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Association of HLA-B40 and DRW9 with Japanese Alcoholic Liver Cirrhosis

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MIYAMOTO, K., H. ISHII, H. TAKATA, S. TAKAGI, Y. SHIGETA, S. SEKIGUCHI, K. SUYAMA, H. KOHNO AND M. TSUCHIYA. *Association of HLA-B40 and DRW9 with Japanese alcoholic liver cirrhosis.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 467–471, 1983.—Seventy-seven chronic alcoholics with liver disease were studied to evaluate the HLA antigen association. There were no significant differences of HLA antigen phenotype frequencies (PF) between the patients and controls regarding A and C loci, (62 healthy Japanese). Prevalences of HLA-B40 complex (B40·48·13) and DRW9 tended to increase among chronic alcoholics. When chronic alcoholics were divided according to whether they had liver cirrhosis or not, the cirrhosis group (42 cases) revealed a significantly higher frequency of HLA-DRW9 ($\chi^2=10.88$, $p<0.001$, corrected $p<0.05$, relative risk (R.R.)=4.17) as compared to controls. There was also a tendency of B40 complex to increase in frequency ($\chi^2=5.51$, $p<0.05$, R.R.=2.65) in the cirrhosis group. Haplotype frequency and linkage disequilibrium parameters of HLA-B40·48-DRW9 were significantly higher than those of controls. Moreover, the increased frequency of DRW9 in the cirrhosis group was similar to that in autoimmune disease like ulcerative colitis or SLE. These data suggest that HLA-DRW9 and/or HLA-B40-DRW9 might be closely associated with susceptibility to developing alcoholic cirrhosis and that autoimmune mechanisms might be involved partly in its etiology.

HLA antigen Immunogenetic marker Alcoholism Alcoholic cirrhosis

AN individual susceptibility to the development of chronic alcoholism seems greatly different under the same condition of alcohol abuse. It is still not known, however, on what this biological feature is dependent. On the other hand, various degrees of liver damage result from prolonged and excessive consumption of alcohol and a number of statistical and experimental studies have clarified the direct toxicity of alcohol or its metabolite to liver. However it is unequivocal that only a relatively small portion (10–30%) of alcoholics develop cirrhosis. These facts lead us to the assumption that, among other mechanisms including malnutrition, there may also be an immunogenetic mechanism in the etiology of alcoholism or alcoholic cirrhosis.

Recently, a variety of immunologic abnormalities have been described in chronic alcoholics [15, 17, 18]. Although HLA antigen as immunogenetic marker is very important to clarify individual susceptibility to disease, the association between HLA antigen and alcoholic liver disease is controversial. The present study was designed to elucidate the relationship between the pattern of HLA antigen and chronic alcoholism or alcoholic cirrhosis in Japan.

METHOD

Seventy-seven chronic alcoholics with liver disease were studied (75 males and 2 females, all hospitalized in National Institute on Alcoholism or Keio University Hospital). They were divided into those with cirrhosis (42 cases) and non-cirrhotics (fatty liver and alcoholic hepatitis, 35 cases). Their average daily alcohol intake was 149±8 g, and the average period of alcohol intake was 20.7±7.8 years. There was no significant difference between these two groups. The average ages were 49.5 (29–67) years in the cirrhosis group and 45.0 (30–60) years in non-cirrhosis group. Diagnosis of liver disease was made by needle biopsy or laparoscopy. All of the subjects were negative for blood HBs antigens and had no history of major surgery. Sixty-two healthy Japanese were selected for the control group.

