# *LIST OF PUBLICATIONS*

# *Publications*

1. Sikta Bandopadhyay, Bisu Singh, Nirmal Kumar Bera, Chitta R Nayak, Tapas Kumar Chaudhuri (2008).Analysis of the role of Dopamine Receptor Genes in the susceptibility of Delusional Disorder. NBU J Anim. Sc. 2(1): 90-94.

2. Bisu Singh, Sikta Banerjee, Nirmal Kumar Bera, Chitta R Nayak, Tapas Kumar Chaudhuri (2008). Impact of HLA antigens and other risk factors on the etiopathology of Schizophrenia. NBU J Anim. Sc. 2(1):61-66.

3. Bisu Singh, Sikta Banerjee, Nirmal Kumar Bera, Chitta R Nayak, Tapas Kumar Chaudhuri (2008).Elevated level of C-reactive protein in drug naïve patients with schizophrenia. Int. J Chem. Sci. 6(3):1276-1282.

4. Bisu Singh, Sikta Banerjee, Nirmal K. Bera, Chitta R Nayak, Tapas K. Chaudhuri (2008). Analysis of the role of human leukocyte antigen class-I genes to understand the etiopathology of schizophrenia. Indian Journal of Psychiatry 50 (3): 166- 170.

5. Sikta Bandopadhyay, Bisu Singh, Nirmal Kumar Bera, Chitta R Nayak, Tapas Kumar Chaudhuri (2008). Dopamine receptors and the dopamine hypothesis in Schizophrenia. NBU J Anim. Sc. 2(2):68-71.

6. Bisu Singh, Sikta Banerjee, Nirmal K. Bera, Chitta R Nayak, Tapas K. Chaudhuri (2008). Immune dysregulation: Can it be an etiological factor for Schizophrenia? . NBU J Anim. Sc. 2(2):60-77.

7. Sikta Bandopadhyay, Bisu Singh, Nirmal K. Bera, Sujit K. Das, Chitta R. Nayak, T.K. Chaudhuri (2009).Plasma homovanillic acid in delusional disorder: Implications for dopamine dysfunction. Int. J Chem. Sci. (In press).

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3. Sikta Bandopadhyay, Bisu Singh, Nirmal K Bera, Chitta R Nayak Taps K Chaudhuri. Association of elevated serum levels of C-reactive protein in delusional disorder: clinical importance for dopamine dysregulation. Indian journal of Psychiatry, 2009.

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# Analysis of the Role of Dopamine Receptor Genes in the Susceptibility of Delusional Disorder

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## ABSTRACT

Delusional disorder is characterized by monosymptomatic paranoid symptoms comprises of an uncommon and probably heterogenous group of illness. The underlying etiological mechanism is poorly understood, though involvement of biological factors has long been suspected. Several investigators have suggested that delusional disorder is a naturally occurring model psychosis based on abnormalities of the dopaminergic temperolimbic system. In the present study, we examined the incidence of dopamine receptor genes in patients with delusional disorder. Significant negative association was found for D2 long chain variants. This preliminary study of allelic association of dopamine receptor genes with delusional disorder may lead to develop future strategies to understand the neurogenetic basis of this disorder.

***Key words:*** Delusional disorder, dopamine receptor genes, association, polymorphism.

## INTRODUCTION

Delusional disorder is characterized by monosymptomatic paranoid symptoms, and in contemporary classifications of mental disorders, delusions are considered as cornerstone symptoms for the diagnosis of psychotic disorders. Since the beginning of psychiatry, delusional disorder has been a central subject of attention and continues to engender controversy right up to now. Delusion formation is a fascinating and enigmatic psychic process that has been the subject of numerous scientific debates and theoretical models; however, surprisingly few empirical studies have been done (Butler & Braff ,1991; Berrios, 1991).

Delusions are understood to mean intersubjectively disconcerting convictions, with a tendency toward subjective certainty, that lose their disconcerting character when made the object of psychiatric analysis. Delusions involve thought contents and, as such, tend to be idiosyncratic and richly varied. Delusional disorder comprises an uncommon and probably heterogeneous group of illnesses; it is complicated by more than 100 conditions and agents, including neurologic disorders, metabolic and endocrine disorders, infections, pharmacologic agents, alcohol and other substances, and psychiatric disorders (Manchreck, 1999). Although its prevalence is low, delusional disorder is not rare (Manchreck, 1996). Recent studies have revealed that delusional disorder is underdiagnosed, which results in poor anticipation of its

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implications (American Psychiatric Association. 1994).The underlying etiologic mechanism and the pathophysiology of delusional disorder are poorly understood. However, surprisingly very few empirical studies have been done (Butler & Braff,1991; Berrios, 1991). Involvement of biological factors has long been suspected (Manchreck, 1999). Recent epidemiological and clinical studies suggest that certain risk factors like advanced age, sensory impairment, personality features, family history etc. may be relevant to etiology (Miller *et al.*,1988). Genetic or family studies lead to convincing data like increased prevalence of delusional disorder and related personality traits (e.g. suspiciousness, jealousy and secretiveness) in the relatives of delusional disorder probands (Munro,1994) and also indicate possible specific family transmission of delusional disorder. Several attempts have been made to identify genetic markers associated with delusional disorder. Since delusional disorder is characterized by monosymptomatic paranoid symptoms, several investigators have suggested that delusional disorder is a naturally occurring model psychosis based on abnormalities of the dopaminergic temperolimbic system(Kaplan *et al.*, 1994).

A recent study of genetic variation in the DNA sequence coding for Dopamine type 4 (DRD4) Exon 3 strongly suggests the involvement of the relevant gene in conferring susceptibility to delusional disorder (Serretti *et al.*, 2001). However, these studies are not uniformly consistent and needs to be replicated on a large sample size to confirm the tentative results of dopaminergic mechanisms responsible for paranoid symptoms (Kendler & Hays,1981).

In the present study, we examined the incidence of dopamine receptor genes in patients with delusional disorder and in healthy control subjects to investigate if dopamine receptor genes could be used as a genetic marker and to explore a possible neurological etiology for delusional disorder.

## MATERIALS & METHODS

**Subjects:** Subjects were recruited from an India-born Bengali population referred to the psychiatric outpatient department at North Bengal Medical College and Hospital. On an average, 1500 new patients with various psychiatric illnesses and about 4000 recurrent follow-up cases attend the outpatient department every year. 150 unrelated patients were recruited (82 women and 68 men) with the symptom of delusions and studied them for 5 years. All subjects were screened independently by two psychiatrists using the Structured Clinical Interview for DSM-IV (SCID) to determine a diagnosis of delusional disorder (American Psychiatric Association. 1994). After longitudinal follow-up, 100 patients represented genuine cases of delusional disorder of various subtypes. We compared delusional disorder patients with normal healthy individuals of no previous history of psychotic illness. The distribution of delusional disorder subtypes, as defined by DSM-IV criteria, were as follows: 62.5% persecutory, 12.5% mixed, 12.5% jealous, 6.25% somatic, 3.75% erotomanic, and 2.5% grandiose. Most patients were clustered between the ages of 25 and 55 years. Of the initial 150 patients, we excluded from the patient group the 50 patients suffering from psychiatric conditions other than delusional disorder. Hundred healthy individuals belonging to the same ethnic group as the patients were used as control subjects. Control subjects were mainly selected from the university as well as from hospital employees. Written consent from all the patients were taken prior to the collection of blood samples from them. All the patients and control subjects were matched for sex, age, and other socio-economic variables. The subjects were mostly from middle-class urban and semirural society and belonged to a nuclear family. The above-mentioned medical college is one of the rural medical institutes in India.

**PCR analysis:** Approximately 5ml of venous blood was taken from each individual. Molecular typing was done only on the final 100 patients with delusional disorder and the equal number of healthy controls. DNA was extracted from the peripheral mononuclear cells using a salting out procedure (Miller *et al.*,1988). Molecular typing was carried out by using polymerase chain reaction sequence-specific primer (PCR-SSP) technique to detect dopamine receptor genes (Figure 1). The primers, Taq polymerase, nucleotides, and other reagents were used as per the standard method and recommended by Svejgaard *et al.* (1974).

***Statistical Analysis:*** The phenotype frequencies were calculated by direct count. The frequency of each allele among the patients was compared with that of the control population, using the chi-square test followed by Fisher's exact test. Testing for a large number of alleles can reveal at least one positive association where none really exists; to be statistically significant, therefore, the *P* values from each Fisher's exact test had to be less than the Bonferroni *P* (0.05 divided by the number of alleles tested [$n = 37$] minus 2 degrees of freedom [1 for each of the 2 loci examined], which equals 0.0014).We estimated relative risk (RR) as recommended by Svejgaard *et al.* (1974).

## RESULT

Molecular typing was done for 100 patients with delusional disorder. The data are shown in Table-1. The results demonstrate a marked down regulation of the long chain variant of D2 receptor in patients with delusional disorder, compared with healthy control subjects. The *P* value after Bonferroni correction was significant.

**Table1:** Shows phenotype frequency (%), chi square, relative risk(RR) and p values dopamine receptor genes and their alleles in patients compared with healthy controls.

| Dopamine receptor genes | Phenotype frequency (%) | | Chi-square | RR | *p* | Remark |
|---|---|---|---|---|---|---|
| | Patients | Controls | | | | |
| D1 | 75 | 100 | 2.28571 | 0.00000 | 2.33333e-1 | NS |
| D2(L) | 0 | 50 | 5.33333 | 0.00000 | 3.84615e-2* | Significant |
| D5 | 8.75 | 100 | 1.06667 | 0.00000 | 5.00000e-1 | NS |

*Significant after Bonferoni correction.

