ROLE OF HLA CLASS-I ANTIGENS IN DELUSIONAL DISORDER

MONOJIT DEBNATH, SUJIT K. DAS, PRAVATI GHOSH,
BICHITRA BIRJYA MANDAL & TAPAS K. CHAUDHURI

ABSTRACT

The investigation was conducted to find out whether there is any association between delusional disorder and HLA antigens. The sample comprised 50 patients with delusional disorder and 282 control samples collected from normal controls. Statistical analysis revealed that the frequency of A3 antigen of the locus A are significantly higher. In case of HLA-B locus significantly higher frequency of B5 and B21 antigens have also been observed. The present study shows that there may be some association of HLA class-I antigens with delusional disorder.

Key words : Delusion, HLA antigens, microlymphocytotoxicity assay, linkage disequilibrium, psychopathology

In the field of psychiatry, delusional disorder is a disease where we get delusion as a discrete symptom. Although delusions remain one of the basic problems in psychopathology, attempts to understand its pathogenesis have been dominated by unsubstantiated speculation (Roberts, 1992).

Though most of the earlier theories explaining the mechanism of the etiology of delusion were based on non-organic psychological theories, it is being increasingly realised that some underlying biological factors are clearly responsible in causing delusions. In addition family studies lead to convincing data in the form of increased prevalence of delusional disorders, and related personality traits (e.g. suspiciousness, jealousy and secretiveness) in the relatives of delusional disorder probands (Kaplan et al., 1994). Several studies have demonstrated that there is an association between HLA antigen frequencies and a variety of diseases. Some of these diseases clearly possess a hereditary component while some manifest disturbed immune function as a major feature. As there is no report of disturbed immune function in delusional disorder, the role of genetic factor has been suspected. More recently, the discovery of HLA association with certain psychiatric diseases, viz. schizophrenia which shows an association between HLA-A9, has been regarded as a major breakthrough in our understanding the genetics of these diseases (Guffin & Stuart, 1986). Such association may be mediated by HLA-linked immune response genes. In other words, this relationship may be expressed through close linkage between specific HLA antigens and the genes controlling disease susceptibility. Therefore in the present investigation we tried to find out whether there is any association of HLA antigen(s) and delusional disorder.

MATERIAL AND METHOD

Subject : A total number of 50 patients of delusional disorder were obtained from the Department of Psychiatry, North Bengal Medical College in a period of about 3 years. The patients
were considered for the study after proper screening by a psychiatrist. Persons not suffering from any kind of psychiatric illness were considered as controls. Control samples were collected from the relatives of the patients attending other O.P.Ds of the same hospital. DSM-IV criteria of delusional disorder were used for the diagnosis of the patients. Moreover, response of the patients to antipsychotics, specially pimozide, which is claimed to have a specific therapeutic efficacy on delusional disorder were also taken into account for the present study (American Psychiatric Association, 1994). Patients suffering from paranoid schizophrenia, paranoid personality disorder, dementia presenting with paranoid features, patients with substance abuse disorder, patients presenting with delusion with any other comorbidity, both physical and psychiatric, were excluded from the present study. In our study it was observed that maximum patients were clustered between 25 to 55 age group. Patients were mostly from middle class urban society belonging to a nuclear family. As patients with delusional disorder are apparently normal and not considered as harbouring any psychiatric illness by themselves as well as by their relatives, a method of routine enquiry was made to all persons attending Psychiatry O.P.D. to find any case in the family who is suspicious and/or jealous. This resulted in attendance of at least 25% of total number of our cases who otherwise would not have consulted a psychiatrist. HLA Typing: Approximately 5ml blood sample was obtained from each individual in a clean heparinised tube with the help of disposable syringes. Collected blood samples were diluted with phosphate buffered saline (PBS) in a sterile clean tube. Diluted blood samples were then layered on to Ficoll-Hypaque carefully with the help of a pasteur pipette and then centrifuged at 2000 rpm for 20 minutes at room temperature. The white foggy layer of the mononuclear cells were aspirated from the interface with a clean pasteur pipette in clean centrifuge tubes. Cells were then washed with PBS for 10 mins. at 1000 rpm for 2-3 times. Cell suspension was counted in Neubauer haemocytometer and adjusted to a final concentration of 2x10⁶ cells/ml. Viability of cells in suspension was checked by Trypan Blue Dye exclusion test.

