

Mutations of the *HFE* Gene and the Risk of Hepatocellular Carcinoma

Submitted 11/09/99; revised 11/19/99
(communicated by Ernest Beutler, M.D., 11/19/99)

Omar Racchi,^{1,2} Rosa Mangerini,^{1,2} Davide Rapezzi,^{1,2} Gian Franco Gaetani,^{1,2} Maria Teresa Nobile,² Antonino Picciotto,³ Anna Maria Ferraris^{1,2}

ABSTRACT: The discovery of the C282Y and H63D point mutations in the hereditary hemochromatosis-associated *HFE* gene allows us to study the molecular basis of congenital and acquired iron overload disorders. In hereditary hemochromatosis an increased frequency of the C282Y and, to a lesser extent, of the H63D mutations has been established, but their role in other conditions associated with iron overload and their prevalence in the normal population are still under investigation. We sought to determine the presence of such mutations, and their possible involvement in the multi-step neoplastic transformation of the hepatocytes, in patients diagnosed with hepatocellular carcinoma, a frequent complication of iron-induced liver cirrhosis occurring in untreated hereditary hemochromatosis subjects. The frequency of the C282Y and H63D mutations was determined in DNA from 12 patients with hepatocellular carcinoma and with no clinical signs of hereditary hemochromatosis. The frequency of the mutations was also determined in 130 normal subjects. A germline C282Y mutation was found in none of the hepatocellular carcinoma patients; the frequency of the H63D mutation was not increased, compared to the 130 controls. The allele frequencies of the C282Y and H63D mutations in the normal population were 0.042 and 0.185, respectively. In conclusion, we suggest that the hereditary hemochromatosis-related mutations of the *HFE* gene do not play a significant role in the pathogenesis of hepatocellular carcinoma.

Keywords: hereditary hemochromatosis, *HFE*, C282Y, H63D, iron overload, hepatocellular carcinoma

INTRODUCTION

The recent discovery of the C282Y and H63D point mutations in the hereditary hemochromatosis-associated *HFE* gene has been a major advance in the understanding of the role of iron overload in human diseases (1). The C282Y mutation is present in 80 to 100% of hereditary hemochromatosis patients, mainly in the homozygote state (1-5), while the H63D mutation seems to have a poorer correlation with hereditary hemochromatosis, possibly only in the condition of compound heterozygosis with the C282Y mutation (6).

Hepatocellular carcinoma is a dramatic consequence of iron overload in many untreated hereditary hemochromatosis patients. It develops in the large majority of cases in cirrhotic livers,

but far more frequently in cirrhosis from hereditary hemochromatosis than from other causes, with a risk up to 45% in some hereditary hemochromatosis series (7). The risk seems to correlate with the amount of iron overload (8), although once cirrhosis has developed, the risk persists despite iron depletion. This strong association between iron overload in hereditary hemochromatosis patients and hepatocellular carcinoma led us to investigate the prevalence of germline *HFE* mutations in patients with hepatocellular carcinoma and no clinical signs of hereditary hemochromatosis, in order to ascertain if the *HFE* mutations, by inducing a moderate but persistent iron excess, might be involved at some stage of the transformation of chronic hepatitis/cirrhosis into overt hepatocellular carcinoma.

¹Dipartimento di Oncologia, Biologia e Genetica, Università di Genova, Genoa, Italy.

²Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy.

³Dipartimento di Medicina Interna, Università di Genova, Genoa, Italy.

Reprint requests to: Dr. Anna Maria Ferraris, Ematologia Oncologica, IST, Largo Rosanna Benzi, 10, 16132 Genoa, Italy. Fax: +39 010 509052. E-mail: gaetani@mbox.ulisse.it.

