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Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes mellitus

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Abstract *Aims/hypothesis:* Fulminant type 1 diabetes, which is characterised by a markedly acute onset of diabetes and an absence of islet-related autoantibodies, accounts for 20% of type 1 diabetes in Japan. We aimed to clarify the contribution of the HLA subtype to fulminant type 1 diabetes in Japanese. *Methods:* We determined the serological subtypes of HLA-A, -DR and -DQ in 115 patients with fulminant type 1 diabetes, 98 patients with typical type 1A diabetes and 190 normal control subjects. *Results:* The frequency of HLA-DR4, but not DR9, was significantly higher in fulminant type 1 diabetes, while those of HLA-DR1, DR2, DR5 and DR8 were significantly lower than those in controls. In contrast, DR9 but not DR4 was more frequent and DR2 was extremely rare in typical type 1A diabetes. Haplotype analysis revealed that DR4-DQ4 was significantly more frequent, and both DR2-DQ1 and DR8-DQ1 were less frequent in fulminant diabetes. In type 1A diabetes, DR2-DQ1 was extremely rare while DR9-DQ3 was significantly more frequent. In the combination analysis, the homozygotes of DR4-DQ4 in fulminant type 1 diabetes and DR9-DQ3 in typical type 1A diabetes indicated high odds ratios (13.3 and 13.3, respectively). *Conclusions/interpretation:* Our results suggest that class II HLA contributes to the development of fulminant type 1 diabetes. Susceptibility and resistance of the HLA subtype to type 1 diabetes are distinct between fulminant and typical autoimmune type 1 diabetes.

Keywords Type 1B diabetes · Fulminant · GAD

Abbreviations ADA: American Diabetes Association · GADAb: Anti-glutamic acid decarboxylase antibodies · IA-2: Insulinoma-associated antigen 2 · IA-2Ab: Anti-IA-2 antibodies · IAA: Insulin autoantibodies · ICA: Islet cell antibodies · OR: Odds ratio

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Introduction

Type 1 (insulin-dependent) diabetes mellitus, one of the two major categories of diabetes mellitus, is characterised by insulin deficiency due to the destruction of pancreatic beta cells [1, 2]. Absolute insulin deficiency in this disease leads to ketosis or ketoacidosis, a life-threatening metabolic disorder, without appropriate treatment.

Susceptibility to type 1 diabetes is determined by a combination of genetic and environmental factors. In both animal models and humans, type 1 diabetes is a polygenic trait, with a major locus encoded by the MHC on chromosome 6p21.3 in humans and referred to as *IDDM1*, along with several other loci that can contribute to disease susceptibility [3, 4]. Both HLA-DR and DQ have been favoured as primary susceptibility factors, but population studies have shown that HLA associations may vary depending on geographic and ethnic origin [5]. In Caucasian populations, predisposition to type 1 diabetes is most associated with the *DRB1*03-DQB1*0201* and/or *DRB1*04-DQB1*0302* haplotypes, which encodes DR3/4, while the *DRB1*15-DQB1*0602* haplotype, which encodes DR2, confers strong protection against the disease. In the Japanese population, the *DRB1*03-DQB1*0201* haplotype is absent, and DR4-DQ3 (*DRB1*04-DQB1*0302*) is not associated with type 1 diabetes [6]. Instead, three haplotypes, DR4-DQ4 (*DRB1*0405-DQB1*0401*), DR8-DQ1 (*DRB1*0802-DQB1*0302*) and DR9-DQ3 (*DRB1*0901-DQB1*0303*), which are rare in Caucasian populations, confer susceptibility to type 1 diabetes [6–9]. Moreover, the DR2-DQ1 (*DRB1*1502-DQB1*0601*) haplotype, which is rare in Caucasian populations, is a major protective haplotype, in addition to DR2-DQ1 (*DRB1*1501-DQB1*0602*), which is a well-known protective haplotype in almost all ethnic groups.

According to the classification of diabetes by the American Diabetes Association (ADA) and the World Health Organization (WHO), type 1 diabetes can be further divided into two subgroups, i.e. autoimmune (immune-mediated) type 1 (type 1A) diabetes and idiopathic (type 1B) diabetes [10, 11]. In type 1A diabetes, islet-related autoantibodies, such as islet-cell antibodies (ICA), anti-glutamic acid decarboxylase antibodies (GADAb), insulin autoantibodies (IAA) and insulinoma-associated antigen 2 (IA-2)/IA-2 beta antibodies are used as markers [12–14]. However, these autoantibodies are not always positive in type 1 diabetic patients, even at the onset of overt diabetes. These patients are categorised as having idiopathic (type 1B) diabetes according to the ADA/WHO criteria.

In Japan, fulminant type 1 diabetes, which is included in type 1B diabetes, accounts for approximately 20% of acute onset type 1 diabetes and is recognised as a significant type 1 diabetes subtype [15–17]. The clinical characteristics of this subtype are (1) remarkably abrupt onset of the disease; (2) very short (usually less than 1 week) duration of diabetic symptoms, e.g. polyuria, thirst and body weight loss; (3) the presence of acidosis at diagnosis; (4) negative findings for islet-related autoantibodies, such as ICA, GADAb, IAA or anti-IA-2 antibodies (IA-2Ab); (5) virtually no C-peptide secretion (less than 10 μg/day in urine); and (6) elevated serum pancreatic enzyme levels [15–17]. Frequent flu-like symptoms around the time of disease onset suggest the contribution of viral infection in the aetiology of fulminant type 1 diabetes, but both environmental and genetic factors are largely unknown. To determine the contribution of genetic factors to fulminant type 1 diabetes and reevaluate the contribution of this factor to typical autoimmune type 1 (type 1A) diabetes, we investigated the serological subtypes of class I and class II HLA in Japanese patients with type 1 diabetes and healthy control subjects.