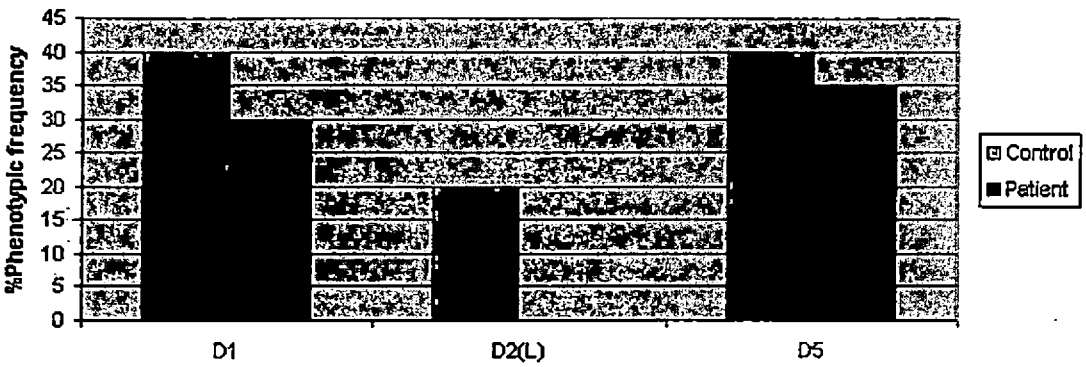
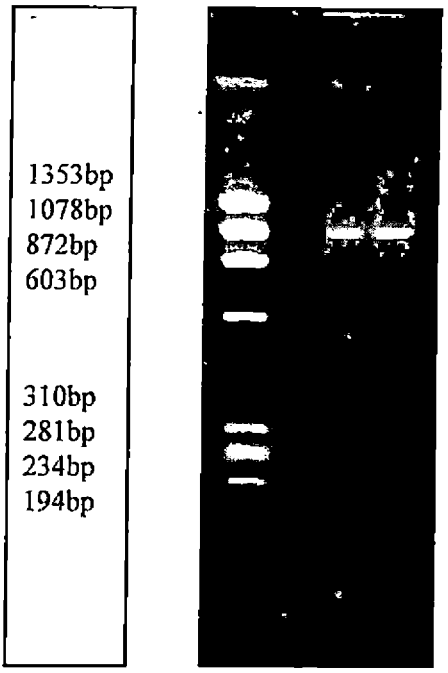



Fig: Phenotype frequency(%) of D1,D2(L) and D5 dopamine receptor genes in patients with delusional disorder and the healthy controls.

## DISCUSSION

A strong negative association has been observed between delusional disorder and dopamine D2 receptor long chain variant (D2L) in the present study. When the strength of negative association is measured by cross product ratio or relative risk of developing a disease, D2L showed RR=0.0000, thus reflecting a negative association. A higher frequency of incidence of D2L in the controls along with total absence of the allele in the patients group,($c^2 = 5.33333$) suggest a strong negative association of that particular allele to delusional disorder. However, the exact nature of the mechanism underlying the empirically observed negative association between D2Land the delusional disorder is not fully understood.

At this point though it is difficult to predict that the D2L is the sole determinant of delusional disorder but this significant negative association might contribute to the disease risk. A comprehensive study is presently going on to understand the pattern of D2L inheritance in the affected families, which may help to determine the validity and specificity of the absence of D2Lgene as genetic marker of delusional disorder.




**LANE:**
1:Marker : φX174
2:D2L(patient)
3:D2L(control-1): 1000bp
4:D2L(control-2): 1000bp

**Fig.2:** Gel photograph of D2L dopamine receptor gene in patients with delusional disorder and the normal healthy controls.

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# Impact of HLA Antigens and Other Risk Factors on the Etiopathology of Schizophrenia

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## ABSTRACT

Schizophrenia is the paradigmatic illness of psychiatry. The involvement of immunological and immunopathological mechanisms along with the environmental factors in the etiopathogenesis of schizophrenia has been a matter of research. We investigated the incidence of HLA Class I antigens to understand the role of HLA genes in schizophrenia. We further studied the birth of the siblings in the family and age of onset to understand the role of environmental factors for triggering the disorder. Some of the HLA antigens are associated with schizophrenia and significant increase was found for HLA-A3 antigen along with the significant decrease in HLA-A25, A31 and HLA-B51.Further, significant value was found after ×2 test between the first child patient and the patient who are not the first child. The study provides the evidence for the possible existence of susceptibility locus for schizophrenia within the HLA region. The study further suggests that in addition to genetic predisposition some environmental factors such as viral infection might play a pivotal role on the onset of the disorder. This preliminary observation may help to understand the etiological basis of this disorder.

***Key words:*** Schizophrenia, etiology, HLA antigens, association

## INTRODUCTION

Schizophrenia is the paradigmatic psychiatric illness. Inspite of its one percent worldwide incidence, it has become a leading public health problem. The etiological process or processes by which causal agents causes the physiopathology is not yet understood. With recent progress in the understanding of the immune system, autoimmune etiology of schizophrenia has become a major focus of research (Ganguli *et al.*, 1993; Knight,1984). Susceptibility to almost all autoimmune diseases is influenced by genes encoded by the human leukocyte antigen (HLA) region, particularly the Class I (A,B and C) and Class II (DR,DQ,DP) antigens. The HLA gene complex is located on the short arm of chromosome 6 (p21.3). It is one of the most gene-rich and polymorphic regions in human genome and traditionally, investigators compare phenotypic or genotypic frequencies between unrelated cases and unaffected controls.

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There have been numerous association studies of HLA antigen and schizophrenia (Mc Guffin and Sturt,1986; Hawi *et al.*,1999). Past association studies with various Class I and Class II alleles yielded inconsistent results (Nimgaonkar *et al.*,1992) except with HLA-A9 (now subdivided into A23 and 24) (Mc.Guffin *et al.*, 1995). In different ethnic populations associations of schizophrenia has been found with HLA-A9 (Goudemand and Goudemand, 1981), HLA-A23 (Ivanyi *et al.*, 1983),HLA-24(Asaka *et al.*,1981) , HLA-A1(Lahdelma *et al.*,1998), HLA-A2, HLA-A3, HLA-A11, HLA-B17, HLA-B27, HLA-B8 and Cw2 (Rudduck *et al.*,1984), HLA-A3(Debnath *et al.*, 2005), DRB1-04(Wright *et al.*, 1996) , DR1 (Sasaki *et al.*,1999) alleles.

Along with genetic factors there are other risk factors for schizophrenia which have been identified. It has been proposed that maternal infection during pregnancy increases the risk of the offspring developing schizophrenia and other developmental neuro-disorders (Wright *et al.*, 1995). Sham *et al.*(1993), using data from a Swedish family study , reported that younger children in a family had a significantly increased risk of later developing schizophrenia if their siblings were 3 to 4 years older at the time when they were in uterus. The researchers suggest that older children can be a source of viral infections to their mother and also to the developing fetus, which they may transmit to their pregnant mothers, and these infections in turn may cause schizophrenia in the offspring.

The present study aimed to investigate the role of HLA antigens in the etiopathology of schizophrenia and evaluate the data with reference to the hypothesis "younger children in a family have a significantly increased risk of later developing schizophrenia."

## MATERIALS AND METHODS

We studied 50 India-born schizophrenic patients residing in and around Siliguri Subdivision of West Bengal, who attended the outpatient department (OPD) of Psychiatry, North Bengal Medical College and Hospital. The patients considered for the present study belonged to Bengali, Nepali, Bihari and some tribal communities. They were diagnosed independently by two psychiatrists with the help of standard diagnostic criteria of DSM IV and assessed by the Brief Psychiatric Rating Scale (BPRS). The age was within the range of 17-58 and male to female ratio was 2.8: 1 were studied. Further, the schizophrenic patients and the family members were made to answer a questionnaire. The questionnaire included self-reported age, caste, sex, medical history, age of onset, month of birth, marital status, education, substance abuse, incidence of any autoimmune disease among patients or in family members etc. A complete pedigree for each of the patient was also drawn.

A total number of 50 healthy individuals, which matched the age, sex and ethnicity of schizophrenic patients, were considered as controls. All control subjects were screened for a recent history of intercurrent infections and allergies. Those with a past history of autoimmune or psychiatric disorders were excluded. Written consent from all the participants were taken prior to collection of blood samples from them.

Methodology: The blood was drawn by the vein puncture method and collected in EDTA anticoagulant. DNA was collected from peripheral mononuclear cells of the blood by the Phenol Chloroform method. The typing of HLA Class I was performed by PCR-SSP technique. The primers, Taq polymerase, nucleotides etc. were obtained from Bangalore Genei, India and the typing and sequence information of primers were taken from Bunce *et al.* (1995).

Statistical Analysis: The phenotype frequencies were calculated by direct count. Chi-square test was done to compare the frequency of each antigen in the patient and control groups and it was followed by Fisher's exact test. Since testing for a large number of antigens can reveal at least one positive association where none really exists, the p values from each Fisher's exact test had to be less than the Bonferroni p [0.05 divided by the number of antigens tested minus two degrees of freedom (one for each of the two loci examined), which equals to 0.0014] to be called stastically significant. Relative risk was estimated as recommended by Svejgard (1974). Furthermore, Chi-square test was employed for birth of the siblings and Welch's t test for age at onset.

