Most cases of hereditary hemochromatosis are due to a single nucleotide mutation in the hemochromatosis gene (*HFE*) that results in a Cys to Tyr conversion at amino acid 282 (Cys282Tyr) in the protein. Sequencing revealed a second mutation (His63Asp) in the HFE protein, but the penetrance of this mutation is much lower compared to Cys282Tyr. Although a candidate early in the search for the hemochromatosis gene, the transferrin receptor (TFR) was not found to be mutated in hemochromatosis. Several years ago, we discovered a second human transferrin receptor termed transferrin receptor 2 (TFR2). Recently, a hemochromatosis pedigree was discovered in Sicily, where a non–sense mutation at position 250 in the *TFR2* gene was found. Affected individuals lacked the Cys282Tyr *HFE* mutation. The carrier frequency of this mutation is 0.9% among a cohort of southern and central Europeans. Roetto and colleagues recently reported 2 new mutations (at exon 2, 84-88insC, resulting in Glu60Xaa; and at exon 4, Met172Lys) in iron overload patients having what has been termed hemochromatosis type 3. A fourth inactivating mutation of *TFR2* at position 250 in the *HFE* protein, but the penetrance of this mutation is much lower compared to Cys282Tyr. Although a candidate early in the search for the hemochromatosis gene, the transferrin receptor (TFR) was not found to be mutated in hemochromatosis. Several years ago, we discovered a second human transferrin receptor termed transferrin receptor 2 (TFR2). Recently, a hemochromatosis pedigree was discovered in Sicily, where a non–sense mutation at position 250 in the *TFR2* gene was found. Affected individuals lacked the Cys282Tyr *HFE* mutation. The carrier frequency of this mutation is 0.9% among a cohort of southern and central Europeans. Roetto and colleagues recently reported 2 new mutations (at exon 2, 84-88insC, resulting in Glu60Xaa; and at exon 4, Met172Lys) in iron overload patients having what has been termed hemochromatosis type 3. A fourth inactivating mutation of *TFR2* (a 4–amino-acid loss Ala-Val-Ala-Gln at 594-597) has recently been reported.

Here, we investigated the genomic DNA from individuals having atypical hemochromatosis with the aim to look for a correlation between mutations of the *TFR2* gene and an altered iron phenotype. We also asked whether differences in penetrance of the Cys282Tyr mutation were associated with mutations in *TFR2*. A transversion at nt 1391, resulting in a substitution Gln/H11022 to G/A transversion resulting in a substitution at codon 455 of Gln (mutant) for an Arg (normal sequence). The

Figure 1. Polymerase chain reaction–single strand conformation polymorphism analysis of exons 10 and 18 in atypical hemochromatosis. (A) Sample no. 212 (mother of proband) had an aberrantly shifted band in exon 10. Direct nucleotide sequencing found a G>A transversion at nt 1391, resulting in a substitution at codon 455 of Gln (mutant) for an Arg (normal sequence). The
change was verified by sequencing from both the sense and antisense directions. The individual initially identified with the mutation was the mother of the proband. The husband and 8 children were then analyzed. Of the children, 4 were found to have the polymorphism (Figure 1B). One was normal at the HFE locus for the Cys282Tyr mutation, 2 were heterozygous, and 1 (the brother of the proband) was also homozygous for Cys282Tyr. The proband did not have the Arg455Gln mutation. Interestingly, this brother was identified in cohort A as having evidence of liver fibrosis, where his brother (the proband) did not.

In addition, analysis of exon 18 of TFR2 showed an identically shifted band (Figure 1C) in 5 samples (1 from group A, 1 from group B, 2 from group D, and 1 in the normal controls). Direct sequencing of these samples showed a change of G>C in the 3’ untranslated region (3’ UTR) of exon 18. Since this change also was present in the normal control sample, we believe that it represents a previously unreported polymorphism. No correlation was found between this alteration and any of the clinical subtypes.

In summary, a group of individuals selected for unusual iron phenotypes was analyzed for evidence of mutation in the TFR2 gene. None had mutations corresponding to those described in the Italian iron overload pedigrees. We describe a new mutation, Arg455Gln, in exon 10 of TFR2 in a pedigree containing an individual with evidence of liver fibrosis in contrast to his HFE identical brother. This mutation could represent a modifier for penetrance of the hemochromatosis phenotype when present with homozygosity for Cys282Tyr. Unlike TFR1 expression, TFR2 expression is not down-regulated in the liver of iron-loaded mice. Our screening for mutations in all 18 exons of TFR2 in genomic DNA from all of the individuals indicates that mutations of the TFR2 gene are rare. The polymorphism in the 3’ UTR that we detected was found in individuals with abnormal iron metabolism as well as in a healthy control. Further cohort studies are needed to determine if this polymorphism is associated with a subtype of hereditary hemochromatosis.


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References


To the editor:

CXCR4 expression is associated with survival in familial chronic lymphocytic leukemia, but CD38 expression is not

Recent immunogenetic studies of chronic lymphocytic leukemia (CLL) suggest a dichotomy: those developing from naive, pregerminal B lymphocytes exhibiting germline configuration of Ig V_{\text{H}} status (poor outcome) and those stemming from more mature, postgerminal center memory B cells with mutated Ig V_{\text{H}} genes.\textsuperscript{1-3} (good prognosis). Due to the cost and expertise required for this technique, a simpler, inexpensive substitute has been sought. CD38 expression has been proposed as a surrogate marker for the CXCR4 chemokine receptor. We recently reported that CXCR4 expression in mouse liver in the face of iron overload and in hereditary hemochromatosis was associated with murine heavy chain genes amplified by polymerase chain reaction and cloned and sequenced, using a previously described method.\textsuperscript{15} Assays were conducted with laboratory personnel blinded to V_{\text{H}} mutation and clinical characteristics of the patients.

Generalized linear models were used to estimate least-squared mean CD38 expression by heavy chain mutation status on 21 patients using SAS Version 8.0 (SAS, Cary, NC) and to evaluate the association with CXCR4 expression data. Survival was estimated on all 39 familial CLL patients by the Kaplan-Meier method, using SPLUS 2000 (Insightful, Seattle, WA). Median levels of CD38 and CXCR4 expression in the subset of cases with V_{\text{H}} data were used to group the patients into lower and higher risk; differences in survival were tested by the Wilcoxon test. All tests of statistical significance were two-sided.

In agreement with earlier reports,\textsuperscript{1,3} unmutated V_{\text{H}} cases displayed a higher percentage of CD38\textsuperscript{+} cells than mutated cases (23.62% versus 5.80%, \(P = .03\)). We did not observe an association between CD38 expression and survival in the 39 patients (Wilcoxon, \(P = .45\)) (Figure 1A). Rather, expression of the CXCR4 chemokine receptor was more strongly correlated with V_{\text{H}} mutational status (9.53% versus 40.82%, \(P = .004\); adjusted for age) and a better predictor of survival (Wilcoxon, \(P = .02\)) (Figure 1B).

CD38 expression has been proposed as a surrogate marker for V_{\text{H}} mutation status to predict the clinical course in CLL with mixed findings.\textsuperscript{4,12} Although we observed an association between CD38...
Mutation analysis of transferrin-receptor 2 in patients with atypical hemochromatosis