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# HFE mutations in α-1-antitrypsin deficiency: an examination of cirrhotic explants

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**Increased iron deposition is often seen in liver explants with α-1-antitrypsin deficiency, but it remains unclear if this is a nonspecific effect of end-stage liver disease or if individuals with α-1-antitrypsin deficiency and excess iron are at increased risk for HFE mutations. To further examine this question, 45 liver explants with α-1-antitrypsin deficiency and 33 control livers with chronic hepatitis C were examined for histological iron accumulation, graded on a scale of 0 to 4+, and HFE mutations. Interestingly, the α-1-antitrypsin cirrhotic livers showed a bimodal distribution of iron accumulation, with peaks at grades 1 and 3. In contrast, hepatitis C cirrhotic livers showed a unimodal distribution with a peak at grade 2. HFE mutations in livers with α-1-antitrypsin deficiency were as follows: C282Y = 2%, H63D = 42%. H63D mutations were more frequent in α-1-antitrypsin deficiency cases than in controls (42 *vs* 27%), but was not statistically significant, *P* = 0.17. However, there was a significant association with HFE mutations in α-1-antitrypsin deficiency livers with grade 3+ or 4+ iron, *P* = 0.02. In contrast, livers with hepatitis C showed a similar frequency of HFE mutations as the general population: C282Y = 15%, H63D = 27%. A rare S65C mutation and a novel A271S mutation were also found in this study; the latter patient had 4+ iron in the liver and later developed heart failure with cardiac iron. In conclusion, total H63D mutations were high (42%) in cirrhotics with α-1-antitrypsin deficiency and there was a significant association between HFE mutations and high levels of iron accumulation.**
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α-1-Antitrypsin deficiency disease most commonly affects individuals of European ancestry and is found in 14 per 1000 US Caucasians.[1] The gene for α-1-antitrypsin, *SERPINA1*, is located on chromosome 14 and encodes a serine protease inhibitor. Over 100 different alleles have been identified, but the most common allele is designated as M and is not associated with α-1-antitrypsin deficiency. The most common *SERPINA1* mutation alleles are designated as S and Z, and have allele frequencies of approximately 2–3 and 1%, respectively. Mutations in *SERPINA1* interfere with the export of the serine protease inhibitor from hepatocytes. Accumulation of α-1-antitrypsin protein within hepatocytes can result in hepatocyte injury, fibrosis and cirrhosis.[1]

Hereditary hemochromatosis is an autosomal recessive iron-disorder and is one of the most common inherited diseases among individuals of Northern European descent.[2] For individuals with clinical hereditary hemochromatosis, *HFE* mutations are the most common genetic association, and the most common mutations are missense mutations that result in a cystosine to tyrosine substitution at amino acid 282 (C282Y) and a histidine to aspartic acid substitution at amino acid 63 (H63D).[3] Overall, C282Y and H63D *HFE* gene mutations are seen in approximately 11 and 27% of US Caucasians, respectively.[4] Most clinical hemochromatosis is associated with C282Y homozygosity, but even within this group, there is substantial variation in phenotypic expression due to individual variables including both exacerbating factors such as alcohol use and fatty liver disease as well as

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protective factors such as female gender. In those individuals who develop clinical disease, symptomatic organ involvement typically manifests in midlife with predominant involvement of the liver.[2] In individuals with *HFE*-related cirrhosis, the 1- and 5-year survival after liver transplantation is significantly lower than with other diseases, such as chronic hepatitis C. Interestingly, in the post-transplant population, significant hepatic iron overload in the native liver can be associated with decreased survival, even in patients without *HFE* mutations who are transplanted for other diseases, with cardiac complications one of the major contributors to post-transplant mortality.[5]

Despite the high prevalence of both hereditary hemochromatosis and α-1-antitrypsin deficiency in people of European ancestry, previous studies have found no strong evidence that the *HFE* and *SERPINA1* mutations are associated. However, we have anecdotally noted that cases with α-1-antitrypsin deficiency can have marked iron accumulation in the liver, and cases of co-existing hereditary hemochromatosis and α-1-antitrypsin deficiency have been reported.[6] In addition, hepatic iron concentrations in the range of HH have been reported in α-1-antitrypsin deficiency.[7] Furthermore, one study suggested that patients with abnormal α-1-antitrypsin deficiency alleles and genetic hemochromatosis may progress to cirrhosis sooner.[8] These observations prompted us to further examine cases of α-1-antitrypsin deficiency cirrhosis to determine the frequency of iron overload and to investigate whether *HFE* mutations are enriched in α-1-antitrypsin deficiency cirrhosis.