## RESULTS

As summarized in the Table I, there was a significantly higher frequency ($\chi2$=11.45, p<0.001) of HLA-A3 in patients than the in the control group. On the other hand HLA A25 (Fig. 1) ($\chi2$=13.619,p<0.001), A31 ($\chi2$=22.56,p<0.001),B51 ($\chi2$=42.85, p<0.001) showed a significantly lower value after the Bonferroni correction. Besides A2 ($\chi2$=6.05,p<0.008)showed lower frequency and B7 ($\chi2$=4.069, p<0.02) and B42 ($\chi2$=5.47,p<0.033) showed higher frequency but they were not found to be significant after the Bonferroni correction.
Further, as shown in Table II, when the Chi- square test was performed between the first child patients and the patients who were not the first child, the test showed a significant diffrence, on the other hand the age of onset did not show a significant difference between the two groups. It is worth mentioning here that both the groups of the patients were HLA-A3 positive.

**Table I:** Phenotype frequency, Chi square, relative risk (RR) values and probability of HLA-A and B loci alleles in the patients with schizophrenia and healthy controls.

| Antigen | Patients (N=50) | Control (N=50) | Chi square | RR | P value | |
|---|---|---|---|---|---|---|
| A*02 | 24 | 27 | 6.0529 | 0.32 | <0.008 | * |
| A*03 | 50 | 38 | 11.458 | 0.00 | <0.001 | ** |
| A*11 | 36 | 31 | 11.723 | 1.58 | <0.288 | |
| A*23 | 21 | 26 | 0.6423 | 0.67 | <0.317 | |
| A*24 | 32 | 26 | 1.026 | 1.64 | <0.224 | |
| A*25 | 10 | 29 | 13.619 | 0.18 | <0.001 | |
| A*26 | 21 | 17 | 0.382 | 1.41 | <0.410 | |
| A*29 | 33 | 32 | 0.000 | 1.09 | <0.833 | |
| A*30 | 33 | 25 | 2.0114 | 1.94 | <0.105 | |
| A*31 | 0 | 20 | 22.522 | 0.00 | <0.001 | ** |
| B*07 | 47 | 39 | 4.069 | 4.42 | <0.02 | * |
| B*21 | 36 | 38 | 0.0519 | 0.81 | <0.825 | |
| B*4001 | 17 | 13 | 0.428 | 1.47 | <0.513 | |
| B*4201 | 39 | 28 | 0.522 | 2.79 | <0.033 | * |
| B*44 | 14 | 10 | 0.493 | 1.59 | <0.349 | |
| B*5101-5105 | 0 | 30 | 40.047 | 0.00 | <0.001 | ** |

*Significant; ** Significant after the Bonferroni correction Bonferroni's probability is 0.003571

**Table II:**Comparison of demographic characteristics between the first schizophrenic child and the other schizophrenic siblings in different families.

| | N=50 | | Analysis | |
|---|---|---|---|---|
| | patients who are first child | patients who not the first child | Statistic | P value |
| 1. Number of patients | 11 | 39 | $\chi 2$ =15.68 (df=1) | <0.001 * |
| 2. Age of Onset (years) | | | | |
| Mean | 24.45 | 28.03 | | = 0.30 |
| Standard deviation (SD) | 12.84 | 8. 97 | t = -1.056 | |

*** significant**

## DISCUSSION

A significantly higher frequency of HLA-A3 obtained in the present study is in accordance with the previously reported study by Debnath *et al.* (2005). Although the association of HLA-A3 antigen and schizophrenia is found to be significantly higher, the present studying does not corroborate a very strong association. This might be due to the small size of control population the present study.

On the other hand, the present study A25, A31 and B51 shows a significantly low negative value which is the unique finding of the present study. The alleles A31 and B51 show strong negative association (RR=0.000). The increased frequency of A11 found in the previous study has not been reproduced in the present study. Apart form this, several other alleles like B7 and B42 show higher values but are not statistically significant. We have also observed a negative but insignificant association of A2 which corroborates the findings of Debnath *et al.* (2005).

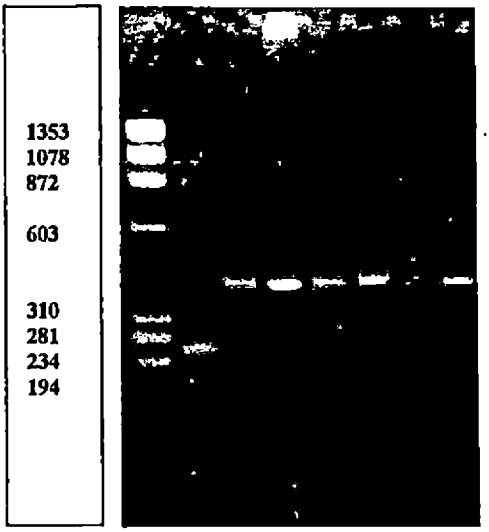


**Fig.1:** Electrophoregram showing the results of HLA-A*25

**Lane:**
1. Marker : Phi X174; 2. Control : 256 bp
3-8. A*25 : 398 bp

The findings in the present study paves the way for the possible association of HLA antigen with schizophrenia but the exact mechanism of the association of HLA with schizophrenia is still obscure. However, the presence of the disease in only a small fraction of the people carrying the marker HLA allele can be explained as the following : (i) Genetic factors not linked with HLA may contribute toward the disease susceptibility; (ii) the disease may be heterogeneous and may have more than one etiology; the observed HLA association is only with a subset of affected individuals; (iii)environmental

factors, such as exposure to viruses, may determine whether individuals with disease susceptibility will manifest the disease clinically; and (iv) if the association is due to a thus-far undetected allele at a closely linked locus, then this allele may occur at a much lower frequency than the one detected at the presently known A,B,C and D/DR loci.( Mc Devitt and Bodmer,1974; Bodmer and Bodmer, 1978).

The present finding of significant association of schizophrenia with the patients who are not the first child was in accordance to the study of Sham *et al.* (1993).It is needless to mention here that all the patients were HLA-A3 positive. Therefore, the present study suggests that in addition to genetic predisposition some environmental factors such as viral infection might play a pivotal role on the onset of the disorder. However, the present study strengthens the hypothesis "younger children in a family had a significantly increased risk of later developing schizophrenia".

The present study supports earlier finding of association of HLA-A3 with schizophrenia along with the negative association of some more alleles, which is the new finding of the present study. However, it is too early to speculate the exact mechanism of association. The result is preliminary and can not be reliably correlated with the birth status, viral infections, prenatal infections and so on. However, this study provides an evidence for the possible existence of a susceptibility locus for schizophrenia within the HLA region. The study also presupposes the idea of requirement of environmental factors along with the genetic predisposition for triggering the disorder. Further study needs to be carried out on larger sample size to decipher the etiopathology of the disorder.

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# ELEVATED LEVEL OF C-REACTIVE PROTEIN IN DRUG NAÏVE PATIENTS WITH SCHIZOPHRENIA

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## ABSTRACT

The well known inflammatory marker C-reactive protein (CRP), was investigated in the drug naïve and antipsychotic medicating patients to understand the role of inflammation in the etiopathology of schizophrenia.

The level of serum CRP was investigated among 64 schizophrenic patients, diagnosed with DSM IV criteria and categorized into different subgroup of schizophrenia. Latex agglutination test was performed to measure the level of CRP. The limitation of detection of serum CRP was less than 6 mg/L. CRP was treated as categorical variable: normal (6 mg/L) and elevated ( $\geq$ 6mg/L). Further, patients were made to answer a questionnaire, which included self-reported age, sex, medical history, age of onset, substance abuse etc. All subjects came from an India-born Bengali population.

3 Paranoid patients showed the elevated level of CRP ( $\geq$ 6mg/L) whereas rest of the patients had normal CRP ( $<$ 6mg/L). When the findings were compared to the demographic variables, the results showed a significant value for the elevated level of CRP and drug naïve status.

The study suggests that some kind of inflammatory process may be one of the etiological factors for schizophrenia and the antipsychotic drug might play an important role in down regulating this inflammatory process and thereby bringing the level of CRP to the normal state.

**Key words:** Schizophrenia, C-Reactive protein, Inflammation, Drug naïve

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## INTRODUCTION

Schizophrenia is the paradigmatic illness of psychiatry. Although the worldwide prevalence of schizophrenia is about 1%, but it has become a leading public health problem now-a-days and exerts enormous personal and economic costs worldwide. Inspite of tireless research efforts, the etiological process or processes by which a causal agent creates the pathophysiology of schizophrenia is not yet clearly understood. However, a good deal is known about risk factors for developing schizophrenia, which leads to direct inferences regarding possible etiopathophysiologies[1]. A large number of studies have also shown the association between the HLA genes and schizophrenia. In such a study, we have reported the association of HLA A*03 allele with paranoid schizophrenia in Indian Bangalee population[2,3].

The roles of immune dysfunction and inflammation have been described in schizophrenia[4,5]. In the past a number of attempts have been made to identify the inflammatory markers for schizophrenia but with conflicting findings. The inconsistent results in the literature might be explained by the heterogeneity of schizophrenia, difference in illness state (acute versus chronic) and effects of antipsychotic medication[6].

One of the well known inflammatory markers is C-reactive protein (CRP). CRP is a normal alpha globulin, which increases in inflammatory processes. The name CRP is derived from the fact that this protein has the capacity to precipitate the somatic C-carbohydrate of Pneumococcus. Elevated CRP levels are usually observed in a variety of infections and inflammatory conditions where there is tissue destruction. Elevated CRP is known to be the risk factor for the cardiovascular diseases, diabetes and other metabolic dysfunction[7,8]. In addition, it is also known to be associated with the depression[9] and cognitive impairment[10].

