PRINT: ISSN 0972-3757 ONLINE: 2456-6360
Int J Hum Genet, 5(4): 273-277 (2005)
DOI: 10.31901/24566330.2005/05.04.08

Study of HLA-Linked Genes in Paranoid Schizophrenia in an Indian Bengalee Population

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KEYWORDS Paranoid schizophrenia; etiology; HLA antigens; association; paranoid symptoms

ABSTRACT Schizophrenia is a major psychotic disorder with a strong genetic component and molecular etiology remains enigmatic. They form a heterogeneous and poorly understood collection of disorders of which Paranoid Schizophrenia is the best diagnosed and least severe clinical subtype of Schizophrenia. Involvement of biological factors has been suspected for long. In the present investigation, the incidence of HLA Class-I antigens has been studied to understand the role of HLA genes in the patients with paranoid schizophrenia with the objectives to explore a possible immunogenetical etiology of paranoid schizophrenia. A case-control study design was performed. Psychiatric reference data were available for total number of 30,000 cases attended between 1998 and 2004. A total number of 120 patients with paranoid schizophrenia belonging to the India born Bengalee population were initially enrolled for the study and DSM-IV criteria was used for the diagnosis of the patients. Upon longitudinal follow up 70 patients turned out to be the cases of other psychotic conditions and PCR-based molecular typing (PCR-SSP) method was applied to 50 genuine cases of Paranoid Schizophrenia. A total numbers of 100 healthy donors belonging to the same ethnic background were considered as controls. The present investigation shows that some of the HLA antigens are associated with paranoid schizophrenia and especially significant increases are found for HLA-A*03 gene which may influence susceptibility to paranoid schizophrenia. The study reveals important interactions between HLA genes and paranoid schizophrenia. This preliminary observation may help to understand the etiological basis of this disorder.

INTRODUCTION

Schizophrenia is a debilitating mental illness, occurs worldwide and affects 1% of the population (Sawa and Synder 2002). Schizophrenia, as a diagnostic category, is heterogeneous, allowing considerable variations in symptoms, clinical course, prognosis and pathophysiology, and the essential biological pathology of schizophrenia is still only partially understood (APA 1994). Different chromosomes have been pinpointed as harbouring genes involved in the pathogenesis of Schizophrenia (Barondes et al. 1997). A susceptibility locus has been identified on chromosome 6 and some association studies involving human leukocyte antigen (HLA) genes have reported diverse results (Gibson et al. 1999; Schwab et al. 2002). Several researchers have also found evidence for schizophrenia vulnerability genes on chromosomes 6p close to the HLA genetic region by linkage analysis (Schwab et al. 1995). Many psychiatric conditions such as psychosis, depression and anxiety have been considered in autoimmune disorders (Denburg et al. 1997) and it has been postulated that autoimmune mechanisms account for a proportion of schizophrenia and been found that schizophrenic patients with an affected first-degree relative are significantly more likely to have parent or sibling with an autoimmune diseases (Wright et al. 1996). Autoimmune pathology of schizophrenia has been proposed (Ganguli et al. 1987) because of increased levels of antibrain antibodies (Shima et al. 1991).

DSM-IV-TR (APA 2000) recognizes five classic subtypes of Schizophrenia. Amongst them Paranoid Schizophrenia is the commonest type of schizophrenia in most parts of the world. The clinical picture is dominated by relatively stable, often paranoid delusions, usually accompanied by hallucinations, particularly of the auditory variety and perceptual disturbances (ICD-10 1992). Onset tends to be later in life and

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the course of paranoid schizophrenia varies and may be episodic, with partial or complete remissions or chronic (Andreasen 1995).

The first HLA association study of schizophrenia was reported by Cazzullo et al. in 1974. More than 60 association studies have been reported since then. HLA and schizophrenia was first reviewed by McGuffin (1979), who comented 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 (Burch 1964). The results have often been unconfirmed or contradictory when schizophrenia has been regarded as a single uniform disorder. It has been established that the pattern of HLA association was different in the schizophrenic patients across different ethnic group, which may be the result of genetic and geographic origins. More consistent findings have been reported in studies of subtypes of schizophrenia (Rudduck et al. 1984). The consistent findings in HLA studies of clinical subtypes like hebephrenic and paranoid patients have been reported because the diagnostic criteria of these two categories are more strictly defined.

The aim of the present investigation is to study association between HLA Class-I genes and paranoid schizophrenia because it may lead us to further investigations whether different categories of schizophrenia bear similar etiological mechanisms or other factors like infections, environmental factors, neurodevelopmental processes etc. modulate the manifestation of clinical features.

