INSULIN DEPENDENT DIABETES MELLITUS EPIDEMIOLOGY: HLA GENOTYPE STUDY IN 12 NORTH EASTERN ITALIAN FAMILIES WITH TWO SIBLINGS AFFECTED BY TYPE I DIABETES

L. PINELLI*, F. DREI**, E. GONFIANTINI*, A. VISENTIN***, C. ROATA****, S. CIALFONI***** and C. MAFFEIS*

*Centro Regionale per il Diabete Giovanile - Clinica Pediatrica Università di Verona, Policlinico di Borgo Roma - 37134 Verona, Italy.
**Divisione di Pediatria Ospedale Civile di Treviso.
***Divisione di Pediatria, Ospedale Civile di Bolzano.
****Servizio Transfusionale Ospedale Civile Maggiore di Verona.

Key words: HLA and diabetes epidemiology - Families with high prevalence of disease

The purpose of our study was to evaluate the relationship between the histocompatibility antigens and type 1 diabetes mellitus in families living in the north-eastern part of Italy. In each family two siblings were affected by diabetes. HLA-antigens were determined with the lymphocytotoxicity test, utilizing antisera of the series A-B-C-DR.

The phenotypic frequencies were compared with those observed in controls.

We showed that diabetes has a strong association with HLA DR 3 and/or DR 4 antigens. In particular we registered high frequency of compound heterozygous DR 3 - DR 4 subjects, and this fact supports the hypothesis of the existence of two different genes for diabetes associated with these HLA antigens.

Moreover we observed a particular haplotype segregation with a very high percentage of HLA identity between patients belonging to the same family, confirming the association between HLA and genetic susceptibility to insulin dependent diabetes. These results confirm data in the literature and, completed by other data from other patients' families living in our area, will be useful in providing reliable genetic counselling.

INTRODUCTION

Insulin Dependent Diabetes Mellitus (IDDM) has hereditary components (21), but the genetic modes of transmission are not yet clear (20). However, some studies have shown an association between type 1 diabetes and the antigens of the HLA system, a complex of genes located on the short arm of chromosome 6. They control the immune response and are involved in determining the susceptibility to various diseases: IDDM, rheumatoid arthritis, ankylosing spondylitis, Addison's disease, etc. The association between HLA and diabetes was shown to be stronger with HLA Dw/Dr3 and Dw/Dr4 which are in linkage disequilibrium with HLA B8 and B15 respectively (24).

The association between HLA B18/Dr3 and type 1 diabetes has also been reported in southern Europe (12). The aim of the present study was to determine which HLA factors show the strongest association with IDDM in families coming from and living in North-Eastern Italy in order to establish whether the
epidemiological relations reported in the literature exist in this ethnic group as well.

**MATERIALS AND METHODS**

Among the 399 diabetic patients treated in Verona at the Regional Centre for Juvenile Diabetes and in Bolzano and Treviso at the Departments of Paediatrics we selected the families (N. 17) with two or more diabetic children.

Twelve families agreed to take part in the study after being informed about its objectives.

Only type one diabetics (ketosis-prone, insulin-dependent diabetes with onset before the age of 30), were included. Each of the 12 families has two children with type 1 diabetes; a total of 24 subjects were thus studied, 12 males and 12 females. Mean age at diagnosis was 9.3 years (range 1yr.,l_1m - 19yr.,6m). All the parents were healthy (see Tab. 1).

The control data were obtained from 400 blood donors unrelated to the diabetics and living in the same geographical area. Sera from these donors were typed for HLA ABC. One hundred forty eight individuals, typed for HLA Dr, were also included in the control group. All these subjects were studied in the transfusion centre of our hospital. They should be considered as individuals and not as family groups.

HLA antigens were determined with the lymphocytotoxicity test using two Terasaki plates for each subject (HLA ABC 2nd tray and HLA Dr - One lambda Inc.) and covering the specificities A, B, Cw, Dr (15, 16).

Seventeen specificities were tested for the A antigens, 32 for B, 4 for C and 12 for Dr. Only the specificities defined at the VIIIth Histocompatibility Workshop were used.

Frequencies of HLA phenotypes in the subjects included in the study were compared with those of controls.

The statistical significance was analysed by Fisher's exact test (13).

**RESULTS**

Table 1 shows that 18 out of the 24 diabetic children (75%) have HLA B8, B15 or B18 antigens.

In these subjects HLA B8, B15 and B18 antigens have increased, but not significantly (Table 2).

Table 3 shows Dr3 and Dr4 frequencies. These antigens are thought to predispose one to the onset of diabetes (19) and they are present in all diabetic children of this study (Table 1).

The phenotype frequency of these antigens is significantly higher in diabetics than in controls (Table 3). In diabetic subjects the phenotype distribution of Dr3 and Dr4 (Table 4) shows higher prevalences of Dr4/x heterozygote subjects and, even more, of Dr3/Dr4 if compared with homozygote (25% versus 17% in both cases).

A particular haplotype segregation among diabetic members of the same family was reported as well; 18 out of 24 had identical HLA, 4 were haploidentical and 2 had different HLA (Table 5).

When compared with their diabetic siblings, 3 of the 12 healthy children had identical HLA, 6 were haploidentical and 3 had different HLA.

No recombination was reported in the 36 siblings belonging to the 12 families included in our study.

**DISCUSSION**

Higher HLA frequencies of some alleles have been reported in type 1 diabetic patients, compared with the healthy population as well as in other diseases with autoimmune aetiologies or caused by altered immune function (11). Moreover, IDDM patients show increased frequencies of HLA B8, B15, B18, DR3 and DR4 antigens if compared with the general population (14, 19).

We studied families with two type 1 diabetic children, on the assumption that the number of genes producing susceptibility to the disease would be higher. As J.I. Rotter and D.L. Rimoin (22) have reported in their studies, HLA antigens account for 60% of the genetic defect of type 1 diabetes. So far, no other genetic factor has proved to play a role in the remaining percentage.

Thus, HLA antigens should be considered as the main parameter of the genetic susceptibility to the disease in these subjects.

The results of our survey support the data reported in the literature which show that, within HLA antigens, the alleles Dr3 and Dr4 are more strongly associated with the genes giving the susceptibility to the disease (22, 24).

Frequency of HLA Dr4 is much higher than that of HLA B15 even though these alleles are in linkage disequilibrium with HLA Dr3 (19). The strong association between HLA B18 and Dr3 has already been observed in France (9), England (6) and Sardinia (5). The high frequencies of HLA Dr3/Dr4 in the diabetic members of our families, with high prevalences of the disease, confirm the high relative risk of developing diabetes associated with this combination (24). The excess of HLA Dr3/4 heterozygotes in diabetic patients, with respect to the number expected on the basis of the frequency of both genes and combined with no increase in homozygotes, strongly supports the hypothesis of two diabetogenic genes associated with the two HLA Dr alleles (18). HLA distribution among affected siblings of diabetic multiplex families show, in agreement with other family studies (2 - 4), an excess of HLA identity.

The ratio among siblings with identical, haploidentical and different HLA antigens is 9:2:1 as opposed to the 3:6:3 ratio that can be expected on the basis of Mendel's laws of inheritance. This high frequency of HLA identity supports the hypothesis of the linkage of the susceptibility gene with HLA genes.