However, very few studies have been carried out to investigate the association of CRP and schizophrenia. In one study, elevated serum levels of CRP was found in patients who showed more severe clinical symptoms of schizophrenia as reflected by the PANSS total score[11]. In another study, the elevated serum levels of C-reactive protein in schizophrenia are found to be associated with the severity of cognitive impairment but not of psychiatric symptoms[12].

In the present preliminary first hand study, we have investigated the level of CRP in serum of the patients with schizophrenia and its relation with other demographic variables.

## EXPERIMENTAL

### Materials and methods

India-born Bengali population referred to the psychiatric outpatient Department (OPD) of Psychiatry, North Bengal Medical College and Hospital were considered for the present study. Patients were diagnosed independently by two psychiatrists according to the standard diagnostic criteria of DSM IV and assessed by the Brief Psychiatric Rating Scale (BPRS). After diagnosis, 64 schizophrenic patients were included for the study.

Further, the schizophrenic patients and the family members were made to answer a questionnaire. The questionnaire included self-reported age, cast, sex, medical history, age of onset, month of birth, marital status, education, substance abuse, incidence of any autoimmune disease among patients or in family members etc. All the participants provided their written consent for giving the blood sample after the study procedures were explained.

### Procedure

About 5 mL. of blood samples were collected from the each patient. The samples were allowed to coagulate at the room temperature for 2-3 hrs. Blood clot was cut and centrifuged for separating the serum. The CRP level in the serum was measured by latex agglutination slide test (Ranbaxy Fine Chemicals Ltd., HP, India). The limitation of detection of serum CRP level was less than 6 mg/L. CRP was treated as categorical variable: undetectable or normal($< 6$ mg/L) and detectable or elevated ($\geq 6$ mg/L).

### Statistical analysis

Statistical analysis was performed for the bivariate associations between elevated CRP groups versus normal group by employing one-way analysis of variance. The association between CRP groups and deficit was examined by Chi-square analysis. The association between CRP group and other clinical/demographic variables were also examined by utilizing one way analysis for continuous variables and Chi-square tests for dichotomous variables.

## RESULTS AND DISCUSSION

Sera levels of CRP were measured for 64 schizophrenic patients. Out of them, 57 were paranoid, 2 residual, 3 undifferentiated and 2 were disorganised type. The elevated level of CRP ($\geq 6$ mg/L) was observed in 3 patients and 61 patients were found to have

normal CRP (< 6 mg/L). All the three elevated cases were found to be of paranoid type. No differences were found in CRP level among different subgroups of schizophrenia.

Further, when the level of CRP was compared to the other demographic variables as shown in the Table 1, only the drug naïve status of the patients showed statistically significant value ($\chi^2 = 16.997$, $P$ value $< 3.75 \times 10^5$).

**Table 1. Comparison of demographic and clinical characteristics between the normal/elevated CRP groups**

| | Elevated CRP N = 3 | | Normal CRP N = 61 | | Statistic (Z) | P value |
|---|---|---|---|---|---|---|
| | Mean or N% | S. D. | Mean or N% | S. D | | |
| Age | 37.67 | 21.13 | 34.69 | 9.64 | F[2,60] = 0.24 | >0.62 |
| Gender Men v/s Women | 33.33% | | 70.49% | | $\chi^2$ [1] = 1.84 | >0.17 |
| Drug naïve patients v/s Patients under antipsychotic medication | 100% | | 11.48% | | $\chi^2$ [1] = 17.00 | <0.001 Significant |
| Substance abuse Yes v/s No | 33.33% | | 55.74% | | $\chi^2$ [1] = 0.58 | >0.44 |
| First child Yes v/s No | 33% | | 18.03% | | X2[1] = 0.44 | >0.50 |
| Autoimmune disease in patients or in family Yes v/s No | 0% | | 24.59% | | $\chi^2$ [1] = 0.97 | >0.32 |

This preliminary first hand study provides further evidence of the involvement of inflammatory processes behind the etiopathology of schizophrenia. The elevated level of CRP in our study is in accordance to the findings of Fan et al.[11] and Dikerson et al.[12]. But unlike previously reported findings, we have considered the CRP level of patients with

their medication status, which showed significantly higher value. In one study, the level of CRP was found to be higher in the patient, who was experiencing psychotic symptoms, in the follow up study in the non-psychotic state, the level of CRP was found to be normal[13]. In this respect, the present study suggests that the antipsychotic drug may perhaps down regulate the inflammatory process, which in turn brings the CRP level to the normal state.

Thus, these findings further suggest that the inflammation may be another possible mechanism in the etiopathology of schizophrenia. It is, however, not clearly understood whether the elevation of the level of CRP is the by-product of the pathophysiology of schizophrenia or directly contributes to the clinical manifestations of the disorder[11].

Until now, it is not clearly understood regarding the mechanism of inflammation in schizophrenia. It is suggested that the vascular-structural brain abnormalities may be one of the factors in the etiology of schizophrenia, like psychoses[14-16]. It is proposed that chronic inflammation might damage the micro-vascular system in the brain and cerebral blood flow[17]. Further, scientific evidence suggests that an increase in the stress hormone like norepinephrine may activate the inflammatory arm of the immune system and triggers the expression of genes that cause chronic, low-grade inflammation. This inflammation is characterized by the degree of the levels of CRP[18].

This is possibly the first reported study of association between CRP and schizophrenia in the Indian scenario. The limitations of the present preliminary study are that the psychopathology measures were not considered for the patients, unlike the previous studies. In contrary to the studies conducted by (Fan et al.[11] Dickerson et al.[12](5 mg/μL), the higher cut off value (6 mg/L) was used for the CRP levels. The sample size of the study is small and the patients were attended in the OPD, which has limited the follow up study.

The study provides further evidence that some kind of inflammation may play a role in the etiopathology of schizophrenia. The study further reveals the immunomodulatory effect of the antipsychotic drugs in the patients.

Additional studies with the highly sensitive techniques like ELISA with longitudinal follow up studies in the large sample size would be required to further strengthen the present study.

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**Contributors -**

Bisu Singh designed the study and wrote protocol and final manuscript; Sikta Banerjee contributed to the design of the study and recruitment of participants; Nirmal K. Bera is a psychiatrist and contributed to the diagnosis of patients; Chitta R Nayak provided the laboratory evaluations and the statistical analysis; Tapas K Chaudhuri contributed substantially in the design of the study and writing the final manuscript.

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# Analysis of the role of human leukocyte antigen class-I genes to understand the etiopathology of schizophrenia

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## ABSTRACT

**Background:** Schizophrenia is the paradigmatic illness of psychiatry. The involvement of immunological and immunopathological mechanisms in the etiopathogenesis of schizophrenia has been a matter of research, with recently increasing effort.
**Aims:** In this study, we investigated the incidence of human leukocyte antigen (HLA) Class I antigens to understand the role of HLA genes in schizophrenia.
**Materials and Methods:** India born schizophrenic patients in and around Siliguri who attended outpatient department (OPD) of Department of Psychiatry, North Bengal Medical College and Hospital were considered for the present study. After the longitudinal follow up, 50 patients were enrolled for the study. The same number of age, sex and ethnically matched healthy subjects were considered as control. Low resolution polymerase chain reaction-sequence specific primer method was applied for typing the HLA antigens.
**Statistics:** The phenotype frequencies were calculated by direct count. $\chi^2$ test was done to compare the frequency of each antigen among the patients and control group and it was followed by Fisher's exact test. Relative risk was estimated by using Haldane's method.
**Results:** The result showed that some of the HLA antigens are associated with the schizophrenia and significant increase were observed for HLA A*03 antigen along with the significant decrease for HLA A*25, A*31 and HLA B*51.
**Conclusions:** The study provides the evidence for the possible existence of susceptibility locus for schizophrenia within the HLA region. This preliminary observation may help to understand the etiological basis of this disorder and the study may further strengthen the HLA antigens as the marker for schizophrenia.

**Key words:** Etiology, human leukocyte antigen, schizophrenia

## INTRODUCTION

Schizophrenia is a severely debilitating neuropsychiatric disorder characterized by "disturbances of thought, auditory hallucinations and multiple delusions".[1] It affects 1% of the worldwide population.[2] The essential biological pathology of schizophrenia ia partially understood till to date.[3] However, there is substantial evidence to indicate a major genetic component.[4][5] Different chromosomes have been pinpointed as harbouring genes involved in the pathogenesis of schizophrenia.[6] A susceptibility locus has been identified on chromosome 6.[7][8] Several researches have also found evidence for schizophrenia vulnerability genes on chromosome 6p close to the HLA genetic region by linkage analysis.[9] HLA and schizophrenia was first reviewed by Mc Guffin (1979),[10] who commented that the MHC was a logical place in which to search for genetic markers for schizophrenia because schizophrenia was similar to diseases for which HLA association had been established in that it was familial, had an imperfectly understood etiology, and had a postulated autoimmune pathogenesis.[11]

The first HLA association study of schizophrenia was

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reported by Cazzullo *et al.*, in 1974.[12] More than 60 association studies have been reported since then.[13] The details of past association studies is given in Table 1.