## Materials and methods

Forty-five cases of liver explants with α-1-antitrypsin deficiency in adults were retrieved from the pathology files at the University of California, San Francisco, CA (12 cases), the Johns Hopkins University School of Medicine, MD (17 cases) and the University of Washington, WA (16 cases). The clinical and pathological charts were reviewed. The following clinical features were obtained from patient records in each case: gender, age at time of transplant, pre-operative diagnosis, laboratory data to support a diagnosis of α-1-antitrypsin or hemochromatosis, and post-transplant clinical course. Thirty-three additional cases of hepatitis C-related explant controls were retrieved from the pathology files at the University of Washington.

### Histological Evaluation of Cirrhosis and Iron Stain

Hematoxylin and eosin-stained slides were reviewed for all study cases. All cases showed established cirrhosis. Iron deposition was scored separately for iron in hepatocytes, Kupffer cells, stroma, bile ducts/ductules and vasculature. Iron accumulation was graded as follows: grade 0, absent granules or barely seen at Olympus $\times 40$ objective and $\times 10$ ocular; $1+$, barely seen at $\times 20$ objective and $\times 10$ ocular but easily seen at $\times 40$ objective and $\times 10$ ocular; $2+$, discreet granules seen at $\times 10$ objective and $\times 10$ ocular; $3+$, discreet granules seen at $\times 2$ objective and $\times 10$ ocular; and $4+$, masses visible at $\times 1$ objective and $\times 10$ ocular or naked eye. Iron deposition was also scored as focal ($<50\%$ of all cirrhotic nodules involved) *vs* diffuse ($>50\%$ of all cirrhotic nodules involved). In addition, the location of the iron deposits within the cirrhotic nodules (peripheral or central) was also assessed.

### PASD and Immunohistochemical Analysis for α-1-Antitrypsin Deficiency

The presence of α-1-antitrypsin accumulation was confirmed by PAS with diastase digestion or with immunohistochemical stain for the α-1-antitrypsin globules. Immunohistochemistry was performed by the avidin–biotin–peroxidase complex technique using commercially available antibodies to α-1-antitrypsin antigens (polyclonal at a 1:5dilution; Cell Marque, Rocklin, CA, USA). The sections were pretreated in DAKO Target Retrieval Solution (DAKO, Carpinteria, CA, USA) at pH 6.0, and antigen retrieval was performed in a DAKO Pascal Pressure Cooker. Stains were performed on a DAKO Autostainer. The binding of primary antibodies was detected by use of the DAKO Envision + Dual Link System, Peroxidase. Appropriate positive and negative controls were performed.

### Analysis of HFE Mutations

*HFE* mutational analysis was performed in all cases of α-1-antitrypsin deficiency and hepatitis C. DNA was extracted from 10-$\mu$ thick, formalin-fixed, paraffin-embedded tissues as previously described.[9] The amplicons were analyzed for mutations by direct DNA sequencing. All mutations were confirmed by a separate PCR amplification and sequencing.

### Statistical Analysis

Group means were compared by Student's *t*-tests. Categorical variables were examined by $\chi^2$-tests.

