Title: Genetic Analysis of the HLA Region of Japanese Patients with Type 1 Autoimmune Hepatitis

Short title: Genetic Analysis of Japanese AIH Patients

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Abstract

Background/Aims: Genetic predisposition to type 1 autoimmune hepatitis (AIH) is linked mainly to HLA-class II genes. The aim of this study is to scan the HLA region for additional genes which may contribute to type 1 AIH susceptibility.

Methods: We performed association analysis using HLA class I and II alleles and 18 polymorphic microsatellite markers distributed throughout the HLA region. We specifically assessed tumor necrosis factor (TNF)-α gene polymorphisms.

Results: HLA-DRB1*0405, DRB4 and DQB1*0401 alleles were markedly significant in AIH patients. The association study revealed the presence of three segments in the HLA region showing significantly low *P* (Pc) values. The first segment was located around the HLA-DR/-DQ subregion, the second was around the HLA-B54 allele, and the third was around two microsatellites near the TNF gene cluster. However, stratification analysis for the effect of DRB1*0405 eliminated association of the latter two segments. Haplotype D of the TNF-α promoter gene polymorphisms was weakly associated with susceptibility, but was found to be not significant after stratification analysis.

Conclusions: The most influential gene on type 1 AIH pathogenesis in Japanese is the HLA-DRB1. Other genes in the HLA region, including TNF-α, have little or no association with type 1 AIH susceptibility.

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Keywords: Autoimmune hepatitis; Gene polymorphisms; Genetic susceptibility; HLA class II antigens; Microsatellite markers; Single nucleotide polymorphism; Tumor necrosis factor α

Introduction

Autoimmune Hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver, hypergammaglobulinemia and autoantibodies [1-3]. Several studies from ethnically different countries have clarified strong genetic bases for both disease susceptibility and behavior [4-14]. Recently, molecular analysis by the use of PCR-based DNA typing techniques have shown that the susceptibility to develop type 1 AIH is associated specifically with the DRB1*0301 and DRB1*0401 alleles in Caucasians [6-10], the DRB1*0405 allele in Japanese [11] and Argentine adults [12], DRB1*0404 in Mexicans [13], and DRB1*1301 in Argentine children [12] and Brazilians [14] at the HLA class II DRB1 locus, which encodes a polymorphic β chain of the HLA-DR antigen.

However, the association with these DRB1 antigens and susceptibility to type 1 AIH is not complete because not all AIH patients possess these antigens. This suggests that additional susceptibility genes (either HLA or non-HLA) and/or environmental factors may contribute to the development of type 1 AIH, such that finding candidate genes has been compared to searching for a needle in a haystack [15].Historically, candidate genes were searched for on an individual level. The current study searched for them comprehensively throughout the HLA region on the short arm of human chromosome 6. HLA, which encompasses 3.6 Mb, is divided into 3 regions, class II (1.1Mb), class III (0.7 Mb) and class I (1.8 Mb), from centromere to telomere. More than 200 genes are localized in the HLA region and many genes located at the telomeric end of the class III region are involved in immune and inflammatory responses, and possess a marked degree of polymorphism [16]. Association analysis using microsatellite markers is a powerful method for mapping candidate susceptibility genes of multifactorial genetic diseases [17, 18]. If the frequencies of some microsatellite markers show significant differences between patients and controls, susceptibility genes might exist near them and be analyzed by sequencing. To search for additional genes influencing the development of type 1 AIH in the HLA region, we performed association analysis using HLA class I and class II alleles and 18 microsatellites densely distributed within or just outside the HLA region.

In addition to HLA-DR, the tumor necrosis factor α (TNF-α) gene, which lies in the HLA class III region, is another candidate susceptibility gene. Czaja AJ et al. described that polymorphisms in the promoter region of the TNF-α gene at position -308 was associated with severity of AIH-1 in European and North-American patients in synergy with HLA-DR3 [8, 9, 19, 20]. To our knowledge, this phenomenon in Japanese type 1 AIH patients has not yet been demonstrated. Therefore, we investigated polymorphisms in the promoter region of the TNF-α gene as well.