HLA typing was done by using 60 well Terasaki trays made of non-toxic disposable polystyrene material (NUNC, Denmark) and with the help of standard two stage microlymphocytotoxicity assay (Terasaki & McClelland, 1964). 1 ml antiserum of different HLA specificities were poured in the trays and prior to that wells were filled in with 5 ml light liquid paraffin oil to avoid the evaporation of such little quantity of antiserum. 1 ml cell suspension from 2x10⁶ cells/ml. was added to each well and incubated at 22°C for 30 mins. At the end of incubation period, 5 ml rabbit complement was added to each well with Hamilton six needle repeating dispenser and the trays were further incubated at room temperature for 60 mins. 5 ml water soluble yellow shade eosin was used for 5 mins. for staining the dead cells in each well. 5 ml of 40% formalin of pH 7.0 was added at the end to fix the cells. A cover glass was layered on the wells. Trays were capped tightly and kept at 4°C for scoring the results after a gap of at least 2 hours (Tait et al., 1981).

Statistical Analysis

The results were analysed in the Hewlett Packard 1000 computer using programmes developed in Fortran-IV. The phenotype frequencies were calculated by direct count. Coefficient of linkage disequilibrium (delta values) was calculated according to Mattiuiz et al. (1970).

RESULTS

The present study has been undertaken to investigate the HLA-phenotype frequency in patients with delusional disorder. The phenotype frequencies of HLA class -1 antigens in controls and patients with delusional disorder have been
DELUSIONAL DISORDER AND HLA ANTIGENS

presented in table 1.

**TABLE 1**

| PHENOTYPE | FREQUENCY OF HLA-A AND B LOCUS ALLELES IN PATIENTS COMPARED WITH HEALTHY CONTROLS |
|-----------|----------------------------------------------------------------------------------|
| Phenotype | % frequency | Chi square | Relative risk |
| A1        | 2 6         | 0.003      | 0.938        |
| A2        | 28 6        | 50.216***  | 17.688       |
| A3        | 72 1        | 220.145*** | 722.571*     |
| A9        | 20 0        | 58.151***  | 0.000        |
| A10       | 18 1        | 45.265***  | 61.682       |
| A11       | 2 2         | 81.093***  | 65.882       |
| A19       | 6 0         | 17.074**   | 0.000        |
| A23       | 6 0         | 17.074**   | 0.000        |
| A24       | 14 2        | 28.445     | 22.790       |
| A25       | 10 1        | 22.266**   | 31.222       |
| A26       | 7 1         | 16.735**   | 24.437       |
| A28       | 8 2         | 12.721**   | 12.173       |
| A29       | 6 6         | 2.414      | 2.936        |
| A30       | 6 1         | 11.371**   | 17.936       |
| B5        | 32 5        | 59.772     | 23.742       |
| B7        | 7 2         | 28.445     | 22.790       |
| B8        | 2 0         | 17.074**   | 0.000        |
| B12       | 8 1         | 45.265     | 61.682       |
| B13       | 6 2         | 23.023     | 19.090       |
| B14       | 1 0         | 5.657*     | 0.000        |
| B15       | 6 4         | 16.277**   | 9.477        |
| B16       | 3 0         | 22.635**   | 0.000        |
| B17       | 3 1         | 11.371     | 17.936       |
| B18       | 2 1         | 6.302*     | 11.708       |
| B21       | 10 1        | 51.167***  | 70.250       |
| B22       | 3 0         | 17.074     | 0.000        |
| B27       | 4 0         | 22.635     | 0.000        |
| B35       | 3 1         | 11.371     | 17.936       |
| B37       | 9 2         | 39.635     | 30.731       |
| B39       | 0 1         | 0.177      | 0.000        |
| B40       | 2 1         | 6.302*     | 11.708       |
| B44       | 6 3         | 19.259     | 12.681       |
| B45       | 4 0         | 22.635     | 0.000        |
| B49       | 2 2         | 3.863*     | 5.833        |
| B50       | 3 0         | 11.348     | 0.000        |
| B51       | 2 2         | 3.863*     | 5.833        |
| B53       | 3 0         | 17.074     | 0.000        |
| B62       | 1 0         | 6.657*     | 0.000        |

*p<0.001, **p<0.001, ***p<0.0005

At the first locus i.e. A, the difference in frequency of A3 antigen between patients group and controls was found to be statistically significant. Apart from A3 antigen, the antigen A9 and A11 were also moderately higher in patient group when compared with the controls (Fig.).

When the antigens of B locus were considered, it was found that some antigens show high frequency of occurrence. Among them B5 and B21 were also moderately significant in patients group (Fig.).

Linkage disequilibrium values have been calculated and presented in table-2. Interestingly we did not get even a single haplotype with positive delta values.

DISCUSSION

Guffin & Stuart (1986) and Owen & McGuffin (1991) studied patients with schizophrenia and found the association between schizophrenia and HLA-A9 antigen.

