

# Association of HLA-A3 and HLA-B14 antigens with idiopathic haemochromatosis

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**SUMMARY** The frequency of HLA-A3 and HLA-B14 antigens was significantly higher in a series of 51 patients with idiopathic haemochromatosis than in a control group, being respectively 78.4 versus 27.0% and 25.5 versus 3.4%. This finding strongly supports the suggestion that idiopathic haemochromatosis is a genetic disease and suggests that the gene(s) responsible for the disease may be linked to the histocompatibility genes.

The aetiology of idiopathic haemochromatosis (IH) is controversial, most authors regarding this affection as an inborn error of metabolism (Sheldon, 1935), while others consider it to be an acquired disease (MacDonald, 1964). In this paper, we report the results of the determination of histocompatibility antigens in a series of patients with IH. The investigation showed a significant association of HLA-A3 and HLA-B14 antigens with IH, a finding which strongly supports the notion of hereditary transmission of the disease.

## Methods

### PATIENTS

Fifty-one patients having no family ties, 46 male (mean age: 42.2 years, range: 26-62) and five female (mean age: 53.4 years, range: 46-61) with unequivocal IH were studied. Skin pigmentation and hepatomegaly were present in all of them, diabetes mellitus in 43, gonadal deficiency in 42, heart failure in 13. Serum iron was 35  $\mu\text{mol/l}$  or more, and unsaturated iron binding capacity was 10% or less in all the patients. Stainable iron was demonstrated in the liver specimens from the 49 biopsied patients; in the two non-biopsied patients the desferrioxamine test (1.5 g infused intravenously for four hours) was markedly abnormal with urinary iron excretion of 10 290 and 18 760  $\mu\text{g}$  24 h (normal: less than 2 000  $\mu\text{g}$  24 h). The control group consisted of 204 apparently normal adult blood donors.

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Only two pairs of linked alleles, A and B according to the new nomenclature (WHO-IUIS Terminology Committee, 1975) were investigated. The antigens determined by these genes were typed on lymphocytes according to the method of Dausset (Dausset, 1973) and/or on platelets according to the method of Colombani *et al.* (Colombani *et al.*, 1973), using the sets of antisera provided by the Laboratoire National de Référence (Département d'Immunologie, Hôpital Saint-Louis, Paris).

The percentages of the different antigens in the patients with IH and in the controls were compared by means of the  $\chi^2$  test or Fisher's exact test (Siegel, 1956). The usual correction for multiple comparisons was made by multiplying the level of significance thus found for each test by the total number of antigens considered.

## Results

The results of the typing and their statistical significance are set out in Table 1: it appears that two antigens, and only two, according to the corrected level of significance, HLA-A3 and HLA-B14, had a significantly higher prevalence in the IH patients than in the controls. The association of HLA-A3 and HLA-B14 was likewise more frequent in the IH patients than in the controls, in whom this association was remarkably uncommon (Table 2).

## Discussion

Thus, there is a significant association of HLA-A3 and HLA-B14 antigens with IH; the present results confirm our preliminary findings based on a

Antigen HLA-A	HLA-A locus				P	Antigen HLA-B	HLA-B locus				P
	IH patients (n = 51)		Controls (n = 204)				IH patients (n = 51)		Controls (n = 204)		
	number *	% **	number *	% **			number *	% **	number *	% **	
1	7	13.1	55	27.0	NC	5	7	13.4	29	14.2	NC
2	18	35.3	98	48.0	NC	7	25	49.0	57	27.9	NS
3	40	78.4	55	27.0	< 1 × 10 <sup>-9</sup>	8	8	15.7	54	26.5	NC
9	6	11.8	34	16.7	NC	12	12	23.5	45	22.1	NS
10	2	3.9	15	7.3	NC	13	0	0	21	10.3	NC
11	5	9.8	28	13.7	NC	14	13	25.5	7	3.4	< 1 × 10 <sup>-4</sup>
28	1	2.0	17	8.3	NC	18	1	2.0	20	9.8	NC
29	3	5.9	29	14.2	NC	27	1	2.0	23	11.3	NC
W 24	2	3.9	1	0.5	NS	W 15	9	17.6	12	5.9	NS
W 26	0	0	1	0.5	NC	W 16	0	0	6	2.9	NC
W 30						W 17	0	0	8	3.9	NC
W 31	1	2.0	11	5.4	NC	W 21	0	0	9	4.4	NC
W 32	1	2.0	4	2.0	NC	W 22	0	0	9	4.4	NC
Blank	16	31.3	60	29.4	NS	W 35	5	9.8	35	17.2	NC
						W 37	2	4.0	6	2.9	NS
						W 38	1	2.0	0	0	NS
						W 40	5	9.8	17	8.3	NS
						Blank	13	25.5	50	24.5	NS