MATERIALS AND METHODS

Subjects: The subjects were recruited from India-born Bengalee population referred to the Psychiatric OPD, North Bengal Medical College and Hospital. On an average 1500 new patients with different psychiatric conditions and about 4000 recurrent follow up cases attend the OPD every year. A total number of 50 (37 male and 13 female) unrelated patients with paranoid schizophrenia who attended the 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 were assessed by the Brief Psychiatric Rating Scale (BPRS). The average age was 34.96 ± 1.40 and male to female ratio of 2.85: 1 were studied.

A total number of 100 ethnically matched healthy individuals were considered as controls. All controls subjects were screened for a recent history of intercurrent infections and allergies. Those with a past history of autoimmune or psychiatric disorders were excluded. All the patients and controls gave informed consent and matched for socio-economic variables.

Methodology: DNA was obtained from peripheral mononuclear cells in ethylene diamine tetra acetate anticoagulant, using salting out procedure (Miller et al. 1988). Low resolution molecular typing using PCR-SSP technique was done for detecting HLA-Class I genes. The primers, Taq polymersae, 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. The frequency of each antigen in the patient group as a whole was compared with the control population using χ^2 test, and which 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 statistically significant. Relative risk was estimated as recommended by Svejgard (1974).

RESULTS

The present study has been undertaken to investigate the HLA-Class I phenotype frequency in patients with paranoid schizophrenia and compared with the frequency of HLA class-I alleles of healthy controls. The data shown in Table 1 are the incidence of HLA class I genes in the patients with paranoid schizophrenia and compared to healthy controls. It was found that HLA-A*03 (50% vs. 15%) showed very significant values ($\chi^2 = 20.88$, $p<0.01$) even with Bonferroni correction in the patients. Apart from this, several other alleles like A*11 (32% vs. 12%),

B*07 (22% vs. 10%), B*45 (14% vs. 5%) also showed increased frequency but was not significant with Bonferroni correction. Only A*02 showed significant negative values in the paranoid schizophrenia.

Significant HLA-A and B haplotypes among sporadic patients with paranoid schizophrenia and healthy control subjects are shown in the Table-2 and Table-3 respectively. Some of the prominent haplotypes observed in the patients were not seen amongst healthy population. For example, A*01 – B*13, A*02 – B*07, A*03 – B*5101, A*23 – B*49 and A*26 – B*7 were noticed only in the patients. However, only A*02 – B*07 haplotype was common in both the groups. Similarly, several haplotype combination occurred in healthy population of which the most significant was A*03 – B*37 and A*23 – B*35.

Table 1: Phenotype frequency, Chi Square, relative risk (RR) values and probability of HLA-A and B loci alleles in the patients with paranoid schizo-phrenia and healthy controls.

Antigen	*% Phenotype frequency*		*Chi square*	*RR*	*p*
	Patients (N=50)	*Controls (N= 100)*			
A*01	12	12	0	1	NS
A*02	12	23	2.5862	0.45	NS
A*03	50	15	20.8806**	5.66	Significant
A*23	12	10	0.1399	1.22	NS
A*24	8	16	1.8461	0.45	NS
A*25	8	10	0.1575	0.78	NS
A*26	1	29	0.3333	1.37	NS
A*11	32	12	8.7822**	3.45	NS
A*29	12	8	0.6302	0.99	NS
A*30	10	7	0.4076	1.47	NS
B*5101	16	15	0.0256	1.07	NS
B*07	22	10	3.9867*	2.53	NS
B*08	14	9	0.8745	1.64	NS
B*13	14	6	2.6951	2.55	NS
B*15	6	13	1.714	0.42	NS
B*16	8	1	5.0689*	0.6	NS
B*18	10	10	0	1	NS
B*35	6	10	0.6737	0.57	NS
B*44	8	8	0	1	NS
B*45	14	5	3.6684*	3.09	NS
B*49	12	8	0.6302	1.56	NS

*p<0.05, **p<0.01

NS= Not Significant; p must be <0.0014 to be declared significant

Table 2: Significant haplotypes and delta values per 10000 among patients.

Haplotypes	*HF*	*Delta values*	χ^2
A*01 – B*13	132.33	177.31	2.11
A*02 – B*5101	120.12	171.81	1.52
A*02 – B*07	200.35	272.68	3.11
A*03 – B*5101	447.46	691.98	5.35
A*03 – B*45	224.89	437.64	1.49
A*23 – B*49	144.27	182.60	2.93
A*26 – B*07	200.35	272.68	3.11
A*29 – B*08	132.33	177.31	2.11

Table 3: Significant haplotypes and delta values per 10000 among healthy controls.

