromatosis demonstrated increased intestinal iron absorption,<sup>23</sup> data confirmed in the animal model of hemochromatosis, i.e. *HFE* knock out mice.<sup>22</sup> Although still not completely clarified, this phenomenon has been attributed to the effect of the *HFE* mutated protein on the transferrin receptor 1, which may reduce its iron uptake and cause a situation of iron deficiency within the cell.<sup>39</sup> This mechanism could, in theory, be protective in celiac disease, since the increased uptake of iron could counteract the effect of the reduction in the absorbing surface.

Unlike Butterworth *et al.*<sup>24</sup> we did not find that either *HFE* mutation had a protective effect when hemoglobin, serum iron and ferritin levels at diagnosis were analyzed according to the different *HFE* genotypes. It should be noted that the frequency of *HFE* mutations among patients with genetic hemochromatosis has been reported to be lower in Italy than in Northern Europe,<sup>15</sup> thus suggesting that other genes may play a role in iron accumulation in Italian subjects. This fact could contribute to explain the differences in the effect of *HFE* mutations on iron parameters between the English celiac population<sup>24</sup> and the Italian one reported here. However, the possible protective effect of the *C282Y* mutation observed by Butterworth *et al.*<sup>24</sup> should be corrected for the severity of the intestinal lesion, since a selection bias due to the presence of a less severe histological damage in *C282Y* patients cannot be excluded. In fact, examining the degree of intestinal involvement, we found that hemoglobin and ferritin levels were inversely correlated with the severity of the histological damage, thus supporting the idea that the number of differentiated enterocytes present at the apical part of the villi is an important factor in determining the amount of iron absorbed by the small intestine. Furthermore, inflammation, and in particular the

increased cytokine levels detected in celiac patients, could affect the production of hepcidin which, in turn, could regulate intestinal iron absorption. In fact, it has recently been demonstrated that interleukin-6 can induce hepcidin expression in cell culture systems as well as in humans.<sup>40</sup> Although higher hemoglobin levels were observed in the female *C282Y* carriers after a gluten-free diet, higher body iron stores (as evaluated by the level of serum ferritin) were not detected in *HFE* mutation carriers of either sex on a gluten-free diet. These data are comparable to those reported by Casanelli *et al.*,<sup>41</sup> Jackson *et al.* (in females and first time donors)<sup>25</sup> and Chambers *et al.*<sup>26</sup> in Italian, Welsh and English blood donor populations, respectively. However, it must be noted that discordant results on the effect of *C282Y* heterozygosity on iron parameters in women have been obtained in populations with different genetic backgrounds.<sup>25,42,43</sup>

It must also be considered that, in complex diseases such as celiac disease, multiple genetic and environmental factors contribute to the phenotype, and frequencies of the alleles involved in the pathogenesis could vary according to the population analyzed. The same consideration could be applied to iron metabolism, and explain the differences between our findings and those in the English population,<sup>24</sup> and is in accordance with a recent study by Beutler *et al.*<sup>44</sup> who detected a difference in hemoglobin levels in *C282Y* carriers of Northern or Southern European descent. Moreover, in the same study,<sup>44</sup> differences in the hemoglobin levels were observed only in the mid-range of hemoglobin distribution and not in the anemic range in *C282Y* heterozygous, a fact which prompted the author to suggest that other factors may be involved in the compensatory mechanisms which take place in iron deficiency anemia.

We conclude that *HFE* mutations do not *per se* represent a protective factor against iron deficiency in Italian celiac patients at diagnosis, although the *C282Y* mutation had a minor effect on hemoglobin levels in women on a gluten-free diet. In our population, the severity of histological damage still seems to play a role in determining the development of iron deficiency in celiac disease.

*The order of authorship was based on the contribution of each author to the design of the study, data interpretation and writing of the manuscript. DB contributed to the study design, carried out some experiments, contributed to the data interpretation and wrote the manuscript. SC and SDB carried out the remaining experiments. RM revised the manuscript and contributed to data interpretation. MTB contributed to the study design, recruited and treated patients in the study, interpreted the data and revised the manuscript.*

*All authors approved the version to be published. All authors reported no potential conflicts of interest. There is no submission or previous report that may be regarded as redundant or a duplicate publication.*

*Manuscript received May 27, 2004. Accepted August 13, 2004.*

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