Europ J. Cancer Vol. 16, pp. 809–815
© Pergamon Press Ltd. 1980. Printed in Great Britain
0014-2964/80/0601-0809 $02.00/0

HLA Genotypes in Familial Hodgkin's Disease. Excess of HLA Identical Affected Sibs*

J. HORS,†‡ G. STEINBERG,† J. M. ANDRIEU,† C. JACQUILLAT,† M. MINEV,§ J. MESSERSCHMITT,¶ G. MALINVAUD,|| F. FUMERON,† J. DAUSSET† and J. BERNARD†

†*Centre Hayem, Hôpital Saint-Louis, Université Paris* VII, *Paris* 75010, *France*, § *Emergency Medical Institute Pirogov, Sofia, Bulgaria*, ¶*C. H. R. d'Amiens, Place Victor-Pauchet*, 80030 *Amiens, France*, ||*C. H. R. de Limoges, Hôpital Dupuytren*, 87000, *Limoges, France*

Abstract—*The observations of HLA-genotyped* 13 *family cases of Hodgkin's disease including* 7 *pairs of affected sibs*, 4 *parent/child cases and* 2 *pairs of first cousins are reported.*

Among the pairs of affected sibs, an excess of HLA identical patients (6 *observed out of* 7) (1.75 *expected*) *do not reach the level of statistical significance.*

However when the results are pooled with international data, the increased frequency of HLA identity among the affected sibs reaches a significant level ($P<0.05$). *This observation strongly supports the hypothesis of the role of the HLA complex as one of the factors of susceptibility to Hodgkin's disease.*

INTRODUCTION

HODGKIN'S disease (HD) differs notably from other malignant hemopathies in the high frequency of multiple family cases, the risk having been evaluated as three times higher for close relatives [1, 2] and seven times higher for siblings [3] than for the general population.

The nature of the relationship between HLA antigens and an increased risk of Hodgkin's disease is still being debated. Since the findings by Amiel *et al.* [4] showing an increased frequency of the antigen 4c in the patients, contradictory results have been published. Some of them established a correlation between several antigens and Hodgkin's disease (see review [5]) and others, no correlation at all [6, 7].

However, after a survey of all of these studies, Svejgaard *et al.* [8] have confirmed from the data available for 1500 patients on an international basis, a significant increase in the frequency of HLA-A1, B5, B8 and B18.

Several other observations of familial Hodgkin's disease with regard to HLA typing, have already been published [9–16].

Accepted 24 October 1979.

*This work was supported by a grant from the Ligue Nationale Française contre le Cancer.

‡To whom correspondence should be addressed.

In the present study, we intend to consider the role of haplotypes and genotypes in multiple family cases rather than the frequency of individual markers compared to that of a reference panel.

MATERIALS AND METHODS

Families

There were 13 caucasoid families included in this study. The relationship between the two affected patients was: siblings in seven families, parents/offsprings in four, and first cousins in two. The present series includes ten new families, and three about which preliminary studies had already been published [17]. A fourth family (Family No. 3 of ref. [17]) has not been included because, after checking lymph node slides, one of the 2 patients was found to be suffering from non-Hodgkin lymphoma. The proband was arbitrarily considered as the earliest affected patient in the family.

Out of a total of 2700 cases of HD examined in the Hospital Saint-Louis, between 1958 and 1978, approximately 40 families were found as bearing two affected members, of whom only 13 families were selected for study, both patients being available for HLA typing.

Patients

All patients, after histological examination using Lukes' criteria [18] were examined clinically, including chest X-rays, bipedal lymphangiography and biological analyses according to Ann Harbor's recommendations [19].

All patients were caucasoids and originated from different parts of France, except two families (Pol. and Sal.) which were Bulgarian.

HLA typing was performed on frozen lymphocytes [20] by microlymphocytotoxicity [21] for the following antigens, according to the Workshop nomenclature [22]:

HLA-A1, 2, 3, 9, 10, 11, 25, 26, 28, 29, w19-2 (w30+w31)

B5, 7, 8, 12, 13, 14, 15, 17, 18

w21, w22, 27, w35, w38, 40.

