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ROLE OF HLA CLASS-I ANTIGENS IN DELUSIONAL DISORDER

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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, HLAantigens, 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 antisera 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 $2x10^6$ 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 Mattiuz 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 beer

presented in table 1.

TABLE 1
PHENOTYPE FREQUENCY OF HLA-A AND B LOCUS ALLELES IN PATIENTS COMPARED WITH HEALTHY CONTROLS

Antigen	% frequency		Chi square	Relative risk
	Patient N=50	Control N=282		
A1	2	6	0.003	0.938
A2	28	6	50.216***	17.868
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	22.265**	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	22.835**	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	22.835	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	22.835	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 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	B12	0.005
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	0.008
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	B16	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	B18	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			

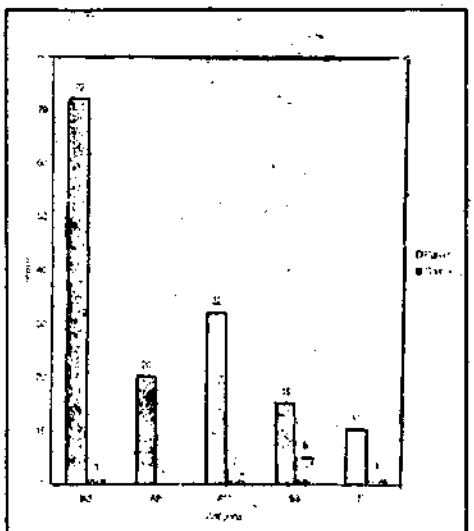

Fig. % Frequency of A3, A9, A11, B5 & B21 in patients and controls

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.

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