Genetic background of autoimmune hepatitis in Japan

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Abbreviations: AIH, autoimmune hepatitis; CTLA-4, cytotoxic T-lymphocyte antigen-4; HLA, human leukocyte antigen; SNPs, single nucleotide polymorphisms; TNF-α, tumor necrosis factor-α

Abstract

Autoimmune hepatitis (AIH) is an organ-specific autoimmune disease characterized by chronic inflammation of the liver. Several studies from ethnically different countries have clarified that the genetic predisposition to type 1 AIH is linked mainly to HLA-class II genes. Recently, molecular analysis using PCR-based DNA typing has revealed that susceptibility to type 1 AIH is primarily associated with the HLA class II DRB1 locus, which encodes a polymorphic β chain of the HLA-DR antigen. However, additional susceptibility genes (either HLA or non-HLA) and/or environmental factors may also contribute to the development of type 1 AIH; in Japanese type 1 AIH patients, although the most influential gene on disease susceptibility is HLA-DRB1*04:05, several other genes have been identified in AIH pathogenesis or resistance and are the currently the focus of single nucleotide polymorphism (SNP) analysis.

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. Specifically, human leukocyte antigen (HLA) class II genes located on the short arm of chromosome 6 are suggested to play a crucial role in the susceptibility to AIH [1, 4]. However, HLA class II genes or gene products cannot completely explain disease predisposition; additional susceptibility genes (either HLA or non-HLA) and/or environmental factors may also contribute to the development of type 1 AIH [1, 4].

This study summarizes the susceptibility genes associated with AIH development that have been published worldwide and incorporates our own findings from association analyses using microsatellite markers distributed throughout the HLA region and the whole genome.

HLA susceptibility genes

Several studies from ethnically different countries have clarified strong genetic bases for both disease susceptibility and behavior [5-8]: Mackay et al. discovered that the HLA-B8-DRw3 haplotype was associated with autoimmune-type chronic hepatitis in Caucasians [5], and Donaldson et al. reported that HLA-DR4 and A1-B8-DR3 were independent risk factors for AIH [6]. In Japan, Seki et al. showed that HLA-Bw54 and DR4 were associated with AIH [7]. Toda et al. showed that positivity for DR4 was seen

in 75% of patients in a national survey of AIH across 101 hospitals in Japan [8]. They also reported that no patients of this nationality existed with the DR3 locus [8].

HLA class II molecules on antigen presenting cells play a crucial role in triggering immune responses, which start with the recognition of peptide antigens in the HLA class II groove by the T cell receptor (TCR) of CD4 T cells through direct contact of both molecules. HLA DR is a heterodimeric glycoprotein that consists of an invariant DRα chain and DRβ chain [9]. Polymorphisms in DRβ chain are clustered in 3 hypervariable regions (HVR), which line the antigen binding groove. HVR3 (residues 67-74) is located on the α-helix of DRβ and influences peptide binding and TCR interaction [9].

Molecular analysis of HLA DR by the use of PCR-based DNA typing techniques have shown that susceptibility to type 1 AIH is associated specifically with the DRB1*03:01 and DRB1*04:01 alleles in Caucasians [10], DRB1*04:05 in Japanese [11] , Koreans [12] and Argentine adults [13], DRB1*04:04 in Mexicans [14], and DRB1*13:01 in Argentine children [15] and Brazilians [15] in the HLA class II DRB1 locus, which encodes the polymorphic β chain of the HLA-DR antigen (Table 1). We earlier performed HLA-genotyping of Japanese AIH patients and showed that the predisposition to type 1 AIH was associated with DRB1*04:05, as well as with DQB1*04:01 (Table 2), which is in strong linkage disequilibrium with DRB1*04:05 [16].