Patients and Methods

Subjects. Seventy seven patients with type 1 AIH and 248 healthy Japanese controls were enrolled for investigation of genetic association with polymorphic genetic markers around the HLA region. Forty-six of these 77 patients were included in our earlier study [11]. They were all residents of Nagano Prefecture, Japan, and their racial backgrounds were all Japanese. All of the patients were diagnosed as probable or definite cases according to the scoring system from the International Autoimmune Hepatitis Group (Table 1) [21]. All patients were classified as having type 1 AIH based on antibody profiles. The average age of the AIH onset in this study was 55.8. The youngest and the oldest patients were 29 and 85 y.o., respectively. (Table 1).No viral markers, such as Hepatitis B surface antigen, anti-hepatitis B core antibody, anti-hepatitis C virus antibody (second generation) or hepatitis C virus RNA were detected in the serum. This study was approved by the Ethics Committee of Shinshu University School of Medicine. Informed consent, in writing, was obtained from each subject.

DNA preparation. Genomic DNA from patients and controls were isolated by phenolic extraction of sodium dodecyl sulfate-lysed and proteinase K-treated cells as described previously[18, 22].

HLA typing. HLA class I and II alleles were determined using Micro SSP^{TM} DNA Typing Kit (One Lambda, Canoga Park, CA). DNA typing of DRB1 and DQB1 alleles was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as previously described [18, 22, 23].

Microsatellite. To determine the number of repeat units of 18 microsatellite loci around the HLA region (Fig. 1), forward primers were synthesized by labeling at the 5’ end with the fluorescent reagents 6-FAM, HEX, or TET (PE Biosystems, Foster City, CA). PCR primers and conditions for amplifying C3-2-11, C2-4-4, C1-3-1, C1-2-5, C1-4-1, MICA-TM(GCT)n (MHC class I chain-related gene A transmembrane region) and C1-2-A were the same as described previously [24- 27]. PCR primers and conditions were comparable to those of the previous papers, for D6S276 [28, 29], MIB [30], TNFa [31, 32], TNFd [32], D6S273 [33], DQ-CARII [34], T16CAR [34], D6S2443 [35], D6S2444 [35], TAP1 [36], and D6S439 [27]. PCR-amplified products were denatured for 5 min. at 100°C, mixed with formamide-containing stop buffer, then electrophoresed on a 4% polyacrylamide denaturing gel containing 8 M urea in a Model 377 automated

DNA sequencer (PE Biosystems). Fragment sizes were determined automatically by means of GeneScan software (PE Biosystems) as described previously [18].

Genotyping of polymorphisms in the 5'-flanking region of the TNF-α gene. Five single-nucleotide polymorphisms at nucleotide positions -1031, -863, -857, -308, and -238 in the promoter region of the TNF-α gene were determined for these samples by direct sequencing as previously reported [18].

Statistical analysis. Gene and phenotype frequencies at polymorphic loci or sites including 18 microsatellites, HLA-DRB1, –DQB1 genes, and promoter haplotypes of the TNF-α gene were estimated by direct counting. The significance of the distribution of alleles between patients with AIH and normal controls was tested by the χ^2 method with continuity correction. The *P* value was corrected by multiplication by the number of alleles observed in each locus tested (corrected *P* value: *P*c value). A *P*c value of less than 0.05 was evaluated as statistically significant. To control for the effect of linkage disequilibrium between loci, Mantel-Haenszel weighted odds ratio (OR) was calculated [37], and again a *P* value of less than 0.05 was accepted as statistically significant.

Results

Tables 2 and 3 show the HLA-DRB1, 3, 4, 5 and DQB1 alleles in type 1 AIH patients and controls. DRB1*0405 (Pc<2.9 x 10^{-8}; OR 4.97), DRB4 (Pc<8.1 x 10^{-7}; OR4.92) and DQB1*0401 (Pc< 5.9 x 10^{-8}; OR 4.70) alleles were markedly significant in the patients.

The association study investigating disease susceptibility to type 1 AIH using polymorphic markers revealed the presence of three segments in the HLA region showing significantly low P (Pc) values (Table 4 and Figure 1). All of the alleles in each microsatellite marker were named on the basis of the amplified fragment size length. The first segment was located around the HLA-DR and -DQ subregion, with the most significant associations observed in the DRB1 gene (DRB1*0405), the DQB1 gene, (DQB1*0401) and allele 193 of the DQCARII microsatellite marker, which is located between the DRB1 and DQB1 loci (Pc<0.00015, OR 3.25).