Owing to the fact that the lymphocytes were kept frozen thus permitting an up-dating of the HLA type, the serology can be considered as homogeneous.

Statistical analysis

The series was too short to permit a gene or haplotype counting in comparison with a normal reference panel.

The observed genotypes of the pairs of affected sibs were classified into 3 relationships: HLA identical, semi-identical and different, and compared to their theoretically expected Mendelian frequencies: 25%, 50% and 25% respectively, by χ^2 square test. Statistical analysis was carried out using our own results; afterwards they were combined with the results from other studies.

RESULTS

The relationship, sex, age and date of onset, clinical staging and histology are summarized for each of the 13 families in Table 1.

The sex of the proband and his related patient was concordant in six cases and discordant in the seven others. The histological type was identical in five pairs of patients.

HLA genotyping was clearly established in five of the seven sib/sib families (Table 2). However it was not possible to perform the HLA typing for the parents of the other two families (Roc. and Mai.), the genotypes of their siblings were assumed to be identical in both cases with a high probability of accuracy. In the Cer. family the two diseased sibs possibly shared in common the haplotypes A2, B12 and were assumed to be HLA haploidentical. In the Via. family, a paternal cross-over has to be postulated, since a healthy daughter has inherited an A3, B27 haplotype instead of A3, Bw35.

In the HD parent/child affected families and in the two pairs of cousins, there was no doubt concerning the HLA genotypes (Table 3).

In the Vdb. family the patients were HLA different and in the Bvc. family they were haploidentical, bearing in common HLA-A3, B18.

Some haplotypes were observed in several families: A1, B8 in 4, A2, B12 in 2, which are known to be in linkage disequilibrium; their frequency is greatly dependent on the ethnic or geographical origins of the tested individuals. If we consider only the 11 French patients, three being A1, B8, the increased frequency of this haplotype is not significant because of the shortness of the series, when compared to a reference panel [23] in which it was present in 12 of the over 106 individuals tested. Among the suspected antigens in previous studies [8], the following ones: Bw35, B5, B18 were observed in two, two and one family respectively, without an excess in frequency, the normal gene frequencies being 10.4, 8.5 and 8% respectively [23].

As shown in Table 4, there are six pairs of HLA identical sibs, one haploidentical and none were HLA different.

These results did not differ significantly from the expected pattern, 1.75, 3.5 and 1.75 pairs respectively. However when our results were combined with those of five other studies concerning a total of nine pairs of affected sibs (9, 12, 13, 14, 15) the significance of the P value reached $P<0.05$ (Table 3).

The previously published data included two pairs of HLA-identical sibs [12], another pair [14], two pairs of HLA haploidentical [15] and two others [9, 13]. Another one is clearly HLA different [15]. The last pair [9] cannot be unequivocally considered as HLA different—as has been done in Table 4—but possibly haploidentical too, according to the index case chosen.

DISCUSSION

The aggregation of Hodgkin's disease in small communities and families has attracted much attention. However, it is very difficult to distinguish between environmental factors and a hypothetical hereditary background of susceptibility to the disease. The results emerging from some recent studies have put forward

Table 1. Familial Hodgkin's disease cases. Clinical data

Family	Relationship	Sex	Age at onset	Date of onset	Clinical stage	Histological type
Pol.	Proband	F	22	10.76	II A	NS
	Brother	M	21	11.77	II B	NS
Sal.	Proband	M	17	12.71	III B	NS
	Brother	M	21	12.74	II A	MC
Lev.	Proband	M	17	9.69	III	Uncl.
	Brother	M	14	11.69	IV A	NS
Bon.	Proband	M	28	11.65	IV B	NS
	Sister	F	28	1.72	II A	NS
Roc.	Proband	F	29	11.66	I A	Uncl.
	Brother	M	32	12.77	II A	NS
Mai.	Proband	F	32	5.76	IV A	MC
	Brother	M	26	8.76	II B	NS
Cer.	Proband	F	29	5.58	II A	Uncl.
	Brother	M	39	3.63	II A	LP
Sai.	Proband	F	47	0.68	I	MC
	Daughter	F	15	0.71	III	NS
Via.	Proband	M	50	0.55	III	Uncl.
	Son	M	18	5.70	II B	MC
Che.	Proband	M	44	7.67	I	LP
	Son	M	21	6.68	II	LP
Cha.	Proband	M	46	5.60	II A	NS
	Daughter	F	24	6.72	II A	NS
Vdb.	Proband	M	27	0.71	III	MC
	Cousin	M	17	0.71	III	MC
Bvc.	Proband	M	18	8.70	II B	LP
	Cousin	F	19	11.71	II B	MC

NS: nodular sclerosis; MC: mixed cellularity, LP: lymphocyte predominance; Uncl.: unclassified.