Genetic susceptibility to type 1 AIH in Caucasians is related to the HLA alleles encoding the six amino acid sequence LLEQKR at position 67-72 of the DRB1 polypeptide [17-19]. DRB1*04:05 and DRB1*04:04 share the same LLEQ-R motif with DRB1*03:01 and DRB1*04:01. DRB1*04:05 and DRB1*04:04 encode arginine

(R) at position 71, which is at the lip of the antigen-binding groove of the HLA DR molecule and influences the interactions between antigen presenting cells and helper T cells. It is likely that this particular motif presents the same or similar auto-antigen(s) in AIH, since lysine (K) at position 71 of DRB1*03:01 and 04:01 is also a basic and highly-charged polar amino acids. Therefore, we believe that disease susceptibility in the Japanese primarily maps to the DRB1 locus, and that the six amino acid sequence LLEQRR at position 67-72 of the DRB1 polypeptide is pivotal in type 1 AIH. Since HLA genome is highly complicated and results were from epidemiologic studies only, it will be crucial to prove the “shared motif” hypothesis of triggering antigenic peptides bound to the HLA-class II groove to reveal these yet uncovered antigens and the exact mechanisms in AIH pathogenesis. In other Japanese studies, AIH patients who were negative for DR4 were mainly DR2, although the frequency of DR2 was similar between patients and controls [11, 16]. Miyake et al. reported that patients with DR2 had a lower frequency of concurrent autoimmune diseases [20]. On the other hand, DRB*501:01-DRB1*15:01 (DR2) was reported to be a protective haplotype against AIH in Caucasians [10]. Ethnic differences among the triggering antigens may explain in part such conflicting results. Recently, Suzuki et al. showed that 23% of Japanese AIH patients after an extended follow-up possessed the DR14 allele, which has been associated with positive treatment response [21]. However, the frequency of DR14 in both patients and controls was very low in our previous study (Table 2) [16], so it is therefore necessary to confirm this in a larger population. Duarte-Rey et al. reported in a meta-analysis study that HLA DQB1*02, DQB1*06:03, DRB1*04:05, and DRB1*13:01 were risk factors for AIH in Latin America, while DRB1*13:02 and DQB1*03:01 were protective factors [22]. Differing genetic associations may be thus

present in type 1 AIH and the antigenic peptides presented by HLA class II molecules may be different for each race [4, 23].

Genetic association analysis in the HLA region

Spanning 3.6 Mb, the HLA human major histocompatibility complex (MHC) is divided into 3 regions from centromere to telomere: class II DR, DQ, and DP (1.1Mb), class III (0.7 Mb), and class I A, B, and C (1.8 Mb). Many genes, including tumor necrosis factor (TNF)-α, TNF-β, C2, C4A, C4B, Bf, and MHC class I chain-related A and B (MICA and MICB), are located at the telomeric end of the class III region, are involved in immune and inflammatory responses, and possess a marked degree of polymorphism [24]. We previously performed an association analysis using HLA class I and II alleles and 18 polymorphic microsatellite markers distributed throughout the HLA region in Japanese type 1 AIH patients [16]. The study revealed the presence of three distinct segments in the HLA region showing significantly low *P* (*P*c) values (Figure 1). The first segment was located around the HLA-DR and -DQ subregion, with the most significant associations being observed for the DRB1 gene (DRB1*04:05), DQB1 gene, (DQB1*04:01), and allele 193 of the DQCARII microsatellite marker, which is located between the DRB1 and DQB1 loci (*P*c<0.00015, OR 3.25). HLA-DRB1*04:05 (*P*c<2.9 x 10^{-8}; 62.3% vs. 25.0%; OR 4.97), DRB4, and DQB1*04:01 alleles were all significant in AIH patients. The second susceptibility region was located around the HLA-B54 allele, and the third segment included two microsatellites near the TNF gene cluster. However, stratification analysis for the effect of DRB1*04:05 eliminated significant associations of the latter two segments [16].

As a key cytokine in the inflammatory response, TNF-α has been reported to be a type 1 AIH candidate gene in Caucasians [25, 26]. In Caucasian AIH patients, the frequency of the -308A allele (TNF*2) was significantly increased [25, 26], but this is not found in Brazilian patients [27]. 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) and not significant. TNF*2 is in strong linkage disequilibrium with HLA DRB1*03:01, which is also very rare in Japanese [16].

