# HL-A System and Diabetes Mellitus

*A. G. Cudworth, M.D., M.R.C.P., and J. C. Woodrow, M.D., F.R.C.P.,*
*Liverpool, England*

*With the technical assistance of N. Usher and Jeannette L. Anderson*

SUMMARY

**HL-A antigens were determined in 100 patients with diabetes mellitus. When the data are combined with that from other studies, there is a definite positive association of acute-onset juvenile diabetes mellitus with HL-A8 and W15. Four families are described in which two or more members with this type of diabetes are present, and in each family, affected individuals share a haplotype including HL-A8 or W15. These findings are consistent with the possible role of immune response genes in the HL-A chromosomal region which might control the immune response to virus infections capable of producing islet cell damage.** DIABETES *24*:345-49, April, 1975.

Studies of the familial aggregation of diabetes mellitus lead to the conclusion that genes at several loci must be involved in producing the genetic predisposition to the disease, involving either the additive effect of genes at different loci or interaction between them. It is likely that in many instances environmental factors operate to precipitate clinical disease. In the case of acute-onset juvenile diabetes the circumstantial evidence for viral infection having a role has been summarized recently.[1]

Genes determining specific immune responses and susceptibility to virus-induced diseases are known to be present in the region of the main histocompatibility locus in the mouse and guinea pig.[2] It is reasonable to ask whether the homologous chromosomal region in man might include a locus at which occur genes determining susceptibility to certain types of diabetes mellitus. Two previous studies on the possible association of HL-A antigens with diabetes have been reported. Finkelstein and co-workers[3] (1972) found no relation with juvenile-onset diabetes in a population of mixed racial-ethnic distribution, whereas Singal and Blajchman (1973)[4] found suggestive evidence of an increased frequency of specificity W15 in insulin-dependent diabetic patients compared with either a control group or with insulin-independent diabetics.

The aim of this report is to present the results of HL-A typing in a series of Caucasian diabetic patients. The data have been analyzed both on their own and in combination with the results from these earlier studies[3,4] and with the data of a simultaneous study performed in Copenhagen.[5] There have been no previous reports of the association of HL-A antigens and diabetes mellitus within families, and details of four families of particular interest are presented.

## PATIENTS AND METHODS

One hundred unrelated Caucasian patients with diabetes were studied. Because it seems reasonable to assume that the underlying genetic factors may differ between different clinical types of diabetes, a broad classification of patients was made by taking those developing clinical disease before the age of thirty years (juvenile onset) and those who developed the disease after this age (late onset). Fifty patients in each group had HL-A typing performed by the two-stage micro lymphocytotoxicity method.[6] The control population (233) was derived from normal blood donors living in the same geographic area. Twenty-three specificities were determined using sera obtained from the National Tissue Typing Reference Laboratory, Bristol, England, and the Serum Bank of the National Institutes of Health, Bethesda, Maryland. Each patient was questioned in regard to the presence of known diabetes in near relatives, and in selected

From the Nuffield Unit of Medical Genetics, Department of Medicine, University of Liverpool, England.

Accepted for publication December 10, 1974.

families where more than one case of juvenile-onset diabetes was known, the HL-A genotypes of the family were determined.

## RESULTS

The frequencies of HL-A antigens in the two groups of diabetic patients and in controls are shown in table 1. There is suggestive evidence of an increase in the frequency of HL-A8 in juvenile-onset diabetic patients when compared with controls. HL-A8 was present in twenty-seven (54 per cent) of this group compared with 74 out of 233 (31.8 per cent) of controls. The calculated relative incidence of juvenile-onset diabetes mellitus in HL-A8 individuals is 2.52 (Fisher-Irwin $p \times 2 = 0.0056$). When multiplied by the number of specificities tested, the probability becomes 0.129. In relation to W15, which has been reported to be increased in insulin-dependent diabetics,[4] nine (18 per cent) of the juvenile-onset patients were positive as against twenty-eight (12 per cent) of controls.