## Results

The average age of the 45 individuals with α-1-antitrypsin deficiency was $50 \pm 12$ years. Thirteen were women (average age $= 50 \pm 10$ years) and 32 were men (average age $= 50 \pm 13$ years), $P = 0.9$. Only one patient with α-1-antitrypsin deficiency had an additional pretransplant clinical diagnosis of

hemosiderosis, but this patient was not found to carry an *HFE* gene mutation in our study. Of the 33 hepatitis C patients, the average age was 54 ± 6 years. Three individuals were women (average age = 53 ± 9 years) and 30 were men (average age = 54 ± 5 years), $P = 0.9$. There were significantly more women in the α-1-antitrypsin deficiency group than the hepatitis C group ($\chi^2$-test, $P = 0.03$), but as female gender has a strong protective effect on iron overload, this gender imbalance will not increase the possibility of finding a false association based on gender but will instead make an association more difficult to find. None of the hepatitis C individuals carried an additional pretransplant clinical diagnosis of hemochromatosis. Additional characteristics of the study groups are given in Table 1.

In the general population, the prevalence of the C282Y and H63D *HFE* gene mutations are approximately 11 and 27% in US Caucasians, respectively.[4,10] The control group of hepatitis C cirrhotics showed mutation frequencies similar to that of the general population, with total frequencies of C282Y = 15% and H63D = 27%: C282Y/wt = 4; C282Y/H63D = 1; H63D/H63D = 2; and H63D/wt = 6. The average age of individuals with hepatitis C and *HFE* mutations (54 years) did not differ from those without mutations (53 years), $P = 0.7$. In cases of α-1-antitrypsin deficiency, mutation frequencies were C282Y = 2% and H63D = 42%: C282Y/wt = 1; H63D/H63D = 2; and H63D/wt = 17. The frequency of H63D mutations was higher in α-1-antitrypsin deficiency as compared with the general population (42 *vs* 27%), but did not reach statistical significance ($P = 0.11$). Similarly, the frequency of H63D mutations in α-1-antitrypsin deficiency was not statistically higher than in hepatitis C controls ($P = 0.11$). Interestingly, individuals with α-1-antitrypsin deficiency were less likely than hepatitis C controls to have C282Y mutations, ($P = 0.03$). Of the α-1-antitrypsin deficiency cases, the average age of all individuals with and without *HFE* mutations was 51 and 51 years of age respectively, $P = 0.9$.

Iron stains on 45 explants with α-1-antitrypsin deficiency showed the following: 0+ ($N = 6$), 1+ ($N = 20$), 2+ ($N = 7$), 3+ ($N = 10$), 4+ ($N = 2$). Iron stains on 33 explants with chronic hepatitis C showed the following: 0+ ($N = 0$), 1+ ($N = 9$), 2+ ($N = 17$), 3+ ($N = 7$), 4+ ($N = 0$). Interestingly, the iron grades in α-1-antitrypsin deficiency cases showed a bimodal distribution, with peaks at 1+ and 3+ (Figure 1). In contrast, hepatitis C cirrhotic livers showed a unimodal peak at 2+ iron (Figure 1). Further analysis showed that *HFE* gene mutations were significantly associated with grade 3+ or higher iron deposition in cases of α-1-antitrypsin deficiency (grades 1 and 2+ *vs* 3 and 4+, $P = 0.02$), but not hepatitis C-related cirrhosis (grades 1 and 2+ *vs* 3 and 4+, $P = 0.1$). The higher grades of iron accumulation in individuals with α-1-antitrypsin deficiency were not associated with an older age (50 ± 10 years in those with 3+ or greater iron, *vs* 51 ± 10 years in those with less than 3+ iron, $P = 0.6$).