Haplotypes	*HF*	*Delta Values*	χ^2
A*01 – B*18	187.72	155.94	8.2
A*02 – B*07	164.35	101.48	1.81
A*02 – B*40	118.65	68.63	1.03
A*03 – B*27	178.23	134.06	4.42
A*03 – B*37	237.89	197.84	10.67
A*23 – B*5101	125.38	85.33	1.96
A*23 – B*35	81.71	55.38	81.71
A*23 – B*37	141.04	120.09	7.30
A*24 – B*18	122.98	80.13	1.62
A*24 – B*40	131.37	97.29	2.99
A*25 – B*5101	181.81	141.76	5.44
A*25 – B*07	81.71	55.38	1.23
A*25 – B*15	81.71	55.38	1.23
A*11 – B*5101	117.42	69.09	1.06
A*11 – B*15	123.55	81.90	1.73
A*11 – B*37	82.89	57.60	1.39
A*11 – B*49	82.89	57.60	1.39

DISCUSSION

Chronic diseases of the central nervous system including schizophrenia are suspected by many investigators of having genetic, immunological, and viral etiology (Michels and Marzuk 1993). Several findings suggest that immunological dysfunctions may have relevant implications for the etiology of schizophrenia. Accumulating evidence suggests that in some cases, schizophrenia is accompanied by changes in the immune system, such as the presence of anti brain antibodies in serum (Henneberg et al. 1994), an altered distribution of T-cell subsets (Muller et al. 1993), reduced mitogen-induced lymphocyte production of interleukin- 2(IL-2), IFNα (Arolt et al. 2000), increased serum levels of interleukin-2 soluble receptor a (IL-2sRα) (Hornberg et al. 1995) and an increased serum levels of interleukin 6(IL-6) (Frommberger et al. 1997). These findings indicate that aberrant immune function in schizophrenia may be associated with the manifestation of the clinical phenotype and with disease processes (McAllister et al. 1997). Strong evidence in favour of autoimmune etiology of Paranoid Schizophrenia came from the recent study by Wilke et al. 1996 where they have shown that a

significantly decreased production of IFN-α in acutely ill paranoid schizophrenics.

Study on the immunogenetic aspect of disease is most useful in identifying not only the mode of inheritance of a particular disease process but also in understanding the immunopathogenic mechanisms underlying it. The discovery of HLA associations with specific disease implies that at least part of their genetic basis lies in the MHC and suggests that it may be possible to determine their etiology (Wright *et al*, 2001). Incidentally, most diseases that show strong HLA association have unknown etiology and mode of inheritance, e.g. various autoimmune and rheumatological diseases.

Our present investigation represents the possible associations between paranoid schizophrenia and HLA antigens. The most of the previous study on the involvement of HLA system in paranoid schizophrenia yielded inconsistent results as most of them focussed on schizophrenia as a whole. The association between HLA-A24 has been reported previously (Asaka et al. 1981). HLA-A9 has been shown to be elevated in paranoid schizophrenia in at least seven studies (McGuffin and Stuart 1986). Studies that have divided HLA-A9 in to subspecies, have found associations to both HLA-A23 (Ivanyi et al. 1983) and HLA-A24 (Crowe et al. 1979). But Alexander et al. 1990 have found no association between either HLA-A23 or A24 and the paranoid subtype, but expressed doubt over an association between HLA-A24 and schizophrenia.

In the present investigation, we found a significant association between paranoid schizophrenia and HLA-A*03 gene. When the strength of association was measured by cross product ratio or relative risk of developing a disease, HLA-A*03 showed a very high value i.e. RR 5.6, thus reflecting a very strong positive association. We also observed moderately strong association of HLA-A*11 with paranoid schizophrenia which coincided with the previous findings of Alexander et al. (1990). However, interestingly it has been noticed that HLA-A*02 showed negative association. We found no association between either HLA-A23 or A24 in our patient group as previously reported by some studies.

However, the exact nature of the mechanism underlying the empirically observed association between HLA-A*03 antigen and the paranoid schizophrenia is not fully understood. Few studies have also correlated the associations of HLA and the influence of prenatal infections, winter birth etc with the Schizophrenia (Narita et al. 2000). It is also to be noted that this result could not be an artifact arising from inadvertent ethnic mismatching of cases and controls, as there is no ethnic group known for which the HLA – A*03 frequency is higher than about 19%. The pattern of HLA-A3 distribution in Indian subcontinent and rest of the countries are as follows: in South African San Population it is 15.5, in Mongolian population 4.8, in Italian population 12.9, in Australian Aborigines 6.6 and in Indian Tribe it is 6.0 (Imanishi et al. 1992). More interestingly the frequency of A3 antigen in two other major populations in our region is 18.52 in Rajbanshi population (Mandal et al. 2000) and 12 in Gurkha population (Chaudhuri et al. 1995).