The second segment was found around the HLA-B locus. Since strong linkage disequilibrium between HLA-B54 and DRB1*0405 had earlier been noted in a Japanese population [5], the significant association of HLA-B54 with AIH was tested for the possible confounding effect of DRB1*0405 and disappeared after stratification (Table 5; 0.88<OR<3.36).

Lastly, there were also significant associations of two microsatellites (TNFa and C1-2-A) giving rise to the third lowest P (Pc) value peak near the TNF gene cluster in the class III region. Within this segment, allele 115 of the TNFa microsatellite marker gave the lowest Pc value (χ^2=9.09, Pc<0.033). However, stratification for the effect of DRB1*0405 showed no significant association for TNFa115 and C1-2-A242 microsatellites with AIH (Table 5).

TNF-α promoter gene polymorphisms were also evaluated by the direct sequence method. Previously, five different haplotypes were assigned in terms of allelic combination at five polymorphic sites in the 5' flanking region in a Japanese population and were named haplotypes A-E [38]. Haplotype D of the TNF-α gene polymorphisms was weakly associated with susceptibility to AIH (OR 2.12, P=0.026, Pc=0.13, Table 6). However, stratification for the effect of DRB1*0405 eliminated this possibility (P=0.075, Table 7).

Discussion

This is the first comprehensive study to search for candidate genes responsible for type 1 AIH susceptibility in the HLA region, and confirms our previous findings that the HLA-DRB1 and/or –DQB1 loci are strongly associated with susceptibility to AIH. We showed that DRB1*0405 (Pc<2.9 x 10^{-8}; OR 4.97), DRB4 (Pc<8.1 x 10^{-7}; OR4.92) and DQB1*0401 (Pc< 5.9 x 10^{-8}; OR 4.70) alleles were markedly significant in the patients. We already reported that a predisposition to type 1 AIH in Japanese was associated with the HLA-B54/ DRB1*0405/ DQB1*0401 haplotype [11]. In Japanese, DQB1*0401 is in very strong linkage disequilibrium with DRB1*0405 and only one patient with the DRB1*0405 allele did not have the DQB1*0401 allele. Therefore, it is not possible to evaluate the association of DQB1*0401 with AIH susceptibility from a statistical point of view. DRB4 is in linkage disequilibrium with DRB1*04, DRB1*0701 and DRB1*0901 and all patients with the DRB1*0405 allele have the DRB4 allele. We previously reported that the DQA1 allele was also associated with AIH, but was not as strongly associated as the serological DR4 antigen. We suggested that its association may be explained by linkage disequilibrium with DR4 because DR4 is tightly linked to DQA1*0301[11].

In our earlier study, we proposed that the basic amino acid at position 13, which is present only on the DR2 and DR4 B1 molecules (arginine on DR2 and histidine on DR4), contributes to the susceptibility to type 1 AIH among Japanese, since all of the 6 DR4-negative patients with AIH (n=53) had DR2 [11]. However, our current study revealed that 10 out of the 77 AIH patients were negative for both DR4 and DR15 (DR2), and 32.5% of AIH patients and 39.1% of controls had DR15 (DRB1*1501; OR 0.44, DRB1*1502; OR 1.03). Therefore, we can no longer conclude that type 1 AIH in Japan is associated with DRB1 alleles encoding arginine or histidine at position 13. Genetic susceptibility to type 1 AIH in Caucasians is related to HLA alleles encoding the six amino acid sequence LLEQKR at position 67-72 of the DRB1 polypeptide [6, 8-10]. In Japan and Argentina, AIH susceptibility is linked to DRB1*0405. In Mexico, it is linked to DRB1*0404. DRB1*0405 and DRB1*0404 encode arginine (R) at position 71, which is at the lip of the antigen-binding groove of the HLA DR molecule and influences the interaction between antigen presenting cells and helper T cells. DRB1*0405 and DRB1*0404 share the LLEQ-R motif with DRB1*0301 and

DRB1*0401. It is likely that this motif presents the same or similar auto-antigen(s) since lysine at position 71 of DRB1*0301 and 0401 and arginine at the same position of DRB1*0405 and 0404 are basic and highly charged polar amino acids. Therefore, we can conclude that type 1 AIH susceptibility in Japanese maps to the DRB1 locus, and that arginine at position 71 of DRB1 is primarily associated with type 1 AIH. The same role of lysine at position 71 in European and North-American patients was proposed by Doherty DG et al. [6].