Table 2. Familial Hodgkin's disease. HLA haplotypes of families in which both patients were siblings

	Patients		Healthy relatives		
Family	Proband	Other related	Father	Mother	Others
Pol.	Aw30 B13 / A2 B5	Aw30 B13 / A2 B5 brother	Aw30 B13 / A1 B40	A2 B5 / Aw24 B12	
Sal.	A1 or-, Bw21 / A1 B8	A1 or-, Bw21 / A1 B8 brother	NT*	A1 B8 / A1 or-, B22	
Lev.	A2 B8 / A10 B22	A2 B8 / A10 B22 brother	A2 B8 / A3 B7	A9 B12 / A10 B22	
Bon.	A29 B12 / Aw30 B5	A29 B12 / Aw30 B5 sister	A29 B12 / A3 Bw35	Aw30 B5 / A3 B7	
Roc.	A3, A2, B7, B27	A3, A2, B7, B27 brother	NT	NT	
Mai.	A1, A26, B8, B40	A1, A26, B8, B40 brother	NT	NT	A1, A26, B8, B40 brother
Cer.	A1 B8 / A2 B12	A11 — / A2 B12 brother	NT	NT	A11 — / A2 B12 brother

*NT = Not tested.
In the families Roc. and Mai. the two affected sibs are only postulated to be HKA identical, since the two parents were not available for the HLA typing.

Table 3. Familial Hodgkin's disease. HLA haplotypes of families in which both patients were parent/child or cousins

Family	Patients		Healthy relatives				
	Proband	Other related	Father	Mother	Others		
Sai.	A2 / A2 or- ; B12 / B12 or-	A28 / A2 ; B14 / B12 daughter	NT*	—	A28 / A2 ; B14 / B12 daughter		
Via.	— / A3 ; B27 / Bw35	— / Aw30 ; B27 / B7 son	—	Aw30 / A3 ; B7 / B7	— / Aw30 ; B27 / B7 3 sons	A3 / Aw30 ; B27 / B7 daughter†	A3 / A3 ; B35 / B7 son
Che.	A10 / A1 ; — / —	A10 / A9 ; — / B40 son	— / —	A2 / A9 ; — / B40	A1 / A9 ; — / B40 2 daughters, 1 son		
Cha.	A1 / A2 ; B8 / B15	A10 / A2 ; B15 / B15 daughter	—	A10 / A9 ; B15 / B40			
Vdb.	A2, A10, B7, B22	A2, A11, B12, B35 cousin	NT	NT			
Bvc.	A3 / A9 ; B18 / B18 or-	A3 / A29 ; B18 / B12 cousin	NT	NT			

†In the Via family, there is a paternal HLA-A/B cross-over in one healthy daughter of the proband.
*NT = not tested.

Table 4. HLA-relationship in multiplex cases of affected sibs

	HLA genotype (pairs of sibs)		
	Identical	Semi-identical	Different
Personal data			
Observed*	6	1	0
Expected	1.75	3.5	1.75
International pooled data (9, 12, 13, 14, 15)			
Observed†	9	5	2
Expected	4	8	4

*Not significant.
†$P<0.05$.

the following as recurrent features of Hodgkin's disease.

The role of environment is suspected due to the clustering which exists not only in family cases but also amongst unrelated individuals living in the same geographical area [24, 25]. The family cases include first relatives, parent/child, sibs in most cases, but also cousins [15, 16] primarily in families bearing a high degree of inbreeding, in the Newfoundland [10] or the Amish populations [26].