At this moment, we are not in a position to propose autoimmune pathogenesis of paranoid schizophrenia. The result is preliminary and so far not correlated with the parameters like birth status, viral infections, prenatal infections etc. But we can assume the possible existence of a susceptibility locus within the HLA region and it may be that HLA-A*03 gene is the sole determinant of paranoid schizophrenia. However, this significant association might contribute to the disease risk or else that there might be a separate susceptibility gene in strong linkage disequilibrium with A*03 gene.

REFERENCES

Alexander RC, Coggiano M, Daniel DG, Wyatt RJ 1990. HLA antigens in Schizophrenia. *Psychiatry Research*, **31:** 221-233.

Andreasen NC 1995. Symptoms, signs and diagnosis of Schizophrenia. *Lancet*, **346:** 477-481.

American Psychiatric Association 1994. *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)*. 4th Edition, Washington, DC: APA.

American Psychiatric Association 2000. *Diagnostic and Statistical Manual of Mental Disorders*. 4th Edition, Text Revision. Washington DC: APA.

Arolt V, Rothermundt M, Wandinger KP, Kirchner K 2000. Decreased *in vitro* production of interferon–gamma and interleukin-2 in whole blood of patients with schizophrenia during treatment. *Molecular Psychiatry*, **5:** 150-158.

Asaka A, Okazaki Y, Namura I, Juji T, Miyamajo M, Ishakawa B. 1981. Study of HLA antigens among Japanese Schizophrenics. *British Journal of Psychiatry*, **138:** 498-500.

Barondes SH, Alberts BM, Andreasen NC, Bargmann C, Benes F, Goldman-Rokic P, Gottesman I, Heinemann SF, Jones EG, Kirschner M, Lewis D, Roff M, Roses A, Rubenstein J, Snyder S, Watson

SJ, Weinberger DR, Yolken RH 1997. Workshop on Schizophrenia. *Proc Natl Acad Sci USA,* **94:** 1612-14.

Bunce M, O'Neill CM, Barnardo MCNM, Krausa P, Browning MJ, Morris PJ et al.1995. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 and DQB1 by PCR with 144 primer mixes utilizing sequence specific primers (PCR-SSP). *Tissue Antigens,* **46:** 355-367.

Burch PRD 1964. Schizophrenia: some new etiological considerations. *British Journal of Psychiatry,* **110:** 818-896.

Cazzullo Cl, Smeraldi E, Penati G 1974. The leukocyte antigenic system HL-A as possible genetic marker of schizophrenia. *British Journal of Psychiatry,* **125:** 612-615.

Chaudhuri TK, Mandal TK, Sen TK, Taneja V 1995. Distribution of HLA antigens in Indian Gurkha population. *Indian Journal of Medical Research,* **101:** 170-172.

Crowe R, Thompson J, Flink R, Weinberger B 1979. HLA antigens and Schizophrenia. *Archives of General Psychitry,* **36:** 231-233.

Denburg SD, Carbotte RM, Denburg JA 1997. Psychological aspects of systemic lupus erythematosus: cognitive function, mood, and self-report. *J Rheumatology*, **24:** 998-1003.

Frommberger UH, Bauer J, Haselbauer P, Fraulin A, Riemann D, Berger M 1997. Interleukin-6-(IL-6) plasma levels in depression and schizophrenia: comparison between the acute state and after remission. *Eur Arch Psychiatry Clin Neurosci,* **247:** 228-233.

Ganguli R, Rabin BS, Kelly RH, Lyte M, Raghu U 1987. Clinical and laboratory evidence of autoimmunity in acute schizophrenia. *Ann NY Acad Sci USA,* **496:** 676-685.

Gibson S, Hawi S, Straub RE, Walsh D, Kendler KS and Gill M 1999. HLA and Schizophrenia; refutation of reported associations with A9 (A23/24), DR4 and DQB1*0602. *Am J Med Genet*, **88:** 416-421.

Henneberg AE, Horter S, Ruffert SS 1994. Increased prevalence of antibrain antibodies in the sera from schizophrenic patients. *Schizophrenia Research,* **14:** 15-22.

Hornberg M, Arolt V, Kruse A. and Kirchner H 1995. Lymphokine production in leukocyte cultures of patients with schizophrenia. *Schizophrenia Research*, **15:** 237-242.

ICD-10 *Classification of Mental and Behavioural Disorders.* 1992. WHO, Geneva.New Delhi : Oxford University Press,pp. 86-109.