In Caucasians, patients with DRB1*0301 present with disease at a significantly younger age than patients with DRB1*0401 [7, 9, 10]. DRB1*0301 is in strong linkage disequilibrium with DRB3*0101, which encodes the LLEQKR motif at DRβ position 67-72. DRB1*0401 is in strong linkage disequilibrium with DRB4*0103, which encode the LLERRR at DRβ position 67-72. It has therefore proposed that number of LLEQKR motifs on the antigen presenting cells determines the clinical expression of AIH, called dose effects [7, 9, 10]. Japanese patients having the DRB1*0405-DRB4 haplotype express similar clinical features as Caucasoid patients with DRB1*0401; Disease onset is late and response to corticosteroid therapy is good. Only 6 out of the 77 patients were homozygous for DRB1 alleles (4 for DRB1*0405, and 1 each for 1302 and 1502). As a result, it was difficult to analyze the dose effects and clinical feature differences between patients with homozygous alleles and heterozygous ones.

Type 1 AIH in Brazilian patients and Argentine children is associated with DRB1*1301, which runs contrary to the shared motif model. Position 71 of DR13 is glutamine, which belongs to a different group of amino acids from arginine or lysine. The only amino acid difference between DRB1*1301 and 1302 is in position 86, so researchers in the above studies proposed an alternative hypothesis of susceptibility based on a valine/glycine dimorphism at position 86 of DRβ [14]. Of the 16 Japanese patients without DR4 (20.8%), 5 had DR13 (4 had 1302 and 1 had 1301). One of them was homozygous for the 1302 allele. Position 86 of DRβ of DRB1*0405 is also glycine. Therefore, valine at position 86 is not considered to be related to AIH in Japanese patients.

We also showed that a large number of oligoclonal αβT cells infiltrated into the liver of type 1 AIH patients [39]. T cells recognize antigens presented by MHC class I or class

II molecules via a specific T cell receptor and clonally expand. Therefore, specific T cells of AIH patients might be stimulated by unknown auto-antigen(s) in the groove of the HLA class II molecule. Taken together, our data strongly suggest that HLA-DRB1 and/or -DQB1 genes alone are the susceptibility genes in the HLA region.

TNF-α is a key cytokine in the inflammatory response. In Caucasian patients with type1 AIH, the frequency of the -308A allele (TNF*2) was significantly increased in cases with AIH [19, 20]. The TNF*2 allele is carried on the extended 8.1 ancestral haplotype (A1-B8-DRB1*0301) of northern Europeans [40], and previous reseachers described that TNF*2 allele may work in synergy with HLA DR3 to affect clinical manifestations and disease severity (autoimmune promoter hypothesis) [9, 10]. However, AIH in Brazilian patients was found not to be linked to TNF-α gene polymorphisms at position -308 [41]. Type 1 AIH in Brazil is also not associated with HLA DR3. It is therefore suggested that region-specific etiologic factors may affect disease susceptibility [42]. In our study, the frequency of the -308A allele (TNF*2) was very rare in both Japanese patients and controls (1.8% in AIH patients and 1.4% in controls, Table 4). TNF*2 is found in strong linkage disequilibrium with HLA DRB1*0301, which is also very rare in Japanese [11].

Lastly, we analyzed the TNF-α polymorphisms in the 5' flanking region to investigate whether other polymorphisms influenced the development of type 1 AIH in Japanese. Haplotype D of the TNF-α gene polymorphisms was weakly associated with susceptibility to AIH, but was found to be not significant after stratification analysis.

In conclusion, microsatellite analysis of the HLA region on the short arm of human chromosome 6 clearly shows that there are no further genes associated with the development of type 1 AIH besides HLA-DRB1 in Japanese. We are currently searching for other candidate susceptibility genes outside of the HLA region using microsatellite and single nucleotide polymorphism analysis. Future studies are needed to confirm the shared motif model and the autoimmune promoter hypothesis in Japanese patients, and to search for the auto-antigen(s) that trigger the disease. Taken together, these results may provide the specific tools for therapeutic intervention.