In table 2, the results of this study in respect of HL-A8 and W15 juvenile-onset diabetes are combined with those from two other series.[3,5] (Only the Caucasian element of the Finkelstein et al.[3] study has been incorporated for combined analysis.) The methods of analysis are those of Woolf.[7] The relative risk (x) for this type of diabetes with HL-A8 in these other studies is 1.67 and 2.62, respectively (table 2a). The combined relative risk is 2.41, which is highly significant, and there is no evidence of heterogeneity between centers. Similarly, in regard to W15 there is evidence of an increased relative risk (2.34) when the data are combined, with a high degree of significance and again no evidence of heterogeneity (table 2b).

TABLE 1

HL-A Frequencies in Diabetics and Controls

| HL-A Antigens | Diabetes Juvenile (n = 50) | Diabetes Maturity onset (n = 50) | Controls (n = 233) |
|---|---|---|---|
| 1st segregant series | | | |
| HL-A 1 | 22 (44%) | 21 (42%) | 82 (35.2%) |
| HL-A 2 | 23 (46%) | 25 (50%) | 109 (46.8%) |
| HL-A 3 | 13 (26%) | 18 (36%) | 65 (27.9%) |
| HL-A 9 | 6 (12%) | 5 (10%) | 45 (19.3%) |
| HL-A10 | 3 ( 6%) | 2 ( 4%) | 19 ( 8.2%) |
| HL-A11 | 2 ( 4%) | 3 ( 6%) | 37 (15.9%) |
| W 28 | 2 ( 4%) | 4 ( 8%) | 12 ( 6.2%)* |
| W 29 | 5 (10%) | 4 ( 8%) | 16 ( 6.9%) |
| W30, W 31 | 2 ( 4%) | 3 ( 6%) | 12 ( 6.0%)† |
| W32 | 1 ( 2%) | 3 ( 6%) | 12 ( 5.2%) |
| 2nd segregant series | | | |
| HL-A 5 | 0 ( 0%) | 3 ( 6%) | 21 ( 9.0%) |
| HL-A 7 | 8 (16%) | 18 (36%) | 75 (32.2%) |
| HL-A 8 | 27 (54%) | 18 (36%) | 74 (31.8%) |
| HL-A 12 | 15 (30%) | 12 (24%) | 63 (27.0%) |
| HL-A 13 | 6 (12%) | 3 ( 6%) | 12 ( 5.2%) |
| HL-A 14 | 3 ( 6%) | 5 (10%) | 17 ( 7.3%) |
| HL-A 17 | 0 ( 0%) | 3 ( 6%) | 14 ( 6.0%) |
| HL-A 27 | 1 ( 2%) | 5 (10%) | 19 ( 8.2%) |
| W 5 | 7 (14%) | 5 (10%) | 32 (13.7%) |
| W 10 | 7 (14%) | 5 (10%) | 23 ( 9.9%) |
| W 15 | 9 (18%) | 6 (12%) | 28 (12.0%) |
| W 18 | 4 ( 8%) | 3 ( 6%) | 10 ( 4.3%) |
| W 22 | 2 ( 4%) | 3 ( 6%) | 13 ( 5.6%) |

*194 tested; †199 tested

Diabetic patients may also be divided into insulin-dependent and insulin-independent, respectively, and the data in respect of HL-A8 and W15 are given in table 3, which also gives the data for three other studies.[3-5] The combined value for the relative risk (x) for HL-A8 is 2.12 (table 3a) and for W15 is 2.60 (table 3b), both being highly significant, and there is no evidence of heterogeneity between centers.

In relation to maturity-onset diabetes, the relative risk for HL-A8 was 0.92 and for W15, 1.07. The corresponding values for the Copenhagen series[8] are 1.24 and 1.92. None of these results, individually or when combined, are significantly different from unity.

*Family studies.* Of the forty-five HL-A8 positive patients, sixteen (35.6 per cent) gave a positive family history of diabetes in near relatives. The corresponding figures for W15 positive patients was five out of fifteen (33.3 per cent) and for those patients who had neither HL-A8 nor W15, sixteen out of forty-six (34.8 per cent). There was thus no evidence of an association between a positive family history and HL-A types.

Figure 1 (a-d) gives the genotypes in four families in which two or more cases of acute-onset juvenile diabetes had occurred in siblings.

