HLA Alleles, Hemochromatosis Allele, and Serum Ferritin in Hemodialysis Patients

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Dear Sir,

In a recent article published in Nephron Quereda et al. [1] studied the influence of certain HLA antigens (A3, B7, B14) on the development of iron overload in long-term hemodialysis patients. They concluded that the presence of these antigens was associated with an increase in serum ferritin levels in patients treated with parenteral iron either in the form of intravenous dextran-iron or transfusions but not in patients receiving oral iron. They explained their observation by suggesting that in patients carrying these antigens the iron overload induced by parenteral iron does not inhibit intestinal iron absorption as it does in noncarriers. Numerous articles, cited by Quereda et al. [1], also reported an association between these HLA antigens and an increase in ferritin levels in hemodialysis patients. Other authors [2,3], however, have not found such an association.

One can hardly get a clear picture of this difficult problem, or in general of the implication of the idiopathic hemochromatosis allele (or gene) in secondary iron overload, without considering a certain number of well-established facts concerning this allele and without avoiding detrimental approximations in methodology.

Listed below there are some of the well-established facts concerning the idiopathic hemochromatosis allele [4–6].

Despite what is generally repeated, since a sort of 'Original sin' by Bregman et al. [7], in the nephrology literature, the HLA alleles A3, B7 and B14 are not 'hemochromatosis alleles' but simply mark the hemochromatosis allele located on chromosome 6 near the HLA A locus. The only independent marker of the hemochromatosis gene is the A3 allele; alleles B7 and B14 are only indirect markers dependent on their own haplotypic linkage to A3. Consequently, when the B7 and B14 alleles are

present without A3, they in no way indicate the presence of the hemochromatosis gene. Thus, it is incorrect to intermingle carriers of A3, B7 or B14 in one hemochromatosis allele group.

Quereda et al. [1] provide an example of this confusion: only 29 of their patients in group 1 were A3 carriers and thus had an increased risk of carrying the hemochromatosis allele (and not the 37 patients who carried A3, B7 or B14).

Accepting the frequency of the hemochromatosis allele to be 0.06 (estimated in Brittany, France, where the disease is frequent), the probability that a chromosome 6 carrying A3 would also carry the hemochromatosis allele is about 0.19 [6]; this risk decreases to 0.04 if the chromosome 6 does not carry A3. Thus a subject carrying just one A3 allele has a risk of being heterozygous for hemochromatosis equal to about 0.23 (0.19 + 0.04) and a patient carrying two A3 alleles has a

risk of about 0.38 ($0.19 + 0.19$). In general, about 8% of A3-positive subjects carry two A3 alleles and 92% just one. It can be estimated that about 7 of the A3-positive patients of Quereda et al. [1] [$(29 \times 0.08 \times 0.38) + (29 \times 0.92 \times 0.23)$] were heterozygous for the hemochromatosis allele. In the same manner, it can be estimated that the probability of at least 1 patient homozygous for the hemochromatosis allele occurring in this group of 29 A3-positive patients is about 0.22. Likewise, the group of Quereda et al. of 74 patients without A3 can be expected to include 6 patients [$(0.04 + 0.04) \times 74$] who are heterozygous for hemochromatosis and has a risk of including at least 1 homozygous individual of about 0.12. It appears that in terms of a risk for iron overload the two groups of Quereda et al. are not a priori highly different. While an increased intestinal absorption of iron in relation to the iron stores of the organism is generally encountered in homozygous subjects, it is occasionally observed in heterozygous subjects [8], among whom only 15–30% show an increase in biochemical expression (in-

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cluding serum ferritin level) of iron load [for a review, see 9]; in addition, biochemical expression rapidly reaches a plateau, then remains unchanged [10]. It is not, however, impossible that, in the particular situation of hemodialysis patients who are at risk of increased iron intake, certain modifications in these phenomena could occur.

In our opinion, this article by Quereda et al. [1], after others and like another one published in 1987 [11], has not avoided bias in a certain number of points which hinder the interpretation of results, repeating again the confusion concerning the so-called ‘hemochromatosis alleles’. For example at the end of each year, the same transfusion patient was included several times, and thus an apparent difference possibly based on very few patients was observed (since a patient with an iron overload at the end of the 1st or 2nd year is likely also to have one at the end of the following; these increases included several times actually represent consecutive elements of a single difference) or the Student test for a variable (ferritin level) was used with a skewed distribution, even if it is assumed to become normal after logarithmic transformation (a distribution-free test such as Wilcoxon’s test would give a more adapted analysis of the data: using this test to compare the data shown in figure 4 gives $p > 0.05$, while the authors mention $p < 0.005$). Finally, only one thing is strongly documented: iron overload progresses with the number of transfusions and the amount of dextran-iron given.

Despite its shortcomings, this article presents a large series with carefully collected data. It would be interesting to know if there are any differences in the iron overload between the two groups when they are defined on the basis of the presence or absence of the A3 allele alone and when each patient is included only once (at the end of his follow-up and not at the end of each year). The data should be adjusted for the amount of iron administered either as dextran-iron or through transfusions, and compared by using a distribution-free test. The results could be interesting for planning hemodialysis management. They could also be useful in better understanding hemochromatosis. Actually, it is highly unlikely, considering the points raised above, that a difference between the groups, if it were induced by a large number of patients, would result from the effect of the hemochromatosis allele encountered in the patients with idiopathic hemochromatosis. If such a difference could be substantiated, it would implicate the possible existence, in addition to the idiopathic hemochromatosis allele, of another HLA-A3-linked allele with a weak expression (requiring environmental conditions favorable to secondary iron

overload for expression) and with a frequency greater than that of the major allele [4].

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