Lymphocytes were isolated by Ficoll-Isopaque gradient centrifugation within a few hours of venipuncture. B-Lymphocytes for DR typing were isolated by the modified thrombin-anti Fab monolayer method [5,7]. HLA typing was done by the NIH standard microcytotoxicity method, using the anti-HLA-A, -B, -C and -DR antisera of the 8th Interna-

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TABLE 1

HLA-A ANTIGEN PHENOTYPE FREQUENCIES (PF) IN CHRONIC ALCOHOLICS

HLA antigen	Controls (62 cases) No.	PF (%)	Chronic Alcoholics (77 cases) No.	PF (%)	χ^2	p	R.R.
A1	1	1.6	3	3.9		ns	
A2	28	45.2	34	44.2		ns	
A3	1	1.6	1	1.3		ns	
A11	12	19.4	15	19.5		ns	
AW24 (9)	37	59.7	37	48.1		ns	
A26 (10)	12	19.4	19	24.7		ns	
AW31	10	16.1	15	19.5		ns	
AW33	11	17.7	20	26.0		ns	
Blank	12	19.4	13	16.9		ns	

p: Uncorrected.
R.R.: Relative risk.
ns: Not significant.
HLA-AW23 (9), A25 (10), A28, A29, AW30, AW32, AW34, AW36 and AW43 showing 0% of PF in controls and chronic alcoholics are excluded.

TABLE 2

HLA-B ANTIGEN PHENOTYPE FREQUENCIES (PF) IN CHRONIC ALCOHOLICS

HLA antigen	Controls (62 cases) No.	PF (%)	Chronic Alcoholics (77 cases) No.	PF (%)	χ^2	p	R.R.
B7	9	14.5	9	11.9		ns	
B8	1	1.6	0	0		ns	
B13	0	0	5	6.5	2.51	*	9.48
BW35	9	14.5	15	19.5		ns	
BW39 (16)	3	4.8	6	7.8		ns	
BW44 (12)	11	17.7	14	18.2		ns	
BW46	3	4.8	1	1.3		ns	
BW48	1	1.6	3	3.9		ns	
BW51 (5)	10	16.1	19	24.7		ns	
BW52 (5)	15	24.2	13	16.9		ns	
BW54 (22)	11	17.7	10	13.0		ns	
BW55 (22)	5	8.1	3	3.9		ns	
BW56 (22)	2	3.2	1	1.3		ns	
BW58 (17)	1	1.6	1	1.3		ns	
BW59	4	6.5	1	1.3		ns	
BW60 (40)	6	9.7	14	18.2		ns	
BW61 (40)	10	16.1	14	18.2		ns	
BW62 (15)	9	14.5	15	19.5		ns	
8W57 (SN2)	1	1.6	4	5.2		ns	
Blank	9	14.5	7	9.1		ns	
B5 complex							
BW51·52	24	38.7	31	40.3		ns	
B5·35	32	51.6	42	54.5		ns	
B40 complex							
B40·48	17	27.4	31	40.3		ns	
B40·48·13	17	27.4	35	45.5	4.77	<0.05	2.21

p: Uncorrected.
R.R.: Relative risk.
ns: Not significant.
HLA-B14, B18, B27, B37, BW38 (16), BW41, BW42, BW45 (12), BW47, BW49 (21), BW50 (21), BW53, BW57 (17), BW63 (15) and 8W59 (Bu) are excluded because of their rare frequencies.
BW51·52: BW51 or BW52, B5·35: BW51 or BW52 or BW35. B40·48: BW60 or BW61 or BW48, B40·48·13: BW60 or BW61 or BW48 or B13.
*$0.05<p<0.01$.

tional Histocompatibility Workshop and the 2nd Asia and Oceania Histocompatibility Workshop, and the antisera kindly supplied by Prof. Terasaki, UCLA. The numbers of the tested antigens were HLA-A locus:17, -B: 34, -C: 6 and -DR: 13, respectively. The specificities of these sera were tested and confirmed by the established Japanese panel cells. HLA-B5 (BW51, BW52) and BW35, HLA-B40 (BW60, BW61) and BW48 and B13 have strong cross-reactivity; therefore, these were analyzed as B5 or B40 complex, respectively.

Statistical analysis was performed using the χ^2 test with Yates' correction for the small number of samples. The corrected p value was calculated by multiplication of the number of antigens tested in each locus. The haplotype frequencies (HF) and coefficients of linkage disequilibrium (Δ) were calculated by the modified method of Mittal *et al.* [11], since the family study was not done.