Imanishi T, Akaza A, Kimura A, Tokunaga K, Gojabori T 1992. Allele and Haplotype frequencies of HLA and complement loci in various ethnic groups. In: K Tsuji, M Aizawa, T Sasazuki (Eds.): *HLA 1991. Proceedings of the 11th International Histocompatibility Workshop and Conference*. Oxford: Oxford University Press, Vol. 1, pp. 1064-74.

Ivanyi P, Droes J, Schreuder GMT, D'Amaro J and Van Rood JJ 1983. A search for association of HLA antigens with paranoid schizophrenia. *Tissue Antigens*, **22:** 186-193.

Mandal BB, Chaudhuri TK 2000. HLA profile of Rajbanshi and Gurkha: A comparative Account. In: Sudhanshu Ghosal and Dipak Ray (Eds.): *Recent Advances of Animal Science Research* . Vol. 1. WB: Orion Press International, pp. 121-125.

McAllister CG, van Kammen DP, Kelley ME 1997. Relapse prediction in schizophrenia: from monoamines to cytokines. In: D Ebert, KP Ebmeier, WP Kasschka, T Rechlin (Series Eds.): *Advances in Biological Psychiatry.* vol. 18. AE Henneberg, WP Kaschka (Eds.). *Immunological Altercations in Psychiatric Diseases*. Karger, Basel, pp. 44-49.

McGuffin P 1979. Is schizophrenia an HLA associated disease? *Psychol Med,* **9:** 721-728.

McGuffin P, Stuart E 1986.Genetic markers in Schizophrenia. *Human Heredity,* **36:** 65-88.

Michels R and Marzuk PM 1993. Progress in psychiatry. *N Engl J Med,* **329:** 552-560

Miller SA, Dikes DED, Polesky HF 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Research,* **16:** 1215.

Muller N, Hofschuster E, Ackenheil M and Eckstein R 1993. T-cells and psychopathology in Schizophrenia: relationship to the outcome of neuroleptic therapy. *Acta Psychiatr Scand,* **87:** 66-71.

Narita K, Sasaki T, Akaho R, Okazaki Y, Kusumi I, Kato T, Hashimoto O, Fukada R, Koyama T, Matsuo K, Okabe Y, Nauko S, Hohjoh H, Tokunaga K 2000. Human Leukocyte Antigen and season of birth in Japanese patients with Schizophrenia. *American Journal of Psychiatry*, **157:** 1173-1175.

Rudduck C, Franzen G, Low B and Rorsman B 1984. HLA antigens and clinical subgroups of schizophrenia. *Human Heredity,* **34:** 18-26.

Sawa A, Synder SH 2000.Schizophrenia: diverse approaches to a complex disease. *Science*, **296:** 692-695.

Schwab SG, Albus M, Hallmeyer J, Honig S, Borrmann M, Lichtermann D, Ebstain PR, Akenheil M, Lerer B, Risch N. 1995 Evaluation of susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. *Nature Genetics,* **11:** 325-7.

Schwab SG, Hallmayer J, Freimann J, Lerer B, Albus M, Borrmann-Hassenbach M, Segman RH, Trixler M, Rietschel M, Maaier W and Wildenaauer DB 2002. Investigation of Linkage and Association / Linkage Disequilibrium of HLA A-, DQA1-, DQB1-, and DRB1-Alleles in 69 sib-pair – and 89 Trio Families with Schizophrenia. *American Journal of Medical Genetics (Neuropsychiatric Genetics),* **114:** 315-320.

Shima S, Yano K, Sagiura M and Tokunaga Y 1991. Anticerebral antibodies in functional psychoses. *Biol Psychiatry,* **29:** 322-328.

Svejgaard A, Jersild C, Nielsen LS, Bodmer WF 1974. HLA antigens and disease. Statistical and genetical considerations. *Tissue Antigens,* **4:** 95-105.

Wilke I, Arolt V, Rothermundt M, Weitzsch C, Hornberg M. and Kirchner H 1996. Investigations of cytokine production in whole blood cultures of paranoid and residual schizophrenic patients. *Eur Arch Psychiatry Clin Neurosc,* **246:** 279-284.

Wright P, Sham PC, Gilvary CM, Jones PB, Cannon M, Sharma T, Murray RM 1996. Autoimmune diseases in the pedigrees of schizophrenia and control subjects. *Schizophrenia Research,* **20(3):** 261-267.

Wright P, Nimgaonkar VL, Donaldson PT, Murray RM. 2001. Schizophrenia and HLA: A review. *Schizophrenia Research,* **47:** 1-12.