**Table 1** Characteristics of cases with α-1-antitrypsin deficiency and chronic hepatitis C

| *Characteristic* | *α-1-Antitrypsin deficiency,* N = *45* | *Hepatitis C,* N = *33* |
|---|---|---|
| *Gender* | | |
| M | 32 | 30 |
| F | 13 | 3 |
| Age at transplantation (mean, ± s.d., years) | 49.7 ± 12 | 53.8 ± 9 |
| *Ethnicity* | | |
| Caucasian | 40 | 21 |
| Hispanic | 3 | 2 |
| African American | 1 | 1 |
| Unknown | 1 | 9 |
| *Other liver diseases* | | |
| Hepatitis C | 5 | 33 |
| Hepatitis B | 2 | 0 |
| Alcohol liver disease | 13 | 9 |
| Hepatitis C and alcohol | 3 | NA |
| Non-alcohol fatty liver disease | 5 | 0 |
| Sarcoidosis | 1 | 0 |
| Autoimmune hepatitis | 1 | 0 |
| Primary biliary cirrhosis | 1 | 0 |
| Primary sclerosing cholangitis | 1 | 0 |
| *Iron grade* | | |
| 0 | 6 | 0 |
| 1 | 20 | 9 |
| 2 | 7 | 17 |
| 3 | 10 | 7 |
| 4 | 2 | 0 |
| Median iron grade | 1 | 2 |
| *Allele frequency* | | |
| C282Y | 2% | 15% |
| H63D | 42% | 27% |
| *HFE mutational status* | | |
| C282Y homozygous | 0 | 0 |
| C282Y heterozygous | 1 | 4 |
| C282Y/H63D | 2 | 2 |
| H63D homozygous | 17 | 6 |
| H63D heterozygous | 0 | 1 |
| S65C homozygous | 0 | 1 |
| A271S homozygous | 1 | 0 |
| No mutations | 25 | 20 |

Analysis of the patterns of iron deposition in the liver showed no qualitative differences between cases of hepatitis C and α-1-antitrypsin deficiency. There was a peripheral predominance of iron deposition in cirrhotic nodules in both hepatitis C and α-1-antitrypsin deficiency cases with and without *HFE* gene mutations. The pattern of iron deposition showed patchy iron staining in patients with lower iron grades and more diffuse iron staining in cirrhotic nodules as the grade of iron deposition increased. Similarly, as the grade of