In our studies, the strongest association has been observed between delusional disorder and HLA-A3 and A11 antigens. The exact nature of the mechanism underlying the empirically observed association between these HLA antigens and the delusional disorder is not yet fully understood.

Linkage disequilibrium values have been calculated and presented in table-2. Interestingly we did not get even a single haplotype with positive delta values.

Guffin & Stuart (1986) and Owen & McGuffin (1991) studied patients with schizophrenia and found the association between schizophrenia and HLA-A9 antigen.

In our studies, the strongest association has been observed between delusional disorder and HLA-A3 and A11 antigens. The exact nature of the mechanism underlying the empirically observed association between these HLA antigens and the delusional disorder is not yet fully understood.

Linkage disequilibrium between the alleles of the loci is one of the important characteristic of HLA antigen in a random mating population. When there is a difference between observed and expected values of haplotype of two specific alleles of different loci, it is said to be linkage disequilibrium. In case of higher observed frequency the linkage disequilibrium will become positive. In our present study we have observed lower frequency in some cases and hence negative linkage disequilibrium. Interestingly we did not get even a single haplotype with positive linkage disequilibrium. Therefore only the haplotypes with negative values have been presented in table 2. It may be because of the small sample size, or this negative association may be due to other disease susceptibility genes present closely to the HLA gene complex. Further extensive study is required to be carried out, before it can be concluded that this particular HLA locus is the sole determinant of delusional disorder. A clear understanding of this type of genetic relationship could be informative to identify individuals at risk of disease, to
**Table 2**

**Delta Value of HLA-A and HLA-B Antigens in Patients**

| Antigen A | Antigen B | Delta Value | Antigen A | Antigen B | Delta Value | Antigen A | Antigen B | Delta Value |
|-----------|-----------|-------------|-----------|-----------|-------------|-----------|-----------|-------------|
| A1        | B15       | 0.009       | A10       | B45       | 0.026       | A26       | B21       | 0.002       |
| A1        | B21       | 0.008       | A10       | B49       | 0.018       | A26       | B37       | 0.003       |
| A2        | B17       | 0.013       | A10       | B53       | 0.005       | A26       | B44       | 0.005       |
| A2        | B7        | -0.025      | A11       | B5        | 0.035       | A26       | B45       | 0.018       |
| A2        | B5        | -0.012      | A11       | B7        | -0.010      | A26       | B49       | 0.009       |
| A2        | B8        | 0.001       | A11       | B7        | 0.001       | A28       | B5        | -0.002      |
| A2        | B12       | -0.006      | A11       | B12       | -0.013      | A28       | B7        | 0.004       |
| A2        | B13       | 0.007       | A11       | B13       | 0.002       | A28       | B12       | 0.003       |
| A2        | B15       | 0.004       | A11       | B14       | 0.004       | A28       | B13       | 0.005       |
| A2        | B21       | 0.019       | A11       | B15       | -0.010      | A28       | B22       | 0.008       |
| A2        | B27       | 0.010       | A11       | B21       | -0.013      | A28       | B37       | 0.003       |
| A2        | B37       | 0.006       | A11       | B22       | 0.134       | A28       | B40       | 0.009       |
| A3        | B5        | 0.004       | A11       | B27       | 0.009       | A28       | B44       | 0.005       |
| A3        | B7        | -0.015      | A11       | B35       | 0.013       | A29       | B5        | 0.001       |
| A3        | B8        | -0.039      | A11       | B37       | 0.004       | A29       | B7        | 0.006       |
| A3        | B12       | -0.010      | A11       | B40       | 0.005       | A29       | B9        | 0.001       |
| A3        | B13       | -0.027      | A11       | B44       | 0.002       | A29       | B13       | 0.007       |
| A3        | B15       | -0.006      | A11       | B50       | 0.005       | A29       | B16       | 0.008       |
| A3        | B16       | 0.002       | A11       | B51       | 0.005       | A29       | B27       | 0.008       |
| A3        | B17       | 0.016       | A11       | B62       | 0.004       | A29       | B45       | 0.005       |
| A3        | B18       | 0.016       | A19       | B5        | 0.001       | A30       | B5        | 0.001       |
| A3        | B21       | -0.031      | A19       | B7        | 0.006       | A30       | B15       | 0.007       |
| A3        | B22       | 0.016       | A19       | B15       | 0.007       | A30       | B16       | 0.008       |
| A3        | B27       | 0.021       | A19       | B15       | 0.018       | A30       | B18       | 0.009       |
| A3        | B35       | 0.010       | A19       | B37       | 0.016       | A30       | B37       | 0.005       |
| A3        | B37       | -0.010      | A19       | B62       | 0.009       | A30       | B62       | 0.009       |
| A3        | B40       | 0.010       | A23       | B5        | 0.001       | A32       | B7        | 0.009       |
| A3        | B44       | -0.006      | A23       | B7        | 0.006       | A32       | B16       | 0.009       |
| A3        | B45       | 0.021       | A23       | B8        | 0.019       |           |           |             |
| A3        | B50       | 0.010       | A23       | B13       | 0.007       |           |           |             |
| A3        | B51       | 0.010       | A23       | B16       | 0.009       |           |           |             |
| A3        | B53       | 0.016       | A24       | B5        | 0.011       |           |           |             |
| A9        | B5        | -0.008      | A24       | B7        | 0.002       |           |           |             |
| A9        | B7        | -0.005      | A24       | B12       | 0.020       |           |           |             |
| A9        | B8        | 0.016       | A24       | B15       | 0.010       |           |           |             |
| A9        | B12       | 0.025       | A24       | B21       | 0.030       |           |           |             |
| A9        | B13       | -0.002      | A24       | B37       | -0.003      |           |           |             |
| A9        | B15       | 0.021       | A24       | B44       | 0.013       |           |           |             |
| A9        | B18       | 0.005       | A24       | B45       | 0.005       |           |           |             |
| A9        | B21       | 0.012       | A24       | B50       | 0.008       |           |           |             |
| A9        | B37       | 0.002       | A25       | B12       | 0.001       |           |           |             |
| A9        | B44       | 0.021       | A25       | B16       | 0.006       |           |           |             |
| A9        | B50       | 0.007       | A25       | B17       | 0.007       |           |           |             |
| A10       | B7        | -0.003      | A25       | B21       | 0.011       |           |           |             |
| A10       | B12       | 0.015       | A25       | B35       | 0.007       |           |           |             |
| A10       | B14       | 0.009       | A25       | B37       | 0.001       |           |           |             |
| A10       | B16       | 0.013       | A25       | B45       | 0.006       |           |           |             |
| A10       | B17       | 0.005       | A25       | B49       | 0.008       |           |           |             |
| A10       | B18       | 0.005       | A25       | B53       | 0.007       |           |           |             |
| A10       | B21       | 0.015       | A26       | B7        | 0.005       |           |           |             |
| A10       | B35       | 0.003       | A26       | B12       | 0.014       |           |           |             |
| A10       | B37       | 0.005       | A26       | B14       | 0.009       |           |           |             |
| A10       | B44       | 0.003       | A26       | B18       | 0.009       |           |           |             |

