Variation in the phenotypic expression of C282Y hemochromatosis in an Italian family

Sir,

Although genetic hemochromatosis (GH) is genetically homogeneous, its phenotypic expression is variable, depending on both environmental and genetic factors. Studying 3 members of the same family carrying C282Y with different clinical presentations, we speculate that the HLA-A3 genetic constitution is associated with a more severe disease and that modifier genes are close to, or identifiable with, HLA-A3.

GH is an autosomal recessive disorder leading to iron overload.[1] Although GH is caused by a prevalent mutation (C282Y) in the HFE gene,[2] its clinical expression is variable.[3-6] The effect of age, sex, dietary iron, blood losses and blood donations on iron accumulation is well known. Iron burden may also be modulated by genetic determinants, particularly by the ancestral haplotype,[3,4] the ancient chromosomal background on which C282Y mutation occurred in the HFE gene. This haplotype is defined by HLA-A3, D6S105 allele-8 and D6S265 allele-1.[7] We describe an Italian family in which all members are affected by GH (Figure 1). The proband, II-1, presented with weakness, arthritis and an altered glucose tolerance test, when aged 29. The parents were both symptomless and diagnosed because of family screening. I-2 was a 53-year-old woman with slight hypertransaminasemia without apparent cause. I-1 was a symptomless 60-year-old man, who had been a blood donor since the age of 35. Clinical data and iron status are summarized in Table 1. Liver biopsy was not performed in any of these patients because of the absence of significant alterations of liver function tests. Iron depletion (ferritin < 50 µg/L) was achieved by weekly phlebotomies; the amount of iron removed is shown in Table 1. All patients had the same HFE genotype (C282Y/C282Y). HLA A3 was present in the heterozygous state in I-1 and I-2 and in the homozygote state in II-1. I-2 had a typical ancestral haplotype, whereas the HLA-A3-associated haplotype in I-1 was not of the *ancestral* type (D6S265 allele-3 and D65105 allele-6) (Figure 1).

The expression of the disease in I-1 was unusually mild. However, blood donations could have had a protective effect (calculated iron removal = 18 g), resulting in a slower iron accumulation. The expression of the disease in I-2 was that expected in a postmenopausal woman. By contrast, II-1 was the first family member in whom the disease was diagnosed. Her age at diagnosis correlates with the severity of the iron burden.[1,6] Confirming the more severe disorder, II-1 showed the greatest IR/age value in the family. IR/age is a good estimate of iron overload in the absence of liver biopsy.[1,4,5] The severity of the disorder in II-1 suggests the effect of modifier genes. II-1 has the same HFE genotype as her parents and carries a single copy of the ancestral haplotype, as does I-2. She is, however, A3 homozygous at the HLA-A locus. It has been hypothesized that modifier genes are associated with the ancestral haplotype, but the markers which characterize the haplotype are spread over 3 megabases on 6p.[2,8] Since II-1 is homozygous for HLA-A3, but is heterozygous for the other markers, this haplotype constitution allows a dissection of the different genetic components, suggesting a major contribution – as a modifier – of the region close to HLA-A3. We cannot at present define whether this is due to a direct role played by HLA-A, as suggested by others,[9,10] or indicates the effect of other nearby genes.

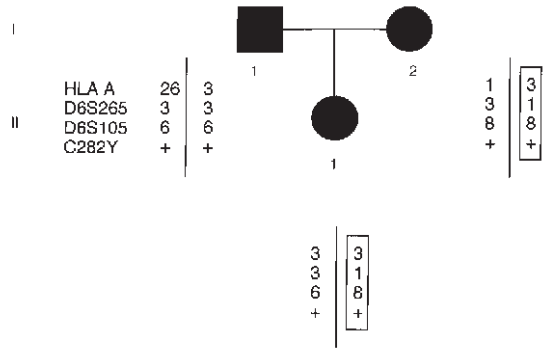

Figure 1. Pedigree of the family studied. Results of HLA typing and of microsatellite marker analysis are under the pedigree. Allele numbers are according to ref. #10. The ancestral haplotype is boxed.

Table 1. Clinical, hematologic and biochemical data of family members.

	I-2	*I-1*	*II-1*
Age (yr)	53	60	29
Hemoglobin (g/dL)	13	14.7	12.9
Alanine transaminase (IU/L)	40	22	6
Oral glucose tolerance test (OGTT)	N	N	A
Transferrin saturation (%)	83.3	55	82.3
Ferritin (µg/L)	626	827	213
Iron removal (IR) (g)	3,150	2,625	2,100
IR/age	0.06	0.043	0.073

N = normal; A = OGTT of diabetic type.