MATERIALS AND METHODS

The prevalence of germline C282Y and H63D mutations was investigated in 12 patients from Northern Italy (7 males and 5 females; mean age 63 years) with histologically confirmed diagnosis of hepatocellular carcinoma. All cases had a past history of liver cirrhosis, in 1 case following alcohol abuse, in 6 cases HCV infection, in 1 case HBV infection and in 4 cases of unspecified origin. The control group included 130 healthy individuals (44 males and 86 females; mean age 53 years) also originating from Northern Italy.

Mutation analysis was performed on DNA extracted by standard methods from peripheral blood leukocytes. To detect the presence of the C282Y and H63D mutations, a PCR amplification of the *HFE* region was performed according to Feder et al. (1). The accuracy of the PCR assay was verified by testing several positive controls, namely 5 subjects known to have hereditary hemochromatosis and one case of Porphyria Cutanea Tarda (PCT), a cutaneous disorder with iron overload in which a high frequency of C282Y mutation has been consistently found (9,10). Four relatives of 2 hereditary hemochromatosis patients were also analyzed. Informed consent was obtained from all subjects.

RESULTS

Table 1 summarizes the results. As to the C282Y mutation, 11 (8.5%) heterozygotes were found in the normal control group (130 subjects), with an allele frequency of 0.042 and an expected frequency of homozygotes of about 0.0017. As to the prevalence of the H63D mutation in the control group, 42 (32.3%) heterozygotes and 3 (2.3%) homozygotes were found, giving an allele frequency of 0.185. The expected frequency of H63D homozygotes of 0.034 fits nicely with that observed.

Table 1. Prevalence of the C282Y and H63D Mutations in Patients with Hepatocellular Carcinoma and Controls

		Hepatocellular carcinoma (n = 12)	Controls (n = 130)	p*
C282Y	-/-	12 (100%)	119 (91.5%)	N.S.
	-/+	-	11 (8.5%)	
H63D	-/-	9 (75%)	85 (65.4%)	N.S.
	-/+	3 (25%)	42 (32.3%)	
	+/+	-	3 (2.2%)	

Note. -/-, wild type; -/+, heterozygote; +/+, homozygote. * Chi-square test. N.S., not significant

Since no C282Y mutation ($\chi^2 = 0.918$; $p = 0.34$) and 3 H63D mutations ($\chi^2 = 0.276$; $p = 0.87$) were found in the 12 hepatocellular carcinoma examined, no increased frequency of the *HFE* gene mutations was evident in the hepatocellular carcinoma group (Table 1).

Analysis of the C282Y mutation in the 5 hereditary hemochromatosis subjects, their 4 relatives and one PCT case (positive controls) showed 10 mutations out of 18 examined chromosomes, giving an allele frequency of 0.55. No H63D mutation was found.

DISCUSSION

It has been suggested that the *HFE* gene may play a role in conditions other than hereditary hemochromatosis, characterized by much less severe iron overload and in which the presence of the C282Y and/or H63D mutations might increase the risk of developing a disease or modify clinical and laboratory findings (11). PCT is the only condition in which such a correlation has been demonstrated (9,10). In sideroblastic anemia (12), thalassemia major (13), and chronic liver disease (14) no significant association with the *HFE* mutations has been found.

The close relationship between iron overload in hereditary hemochromatosis patients and hepatocellular carcinoma is indicated by the direct correlation between hepatic iron content

and the risk of developing the tumor (8). Liver cancer complicates other forms of cirrhosis, but its incidence in hereditary hemochromatosis is much higher and it is not clear if the causative role of iron is related to its ability to induce chronic inflammation and consequently cirrhosis, or if the iron molecule itself is involved directly in the malignant transformation, or both (15-17).

Our findings do not indicate a significant role of the *HFE* gene mutations in the pathogenesis of hepatocellular carcinoma, since no increased frequency of germline C282Y and H63D mutations was observed in the hepatocellular carcinoma patients, in contrast to two preliminary reports (18,19) that suggested a limited increase of the C282Y mutation in such patients.