Past association studies with various Class I and Class II alleles yielded inconsistent results[14] except HLA-A*9 (now subdivided into A*23/A*24).[15] In different ethnic population, associations have been found for HLA-A9,[16] HLA-A23,[17] HLA-A*24,[18] HLA-A*1,[19] HLA-A*2, HLA-A*3, HLA-A*11, HLA-B*17, HLA-B*27, HLA-B*8 and Cw 2,[20] HLA-A*3.[21] The reason for the inconsistencies include the diagnostic methods, particularly in early studies , are imprecise and vary greatly.[22] The majority of the previous association studies were carried out using serological typing techniques [microlymphocytotoxicity testing][23], which have been found to be inaccurate, with 7-25% misassignment errors[24] compared with the DNA based techniques (polymerase chain reaction (PCR) and sequence specific oligonucleotide probes (SSOP). The source of controls is not always described in sufficient detail to ensure that results are not simply due to population stratification. Significant results are not always corrected for the number of statistical tests performed.[25]

The present study has been carried out to investigate the association of HLA Class I alleles in Schizophrenia with the help of DNA-based typing method in well-characterized sample of ethnically matched patients and controls. The study may help to identify disease-specific susceptibility (risk) and protective markers that can be used in immunogenetic profiling, risk assessment and therapeutic decisions. Further, the study may refine already known associations in the light of modern DNA based HLA typing method.

## MATERIALS AND METHODS

We studied 50 India-born schizophrenic patients residing in and around Siliguri subdivision of West Bengal, referred to the psychiatric outpatient department (OPD) of Psychiatry, North Bengal Medical College and Hospital. Three major selection criteria were considered for selection of schizophrenic group; (i) unrelatedness of individuals from each other, (ii) resident of the state of West Bengal and (iii) subjects satisfying DSM IV[3] diagnostic criteria for schizophrenia . The exclusion criteria followed in the present study include; (i) history of substance abuse, (ii) presence personality disorder, (iii) presence of dementia and mental retardation. The patients considered for the present study were belonging to the Bengali, Nepali, Bihari and some tribal community. They were diagnosed independently by two psychiatrists using Structured Clinical Interview[26] and according to the standard diagnostic criteria of DSM IV and assessed by the Brief Psychiatric Rating Scale (BPRS).[27] The present study comprise of 45 Paranoid, 2 Residual, 2 Undifferentiated and 1 disorganised schizophrenic patients. Considering the small number of different subtypes of schizophrenic patients in this study (except Paranoid), we have considered schizophrenic patients as a whole. Soı the demographic variables which have been studied i patient group are given in the table 2.

A total number of 50 ethnically matched healthy indivi were considered as controls. To avoid the spu associations resulting from population stratification care was taken. The following criteria were strictly foll for the selection of controls, (i) same ethnic group a patients, (ii) sex and age matched with patients, (iii) ab: of family history of autoimmune or psychiatric disc (iv) recent history of intercurrent infection and alle (v) unrelatedness of individuals from one another, (\ history of any substance abuse. All the participants pro their written consent for giving the blood sample aft study procedures were explained.

### Methodology

The blood was drawn by vein puncture method and EDT added as anticoagulant. DNA was extracted from perip mononuclear cells of the blood by the Phenol Chlorc method. The typing of HLA Class I was performed by PC technique. The typing and sequence information of pr were taken from Bunce *et al.*, (1995).[28] The primers polymerase, nucleotides etc. were obtained from Bang Genei, India. In general 25$\mu$l of reaction mixture in 1x PCR buffer, 200$\mu$M of each of dNTP, 1.5mM M 0.4$\mu$M of forward and reverse primers, 100ng of ger DNA and 1unit of Taq polymerase. The amplifications accomplished on a thermal cycler (Perkin Elmer, PCR reaction are subjected to 30cycles, each consist 94°C for 30s, 60°C for 1min. and 72°C for 1min. with denaturation step of 2min and final extension of 2min

### Statistical analysis

The phenotype frequencies were calculated by direct c

**Table 2: Psycho-socio-demographic characteristic the schizophrenic patients**

| | | Standard deviation | |
|---|---|---|---|
| Gender | | | |
| Male | P= 0.78 (78%) | | Z= |
| Female | p<0.001 | | |
| Age | | | |
| Mean | 34.06 | 9.46 | |
| Disease duration | | | |
| (in years) mean | 5.77 | 6.37 | |
| Substance abuse | | | |
| Yes | P=0% | | |
| No | | | |
| Marital status | | | |
| Married | P=0.64 (64%) | | Z= |
| Unmarried | p<0.05 | | |
| Ethnicity | | | |
| Bengali | 68% | | $X^2$= |
| Nepali | 10% | | (d. |
| Tribal | 18% | | p< |
| Bihari | 4% | | |

**Table 1: HLA association studies of schizophrenia: Class I (A, B and C) antigens***

| Investigator, year† | Ethnicity Diagnosis (number) | Patient subjects Origin (number) | Comparison subjects | Result (comment) |
|---|---|---|---|---|
| Cazzullo *et al.*, 1974 | Caucasian | Feignner (52) | Population (386) | no association |
| Eberhard *et al.*, 1975 | Caucasian | Bleuler (47) | -(1263) | A9 (RR=2.9) |
| Ivanyi *et al.*, 1976 | Caucasian | -(148) | Population (1200) | A28(RR=3.4) |
| Smeraldi *et al.*, 1976a | Caucasian | Feighner (70) | Popualtion (386) | No association |
| Smeraldi *et al.*, 1976b | Caucasian | Feighner (144) | Population (386) | A10 (RR=0.4) |
| Julien *et al.*, 1977 | Caucasian | -(65) | Population (250) | A9 (RR=2.5) |
| Ivanyi *et al.*, 1977 | Caucasian | -(40) | Population (438) | Cw4 with paranoid schizophrenia (RR=3.7) |
| | | | Population (1200) | B18 with paranoid schizophrenia (RR=3.4) |
| Bennahum *et al.*,1977 | Caucasian | Feighner (38) | -(102) | No association |
| Kyner *et al.*,1978 | Caucasian | Feighner (20) | -(67) | No association |
| Ivanyi *et al.*,1978 | Caucasian | -(200) | Population (1200) | A28(RR=3.0) |
| Mc Guffin *et al.*, 1978 | Caucasian | ICD-9 (80) | Blood donors(458) | No association |
| Perris *et al.*, 1979 | Caucasian | -(50) | Blood donors (449) | No association |
| Crowe *et al.*, 1979 | Caucasian | Feighner (45) | Population(1263) | Aw 10 (A26 subtype) with hebephrenia (RR=6.6) |
| Luchins *et al.*, 1980 | Caucasian | RDC(38) | Published data (743) | No association |
| | African-USA | RDC(92) | Published data (563) | A2 (RR=2.3) |
| Gattaz and Beckmann, 1980 | Caucasian | Feighner (100) | -(472) | B27 with poor prognosis patients |
| Mendlewicz *et al.*,1981 | Caucasian | Feighner (64) | Blood donors (113) | No association |
| Asaka *et al.*, 1981 | Japanese | -(136) | Blood donors (187) | A9(Aw24 subtype ) (RR=2.0) A10(A26 subtype) (RR=1.9) |
| Goudemand *et al.*,1981 | Caucasian | -(51) | Blood donors (94) | No association |
| Singer *et al.*, 1982 | Caucasian | -(75) | Blood donors (184) | No association |
| Ivanyi *et al.*, 1983 | Caucasian | Feighner (62) | -(1018) | No association |
| Rosler *et al.*, 1980 | Caucasian | Feighner (107) | Blood donors (600) | A28 (RR=3.1) |
| Miyanaga *et al.*,1984 | Japanese | DSM-III (77) | Blood donors (1252) | No association |
| Rudduck *et al.*1984a | Caucasian | DSM-III (100) | Blood donors (919) | No association |
| Rudduck *et al.*1984b | Caucasian | DSM-III (116) | Blood donors (919) | No association |
| Adler *et al.*,1985 | Caucasian | RDC (14) | -(365) | No association |
| Amar *et al.*, 1988 | Jewish | -(32) | -(151) | No association |
| Metzer *et al.*, 1988 | Caucasian | DSM-III (53) | Blood donors (114) | No association |
| Alexander *et al.*, 1990 | Caucasian | DSM-III (55) | Published data (1029) | No association |
| DiMichele *et al.*1990 | Caucasian | DSM-III (36) | - (500) | No association |
| Campion *et al.*, 1991 | Caucasian | DSM-III (107) | Relatives (174) | No association |
| Wright *et al.*, 1995 | Caucasian | DSM-III-R (93) | Screened controls (141) | A9 (RR=1.94) A24 subspecificity Of A9 (RR=2.76) |
| Blackwood *et al.*, 1996 | Caucasian | RDC&DSM-III-R (107) | -(133) | B35(corrected P=0.004, RR=0.06) Cw5 (corrected P=0.05, RR=0.38) |
| | | Blood donors (264) | B35 | |
| Ozcan *et al.*,1996 | Caucasian | -(75) | -(3731) | No association |
| Jocobsen *et al.*, 1998 | Caucasian | DSM-IV(28 children) | Population controls (51) | No association |
| Gibson *et al.*, 1999 | Caucasian | DSM-III-R (256) | Blood donors (261) | No association |
| Debnath *et al.*,2005 | Indian (Bengalee) | DSM-IV-TR (50) | Blood donors (100) | A3 (RR=5.66) |
| *Total studies* | *Total patients per ethnic group* | *Total controls per group§* | *Associations reported more than once* | |
| 35 serotyping studies | Caucasian 3146 (including 28 children | 7802 unknown | 4 studies:A9 or A24 subspecificity of A9 | |
| Igenotyping study | Japanese 213 | 4895 blood donors | 3 studies: A28 | |
| | African-USA 92 | 4788 population | 3 studies: A10 | |
| | Jewish 32 | 2335 published data | | |
| | 174 relatives | | | |
| | 141 screened controls | | | |

* Table based on data from Nimgaonkar *et al.*,(1992), Hawi *et al.*, (1999), Index Medicus, MEDLINE and EMBASE searches from 1974 to 2000, and personal communications, † RR=relative risk when significant association remains after correction for multiple comparisons; n=number of schizophrenic patients or number of controls; diagnostic criteria utilized in the above studies are those of Feighner *et al.*, (1972) and Bleuler (1950), the International Classification of Diseases 9 (WHO,1978), the Diagnostic and Statistical Manual III, III-R and IV (American Psychiatric Association, 1980, 1987, 1994) and the Research Diagnostic Criteria of Spitzer *et al.*, 1978), ‡ All studies utilized HLA serotyping, except that of Gibson *et al.*, (1999) which utilized genotyping, § Total controls per group is not equal to total number of controls, because the same control groups were used by some investigators. [This table has been reproduced (with slight modification) by seeking permission from the Review , by Padraig Wright *et al.*, title "Schizophrenia and HLA: a review", Volume 47, pg no.4-5, Copyright Elsevier ,2001.]