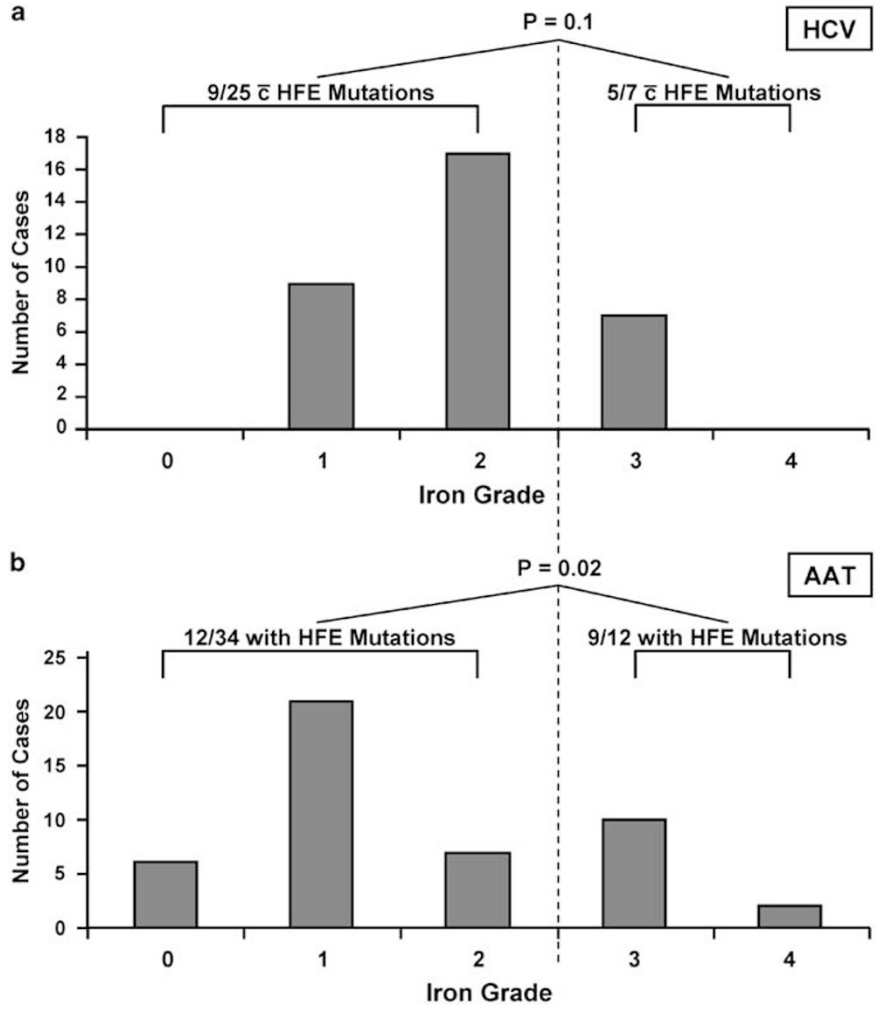


**Figure 1** (**a**) In hepatitis C cirrhotic livers, iron grades showed a unimodal distribution with a peak at grade 2. Mutations were more common in higher grades of iron accumulation, but the frequency did not reach statistical significance, $\chi^2$-test. (**b**) In contrast, in cases of α-1-antitrypsin deficiency, the iron grades showed a bimodal distribution and cases with 3+ or greater irons were more likely to have *HFE* gene mutations, $\chi^2$-test.

iron deposition increased, iron deposition in bile duct epithelium and endothelial cells increased in all patient groups. Likewise, there was also a trend toward increased Kupffer cell iron deposition as the overall hepatic iron grade increased. However, the maximum Kupffer cell iron deposition did not exceed 2+, and there was no apparent differences between cases with and without *HFE* gene mutations. Overall, the pattern, location and cellular distribution of iron deposition were not qualitatively predictive for the presence of *HFE* gene mutation in either α-1-antitrypsin deficiency or hepatitis C-associated liver cirrhosis.

Other rare mutations identified include a S65C mutation in one hepatitis C liver and a novel A271S mutation in one α-1-antitrypsin deficiency liver (Figure 2). The individual with the novel A271S mutation had 4+ iron in the liver explant and later developed heart failure with cardiac iron deposition (Figure 3).

## Discussion

In this study, a bimodal distribution of iron was found in cases of cirrhosis with α-1-antitrypsin deficiency, and the second peak was associated with an increased frequency of *HFE* mutations. There also is a trend toward increased numbers of H63D mutations in the overall α-1-antitrypsin deficiency study group (42%) as well as fewer than expected C282Y mutations. These findings suggest a relationship between α-1-antitrypsin deficiency and *HFE* mutations in the setting of cirrhosis and iron overload. Our results do not support the generally accepted idea that α-1-antitrypsin deficiency and hereditary hemochromatosis are completely independent disease processes, at least in cirrhotic livers. In a previous report, Fargion *et al*,[11] studied the relationship between hemochromatosis and α-1-antitrypsin deficiency by investigating whether genetic hemochromatosis is a risk factor