Finally, our control group contributes to the determination of the frequency of these mutations in the population of Northern Italy. Since we found 8.5% of heterozygotes, with an allele frequency of 0.042, the frequency of the C282Y mutation is in agreement with data reported in other series from Northern Europe (20), and higher than the 0.01 previously reported in Italy (3). As to the H63D mutation, roughly one third of the control subjects were heterozygotes, with an allele frequency of 0.185.

ACKNOWLEDGMENTS

This work was supported by funds from MURST 1998-1999 and 1999-2000; and P.F. Biotechnologie (CNR, Target Project on Biotechnology). O.R. was supported by a fellowship from FIRC.

REFERENCES

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 13:399–408, 1996.
2. Beutler E, Gelbert T, West C, et al. Mutation analysis in hereditary hemochromatosis. *Blood Cell Mol Dis* 22:187–194, 1996.
3. Carella M, D'Ambrosio L, Totaro A, et al. Mutation Analysis of the HLA-H gene in Italian hemochromatosis patients. *Am J Hum Genet* 60:828–832, 1997.
4. Ryan E, O'Keane C, Crowe J. Hemochromatosis in Ireland and *HFE*. *Blood Cells Mol Dis* 24:428–432, 1998.
5. Nielsen P, Carpinteiro S, Fischer R, Cabeda JM, Porto G, Gabbe EE. Prevalence of the C282Y and H63D mutations in the *HFE* gene in patients with hereditary haemochromatosis and in control subjects from Northern Germany. *Br J Haematol* 103:842–845, 1998.
6. Beutler E. The significance of the 187G (H63D) mutation in hemochromatosis. *Am J Hum Genet* 61:762–764, 1997.
7. Schafer DF, Sorrell MF. Hepatocellular carcinoma. *Lancet* 353:1253–1257, 1999.
8. Niederau C, Fischer R, Sonnenberg A, Stremmel W, Trampisch HJ, Strohmeyer. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. *N Engl J Med* 313:1256–1262, 1985.
9. Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH. Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. *Lancet* 349:321–323, 1997.
10. Santos M, Clevers HC, Marx JJM. Mutations of the hereditary hemochromatosis candidate gene HLA-H in porphyria cutanea tarda. *N Engl J Med* 336:1327–1328, 1997.
11. Beutler E. Genetic irony beyond haemochromatosis: clinical effects of HLA-H mutations. *Lancet* 349:296–297, 1997.
12. Beris P, Samil K, Darbellay R, et al. Iron overload in patients with sideroblastic anaemia is not related to the presence of the haemochromatosis Cys282Tyr and His63Asp mutations. *Br J Haematol* 104:97–99, 1999.
13. Borgna-Pignatti C, Solinas A, Bombieri C, et al. The haemochromatosis mutations do not modify the clinical picture of thalassaemia major in patients regularly transfused and chelated. *Br J Haematol* 103:813–816, 1998.
14. Aldersley MA, Howdle PD, Wyatt JI, Robinson PA, Markham AF. Haemochromatosis gene mutation in liver disease patients. *Lancet* 349:1025–1026, 1997.
15. Cox TM. Haemochromatosis. *Blood Reviews* 4:75–87, 1990.
16. Kew MD. Pathogenesis of hepatocellular carcinoma in hereditary hemochromatosis: occurrence in noncirrhotic patients. *Hepatology* 11:1086–1087, 1990.
17. Bacon B, Britton RS. The pathology of hepatic iron overload: a free radical-mediated process? *Hepatology* 11:127–137, 1990.

18. Willis G, Wimperis JZ, Lonsdale R, Jennings BA. Haemochromatosis gene mutation in hepatocellular cancer. *Lancet* 350:565–566, 1997.
19. Bralet MP, Degott C, Belghiti J, Terris B. Prevalence of haemochromatosis gene mutation in non-cirrhotic liver with hepatocellular carcinoma. *J Hepatol* 28:740–741, 1998.
20. Lucotte G. Celtic Origin of the C282Y mutation of hemochromatosis. *Blood Cells Mol Dis* 24:433–438 1998.