AIH susceptibility genes in non-HLA regions

Similarly to polymorphisms in the HLA region, variability among racial groups seems to exist among non-HLA gene AIH susceptibility candidates as well. For instance, polymorphisms of cytotoxic T-lymphocyte antigen-4 (CTLA-4), which down-regulates peripheral T cell immune responses, have been found in Caucasian patients with type 1 AIH [28]. However, similar associations with CTLA-4 polymorphisms were not seen in Japanese [29] or Brazilian [30] patients.

Promoter polymorphisms (position -670) of Fas (CD95/Apo-1), a member of the TNF receptor superfamily that mediates programmed cell death, were found to influence susceptibility to AIH in Japanese [31]. In Caucasians, however, they affect the early development of cirrhosis, with no reported association to AIH susceptibility [32].

The vitamin D receptor was reported to be another candidate gene of AIH susceptibility in Germany [33] and China [34], but no such reports have surfaced in Japan.

Polymorphisms of the interleukin (IL)-1 family (IL-1B and IL-1RN) and IL-10 have been investigated, but no association with AIH susceptibility was found in Caucasians [25]. We obtained the same results with regard to IL-10 in Japan (unpublished data).

Recently, transforming growth factor (TGF)-β1 polymorphisms were reported to be associated with pediatric and adult AIH in Latin American Caucasians [35]. Sakaguchi et al. reported that serum level TGF-β1 was higher and the expression of its type II receptor in peripheral blood mononuclear cells was lower in Japanese AIH patients than controls, although specific polymorphisms were not investigated [36]. TGF-β1 plays a crucial role in the differentiation and function of CD4+CD25+ forkhead box P3 (Foxp3)+ regulatory cells (Treg) [37]. Tregs are closely associated with the development of autoimmune liver diseases, and the frequency of peripheral Tregs is decreased in patients with AIH [38]. Therefore, we should validate TGF-β1 SNP and its function in AIH patients.

Genome-wide study

There are several ways to search for new genes associated with AIH susceptibility and pathogenesis in the whole human genome. Honda et al. analyzed the gene expression profiles of liver tissues in patients with AIH and several other diseases using cDNA microarrays and showed several genes that were up-regulated in AIH patients, although each gene function was not investigated [39].

We performed a case-control association study using 400 polymorphic microsatellite markers distributed throughout the whole genome [40]. Two markers, one on chromosome 11 and one on chromosome 18, were revealed to have statistically significant associations with AIH. An additional seven markers were also found to be candidate susceptibility regions for AIH and our results showed another 17 regions in which resistance genes may exist. We are currently attempting to replicate these findings in an association study using SNPs. Moreover, we are underway with a

genome-wide association study (GWAS) in the Japanese population using 500,568 SNPs from the Affimetrix GeneChip Human Mapping 500K set. This new technology is expected to uncover new genes related to the pathogenesis and progression of AIH. Genetic studies of AIH have been based on case-control association studies, of which the strict limiting factor is sample size. Since AIH is not a common disease, the enrolled cohort in each study is under 200. Replication and/or nation-wide studies with larger number of patients and controls are needed, especially for searching the multiple genetic factors in uncommon diseases like AIH.

Conclusion

The most influential gene on type 1 AIH pathogenesis is HLA-DRB1, but several other genes are suspected in AIH pathogenesis both inside the HLA region and out. We anticipate our genome-wide association study with SNPs to be an excellent tool in revealing new genes associated with AIH, whose functions and gene products are also require further study.