(a) Patients II 4 and II 6 developed acute-onset diabetes mellitus simultaneously (aged twenty-one and thirteen years, respectively) following a recent influenza-like illness. Patient II 5 developed diabetes two years previously. Standard 50 gm. oral glucose tolerance tests were normal in the remaining siblings apart from II 2, who showed the following half-hourly blood glucose (milligrams per 100 milliliters) results: 123 (fasting); 192; 152; 174; 166. This individual and the three with clinical diabetes share the W 29, 8 haplotype.

(b) The proband II 4, now aged forty years, has had insulin-dependent diabetes from the age of eleven years. The onset was simultaneous with diabetes in a sister (II 3) who is seven years older. These two siblings were living in the same house at the time of onset. The other diabetic sibling (II 2) has had

TABLE 2

HL-A8 and W15 in Juvenile-Onset Diabetes

| Study | a* | b | c | d | x† | y‡ | w§ | chi-squared Wy² | chi-squared Ordinary |
|---|---|---|---|---|---|---|---|---|---|
| **(a) HL-A8** | | | | | | | | | |
| Finkelstein et al.[3] | 10 | 18 | 25 | 75 | 1.67 | 0.51 | 4.79 | 1.20 | 0.78 |
| Nerup et al.[5] | 35 | 43 | 466 | 1,501 | 2.62 | 0.96 | 18.30 | 17.00 | 18.20 |
| Present study | 27 | 23 | 74 | 159 | 2.52 | 0.92 | 9.97 | 8.35 | 7.93 |
| | | Combined y = 0.88 | | | Combined × = 2.41 | | | ($\chi^2$ = 25.6, 1 d.f.) | |
| | | | | $\chi^2$ for heterogeneity = 0.96, 2 d.f. (p>0.5) | | | | | |
| **(b) W15** | | | | | | | | | |
| Finkelstein et al.[3] | 5 | 23 | 7 | 93 | 2.89 | 1.06 | 2.52 | 2.83 | 1.89 |
| Nerup et al.[5] | 28 | 50 | 352 | 1,615 | 2.57 | 0.94 | 16.90 | 15.05 | 14.90 |
| Present study | 9 | 41 | 28 | 205 | 1.61 | 0.47 | 5.68 | 1.28 | 0.82 |
| | | Combined y = 0.85 | | | Combined × = 2.34 | | | ($\chi^2$ = 18.1, 1 d.f.) | |
| | | | | $\chi^2$ for heterogeneity = 1.06, 2 d.f. (p>0.5) | | | | | |

*a and b are the number of patients positive and negative, respectively, for the HL-A antigen. c and d are the corresponding numbers for controls.

†x = relative incidence (Woolf)[7]; ‡y = variance; §w = 1/y

insulin-dependent diabetes since the age of forty years. The oldest sibling, who has no symptoms of diabetes, had severe tropical sprue after returning from the Far East in 1945. In these sibships, the three diabetics share the 2,8 haplotype.

(c) The two affected brothers (II 2 and II 3) developed acute symptoms of diabetes within a three to four week period at the age of fifteen and twelve years, respectively. The sister (II 1) had a glucose tolerance test during a recent pregnancy, and this was normal. The two diabetic brothers in this sibship share the 2, W15 haplotype.

(d) The father (I 1) developed diabetes at age twenty-three years and died from complications of the disease at age forty-six years. Patient II 1 developed diabetes at the age of seven years, and II 4 and II5 both developed diabetes at the age of twenty-one years. Patient II 2 has celiac disease. Notable is that the diabetic parent was W15 positive, the other parent having HL-A8. One diabetic child has inherited both W15 and HL-A8, a second W15 and a third HL-A8. The sibling with celiac disease is HL-A8 positive.

## DISCUSSION

Where the association between a disease and the HL-A system is strong, e.g. HL-A27 with ankylosing spondylitis,[9] a relatively small number of patients and controls will demonstrate that the association is real, and problems of sampling are relatively unimportant.