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References

1. Pietrangelo A, Camaschella C. Molecular genetics and control of iron metabolism in hemochromatosis. Haematologica 1998; 83:456-61.
2. 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.
3. Crawford DG, Powell LW, Leggett BA, et al. Evidence that the ancestral haplotype in Australian hemochromatosis patients may be associated with a common mutation in the gene. Am J Hum Genet 1995; 57:362-7.
4. Piperno A, Arosio C, Fargion S, et al. The ancestral hemochromatosis haplotype is associated with a severe phenotype expression in Italian patients. Hepatology 1996; 24:43-6.
5. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy. Gastroenterol 1998; 114:996-1002.
6. Adams PC, Valberg LS. Evolving expression of hereditary hemochromatosis. Semin Liv Dis 1996; 15:47-54.
7. Jazwinska EC, M Cullen L, Busfield F, et al. Haemochromatosis and HLA-H. Nature Genet 1996; 14:249-50.
8. Totaro A, Roetto A, Rommens JM, et al. Generation of a transcription map of a 1 Mbase region containing the HLA-H gene on 6p22. Eur J Hum Genet 1998; 6:105-13.
9. Porto G, Vicente C, Teixeira MA, et al. Relative impact of HLA phenotype and CD4-CD8 ratios on the clinical expression of hemochromatosis. Hepatology 1997; 25:397-402.
10. Camaschella C, Roetto A, Piperno A, et al. Allelic association of microsatellite of 6p in Italian hemocromatosis patients. Hum Genet 1996; 97:476-81.

Pilot study of combined therapy with interferon-α, arabinosyl cytosine and all-trans retinoic acid in patients with chronic myeloid leukemia in the chronic phase

Sir,

The beneficial effect of IFNα on survival of Ph+ CML patients is known to be associated with the achievement of cytogenetic remission.[1-3] Low-dose arabinosyl cytosine (LDAC)[4] and all-trans retinoic acid (ATRA)[5-10] can increase the response rate to IFNα. This study was designed to evaluate the feasibility of treatment with IFNα, LDAC and ATRA in patients with Ph+ CML in the chronic phase with special attention focused on dose adjustment and side effects. Our observations suggest that if one gives and maintains IFNα at 9 MU/daily or at the maximum tolerated dose, LDAC and ATRA cannot be given at the dose and schedule that were tested in this study.

Eleven consecutive patients received IFNα at a dose of 9 MU/day s.c. and in addition, monthly courses of LDAC (40 mg/day s.c., for 10 days every month, from day 1 to day 10) and ATRA (80 mg/sqm/day p.o., for 10 days every month, from day 20 to day 30) (Table 1). Treatment adjustment was decided every 30 days with the purpose of maintaining IFNα at the maximum tolerated dose. When WBC was 3-3.9$\times10^9$/L or PLT 75-99$\times10^9$ (grade I toxicity), IFNα and ATRA were continued at full dose while the next course of LDAC was purposely omitted; if WBC was 2-2.9$\times10^9$/L or PLT 50-74$\times10^9$ (grade II toxicity), IFNα was reduced to 3 MU/day for the next 30 days, ATRA was kept at full dose and the next course of LDAC was skipped; in case of WBC < 2×10^9 or PLT < 50×10^9 (grade III toxicity), IFNα, LDAC and ATRA were discontinued for the next 30 days, and if the recovery did not take place within 90 days LDAC was stopped permanently. Treatment adjustment for non hematologic toxicity was based on IFNα, LDAC and ATRA related side-effects which were graded according to the WHO scale. In case of grade II toxicity, the dose was reduced by 50% for the next month, after which the full dose was restored. In case of grade III toxicity, the drug was discontinued for the next month and then, if complete recovery occurred, 50% of the dose was given. In case of grade IV toxicity or refusal the drug was discontinued permanently.

During the first 12 months of therapy we observed that: i) LDAC had to be discontinued in the majority of patients (72%) because of persistent leukopenia and/or thrombocytopenia (grade III); ii) ATRA had to be discontinued in 45% of patients, mainly due to headaches (WHO grade III-IV); iii) IFNα was never discontinued and was maintained at a dose of 9 MU/day in 60% of patients. By the 3rd month, all of the drugs had had to be reduced in 5/11 patients (45%) (Table 2). None of the patients experienced bleeding or infectious episodes, or required blood transfusions (the lowest hemoglobin level was 8.2 g/dL). As for the hematologic effects, the majority of patients (82%) achieved and maintained a complete hematologic response[1] and five (45%) obtained a cytogenetic response (Table 3). Two out of the 4 patients who displayed a major cytogenetic response (Ph-neg > 66%) where those who received the combined therapy for a prolonged period of time.

These observations suggest that this combination could be potentially effective in the treatment of Ph+ CML. If one gives and maintains IFNα at 9 MU/daily or at the maximum tolerated dose, LDAC and ATRA[9] cannot be given at the dose and schedule that were tested in this study. To administer this drug combination for a longer time a reduction of either