$\chi^2$ test was performed to compare the frequency of each antigen among the patient and control group followed by Fisher's exact test. Since testing for a large number of antigens can reveal at least one positive association where none really exists, the *p* values from each Fisher's exact test had to be less than the Bonferroni *p* [0.05 divided by the number of antigens tested which equals to 0.003125] to be called statistically significant.[21] Relative risk was estimated by using Haldane's method (1956).[29]

## RESULTS AND DISCUSSION

The incidence and frequency of HLA Class I antigens among patients and control has been presented in table 3. There was a significantly higher frequency of HLA-A*03 ($\chi^2$=11.458, *p*=1.155e-4) in patients than the control groups. On the other hand HLA-A*25 ($\chi^2$=13.619, *p*=9.185e-5), A*31 ($\chi^2$=22.562, *p*=8.793e-8) and B*51 ($\chi^2$=40.047, *p*=1.604e-12) showed lower value significantly even after the Bonferroni correction. Though A*02 ($\chi^2$=6.052, *p*=6.699e-3)showed lower frequency and B*07 ($\chi^2$=4.069, *p*=2.035e-2) and B*42 ($\chi^2$=4.522, *p*=1.632e-2) showed higher frequency they were not found to be significant after the Bonferroni correction.

A significant higher frequency of HLA-A*03 observed in the present study is in accordance with the previously reported study by Debnath *et al.*[21] which is also in accordance with the study of Rudduck *et al.*, (1984a, 1984b)[30] in Swedish population. Although the association was found to be significantly higher, the present study did not reveal very strong association as it has been reported earlier.

On the other hand, in the present study A*25, A*31 and B*51 showed significantly lower negative value which is the unique finding of the present study. Among these alleles, A*31 and B*51 showed strong negative associations (RR=0.014 and RR=0.006 respectively). The increased frequency of A*11 found in the previous study was not reproducible in the present study. Apart from this, several other alleles like B*7 and B*42 showed higher value but were not statistically significant. We also observed a negative association of A*2 but the association was not found to be significant which was in accordance with the findings of Debnath *et al.*[21] But the finding was unlike the previous findings by Luchins *et al.*, (1980)[31] which showed positive association of A*02 with schizophrenia in African-USA population. However, we have not found association between HLA-A*23 and A*24 in our study as has been reported by previous studies.

Many microbial factors have been implicated in the pathogenesis of schizophrenia, but so far each microbial factor has been identified in a relatively small subgroup of patients.[32][33] The heterogeneity of these microbial factors is also reflected by the associations with different HLA loci and their alleles.[34] Polymorphic HLA molecules process, select and present degraded microbial proteins.[35][36] The set of inherited HLA alleles determines susceptibility or resistance to particular microbes.[34]

**Table 3: Phenotype frequency, Chi square, relative risk (RR) values and probability of HLA-A and B loci alleles in the patients with schizophrenia and healthy controls**

| Antigen | Patients (N=50) | Control (N=50) | Chi-square | Chi square (y) | RR | *P* value |
|---|---|---|---|---|---|---|
| A*02 | 24 | 37 | 7.103 | 6.052 | 0.332 | 6.699e-3* |
| A*03 | 50 | 38 | 13.636 | 11.458 | 32.792 | 1.155e-4† |
| A*11 | 36 | 31 | 1.130 | 0.723 | 1.558 | 1.975e-1 |
| A*23 | 21 | 26 | 1.003 | 0.642 | 0.673 | 2.115e-1 |
| A*24 | 32 | 26 | 1.477 | 1.026 | 1.624 | 1.555e-1 |
| A*25 | 10 | 29 | 15.174 | 13.619 | 0.188 | 9.185e-5† |
| A*26 | 21 | 17 | 0.679 | 0.382 | 1.395 | 2.684e-1 |
| A*29 | 33 | 32 | 0.043 | 0.000 | 1.089 | 5.000e-1 |
| A*30 | 33 | 25 | 2.627 | 2.011 | 1.914 | 7.787e-2 |
| A*31 | 0 | 20 | 25.000 | 22.562 | 0.014 | 8.793e-8† |
| B*07 | 47 | 39 | 5.315 | 4.069 | 3.951 | 2.035e-2* |
| B*21 | 36 | 38 | 0.207 | 0.051 | 0.817 | 4.099e-1 |
| B*4001 | 17 | 13 | 0.761 | 0.428 | 1.451 | 2.565e-1 |
| B*4201 | 39 | 28 | 5.472 | 4.522 | 2.711 | 1.632e-2* |
| B*44 | 14 | 10 | 0.877 | 0.493 | 1.532 | 2.414e-1 |
| B*5101-5105 | 0 | 30 | 42.857 | 40.047 | 0.006 | 1.604e-12† |

y= Yates's correction, * Significant, † Significant after the Bonferroni correction Bonferroni's probability is 0.003125, Note= The abbreviation 1.156 e-4 means 1.156 x 10-4, like wise the other values may be interpreted.

The analysis of the demographic variables suggests the present schizophrenic population is not in equal composition for the different ethnic group. The study comprises more number of Bengali populations. However, as mentioned earlier they were strictly matched according to their ethnicity, age and sex with the patients. The study comprise of higher number of male schizophrenics, which suggest the higher vulnerability of men to this disorder, at least in this region. The higher number of married patients in this study may be due to the strict social customs and strong social bondage of the Indian society.

The present study supports earlier finding of association of HLA-A*03 with schizophrenia along with the negative association of some more alleles, which is the new finding of the present study. However, it is too early to speculate the exact mechanism of the association. The result is preliminary and so far not correlated with the parameters like birth status, viral infections, prenatal infections, etc. However, this study provides the evidence for the possible existence of a susceptibility locus for schizophrenia within the HLA region. Given the size of our sample the result of our finding should be interpreted with caution. The present study needs to be replicated in the large sample size to strengthen our hypothesis of genetic association of HLA Class I antigen with schizophrenia.

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# Dopamine Receptors and the Dopamine Hypothesis in Schizophrenia

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## ABSTRACT

Alteration in dopaminergic activity may play an important role in the pathogenesis of schizophrenia. In the present study we investigated the incidence of dopamine receptor genes in the schizophrenic patients of Siliguri sub-division and adjacent areas to understand the dopamine hypothesis of schizophrenia in the Indian context. We used a nested case-control study design.Low resolution PCR-SSP method was applied for typing the dopamine receptor genes. The result showed the significant increase of dopamine D1 receptor gene along with the significant decrease of dopamine D3 receptor gene. The results suggest the possible association of dopamine D1 receptor gene with the schizophrenia.

*Key words*: dopamine, schizophrenia, dopamine receptor gene, etiopathology.

## INTRODUCTION

The term schizophrenia, which literally means "split mind", was first applied by a Swiss psychiatrist, Eugen Bleuler, in 1911(Calkins and Iacono, 2003). It is a severely debilitating neuropsychiatric disorder characterized by 'disturbances of thought', auditory hallucinations and multiple delusions (Shaw et al., 1998). Although the worldwide prevalence of schizophrenia is about 1%, (Sawa and Syndar, 2000), it has become a leading public health problem now-a-days and exerts enormous personal and economic costs worldwide. In spite of tireless research efforts, the etiological process or processes by which a causal agent creates the pathophysiology of schizophrenia is not yet clearly understood (Buchanan and Carpenter, 2000).

Several etiological theories have been proposed for schizophrenia like developmental (Horning *et al.*, 2002) or neurodegenerative processes, neurotransmitter abnormalities (Aghajanian et al., 2000), viral infection and immune dysfunction or autoimmune mechanisms. In addition, there is substantial evidence for genetic predisposition in case of schizophrenia (Mueser and Mc Gurk, 2004). Different chromosomes have been pinpointed as harboring genes involved in the pathogenesis of schizophrenia (Barondes *et al.*, 1997).

In recent studies dopamine hypothesis continues to provide the principal conceptual gateway into the mysteries of schizophrenia. Dopamine hypothesis of schizophrenia was first suggested by Van Rossum in 1966 (Seeman, 2004). The central effects of dopamine are mediated by at least five G protein-coupled receptors, Dl, D2, D3, D4 and D5. Molecular genetic evidence for this dopamine hypothesis has been supported by several studies. However, past association studies mainly with dopamine receptor D1, D2 and D3 yielded inconsistent results. A large number of studies have also shown the association between the dopamine D3

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and D2 receptor genes and schizophrenia in Chinese (Liao *et al.*, 2001) and Swedish population (Jönsson et al., 2003), while in other studies neither any linkage of D2 receptor has been observed among Italian (grassi et al, 1996), Swedish and Californian populations (Moises *et al.*, 1991) nor any significant association has been found with dopamine D3 receptor gene (Yang *et al.*, 2005). the study of Jensen *et al.*, (1993) suggested that dopamine D1 receptor gene is unlikely to be associated with schizophrenia. The reason may be due to the diagnostic method which particularly in earlier studies, are imprecise and vary greatly (Singer *et al,* 1982). The source of controls is not always described in sufficient details to ensure that results are not simply due to population stratification. Significant results are not always corrected for the number of statistical tests performed (Hawi *et al.*, 1999).