RESULTS

Phenotype frequencies of HLA-A, -B, -C and -DR antigens in controls coincided with the Japanese data of the 8th International Histocompatibility Workshop. Regarding HLA-A and -C locus antigens, HLA-A2, AW24 (9), CW1 and CW3 were more prevalent in chronic alcoholics but were not significantly different from controls (Tables 1, 3). A group of HLA-B40 associated antigens (B40·48·13) in chronic alcoholics showed a higher frequency as compared to controls ($p<0.05$, corrected $p>0.05$), while HLA-B13 in chronic alcoholics showed a high relative risk value (Table 2). As shown in Table 4, a higher frequency of HLA-DRW9 in chronic alcoholics was observed; however this was not statistically significant after correction of the p value.

When chronic alcoholics were divided according to hepatic morphological features, the cirrhosis group revealed a significantly higher frequency of HLA-DRW9 ($\chi^2=10.88$, $p<0.001$, corrected $p<0.005$, relative risk=4.17) as compared to controls (Table 5). There were also an increase in frequency of HLA-B40 complex (B40·48·13) and a decrease in that of CW1 in the cirrhosis group, but these findings were not statistically significant when corrected. Markedly high relative risk value (13.46) of HLA-B13 was found in the non-cirrhosis group. This seemed to contribute to the increased value in all alcoholics (Table 2).

Furthermore, haplotype frequency and coefficient of linkage disequilibrium (Δ) were investigated to see whether HLA-B40 complex and DRW9 were closely associated with each other (Table 6). A significantly higher frequency of HLA-B40·48-DRW9 was found in cirrhosis group.

Table 7 shows characteristic phenotype frequencies of HLA antigens in various diseases which were detected using the same antisera as those in this series. A higher frequency of HLA-DRW8 was found in both groups of non-alcoholic

TABLE 3

HLA-C ANTIGEN PHENOTYPE FREQUENCIES (PF) IN CHRONIC ALCOHOLICS

HLA antigen	Controls (62 cases) No.	PF (%)	Chronic Alcoholics (77 cases) No.	PF (%)	χ^2	p	R.R.
CW1	22	35.5	17	22.1		ns	
CW2	1	1.6	0	0		ns	
CW3	32	51.6	43	55.8		ns	
CW4	3	4.8	8	10.4		ns	
CW5	1	1.6	0	0		ns	
CW6	1	1.6	1	1.3		ns	
Blank	61	98.4	87	113.0		ns	

p: Uncorrected.
R.R.: Relative risk.
ns: Not significant.

TABLE 4

HLA-DR ANTIGEN PHENOTYPE FREQUENCIES (PF) IN CHRONIC ALCOHOLICS

HLA antigen	Controls (62 cases) No.	PF (%)	Chronic Alcoholics (73 cases) No.	PF (%)	χ^2	p	R.R.
DR1	8	12.5	6	8.2		ns	
DR2	22	35.5	24	32.9		ns	
DR3	0	0	0	0		ns	
DR4	30	48.4	30	41.1		ns	
DR5	4	6.5	5	6.8		ns	
DRW6	5	8.1	8	11.0		ns	
DR7	0	0	0	0		ns	
DRW8	7	11.3	5	6.8		ns	
DRW9	13	21.0	34	46.6	9.69	<0.01	3.29
DRW10	1	1.6	1	1.4		ns	
8WDRW6Y	15	24.2	20	27.4		ns	
8WDRW17	1	1.6	2	2.7		ns	
Se-11*	3	4.8	2	2.7		ns	
Blank	15	24.2	13	17.8		ns	

*See [12].
p: Uncorrected.
R.R.: Relative risk.
ns:Not significant.