TABLE 3

HL-A8 and W15 in Insulin-Dependent Diabetes

| Study | a | b | c | d | x | y | w | chi-squared wy² | chi-squared ordinary |
|---|---|---|---|---|---|---|---|---|---|
| **(a) HL-A8** | | | | | | | | | |
| Finkelstein et al.[3] | 10 | 18 | 25 | 75 | 1.67 | 0.51 | 4.78 | 1.20 | 0.78 |
| Singal and Blajchman[4] | 12 | 38 | 20 | 80 | 1.26 | 0.23 | 5.81 | 0.32 | 0.12 |
| Nerup et al.[5] | 46 | 63 | 466 | 1,501 | 2.46 | 0.90 | 24.68 | 19.95 | 20.06 |
| Present study | 32 | 31 | 74 | 159 | 2.22 | 0.80 | 12.00 | 7.62 | 7.01 |
| | | Combined y = 0.75 | | | Combined × = 2.12 | | | ($\chi^2$ = 26.7, 1 d.f.) | |
| | | | | $\chi^2$ for heterogeneity = 2.35, 3 d.f. (p>0.5) | | | | | |
| **(b) W15** | | | | | | | | | |
| Finkelstein et al.[3] | 5 | 23 | 7 | 93 | 2.89 | 1.06 | 2.52 | 2.83 | 1.89 |
| Singal and Blajchman[4] | 18 | 32 | 10 | 90 | 5.06 | 1.62 | 5.05 | 13.29 | 13.18 |
| Nerup et al.[5] | 38 | 71 | 352 | 1,615 | 2.46 | 0.90 | 22.80 | 18.40 | 18.39 |
| Present study | 13 | 50 | 28 | 205 | 1.90 | 0.64 | 7.27 | 3.01 | 2.41 |
| | | Combined y = 0.96 | | | Combined × = 2.60 | | | ($\chi^2$ = 34.5, 1 d.f.) | |
| | | | | $\chi^2$ for heterogeneity = 3.05, 3 d.f. (p>0.3) | | | | | |

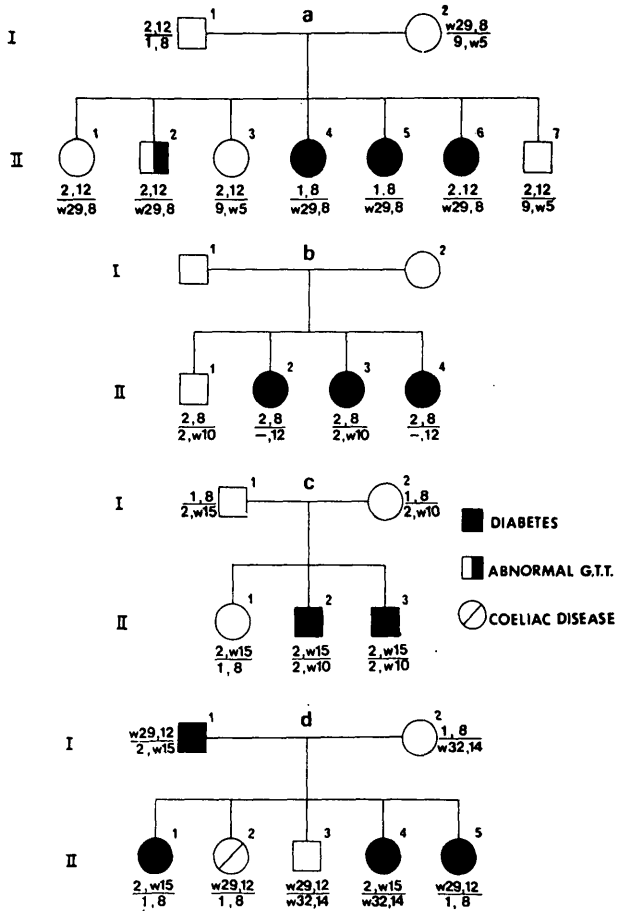


FIG. 1. HL-A genotypes in four families, each containing two or more juvenile-onset diabetic patients.