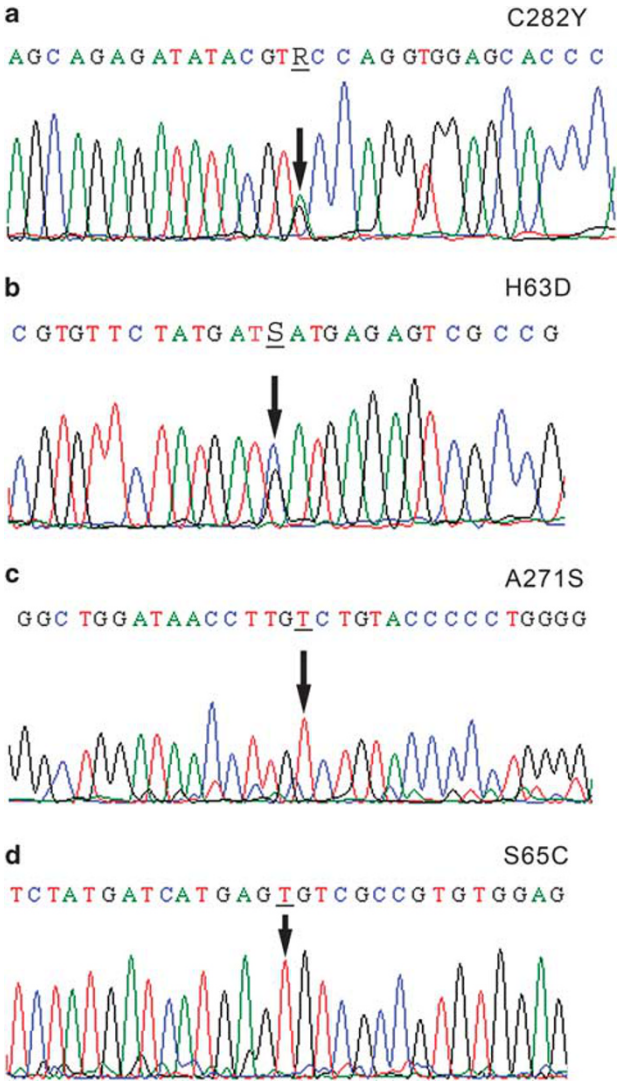


**Figure 2** Sequencing electropharograms of *HFE* mutations. Panel a is representative case of C282Y heterozygote; panel b is representative case of H63D heterozygote; panel c shows novel A271S homozygote; and panel d shows S65C homozygote.

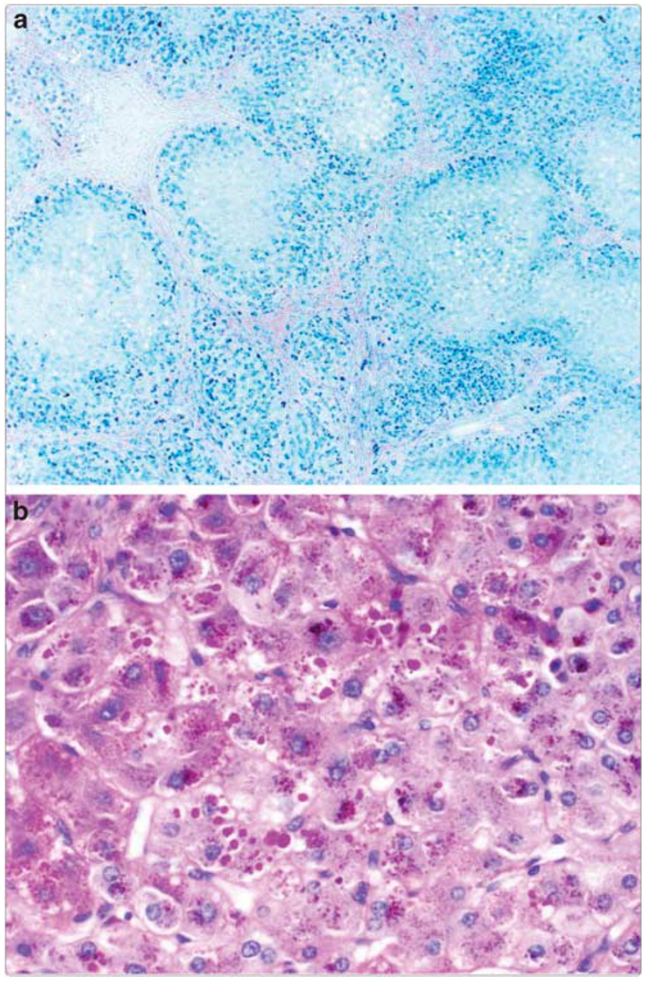


**Figure 3** (**a**) Liver explant with 4+ iron deposition in an α-1-antitrypsin deficiency case with a novel A271S *HFE* gene mutation, ×40 original magnification. (**b**) PASD stain with eosinophilic globules in same case, ×400 original magnification.