TABLE 5

HLA ANTIGEN PHENOTYPE FREQUENCIES (PF) IN CHRONIC ALCOHOLICS WITH OR WITHOUT LIVER CIRRHOSIS

HLA antigen	Controls (62 cases) No.	PF (%)	Chronic Alcoholics with Liver Cirrhosis (42 cases)* No.	PF (%)	Chronic Alcoholics without Liver Cirrhosis (35 cases)† No.	PF (%)
A2	28	45.2	15	35.7	19	54.3
AW31	10	16.1	10	23.8	5	14.3
B13	0	0	2	4.8	3	8.6‡
BW35	9	14.5	8	19.0	7	20.0
BW48	1	1.6	2	4.8	1	2.9
BW51 (5)	10	16.1	12	28.6	5	14.3
BW52 (5)	15	24.2	10	23.8	3	8.6
BW54 (22)	11	17.7	4	9.5	6	17.1
BW55 (22)	5	8.1	0	0	3	8.6
BW60 (40)	6	9.7	8	19.0	6	17.1
BW61 (40)	10	16.1	9	21.4	5	14.3
B5 complex						
BW51·52	24	38.7	21	50.0	10	28.6
B5·35	32	51.6	26	61.9	16	45.7
B40 complex						
B40·48	17	27.4	18	42.9	9	25.7
B40·48·13	17	27.4	21	50.0§	14	40.0
CW1	22	35.5	7	16.7¶	10	28.6
CW3	32	51.6	23	54.8	20	57.1
DR1	8	12.9	1	2.5	5	15.2
DR2	22	35.5	12	30.0	12	36.4
DR5	4	6.5	1	2.5	4	12.1
DRW9	13	21.0	21	52.5#	13	39.4
8WDRW6Y	15	24.2	12	30.0	8	24.2

*40 cases in HLA-DR antigen.
†33 cases in HLA-DR antigen. Other HLA phenotypes which showed no remarkable differences between the both groups are excluded.
‡$\chi^2=3.00$, $0.05<p<0.1$, R.R.=13.46.
§$\chi^2=5.51$, $p<0.05$, R.R.=2.65.
¶$\chi^2=4.41$, $p<0.05$.
#$\chi^2=10.88$, $p<0.001$, corrected $p<0.05$, R.R.=4.17.

liver cirrhosis and hepatocellular carcinoma, and this was different from HLA-DRW9 in chronic alcoholics with liver cirrhosis. The characteristic pattern of HLA antigen in chronic alcoholics with liver cirrhosis was similar to ulcerative colitis (HLA-DRW9) and systemic lupus erythematosus (HLA-B40·48·13 and -DRW9).

DISCUSSION

Recently an immunogenetic approach using HLA antigens has been prevailing among many researchers to find clues to the pathogenesis of certain diseases. The reason arises from the hypothesis that the disease susceptibility genes may be very near or included in the HLA region and transmitted holding strong linkage disequilibrium with HLA antigens [4].

As already reported, there appears to be significant association between HLA-A1 and B8 and autoimmune chronic active hepatitis [8], while association between HLA antigens and alcoholic liver disease is controversial [2, 3, 9, 10].

In alcoholic cirrhosis, increased frequencies of HLA-B8 [2,9], BW40 [3] and B13 [10] were reported. Meléndez [10] further described that association of HLA-BW40 in Norway [3] and B13 in Chile could be explained by a linkage disequilibrium between genes of the B loci. Interestingly, also in our results, HLA-B40 complex (B40·48·13) tended to be increased in chronic alcoholics with liver cirrhosis. Therefore it could not be ruled out that HLA-B40 associated antigens

TABLE 6
SIGNIFICANT LINKAGE IN CHRONIC ALCOHOLICS WITH LIVER CIRRHOSIS

	Haplotype	HF	Δ	χ^2	p
Chronic Alcoholics with Liver Cirrhosis (N=40)	B40·48-DRW9	117	37	5.55	<0.02
Controls (N=62)	B40·48-DRW9	33	17	—	—

HF: Haplotype frequency per 1000.
Δ:Linkage disequilibrium parameter per 1000.

might show common antigenicity beyond the racial differences for developing alcoholic cirrhosis.

On the contrary, Scott [13] and Gluud [6] found no significant association between HLA antigens and alcoholic cirrhosis. Gluud also emphasized a pitfall in statistical analysis and the importance of corrected p values.