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Table 1. Susceptibility HLA class II genes in type 1 AIH

Gene	Race	Reference
HLA-DRB1*03:01	Caucasian	5, 6, 10
HLA-DRB1*04:01	Caucasian	6, 10
HLA-DRB1*04:04	Mexican	14
HLA-DRB1*04:05	Japanese, Korean, Argentine	7, 8, 11, 12, 13
HLA-DRB1*13:01	Brazilian, Argentine children	13, 15

Table 2. HLA-DRB1 alleles in type 1 AIH patients and controls

DRB1*	AIH n=77 (%)	Control n=248 (%)	OR	χ^2	P	Pc
01:01	5 (6.5)	28 (11.3)	0.55	1.48	0.22	
15:01	5 (6.5)	34 (13.7)	0.44	2.90	0.089	
15:02	20 (26.0)	63 (25.4)	1.03	0.01	0.92	
16:02	2 (2.6)	2 (0.8)	3.28	1.55	0.21	
04:01	2 (2.6)	3 (1.2)	2.18	0.75	0.39	
04:03	5 (6.5)	7 (2.8)	2.39	2.23	0.14	
04:05	48 (62.3)	62 (25.0)	4.97	36.6	$1.5x10^{-9}$	$2.9x10^{-8}$
04:06	4 (5.2)	13 (5.2)	0.99	0.00	0.99	
04:10	1 (1.3)	8 (3.2)	0.39	0.81	0.37	
10:01	1 (1.3)	2 (0.8)	1.62	0.16	0.69	
11:01	3 (3.9)	15 (6.0)	0.63	0.52	0.47	
12:01	11 (14.3)	16 (6.5)	2.42	4.73	0.030	
12:02	2 (2.6)	5 (2.0)	1.30	0.09	0.76	
13:01	1 (1.3)	2 (0.8)	1.62	0.16	0.69	
13:02	7 (9.1)	33 (13.3)	0.65	0.97	0.33	
14:03	1 (1.3)	6 (2.4)	0.53	0.35	0.55	
14:06	2 (2.6)	13 (5.2)	0.48	0.93	0.33	
08:02	4 (5.2)	18 (7.3)	0.70	0.40	0.53	
08:03	12 (15.6)	39 (15.7)	0.99	0.00	0.98	
09:01	15 (19.5)	76 (30.6)	0.55	3.63	0.057	