Table 1 Distribution of histocompatibility antigens in patients with idiopathic haemochromatosis (IH) and in controls

\*Total = 2 n (two antigens per locus); \*\*total = 200%; NC = not calculated (lower frequency of the antigens in IH patients than in controls, as a direct consequence of higher frequency of HLA-A3 or HLA-B14); NS = not significant (with corrected P).

Allele associations	IH patients (n = 51)		Controls (n = 204)	
	no.	%	no.	%
HLA A-B				
HLA-A3 - HLA-B14	12	23.5	2	1.0
HLA-A3 - HLA-BW15	8	15.7	3	1.5
HLA-A3 - HLA-B7	20	39.2	21	10.3
HLA A-A				
HLA-A3 - different antigens	27	52.9	43	21.1
HLA-A3 - blank	13	25.5	12	5.9

Table 2 Frequency of certain allele associations in patients with idiopathic haemochromatosis and in controls

small number of patients with IH (Genetet *et al.*, 1975; Simon *et al.*, 1975). Therefore, IH is an additional instance of association between histocompatibility antigens and diseases (Dausset *et al.*, 1974; Svejgaard *et al.*, 1975). However, the association of HLA-A3 and HLA-B14 with IH is remarkable for the following reasons: (1) the association of HLA-A3 is close, reaching the same order of magnitude as that of HLA-B27 antigen with ankylosing spondylitis (Brewerton *et al.*, 1973; Schlosstein *et al.*, 1973); (2) one of the antigens associated with IH belongs to the HLA-A locus, whereas most of the antigens associated with other diseases belong to the HLA-B locus; (3) most of the diseases associated with histocompatibility antigens are more or less related to disorders of immunity, whereas IH is apparently unrelated to this type of disorder.

Although most authors accept that IH is an in-born error of iron metabolism (Sheldon, 1935), some have questioned the genetic transmission of the

disease and suggested that IH is an acquired disorder (MacDonald, 1964). Our finding that IH is associated with certain histocompatibility antigens represents a strong argument in favour of the genetic origin of IH, although the adjuvant role of environmental factors cannot be excluded. The histocompatibility alleles themselves might be directly implicated in the transmission of IH; however, the discrepancy between the relatively high frequency of HLA-A3 antigen in the normal population and the relatively low frequency of IH makes this hypothesis unlikely. An alternative hypothesis might be that the gene(s) responsible for IH would be linked to the histocompatibility alleles; in this case, the gene(s) for IH would be located on chromosome 6, to which the major histocompatibility complex is assigned (Lamm *et al.*, 1975), close to the HLA-A locus. With respect to the mode of inheritance of IH, the observation that iron overload is significantly more frequent (Alexandre, 1975) in

the siblings than in the offspring is hardly compatible with the hypothesis of a monogenic dominant pattern (Alexandre, 1975; Saddi and Feingold, 1974). Likewise, a monogenic recessive pattern (Saddi and Feingold, 1974) is not satisfactory for the following reason: the heterozygous state for HLA-A3, and therefore for the gene(s) for IH which would be linked with it, is present in up to 53% of the patients, the frequency of possible homozygosity (25.5%) being frankly lower (Table 2).