The main interest lies in the observations of pairs of affected sibs. As a matter of fact the observed excess of HLA identical pairs of HD patients, in the pooled international data, will give weight to more than a simple association between some HLA antigens and HD but rather a possible linkage between genes coding for HLA and some hypothetical others controlling the susceptibility to the disease.

These observations provide for the first time strong evidence in favor of the major histocompatibility complex, as a factor of susceptibility to the disease, in addition to environmental and perhaps infectious factors [27].

The role played by the HLA complex may be that of an immune response defect. In this respect, of great importance is the frequently observed impairment of the immunological status, not only in the patients but also in healthy relatives: immunological depletion [9], immunodeficiency [10] or other disorders [28, 29].

Interestingly enough, the higher risk associated with sibs having both identical HLA haplotypes is in favor of a possible recessive susceptibility. This is in agreement with the mechanism of the immune surveillance theory in mice, where the H-2 linked susceptibility to virus leukemia is recessive and resistance is dominant [30–33].

We remain quite suspectful of a possible bias, i.e., that only the more compatible relationships between patients have been published. We therefore feel that an international registry should be undertaken.

If the hypothesis is confirmed, the penetrance of the trait is probably low, since healthy sibs identical to the index case are often observed.

In confirming a linkage between HLA and a susceptibility to Hodgkin's disease, it will be easier to establish the other factors certainly involved: either other genes and probably environmental factors, and also whether Hodgkin's disease is involved specifically, or rather, is a part of a larger background of immunological impairment.

Acknowledgements—We are grateful to Mr. B. Bratton and Mrs. F. Bayle for preparing the manuscript.

REFERENCES

1. J. W. Devore and C. A. Doan, Studies in Hodgkin's syndrome. XII. Hereditary and epidemiologic aspects. *Ann. intern. Med.* **47,** 300 (1957).
2. D. V. Razis, H. D. Diamond and L. F. Crauer, Familial Hodgkin's disease. *Ann. intern. Med.* **51,** 933 (1959).