Considering the above mentioned inconsistency of specific dopamine gene association with schizophrenia, we were stimulated to examine the possible association of dopamine receptor gene with the disease in the Indian perspective.

## MATERIALS AND METHODS

***Subjects:*** India-born Bengali psychiatric patient & referred to the psychiatric outpatient Department (OPD) of Psychiatry, North Bengal Medical College and Hospital were considered for the present study. Patients were diagnosed independently by two psychiatrist according to the standard diagnostic criteria of DSM IV and assessed by the Brief Psychiatric Rating Scale (BPRS). After diagnosis, 30 schizophrenic patients were included for the study. Further, the schizophrenic patients and the family members were made to answer a questionnaire containing self-reported age, cast, sex, medical history, age of onset, month of birth, marital status, education, substance abuse, incidence of any autoimmune disease among patients or in family members etc. A total number of 30 unrelated, ethnically matched healthy individuals were considered as controls. All the participants provided their written consent for giving the blood sample after the study procedures were explained.

***DNA isolation***: 5ml blood was drawn by vein puncture method and was collected in EDTA, an anticoagulant for the DNA extraction. DNA was extracted from peripheral mononuclear cells of the blood by the Phenol Chloroform method.

***PCR amplification with Sequence Specific Primer (SSP)***: Polymerase chain reaction was performed for all the dopamine receptor genes using 10ng of DNA, 250mM dNTP, 1.5mM MgCl2, 5µl 10X buffer (Sigma-Aldrich Pvt Ltd), 1.5 units of Taq polymerase (Sigma-Aldrich Pvt Ltd) and 0.1mM each of the primer (Sigma-Aldrich Pvt Ltd) in a total volume of 50ml.

***Statistical analysis:***Phenotypic frequency was calculated by direct count. The frequency of the alleles of all the dopamine receptor genes is compared in the patient group as a whole with that of the control population using $c^2$ test. Relative risk was also been estimated.

## RESULTS

Table II shows the allele profile of the dopamine receptor genes for the patients with schizophrenia, and the healthy control subjects. The results demonstrate a marked elevation in the frequency of dopamine D1 receptor gene in patients with schizophrenia, compared with control subjects ($c^2$=17.55, $p<0.01$). Statistically, this was highly significant even after Bonferroni and Yates correction. In addition, we also noticed the significantly decreased frequency of dopamine D3 receptor gene among the patients.

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# Immune Dysregulation:Can it Be an Etiological Factor for Schizophrenia?

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## ABSTRACT

In the present investigation the incidence of HLA Class I antigens, C-reactive protein (CRP), cytokines were studied along with the demographic variables in the schizophrenic patients of Siliguri sub-division and adjacent areas to enlighten the immune dysregulation and autoimmune etiopathology of schizophrenia in the Indian context. Low resolution PCR-SSP method was applied for typing the HLA antigens. The level of serum CRP was investigated by latex agglutination test. Serum level of cytokines were measured by ELISA method. Further the patients and family members were made to answer a questionnaire to assess the demographic variables. The result showed the significant increase for HLA A*03 antigen along with the significant decrease for HLA-A*25, A*31 and HLA-B*51. Further, the drug naïve patients showed elevated level of CRP, cytokines such as IL-2 showed decrease level in the schizophrenic group. The demographic variables showed significantly increase frequency of schizophrenia among the patients who are not the first child. The results suggest the possible association of HLA antigen with schizophrenia like most of the other autoimmune disorders. The findings are suggestive of immune dysregulation in schizophrenia. The study also supports the hypothesis of increase risk of developing the disorder among the younger children of the family.

*Key words*: Autoimmunity, schizophrenia, HLA, CRP, cytokine,

## INTRODUCTION

Schizophrenia is a debilitating, often chronic, mental disorder characterized by disturbances in thinking, perception, emotion, and social relationships. The term schizophrenia, which literally means "split mind", was first applied by Swiss psychiatrist Eugen Bleuler in 1911.(Calkins and Iacono, 2003). Several etiological theories have been proposed for schizophrenia , including developmental (Horning *et al.*, 2002) or neurodegenerative processes (Lieberman, 1999), neurotransmitter abnormalities (Aghajanian and Muck, 2000), viral infection and immune dysfunction or autoimmune mechanisms (Noy *et al.*, 1994). In addition, there is substantial evidence for a genetic predisposition to schizophrenia (Mueser and MeGurk, 2004). However, none of the current etiological theories can fully explain the varied symptoms observed in different patients. Several lines of evidence indicate that immunological dysfunction may have

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relevant implication for the etiology of schizophrenia. It was suggested that viral infections and/or autoimmune reactions against central nervous structures may play an important role in the pathogenesis of the disease (Kirch,1993)

With recent advances in technology and an increased understanding of the immune system, autoimmune theories of schizophrenia have once again become a major focus of research. Many general immune abnormalities have been reported in schizophrenia. These include increase (Masserini *et al.*, 1990), decrease (Nyland *et al.*, 1980, Coffey *et al.*, 1983) or unchanged (Printz *et al.*, 1999) lymphocyte population, morphological changes in lymphocytes (Fessel and Hirala Hibi1963), altered levels of CD4+, CD45RA+ T cells, CD8+ T cells(Cazzullo *et al.*,1998), CD5+ B cells (Printz *et al.*, 1999), altered levels of IL-2 (Interleukin-2)(Becker *et al.*, 1996, IL-6 (Ganguli *et al.*, 1994, Akiyama ,1999), IFNγ (Interferon γ) (Arolt *et al.*, 2000) and increased levels of antiviral antibodies (Kaufmann *et al* ,1983).In other study elevated level of C-reactive protein (CRP) is also found in schizophrenic patients (Fan *et al.*, 2006).

Since the HLA (Human leukocyte antigens) system governs the immune responses, and many of proven autoimmune disorder had shown association with HLA, this system were implicated in the etiology of schizophrenia. A large number of studies have also shown the association between the class I HLA antigens and schizophrenia viz. HLA-A9. HLA-A23 (Ivany *et al.*,1983) and HLA-A24 (Asaka *et al.*,1981). Recently HLA-A*03 showed positive association among paranoid schizophrenics in the Bengali population of Siliguri (Debnath et al., 2005 ). Association of HLA-A1 and schizophrenia have been reported by Lahdelma *et al.*, (1998). In a similar research conducted in south Sweden the significant increases were found for A2, A3, B17, B27 and CW2 and decrease of A1, A11 and B8 among the schizophrenic patients (Rudduck *et al.*,1984). Past association studies with various HLA Class I alleles yielded inconsistent results (Nimgaonkar *et al.*, 1992), except HLA-A9.

The present study has been undertaken to study whether there is any immune dysregulation is schizophrenia and to investigate the autoimmune basis of schizophrenia in the Indian perspective.

## MATERIALS AND METHODS

India-born Bengali psychiatric patient referred to the psychiatric outpatient Department (OPD), North Bengal Medical College and Hospital were considered for the present study. Patients were diagnosed independently by two psychiatrist according to the standard diagnostic criteria of DSM IV and assessed by the Brief Psychiatric Rating Scale (BPRS). After diagnosis, 64 schizophrenic patients were included for the study. Further, the schizophrenic patients and the family members were made to answer a questionnaire containing self-reported age, cast, sex, medical history, age of onset, month of birth, marital status, education, substance abuse, incidence of any autoimmune disease among patients or in family members etc. A total number of 50 unrelated, ethnically matched healthy individuals were considered as controls. All the participants provided their written consent for giving the blood sample after the study procedures were explained.

Five ml blood was drawn by vein puncture method out of which 2 ml was collected in EDTA, an anticoagulant for the DNA extraction. 3 ml blood was allowed to coagulate at the room temperature for 2-3 hrs. Blood clot was cut and centrifuged for separating the serum. The serum samples were aliquoted and kept at -70 refrigerator. DNA was extracted from peripheral mononuclear cells of the blood by the Phenol Chloroform method.fifty number of

the patients and controls were typed for the HLA Class I antigens by PCR-SSP technique. The typing and sequence information of primers were taken from Bunce *et al.*, (1995). The primers, Taq polymerase, nucleotides etc. were obtained from Bangalore Genei, India. The CRP level in the serum was measured by latex agglutination slide test (Ranbaxy Fine Chemicals Ltd., HP, India). Serum IL-2 levels were measured by enzyme linked immunosorbent assay kit (Endogen Human IL kit, Pierce Biotechnology, Inc. Rockford). The sensitivities were <6pg/ml and <1pg/ml respectively, with inter and intra assay coefficient of variation of <10%. Absorbance was measured by a microtiter plate reader set at 450 nm. For the IL-2 study the patients were grouped into two categories, one under antipsychotic medication and the second without antipsychotic medication i.e. neuroleptic-free, under this category the patients who have not taken antipsychotic for minimum period of 1month were considered.