Where an association is not so strong, however, it is more difficult to establish it with certainty. The problems encountered and appropriate methods of analysis are summarized by Svejgaard et al.[10] In three other studies of the HL-A system and diabetes, one claimed no positive association,[3] one showed evidence of a positive association between W15 and insulin-dependent diabetes,[4] and a third demonstrated definite evidence of association between both HL-A8 and W15 and juvenile-onset insulin-dependent diabetes.[5]

The present data support the association of HL-A8 with juvenile-onset and/or insulin-dependent diabetes, but on their own and taking into account the fact that twenty-three specificities were being tested for, they would not supply conclusive evidence. In relation to W15, although the data suggest that the relative risk of insulin-dependent diabetes is almost doubled in W15 positive individuals, the difference found is not statistically significant. When all the available data are combined there is however strong evidence that the relative risk of juvenile-onset insulin-dependent diabetes is increased for HL-A8 and W15 positive individuals (2.12 for HL-A8 and 2.60 for W15, respectively). If there were no positive association, the expected relative risks would be unity. There is no evidence of heterogeneity between centers and therefore the apparent differences can be attributed to chance variations.

Studies of the genetic predisposition to diabetes suggest that there is considerable genetic heterogeneity.[11,12] It seems likely from the evidence presented here that one of the loci involved in determining some cases of juvenile-onset diabetes is situated within the HL-A chromosomal region. Analysis of the genetics of a disease such as diabetes is most productive of useful hypotheses when the genetic evidence is considered in relation to possible environmental precipitating factors.

A number of viruses have been reported to be capable of producing pancreatic damage.[1] Of particular interest is the evidence that the pattern of increased seasonal incidence of juvenile-onset diabetes shows significant positive correlation with the annual prevalence data for Coxsackie B4 infection.[13] A higher incidence of neutralizing antibodies to Coxsackie B4 has been found in recent-onset juvenile diabetics as compared with control subjects.[14,15] Experimentally, some viruses have been shown to be capable of specifically producing beta-cell necrosis. Thus the "M" variant of the encephalomyocarditis virus has been shown to produce beta-cell necrosis in mice with resulting chemical diabetes.[16,17] It is of interest that some strains of mice were more susceptible to such injury than others, suggesting considerable genetically determined variability in susceptibility. Coxsackie B virus has also been shown to produce islet cell damage in mice[18] but neither this nor any other virus has been isolated from human tissues obtained from patients with acute-onset diabetes. Histologically, however, marked lymphocytic infiltration of the islets[19,20] has been described in some cases of juvenile-onset diabetes which would be compatible with a viral etiology.

Several possible mechanisms have been suggested for the association between the HL-A polymorphism and disease.[21] Detailed studies of the homologous chromosomal region in the mouse and guinea pig show that many loci exist in this region at which genes occur, which could affect susceptibility to disease in several important ways. Thus, genes determining susceptibility to virus-induced disease[22] and specific immune responses[2] have been shown to occur in this region. There is the possibility that the HL-A determinants themselves might be involved in pro-

moting disease susceptibility, but probably more likely, in the case of the associations between HL-A8 and W15 with diabetes, is that genes at loci closely linked to the HL-A loci are involved. The implication would be that such diabetes-promoting genes are in linkage disequilibrium with HL-A8 and W15. In other words a gene or genes predisposing to diabetes, occurring at a locus closely linked to HL-A, would be particularly associated in the population with haplotypes containing HL-A8 and W15. Thus the increase in the frequency of these HL-A antigens would merely be reflecting an increase in the presence of such linked genes.

It is of interest that in the four families reported here, the affected siblings in each family possess a common haplotype including either HL-A8 or W15. This is consistent with the possibility that a necessary condition for the development of diabetes in the members of these families is the inheritance of an immune response gene closely linked to the HL-A region. The family shown in figure 1 (d) is of particular interest as such a gene could be linked to HL-A8 in one parent and to W15 in the other. The very close temporal relationship of the onset of diabetes in two siblings, seen in three of the families, suggests the possibility that virus infection may have occurred just preceding the onset of clinical disease.

The suggestion has been made that a viral cause is present in approximately 15 per cent of juvenile insulin-requiring diabetics.[1] If this proves to be correct, it might be particularly in these cases that immune response genes in the HL-A chromosomal region have an important role. These genes might influence the virus receptor sites or modify adversely the immune response to such viruses.[2] It is of interest that in a series of identical twins studied by Tattersall and Pyke (1972)[12] where one twin had developed diabetes before the age of forty years, only approximately half of the twins were concordant in respect of diabetes. This suggests that the genetic and environmental factors in the two groups may differ in an important way. A study of the frequencies of HL-A8 and W15 in the two types of twins might be very informative. Prospective studies involving the search for evidence of recent Coxsackie B4 infection coupled with HL-A typing and further family studies might also provide further evidence for viral mediated diabetes.