*Fig. % Frequency of A3, A9, A11, B5 & B21 in patients and controls*
DELUSIONAL DISORDER AND HLA ANTIGENS

understand the pathophysiologic processes which occur in high risk individuals that precede clinical disease development, and to distinguish subgroups within the disease category that are associated with a different prognosis.

REFERENCES

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, Edn. 4, Washington D.C.:APA.

Guffin, M.C. & Stuart, E. (1986) Genetic markers in schizophrenia. Human Heredity, 36, 65-88.

Kaplan, H.I., Sadock, B.J. & Grebb, J.A. (1994) Delusional Disorder. In: Synopsis of Psychiatry, Edn. 7, (Eds) Kaplan, H.I. & Sadock, B.J., pp 503-509, New Delhi: B.I. Waverly Pvt. Ltd.

Mattiuz, P.L., Inde, D., Piazza, A., Cepallini, R. & Bodmer, W.F. (1970) New approaches to the population genetics and segregation analysis of the HLA system. In: Histocompatibility Testing, Copenhagen, 193.

Owen, M.J. & McGuffin, P. (1991) DNA and Classical genetic markers in Schizophrenia. European Archives of Psychiatry and Clinical Neurosciences, 240, 169-173.

Roberts, G. (1992) Origin of Delusion. British Journal of Psychiatry, 161, 248.

Tait, B.D., Finlay, R.F. & Simons, M.J. (1981) Serum HLA typing. Tissue Antigens, 17, 129-135.

Terasaki, P.I. & McClelland, J.D. (1964) Microdroplet assay of human serum cytotoxins. Nature, 204, 998.

MONOJIT DEBNATH, Junior Research Fellow, Cellular Immunology Lab., Deptt. of Zoology, SUJIT K. DAS, Prof. & Head, Deptt. of Psychiatry. PRAVATI GHOSH, Senior Research Fellow. BICHITRA BIRYA MONDAL, Senior Research Fellow. TAPAS K. CHAUDHURI*, Reader & Incharge, Cellular Immunology Lab., Deptt. of Zoology, North Bengal University, Siliguri, West Bengal.

*Correspondence