## RESULTS

The incidence and frequency of HLA Class I antigens among patients and control has been presented in Table I. There was a significantly higher frequency of HLA-A*03 ($X^2$=11.458, p=1.155e-4) in patients than the control groups. On the other hand HLA-A*25 ($X^2$=13.619, p=9.185e-5), A*31 ($X^2$=22.562, p=8.793e-8) and B*51 ($X^2$=40.047, p=1.604e-12) showed lower

**Table I:** Phenotype frequency, Chi square , relative risk (RR) values and probability of HLA-A and B loci alleles in the patients with schizophrenia and healthy controls.

| Antigen | Patients (N=50) | Control (N=50) | Chi-square | Chi square (y) | RR | P value | |
|---|---|---|---|---|---|---|---|
| A*02 | 24 | 37 | 7.103 | 6.052 | 0.332 | 6.699e-3 | * |
| A*03 | 50 | 38 | 13.636 | 11.458 | 32.792 | 1.155e-4 | † |
| A*11 | 36 | 31 | 1.130 | 0.723 | 1.558 | 1.975e-1 | |
| A*23 | 21 | 26 | 1.003 | 0.642 | 0.673 | 2.115e-1 | |
| A*24 | 32 | 26 | 1.477 | 1.026 | 1.624 | 1.555e-1 | |
| A*25 | 10 | 29 | 15.174 | 13.619 | 0.188 | 9.185e-5 | † |
| A*26 | 21 | 17 | 0.679 | 0.382 | 1.395 | 2.684e-1 | |
| A*29 | 33 | 32 | 0.043 | 0.000 | 1.089 | 5.000e-1 | |
| A*30 | 33 | 25 | 2.627 | 2.011 | 1.914 | 7.787e-2 | |
| A*31 | 0 | 20 | 25.000 | 22.562 | 0.014 | 8.793e-8 | † |
| B*07 | 47 | 39 | 5.315 | 4.069 | 3.951 | 2.035e-2 | * |
| B*21 | 36 | 38 | 0.207 | 0.051 | 0.817 | 4.099e-1 | |
| B*4001 | 17 | 13 | 0.761 | 0.428 | 1.451 | 2.565e-1 | |
| B*4201 | 39 | 28 | 5.472 | 4.522 | 2.711 | 1.632e-2 | * |
| B*44 | 14 | 10 | 0.877 | 0.493 | 1.532 | 2.414e-1 | |
| B*5101-5105 | 0 | 30 | 42.857 | 40.047 | 0.006 | 1.604e-12 | † |

y= Yates's correction

* Significant

† Significant after the Bonferroni correction

Bonferroni's probability is 0.003125

(Note: The abbreviation 1.156 e-4 means $1.156 \times 10^{-4}$, like wise the other values may be interpreted)

**Table II.** Comparison of demographic and clinical characteristics between the normal / elevated CRP groups.

| | Elevated CRP N=3 | | Normal CRP N=61 | | | |
|---|---|---|---|---|---|---|
| | Mean or N% | Standard deviation | Mean or N% | Standard deviation | Statistic (Z) | *P* value |
| Age | 37.67 | 21.13 | 34.69 | 9.64 | F[2,60]=0.24 | >0.62 |
| Gender<br>Men<br>Women | 33.33% | | 70.49% | | $X^2$ [1]=1.84 | >0.17 |
| Drug naïve patients<br>Patients under antipsychotic medication | 100% | | 11.48% | | $X^2$ [1]=17.00 | <0.001 Significant |
| Substance abuse<br>Yes<br>No | 33.33% | | 55.74% | | $X^2$[1]=0.58 | >0.44 |
| First child<br>Yes<br>No | 33% | | 18.03% | | $X^2$[1]=0.44 | >0.50 |
| Autoimmune disease in patients or in family<br>Yes<br>No | 0% | | 24.59% | | $X^2$[1]=0.97 | >0.32 |

**TableIII:** Comparison of serum IL-2 levels between psychotropic medication free, antipsychotic medicating schizophrenic patients with the normal controls.

| Variables | Psychotropic medication free schizophrenic group (n=20) | Control (n=30) | P value | |
|---|---|---|---|---|
| IL-2 (pg/ml) | Mean= 38.76<br>SD=27.23 | Mean=56.04<br>SD=18.82 | P<0.05 * | t= - 2.656<br>d.f.=48 |

| Variables | Antipsychotic medicating schizophrenic group (n=30) | Control (n=30) | P value | |
|---|---|---|---|---|
| IL-2 (pg/ml) | Mean=34.54<br>SD=22.09 | Mean=56.04<br>SD=18.82 | P<0.001* | t= - 4.058<br>d.f.=58 |

* = significant

**Table IV.** Comparison of demographic characteristics between the first schizophrenic child and the other schizophrenic siblings in different families.

| | N=50 | | Analysis | |
|---|---|---|---|---|
| | patients who are first child | patients who not the first child | Statistic | P value |
| 1. Number of patients | 11 | 39 | $\chi 2$ =15.68 (df=1) | <0.001 * |
| 2. Age of Onset (years) | | | | |
| Mean | 24.45 | 28.03 | t = -1.056 | = 0.30 |
| Standard deviation (SD) | 12.84 | 8. 97 | | |

value significantly even after the Bonferroni correction. Though A*02 ($X^2$=6.052,p=6.699e-3) showed lower frequency and B*07 ($X^2$=4.069, p=2.035e-2) and B*42 ($X^2$=4.522, p=1.632e-2) showed higher frequency they were not found to be significant after the Bonferroni correction.

Table II shows the comparison of demographic and clinical characteristics between the normal / elevated CRP groups. Sera levels of CRP were measured for 64 schizophrenic patients. The elevated level of CRP (e"6mg/l) was observed in 3 patients and 61 patients were found to have normal CRP (<6mg/l). All the three elevated cases were found to be of paranoid type. Further, when the level of CRP was compared to the other demographic variables as shown in the Table I, only the drug naïve status of the patients showed statistically significant value ($X^2$ = 16.997, *P* value <3.75x $10^5$).

The results of IL-2 assay is summarized in Table III. The serum IL-2 level in antipsychotic medicating patients were found to be significantly lower (34.54±22.09 pg/ml) than the control subjects (56.04±18.82,t= -4.058; df=58; p<=0.00015). Whereas the serum IL-2 level in drug naïve patients (45.69±51.49pg/ml) and controls (56.04±18.81pg/ml) were found to have no significant difference with each other.

Further, as shown in Table IV, when the Chi- squire test was performed between the first child patients and the patients who were not the first child, the test showed a significantly high incidence of schizophrenia among the patients who are not the first child. It is worth mentioning here that both the groups of the patients were HLA-A3 positive.

## DISCUSSION

A significantly higher frequency of HLA-A3 obtained in the present study is in accordance with the previously reported study by Debnath *et al.* (2005) which is also in accordance with the study of Rudduck *et al.*(1984). Although the association of HLA-A3 antigen and schizophrenia is found to be significantly higher, the present studies do not reveal a very strong association as has been reported earlier.

On the other hand, in the present study A*25, A*31 and B*51 showed significantly lower negative value which is the unique finding of the present study. Among these alleles, A*31 and B*51 showed strong negative associations (RR=0.014 and RR=0.006 respectively). The increased frequency of A*11 found in the previous study was not reproducible in the present

study. Apart from this, several other alleles like B*07 and B*42 showed higher value but were not statistically significant. We also observed a negative association of A*02 but the association was not found to be significant which was in accordance with the findings of Debnath *et al.*, (2005).But the finding was unlike the previous findings by Luchins et al., (1980) which showed positive association of A*02 with schizophrenia in African-USA population. However, we have not found association between HLA-A*23 and A*24 in our study as has been reported by previous studies.

The elevation of CRP in this study provides further evidence of the involvement of inflammatory processes behind the etiopathology of schizophrenia. The elevated level of CRP in our study is in accordance to the findings of Fan and Dikerson (2007). But unlike previously reported findings, we have considered the CRP level of patients with their medication status, which showed significantly higher value. In one study, the level of CRP was found to be higher in the patient who was experiencing psychotic symptoms, in the follow up study in the non-psychotic state, the level of CRP was found to be normal (Ohaeri *et al.*, 1993). In this respect the present study suggests that the antipsychotic drug may perhaps down regulate the inflammatory process which in turn brings the CRP level to the normal state. Thus, these findings further suggest that the inflammation may be another possible mechanism in the etiopathology of schizophrenia. It is however not clearly understood whether the elevation of the level of CRP is the by-product of the pathophysiology of schizophrenia or directly contributes to the clinical manifestations of the disorder (Fan *et al.*, 2007).

To our knowledge this is the first attempt to study the role of IL-2 in the Indian Bengalee schizophrenic subjects. The major findings of the present study are the decreased level of IL-2 in the schizophrenic group who were under antipsychotic medication. The finding is new for the lower level of IL-2 in the antipsychotic medicating patients. Our finding is in contrast to previous findings by Ebrinc et al., (2002) and Zhang *et al.*, (2005) who have found elevated level of IL-2. Also the finding is in contrast to the studies by Kim et al.,(2000) who found an increase of IL-2 serum level in Korean schizophrenic patients. On the other hand our finding is in agreement to Theodoropoulou *et al.*, (2001) who found that IL-2 serum levels were significantly lower in both medicated and non-medicated schizophrenic patients.

At this moment it is too early to speculate the autoimmune etiopathology of schizophrenia but the result of our present investigation definitely strengthens the hypothesis of immune dysregulation in schizophrenia which may be one of the etiological factor for the disorder. Our study also supports the hypothesis of increase risk of developing the disorder among the younger children of the family. Further, studies are needed to reveal the mechanism of the changes in the immune system in the disorder.

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