Finally, a plausible hypothesis might be that IH is inherited according to a polygenic pattern, or, more probably, an oligogenic pattern, since the HLA alleles or the gene(s) linked with them play a predominant role; the high frequency of IH over one generation might result from an interaction effect.

The incidence of IH is remarkably high in Brittany. Our finding of the association of certain histocompatibility antigens with IH might be peculiar to the form of IH observed in Brittany. Observations in patients with IH from other geographical areas are needed to extend the concept of association of certain histocompatibility antigens to all the patients suffering from the disease.

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#### References

- Alexandre, J.-L. (1975). *Etude de l'Hérédité dans l'Hémochromatose, à Propos de 106 Enquêtes Familiales*. Thesis: Rennes.
- Brewerton, D. A., Caffrey, M., Hart, F. D., James, D. C. O., Nicholls, A., and Sturrock, R. D. (1973). Ankylosing spondylitis and HL-A 27. *Lancet*, **1**, 904-907.
- Colombani, J., D'Amato, J., Gabb, B., Smith, G., and Svejgaard, A. (1973). Micro-technique of platelet complement fixation. In *Manual of Tissue Typing Techniques*, pp. 69-74. Edited by J. G. Ray, D. B. Hare, and D. E. Kayhoe. (DHEW Publication No. (NIH) 74-545): Bethesda, Maryland.
- Dausset, J. (1973). Micro-lymphocytotoxicity technique. In *Manual of Tissue Typing Techniques*, pp. 28-33. Edited by J. G. Ray, D. B. Hare, and D. E. Kayhoe. (DHEW Publication No. (NIH) 74-545): Bethesda: Maryland.
- Dausset, J., Degos, L., and Hors, J. (1974). The association of the HL-A antigens with diseases. *Clinical Immunology and Immunopathology*, **3**, 127-149.
- Genetet, B., Genetet, N., Fauchet, R., Pawlotsky, Y., Oger, J., Simon, M., and Bourel, M. (1975). HL-A antigens and diseases. In *Book of Abstracts of the XIV Congress of the International Society of Blood Transfusion*, p. 37. Finnish Red Cross Blood Transfusion Service: Helsinki.
- Lamm, L. U., Thorsen, I.-L., Petersen, G. B., Jørgensen, J., Henningsen, K., Bech, B., and Kissmeyer-Nielsen, F. (1975). Data on the HL-A linkage group. *Annals of Human Genetics*, **38**, 383-390.
- MacDonald, R. A. (1964). *Hemochromatosis and Hemosiderosis*. Thomas: Springfield, Ill.
- Saddi, R., and Feingold, J. (1974). Idiopathic haemochromatosis: an autosomal recessive disease. *Clinical Genetics*, **5**, 234-241.
- Schlosstein, L., Terasaki, P. I., Bluestone, R., and Pearson, C. M. (1973). High association of an HL-A antigen, W 27, with ankylosing spondylitis. *New England Journal of Medicine*, **288**, 704-706.
- Sheldon, J. H. (1935). *Haemochromatosis*. Oxford University Press: London.
- Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*, MacGraw-Hill: New York.
- Simon, M., Pawlotsky, Y., Bourel, M., Fauchet, R., and Genetet, B. (1975). Hémochromatose idiopathique. Maladie associée à l'antigène tissulaire HL-A3? *Nouvelle Presse Médicale*, **4**, 1432.
- Svejgaard, A., Platz, P., Ryder, L. P., Staub Nielsen, L., and Thomsen, M. (1975). HL-A and disease associations—a survey. *Transplantation Review*, **22**, 3-43.
- WHO-IUIS Terminology Committee (1975). Nomenclature for factors of the HLA system. In *Proceedings of the Sixth International Histocompatibility Workshop Conference*. Munksgaard: Copenhagen. (in press).