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Figure legend

Figure 1. AIH susceptibility gene mapping by association analysis using genetic markers in the HLA region. OR and *P* values obtained by association tests between control and patient groups are displayed with the location of genetic markers used for mapping. The gene map at the bottom indicates the location of these genetic markers in boxes, and representative genes in the HLA region are indicated by black ovals.

Table 1. Clinical Data of Japanese Type 1 AIH Patients

Features	AIH (n=77)
Age (year)	55.8±12.7 (29-85)
Women:men	64:13
ALT (nl: 7-45 U/L)	701.5±589.9 (40-2644)
Bilirubin (nl: 0.3-1.2 mg/dl)	5.4±7.3 (0.5-30.2)
Alkaline phosphatase (nl: 124-367 U/L)	468.5±288.8 (144-1405)
IgG (nl: 800-2000 mg/dl)	3189.9±1122.3 (1312-7248)
IgM (nl: 40-350 mg/dl)	348.2±323.5 (57-1498)

ALT, alanine aminotransferase; AIH, autoimmune hepatitis; nl, normal range

Table 2. HLA-DRB1 alleles in Type 1 AIH Patients and Controls

DRB1*	AIH n=77 (%)	Control n=248 (%)	OR	χ^2	P	Pc
0101	5 (6.5)	28 (11.3)	0.55	1.48	0.22	
1501	5 (6.5)	34 (13.7)	0.44	2.90	0.089	
1502	20 (26.0)	63 (25.4)	1.03	0.01	0.92	
1602	2 (2.6)	2 (0.8)	3.28	1.55	0.21	
0401	2 (2.6)	3 (1.2)	2.18	0.75	0.39	
0403	5 (6.5)	7 (2.8)	2.39	2.23	0.14	
0405	48 (62.3)	62 (25.0)	4.97	36.6	$1.5x10^{-9}$	$2.9x10^{-8}$
0406	4 (5.2)	13 (5.2)	0.99	0.00	0.99	
0410	1 (1.3)	8 (3.2)	0.39	0.81	0.37	
1001	1 (1.3)	2 (0.8)	1.62	0.16	0.69	
1101	3 (3.9)	15 (6.0)	0.63	0.52	0.47	
1201	11 (14.3)	16 (6.5)	2.42	4.73	0.030	
1202	2 (2.6)	5 (2.0)	1.30	0.09	0.76	
1301	1 (1.3)	2 (0.8)	1.62	0.16	0.69	
1302	7 (9.1)	33 (13.3)	0.65	0.97	0.33	
1403	1 (1.3)	6 (2.4)	0.53	0.35	0.55	

1406	2 (2.6)	13 (5.2)	0.48	0.93	0.33
0802	4 (5.2)	18 (7.3)	0.70	0.40	0.53
0803	12 (15.6)	39 (15.7)	0.99	0.00	0.98
0901	15 (19.5)	76 (30.6)	0.55	3.63	0.057

OR, odds ratio; Pc, corrected P

Table 3. HLA-DRB3, 4, 5 and HLA-DQB1 alleles in Type 1 AIH Patients and Controls

DRB3, 4, 5 DQB1*	AIH n=77 (%)	Control n-248 (%)	OR	χ^2	P	Pc
DRB3	24 (31.2)	89 (35.9)	0.81	0.58	0.45	
DRB4	64 (83.1)	124 (50.0)	4.92	26.4	$2.7x10^{-7}$	$8.1x10^{-7}$
DRB5	24 (31.2)	82 (33.1)	0.92	0.10	0.76	
DQB1*0301	14 (18.2)	44 (17.7)	1.03	0.01	0.93	
0302	8 (10.4)	26 (10.5)	0.99	0.00	0.98	
0303	15 (19.5)	69 (27.8)	0.63	2.13	0.14	
0401	47 (61.0)	62 (25.0)	4.70	34.2	$4.9x10^{-9}$	$5.9x10^{-8}$
0402	5 (6.5)	15 (6.0)	1.08	0.02	0.89	
0501	5 (6.5)	29 (11.7)	0.52	1.70	0.19	
0502	2 (2.6)	7 (2.8)	0.92	0.01	0.92	
0503	0 (0)	11 (4.4)	0.13	3.53	0.06	
0601	17 (22.1)	77 (31.0)	0.63	2.30	0.13	
0602	1 (1.3)	28 (11.3)	0.10	7.22	0.0072	0.084
0603	1 (1.3)	1 (0.4)	3.25	0.77	0.38	
0604	4 (5.2)	29 (11.7)	0.41	2.72	0.010	