ACKNOWLEDGMENT

We are indebted to the National Tissue Typing Reference Laboratory, Bristol, England, and the National Institutes of Health, Bethesda, Maryland, for supplying the antisera. The work was supported in part by the Medical Research Committee of the Mersey Regional Health Authority.

REFERENCES

[1]Steinke, J., and Taylor, K.W.: Viruses and the etiology of diabetes. Diabetes *23*:631-33, 1974.

[2]McDevitt, H.O., and Benacerraf, B.: Genetic control of specific immune responses. Adv. Immunol. *11*:31-74, 1969.

[3]Finkelstein, S., Zeller, E., and Walford, R.L.: No relation between HL-A and juvenile diabetes. Tissue Antigens *2*:74-77, 1972.

[4]Singal, D.P., and Blajchman, M.A.: Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with juvenile diabetes. Diabetes *22*:429-32, 1973.

[5]Nerup, J., Platz, P., Andersen, O.O., Christy, M., Lyngsøe, J., Poulsen, J.E., Ryder, L.P., Nielsen, L.S., Thomsen, M., and Svejgaard, A.: HL-A antigens in diabetes mellitus. Lancet *II*:864-66, 1974.

[6]Terasaki, P.I., and McClelland, J.D.: Microdroplet assay of human serum cytotoxins. Nature *204*:998-1000, 1964.

[7]Woolf, B.: On estimating the relation between blood group and disease. Ann. Hum. Genet. *19*:251-53, 1955.

[8]Svejgaard, A.: Personal communication, 1974.

[9]Brewerton, D.A., Caffrey, M., Hart, F.D., James, D.C.O., Nicholls, A., and Sturrock, R.D.: Ankylosing spondylitis and HL-A27. Lancet *I*:904-07, 1973.

[10]Svejgaard, A., Jersild, C., Nielsen, L.S., and Bodmer, W.F.: HL-A antigens and disease. Statistical and genetical considerations. Tissue Antigens *4*:95-105, 1974.

[11]Rimoin, D.L.: Genetics of diabetes mellitus. Diabetes *16*:346-51, 1967.

[12]Tattersall, R.B., and Pyke, D.A.: Diabetes in identical twins. Lancet *II*:1120-25, 1972.

[13]Gamble, D.R., and Taylor, K.W.: Seasonal incidence of diabetes mellitus. Br. Med. J. *3*:631-34, 1969.

[14]Gamble, D.R., Kinsley, M.L., Fitzgerald, M.G., Bolton, R., and Taylor, K.W.: Viral antibodies in diabetes mellitus. Br. Med. J. *3*:627-31, 1969.

[15]Gamble, D.R., Taylor, K.W., and Cumming, H.: Coxsackie viruses and diabetes mellitus. Br. Med. J. *4*:260-62, 1973.

[16]Craighead, J.E., and McLane, M.F.: Diabetes mellitus: Induction in mice by encephalomyocarditis virus. Science *162*:913-14, 1968.

[17]Craighead, J.E., and Steinke, J.: Diabetes mellitus-like syndrome in mice infected with encephalomyocarditis virus. Am. J. Pathol. *63*:119-30, 1971.

[18]Burch, G.E., Tsui, C.Y., Harb, J.M., and Colcolough, H.L.: Pathologic findings in the pancreas of mice infected with Coxsackie virus B4. Arch. Intern. Med. *128*:40-47, 1971.

[19]LeCompte, P.M.: Insulitis in early juvenile diabetes. Arch. Pathol. *66*:450-57, 1958.

[20]Gepts, W.: Pathologic anatomy of the pancreas in juvenile diabetes mellitus. Diabetes *14*:619-33, 1965.

[21]McDevitt, H.O., and Bodmer, W.F.: HL-A, immune response genes and disease. Lancet *I*:1269-74, 1974.

[22]Lilly, F.: The effect of histocompatibility 2 type on response to the Friend leukaemia virus in mice. J. Exp. Med. *127*:465-73, 1968.