for α-1-antitrypsin deficiency: they found no relationship. Although Fargion *et al*[11] did not report mutational data for their cases, the clinical data suggests most of the studied individuals likely had C282Y mutations. Likewise, Sharrard *et al*[12] found no link between *HFE* mutations and α-1-antitrypsin deficiency but looked only at C282Y mutations. Thus, the results of our study and these two previous reports are not necessarily discordant, as a link with α-1-antitrypsin deficiency and H63D mutations would likely have been missed by their study design. Also of note, the C282Y mutation is only rarely found outside of European populations, supporting the generally accepted Celtic origin of this allele. However, the H63D mutation can be found in a much more diverse group of genetic populations, suggesting a separate origin from C282Y.[13] This observation suggests that risk assessment observations for α-1-antitrypsin deficiency phenotype or genotypes in C282Y-affected populations may not be easily extrapolated to H63D-affected populations. There is relatively little additional data that addresses the question of α-1-antitrypsin deficiency and *HFE* genetics, but it is of interest to note that Ludwig *et al*[7] reported that the most likely disease, after hereditary hemochromatosis, to have hepatic iron indexes greater than 1.9 in cirrhotic livers was α-1-antitrypsin deficiency: hereditary hemochromatosis, 100%; α-1-antitrypsin deficiency, 28%; cryptogenic cirrhosis, 19%; alcohol liver disease, 14%; chronic hepatitis C, 7%.[7]

It seems likely that other diseases besides α-1-antitrypsin deficiency with marked iron overload may also be at increased risk for *HFE* mutations. In this study for example, five out of the seven individuals with chronic hepatitis C and grade 3+ iron had *HFE* mutations. Although this did not reach statistical significance, this may be due to relatively small numbers. Also of note, we observed

that individuals with hepatitis C-related cirrhosis were more likely to have C282Y *HFE* mutations. Others have also observed that individuals with chronic hepatitis C and C282Y *HFE* mutations have an increase risk for fibrosis.[14]

It is well recognized that chronic liver disease from many different causes may lead to an increase in hepatic iron stores. For example, in chronic hepatitis C, iron trafficking and metabolism can be effected by both chronic necroinflammatory damage, as well as viral replication itself.[15] As chronic liver injury progresses to end-stage cirrhosis, there can be increased parenchymal iron deposition that is histologically similar to hereditary hemochromatosis patterns of iron deposition, due to decreased transferrin production and decreased hepcidin synthesis by the liver.[16] Decreased transferrin production results in an increase in unbound serum iron, which is taken up by hepatocytes.[17] In addition, hepcidin is a negative regulator of iron absorption; thus decreased synthesis of hepcidin by the liver could lead to increased iron absorption and increased iron deposition in the liver.[2] Our findings suggest that α-1-antitrypsin deficiency could potentially further perturb iron metabolism in individuals with *HFE* mutations, leading to enhanced accumulation of iron. Laboratory studies have identified the endoplasmic reticulum stress response as a potential shared pathway by which these two diseases could synergistically enhance cellular injury.[18]

In this study, a novel A271S *HFE* mutation was also found in one α-1-antitrypsin deficiency case with heart failure and cardiac iron deposition. The systemic iron accumulation suggests that the mutation may have functioned in a similar manner to that of other *HFE* mutations in increasing iron deposition. However, the possibility that A2721S may represent a polymorphism cannot be completely excluded. Novel mutations in the *HFE* gene or other iron metabolism genes may potentially account for a proportion of cases with systemic iron overload that lack the classic C282Y and H63D mutations.[9]

Because this is a retrospective and cross-sectional study, our findings will need to be verified in additional cohorts. The bimodal pattern of iron accumulation in individuals with α-1-antryptin deficiency was an unanticipated finding and should be confirmed by other groups. In addition, there are several other limitations to our study that should be noted. First, our findings are limited in part by the lack of serum testing for α-1-antitrypsin deficiency, so data on homozygosity *vs* heterozygosity for α-1-antitrypsin deficiency are not available. In addition, our study population, while predominately Caucasian, is from different parts of the United States and are likely a genetically mixed cohort, which is relevant when attempting to compare genetic frequencies in this study to that of other studies. Although ethnicity for hepatitis C controls was not available in a third of the cases, the fact that the *HFE* mutation profile in this group was very similar to the expected distribution for a Caucasian population indicates that this is unlikely to have significantly skewed our findings. Finally, it bears emphasis that, while our findings demonstrate that a subgroup of individuals with both α-1-antitrypsin deficiency and increased iron have an increased frequency of *HFE* mutations, we do not have data that would address the question of whether the *HFE* mutations increased the risk of cirrhosis or the rate of fibrosis progression.