3. S. Grufferman, P. Cole, P. G. Smith and R. J. Lukes, Hodgkin's disease in siblings. *New Engl. J. Med.* **296,** 248 (1977).
4. J. L. Amiel, Study of the leucocyte phenotypes in Hodgkin's disease. In *Histocompatibility Testing.* (Edited by J. Dausset and J. Colombani) p. 79, Munksgaard, Copenhagen (1967).
5. J. Dausset, L. Degos and J. Hors, The association of the HL-A antigens with diseases. *Clin. Immunol. Immunopathol.* **3,** 127 (1974).
6. J. Hors, A. Wang, A. Marcelli-Barge, M. Schmid, H. Dastot, E. Csarzar, A. Benajam, N. Chelloul and J. Dausset, Study of biological markers in Hodgkin's disease. In *Histocompatibility Testing.* (Edited by J. Dausset and J. Colombani) p. 567, Munksgaard, Copenhagen (1973).
7. P. J. Morris, S. Lawler and R. T. D. Oliver, Joint report of the Vth International Histocompatibility Workshop. II. HLA and Hodgkin's disease. In *Histocompatibility Testing.* (Edited by J. Dausset and J. Colombani) p. 669. Munksgaard, Copenhagen (1973).
8. A.Svejgaard, P. Platz, L. P. Ryder, L. S. Nielsen and Thomsen M. HL-A and disease association. A survey. *Transplant. Rev.* **22,** 3 (1975).
9. A.McBride and J. J. Fennelly, Immunological depletion contributing to familial Hodgkin's disease. *Europ. J. Cancer* **13,** 549 (1977).
10. W. H. Marshall, J. M. Barnard, S. K. Buehler, J. Crumley and B. Larsen, HLA in familial Hodgkin's disease. Results and a new hypothesis. *Int. J. Cancer* **19,** 450 (1977).
11. E. Perlin, P. H. Levine, J. McCoy, J. Dean and R. Herberman, Hodgkin's disease in siblings: a family study. *Oncology* **33,** 116 (1976).
12. A. Nunez-Roldan, F. Martinez-Guibelalde, P. Gomez-Garcia, C. Gomez-Pereira, G. Nuñez-Ollero and A. Torres-Gomez, Possible HLA role in a family with Hodgkin's disease. *Tiss. Antig.* **13,** 377 (1979.
13. J. E. Maldonado, H. F. Taswell, J. M. Kiely, Familial Hodgkin's disease *Lancet* **ii,** 1259 (1972).
14. J.K. Bowers, C. F. Moldow, C. D. Bloomfield and E. J. Yunis, Familial Hodgkin's disease and the major histocompatibility complex. *Vox sang.* **33,** 273 (1977).
15. M. H. Green, E. McKeen, F. P. Li, W. A. Blattner and J. F. Fraumeni HLA antigens in familial Hodgkin's disease. *Int. J. Cancer* **23,** 777 (1979).
16. H. T. Lynch, V. A. Saldivar, H. A. Guirgis, P. I. Terasaki, W. A. Bardawil, R. E. Harris, J. F. Lynch and R. T. Thomas, Familial Hodgkin's disease and associated cancer. *Cancer (Philad.)* **38,** 2033 (1976).
17. J.Dausset and J. Hors, Some contributions of the HL-A complex to the genetics of human disease. *Transplant. Rev.* **22,** 44 (1975).
18. R. J. Lukes and J. J. Butler, The pathology and nomenclature of Hodgkin's disease. *Cancer Res.* **26,** 1063 (1966).
19. P. P. Carbone, H. S. Kaplan, K. Musshof, D. W. Smithers and M. Tubiana, Report of the committee on Hodgkin's disease staging classification. *Cancer Res.* **31,** 1860 (1971).
20. J. Hors, J. L. Preud'Homme, M. T. Toulze-Zapateria, J. Guillet-Bigot, J. P. Roy, and J. Dausset, A simplified method for freezing lymphocytes in nitrogen vapors. *Transplantation* **15,** 417 (1973).
21. K. K. Mital, M. R. Mickey, D. P. Singal and P. I. Terasaki, Refinement of microdroplet lymphocyte cytotoxicity test. *Transplantation* **6,** 913 (1968).
22. Nomenclature for factors of the HLA system 1977 *Vox sang.* **35,** 111 (1978).
23. J. Dausset, L. Legrand, V. Lepage, L. Contu, A. Marcelli-Barge, I. Wildloecher, A. Benajam, T. Meo and L. Degos, A haplotype study of HLA complex with special reference to the HLA-DR series and to Bf. C2 and glyoxalase I polymorphisms. *Tiss. Antig.* **12,** 297 (1978).
24. R. L. Dworsky, B. E. Henderson, Hodgkin's disease clustering in families and communities. *Cancer Res.* **34,** 1161 (1974).
25. N. J. Vianna, J. N. P. Davies, A. K. Polan and P. Wolfgang, Familial Hodgkin's disease: an environmental and genetic disorder. *Lancet* **ii,** 854 (1974).
26. J. F. Halazun, S. E. Kerr and J. N. Lukens, Hodgkin's disease in three children from an Amish kindred. *J. Ped.* **80,** 289 (1972).
27. H. Doerken, Familial Hodgkin's disease. *Lancet* **ii,** 1463 (1974).
28. E. T. Creagan and J. F. Fraumenti, Jr., Familial Hodgkin's disease. *Lancet* **ii,** 547 (1972).

29. J. F. PLOUFFE, J. SILVA, R. S. SCHWARTZ, J. P. CALLEN, P. KANE and L. A. MURPHY. Abnormal lymphocyte responses in residents of a town with a cluster of Hodgkin's disease. *Clin. exp. Immunol.* **35,** 163 (1979).
30. F. LILLY, E. A. BOYSE and L. J. OLD, Genetic basis of susceptibility to viral leukaemigenesis. *Lancet,* **ii,** 1207 (1964).
31. F. LILLY, The inheritance of susceptibility to the Gross leukemia virus in mice. *Genetics,* **53,** 529 (1966).
32. F. LILLY, Genetic determination of susceptibility to Friend virus in mice. *Proc. Amer. Ass. Cancer Res.* **8,** 41 (1967).
33. J. R. TENNANT, Susceptibility and resistance to viral leukemogenesis in the mouse. Response to the virus relative to histocompatibility factors carried by prospective host. *J. nat. Cancer Inst.* **34,** 633 (1965).