OR, odds ratio; Pc, corrected P

Table 4. Statistically Significant Alleles Associated with Type 1 AIH Patients

Marker	No. of alleles	Significant allele	AIH n=77 %	Control n=248 %	OR	χ^2	P	Pc
D6S2443	8	185	9.1	2.4	4.03	6.81	0.0091	
T16CAR	12	211	9.1	2.4	4.03	6.81	0.0091	
DQB1	12	0401	61.0	25.0	4.70	34.24	$4.9x10^{-9}$	$5.9x10^{-8}$
DQCARII	10	193	70.1	41.9	3.25	18.70	$1.6x10^{-5}$	$1.6x10^{-4}$
DRB1	20	0405	62.3	25.0	4.97	36.58	$1.5x10^{-9}$	$2.9x10^{-8}$
D6S273	7	134	63.6	46.4	2.02	7.01	0.0081	
TNFa	13	115	48.1	29.4	2.22	9.09	0.0026	0.033
C1-2-A	14	242	62.3	42.7	2.22	9.05	0.0026	0.037
MICA-TM(GCT)n	5	179	44.2	29.8	1.85	5.32	0.021	
HLA-B	21	54	33.8	14.5	3.09	14.89	0.00011	0.0023
C1-2-5	21	202	19.5	10.5	2.07	4.31	0.038	
C2-4-4	13	251	19.5	10.1	2.16	4.81	0.028	
C3-2-11	18	213	28.6	14.5	2.36	7.92	0.0049	

OR, odds ratio; Pc, corrected P

Table 5. Association of HLA-B54 and Microsatellites TNFa115 and C1-2-A242 in Type 1 AIH Patients after Stratification for the Effect of DRB1*0405

DRB1*0405	HLA-B54	Control	AIH	Weighted OR	χ^2	P	95% CI
Present	Negative	42	24				
	Positive	20	24				
				1.72	1.91	0.17	0.88<OR<3.36
Absent	Negative	171	27				
	Positive	15	2				

DRB1*0405	TNFa115	Control	AIH	Weighted OR	χ^2	P	95% CI
Present	Negative	32	20				
	Positive	30	28				
				1.50	1.53	0.22	0.85<OR<2.64
Absent	Negative	143	20				
	Positive	43	9				

DRB1*0405	C1-2-A242	Control	AIH	Weighted OR	χ^2	P	95% CI
Present	Negative	25	12				
	Positive	37	36				
				1.55	1.85	0.17	0.88<OR<2.73
Absent	Negative	117	17				
	Positive	69	12				

OR, odds ratio; Pc, corrected P

95% CI, 95% confidence interval

Table 6. TNF-α Promoter Gene Polymorphisms

	Polymorphism in the 5'-flanking region					AIH	Control		
Haplotype	-1031	-863	-857	-308	-238	n=56 %	n=210 %	OR	P
A	T	C	C	G	G	78.6	90.0	0.41	0.042
B	C	A	C	G	G	25.0	28.6	0.83	1.192
C	C	C	C	G	A	5.4	3.3	1.64	0.959
D	T	C	T	G	G	48.2	30.5	2.12	0.026*
E	T	C	C	A	G	1.8	1.4	1.25	1.691