However, most of studies thus far reported were limited to A, B (and partly C) loci. HLA-DR antigen is considered to correspond to murine Ia antigen and to be closely related to immune response. Therefore it was of interest to see whether HLA-DR antigen are associated with alcoholic cirrhosis. Indeed, the most important finding in this study was a significantly increased prevalence of HLA-DRW9 in chronic alcoholics with liver cirrhosis (Table 5). Moreover HLA-DRW9 was linked closely with B40 complex (Table 6). Since it is conceivable that a high frequency of HLA-B40 complex in chronic alcoholics with liver cirrhosis may reflect a secondary phenomenon due to the increased frequency of DRW9, it would be interesting to discern whether or not B13 or BW40 in the other reports [3,10] is also linked with HLA-DR antigens.

It is noteworthy that the HLA antigen pattern in chronic alcoholics with liver cirrhosis, as shown in Table 7, was different from those in non-alcoholic liver cirrhosis and hepatocellular carcinoma. Since the numbers of these illnesses tested were limited, this difference cannot be conclusive. However, such a difference in HLA antigen patterns of these diseases might give a clue to why hepatocellular carcinoma based on alcoholic cirrhosis is less prevalent than that based on posthepatitic cirrhosis.

On the other hand, HLA-DR antigen pattern in chronic alcoholics with liver cirrhosis was similar to that in autoimmune disease such as ulcerative colitis [1] or systemic lupus erythematosus [16], suggesting that autoimmune mechanism may be involved partly in the etiology of alcoholic cirrhosis.

We reported the increased prevalence of HLA-CW3 in chronic alcoholics, especially in alcoholic hepatitis or alcoholic cirrhosis in the previous paper [14], but this time we found no significant difference compared to controls. Regarding this point, unknown specificities of C locus still remain to be determined as shown in the large number of blanks in C locus. Therefore, association between C locus antigens and diseases should be clarified in the future. However HLA-CW3 is known to be linked with B40 complex in Japanese, and the haplotype, HLA-DRW9-B40-CW3 appears important in further clarifying the individual susceptibility to alcoholic liver disease.

A high relative risk of HLA-B13 in the non-cirrhosis group (Table 5) is difficult to be interpreted at present, and further quantitative analysis with a larger number of cases is needed.

Thus, characteristic pattern of HLA antigens associated with susceptibility for alcoholism was not found, while it was suggested that HLA-DRW9 and/or HLA-B40-DRW9 might be closely associated with susceptibility to the development of alcoholic cirrhosis and moreover autoimmune mechanism might play a role in the etiology. In order to further elucidate the contribution of immunogenetic factor in alcoholic liver disease, detection of HLA-D/DR antigens in many laboratories is needed.

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TABLE 7
CHARACTERISTIC HLA ANTIGEN PHENOTYPE FREQUENCIES IN VARIOUS DISEASES

HLA Antigens	Controls (N=62)	Alc-LC (N=42)	LC (N=20)	HCC (N=28)	UC (N=47)	SLE (N=34)
A26 (10)	19.4 (%)	(%)	(%)	39.3*(%)	(%)	38.2*(%)
BW35	14.5		45.0†§		48.9†	
BW52 (5)	24.2					
B5·35	51.6		85.0*			
B40·48	27.4					50.0*
B40·48·13	27.4	50.0* (N=40)	(N=21)		(N=45)	50.0*
DR2	35.5				68.9‡§	
DRW8	11.3		42.9†	35.7†§		
DRW9	21.0	52.5‡§			44.4†	52.9†§

*Uncorrected $p<0.05$.
†Uncorrected $p<0.01$.
‡Corrected $p<0.05$.
§4<R.R.<10. N: Number.
Alc-LC: Chronic alcoholics with liver cirrhosis, LC: Non-alcoholic liver cirrhosis, HCC: Hepatocellular carcinoma, UC: Ulcerative colitis, SLE: Systemic lupus erythematosus.

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