OR, odds ratio; Pc, corrected P

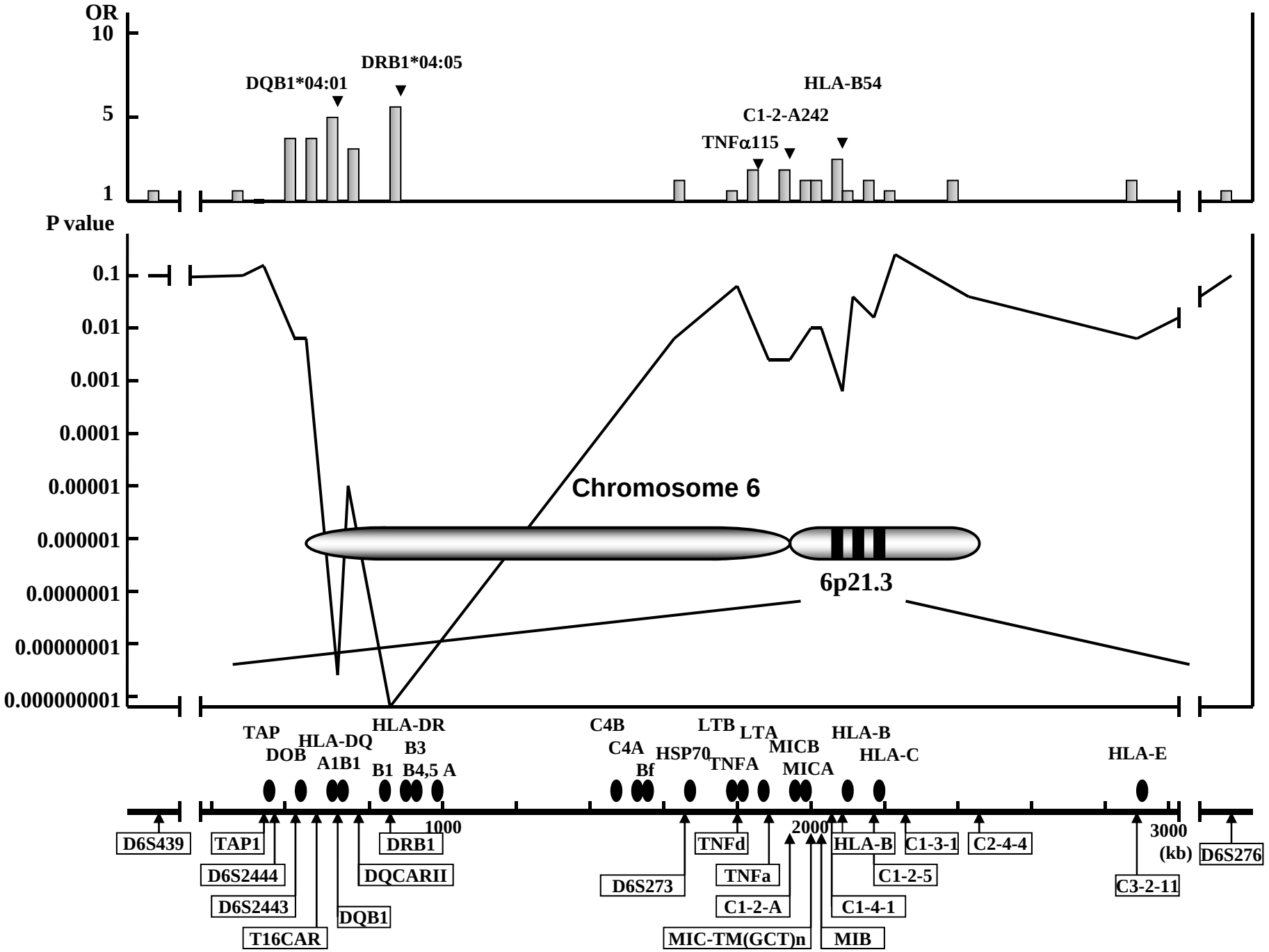
Table 3. HLA-DRB3, -DRB4, and -DRB5 and HLA-DQB1 alleles in type 1 AIH patients and controls

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	2.7×10^{-7}	8.1×10^{-7}
DRB5	24 (31.2)	82 (33.1)	0.92	0.10	0.76	
DQB1*03:01	14 (18.2)	44 (17.7)	1.03	0.01	0.93	
03:02	8 (10.4)	26 (10.5)	0.99	0.00	0.98	
03:03	15 (19.5)	69 (27.8)	0.63	2.13	0.14	
04:01	47 (61.0)	62 (25.0)	4.70	34.2	4.9×10^{-9}	5.9×10^{-8}
04:02	5 (6.5)	15 (6.0)	1.08	0.02	0.89	
05:01	5 (6.5)	29 (11.7)	0.52	1.70	0.19	
05:02	2 (2.6)	7 (2.8)	0.92	0.01	0.92	
05:03	0 (0)	11 (4.4)	0.13	3.53	0.06	
06:01	17 (22.1)	77 (31.0)	0.63	2.30	0.13	
06:02	1 (1.3)	28 (11.3)	0.10	7.22	0.0072	0.084
06:03	1 (1.3)	1 (0.4)	3.25	0.77	0.38	
06:04	4 (5.2)	29 (11.7)	0.41	2.72	0.010	

OR, odds ratio; Pc, corrected P

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.


OR
10
5
1
DQB1*04:01
DRB1*04:05
HLA-B54
C1-2-A242
TNFα115
P value
0.1
0.01
0.001
0.0001
0.00001
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0.0000001
0.00000001
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Chromosome 6
6p21.3
TAP
DOB
HLA-DQ
A1B1
HLA-DR
B1
B3
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
DRB1
DQCARII
D6S273
TNFd
TNFa
C1-2-A
MIC-TM(GCT)n
HLA-B
C1-2-5
C1-4-1
MIB
C1-3-1
C2-4-4
C3-2-11
D6S276

Figure 1