OR, odds ratio; Pc, corrected P

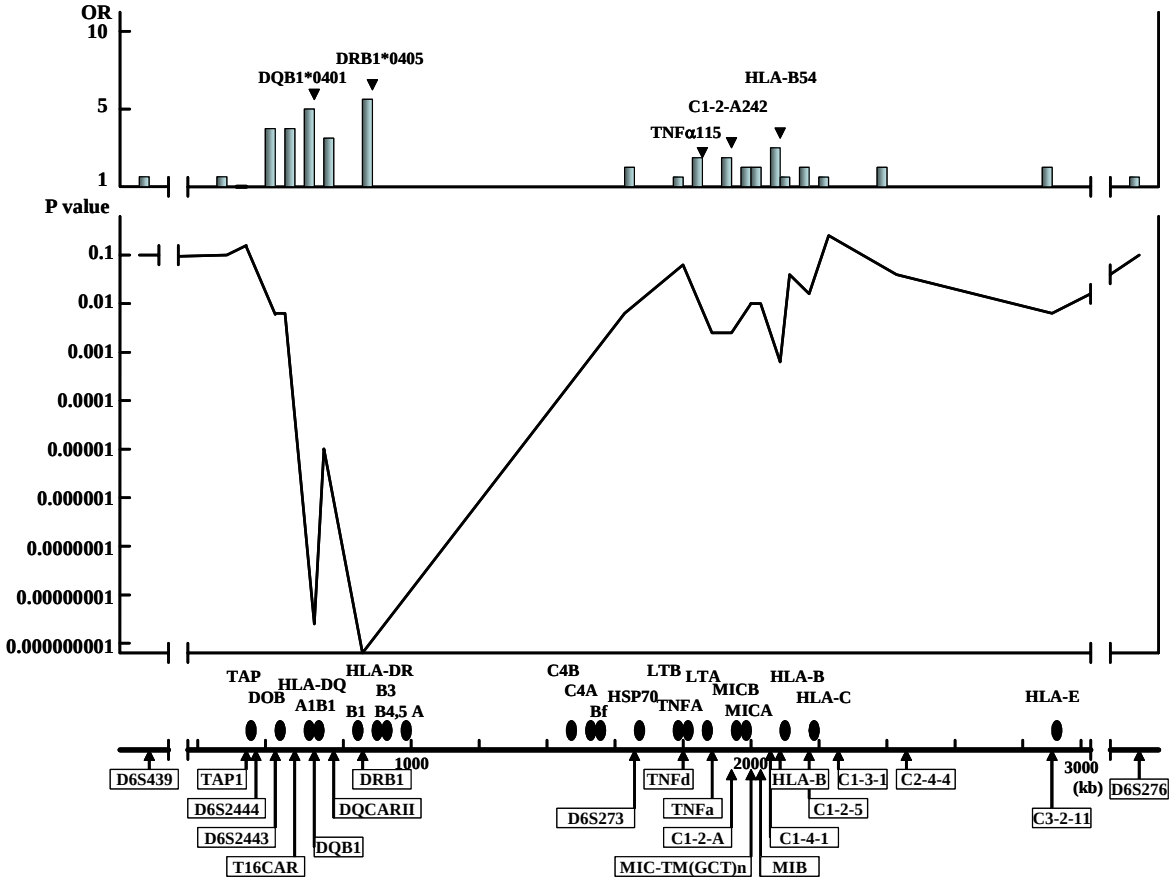
*corrected P= 0.13

Table 7. Association of TNF-α Promoter Gene Haplotype D with Type 1 AIH Patients after Stratification for the Effect of DRB1*0405

DRB1*0405	Allele D	Control	AIH	Weighted OR	χ^2	P	95% CI
Present	Negative	30	10				
	Positive	32	22				
				1.91	3.18	0.075	1.01<OR<3.60
Absent	Negative	116	19				
	Positive	32	5				

OR, odds ratio; Pc, corrected P

95% CI, 95% confidence interval


OR
10
5
1
DQB1*0401
DRB1*0405
C1-2-A242
TNFα115
HLA-B54
P value
0.1
0.01
0.001
0.0001
0.00001
0.000001
0.0000001
0.00000001
0.000000001
TAP
DOB
HLA-DQ
A1B1
HLA-DR
B3
B1
B4,5
A
C4B
C4A
Bf
HSP70
LTB
TNFA
LTA
MICB
MICA
HLA-B
HLA-C
HLA-E
1000
2000
3000
(kb)
D6S439
TAP1
D6S2444
D6S2443
T16CAR
DQB1
DQCARII
DRB1
D6S273
TNFd
TNFa
C1-2-A
MIC-TM(GCT)n
MIB
C1-4-1
HLA-B
C1-2-5
C1-3-1
C2-4-4
C3-2-11
D6S276