In conclusion, cirrhotic livers with α-1-antitrypsin deficiency have a bimodal distribution of iron accumulation, and those cases with grades 3 + or 4 + iron have a higher frequency of *HFE* mutations. Overall, H63D mutations were higher and C282Y mutations lower in cases with α-1-antitrypsin deficiency compared with controls.

## Disclosure/conflict of interest

The authors declare no conflict of interest.

## References

1 Fairbanks KD, Tavill AS. Liver disease in alpha 1-antitrypsin deficien cy: a review. Am J Gastroenterol 2008;103:2136–2141; quiz 42.
2 Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. N Engl J Med 2004;350: 2383–2397.
3 Feder JN, Gnirke A, Thomas W, *et al.* A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399–408.
4 McDonnell SM, Hover A, Gloe D, *et al.* Population-based screening for hemochromatosis using phenotypic and DNA testing among employees of health maintenance organizations in Springfield, Missouri. Am J Med 1999;107:30–37.
5 Kowdley KV, Brandhagen DJ, Gish RG, *et al.* Survival after liver transplantation in patients with hepatic iron overload: the national hemochromatosis transplant registry. Gastroenterology 2005;129:494–503.
6 Shen R, Frankel WL, Wen P. Coexistent hereditary hemochromatosis and alpha1-antitrypsin deficiency. Arch Pathol Lab Med 2004;128:491–492.
7 Ludwig J, Hashimoto E, Porayko MK, *et al.* Hemosiderosis in cirrhosis: a study of 447 native livers. Gastroenterology 1997;112:882–888.
8 Rabinovitz M, Gavaler JS, Kelly RH, *et al.* Association between heterozygous alpha 1-antitrypsin deficiency and genetic hemochromatosis. Hepatology 1992;16: 145–148.
9 Fenton H, Torbenson M, Vivekanandan P, *et al.* Marked iron in liver explants in the absence of major hereditary hemochromatosis gene defects: a risk factor for cardiac failure. Transplantation 2009;87: 1256–1260.
10 Hanson EH, Imperatore G, Burke W. HFE gene and hereditary hemochromatosis: a HuGE review. Human Genome Epidemiology. Am J Epidemiol 2001;154: 193–206.
11 Fargion S, Bissoli F, Fracanzani AL, *et al.* No association between genetic hemochromatosis and

alpha1-antitrypsin deficiency. Hepatology 1996;24: 1161–1164.
12 Sharrard MJ, Durkie M, Tanner MS. Genetic hemochromatosis in alpha1-antitrypsin-deficient liver disease. Hepatology 1997;26:1366.
13 Settin A, El-Bendary M, Abo-Al-Kassem R, *et al.* Molecular analysis of A1AT (S and Z) and HFE (C282Y and H63D) gene mutations in Egyptian cases with HCV liver cirrhosis. J Gastrointestin Liver Dis 2006;15:131–135.
14 Geier A, Reugels M, Weiskirchen R, *et al.* Common heterozygous hemochromatosis gene mutations are risk factors for inflammation and fibrosis in chronic hepatitis C. Liver Int 2004;24:285–294.
15 Pietrangelo A. Hemochromatosis gene modifies course of hepatitis C viral infection. Gastroenterology 2003;124:1509–1523.
16 Deugnier Y, Brissot P, Loreal O. Iron and the liver: update 2008. J Hepatol 2008;48(Suppl 1):S113–S123.
17 Deugnier Y, Turlin B, le Quilleuc D, *et al.* A reappraisal of hepatic siderosis in patients with end-stage cirrhosis: practical implications for the diagnosis of hemochromatosis. Am J Surg Pathol 1997;21: 669–675.
18 Lawless MW, Mankan AK, White M, *et al.* Expression of hereditary hemochromatosis C282Y HFE protein in HEK293 cells activates specific endoplasmic reticulum stress responses. BMC Cell Biol 2007;8:30.