Subjects and methods

Patients We examined 115 patients with fulminant type 1 diabetes, 98 patients with typical autoimmune type 1 diabetes and 190 healthy control subjects in Japan. The detailed characteristics of these subjects are shown in Table 1.

We asked the Japan Diabetes Society to recruit fulminant type 1 diabetic patients. Inclusion criteria for fulminant type 1 diabetes in this study were (1) ketosis or ketoacidosis at the onset of diabetes; (2) the duration of hyperglycaemic symptoms occurred within a week; (3) insulin dependence, both at the onset and 6 months after; (4) the absence of GADAb, ICA, IAA, or IA-2Ab; (5) HbA_1c<8.5% on the first visit; and (6) urinary C-peptide excretion <10 μg/day or fasting serum C-peptide <0.3 ng/ml (0.1 nmol/l) or serum C-peptide <0.5 ng/ml (0.17 nmol/l) after glucagon injection or meal load, soon after the disease onset. These criteria were determined based on the data of the first 11 patients with fulminant diabetes reported by Imagawa et al. [15]. GADAb, IAA and IA-2Ab were measured by radioimmunoassay and ICA were determined by immunohistochemical methods, as described previously [15, 17].

The patients with typical autoimmune type 1 diabetes were recruited from our hospitals. These patients are ketosis-prone and were positive for at least one of the islet-related autoantibodies (GADAb, ICA, IAA or IA-2Ab). The duration of hyperglycaemic symptoms, before the start of insulin therapy was less than 3 months in these patients. Patients with slowly progressive type 1 diabetes

Table 1 Clinical characteristics of type 1 diabetic and control subjects

	Fulminant	Autoimmune	Control
n	115	98	190
Sex (M/F)	54/61	35/63	128/62
Age at disease onset (years)	36 (1–80)	26 (1–75)	50 (28–74)
BMI at disease onset (kg/m^2)	20.3±3.6	18.8±2.8	23.3±2.9
HbA_1c at disease onset (%)	6.3±0.8	12.2±2.2	ND
GADAb-positive (%)	0	92.8[a]	ND

Data are median (range) or mean ± SEM
ND Not determined
[a] Either IA-2Ab or ICA was positive in GADAb-negative patients

Table 2 HLA-A allele frequency in type 1 diabetic patients

	Fulminant (n=128)	Autoimmune (n=152)	Cf. (n=1,023)[a]
1	0.0 (0)	0.0 (0)	0.7
2	19.5 (25)	20.4 (31)	24.4
3	0.0 (0)	0.0 (0)	0.6
24 (9)	40.6 (52)	41.4 (63)	35.1
26 (10)	15.6 (20)	9.2 (14)	10.9
11	8.6 (11)	9.9 (15)	10.4
31 (19)	4.7 (6)	10.5 (16)	8.0
33 (19)	10.9 (14)	8.5 (13)	7.7
28	0.0 (0)	0.0 (0)	0.0

Data are % (n)
[a] Normal control in 11th International Histocompatibility Workshop

or latent-onset diabetes of the adult were excluded from this study.

As a control for the analysis of serotype of HLA-DR and HLA-DQ, we also studied 190 unrelated healthy individuals [18]. They had normal glucose tolerance assessed by a 75-g OGTT, no family history of diabetes, and resided in Japan.

This study was approved by the ethics committee of the Japan Diabetes Society, and informed consent was obtained from all subjects.

HLA typing Serological subtype of HLA-A, HLA-DR and HLA-DQ was determined. In some patients, genotype of *DRB*1 and *DQB*1 was also determined. Allele frequencies were estimated by direct counting [19].

Statistical analysis Statistical analysis was performed with chi-square methods or Fisher's exact probability test. The values were corrected for the number of different alleles tested (pc). Statistical significance was defined as $p<0.05$.

Results

Serological typing of HLA-A Allele frequencies of HLA-A typing are shown in Table 2. There were no significant differences in the frequency of HLA-A subtype between fulminant and autoimmune type 1 diabetes.

Serological typing of HLA-DR The allele frequency of HLA-DR4 was 44.7% in fulminant diabetic patients, 29.2% in autoimmune diabetic patients, and 21.8% in healthy control subjects. HLA-DR4 occurred at a significantly greater frequency in fulminant diabetic patients than in healthy controls [p=2.85E−09, pc=2.57E−08, (OR 2.90)], but there was no significant difference in the frequency of HLA-DR4 between autoimmune type 1 diabetic patients and healthy controls. The HLA-DR2 allele was observed in only 1.1% of autoimmune type 1 diabetes, but in 10.1% of fulminant diabetic patients, with a significant difference in the frequency of HLA-DR2 between these two groups (p=0.0000039, pc=0.000035). HLA-DR2 was less frequent in fulminant diabetes and autoimmune diabetes than in healthy controls [p=0.0027, pc=0.024 (OR 0.47) and p=2.61E−12, pc=2.35E−11 (OR 0.04), respectively]. HLA-DR9 was significantly more frequent in autoimmune type 1 diabetes than in healthy controls [p=3.06E−09, pc=2.75E−08 (OR 4.0)], but was not different in fulminant type 1

Table 3 HLA-DR-DQ haplotype frequency in type 1 diabetic and normal control subjects

	Fulminant (n=182)	Autoimmune (n=162)	Control (n=380)	Fulminant vs. control			Autoimmune vs. control			Fulminant vs. autoimmune	
				p	pc	OR	p	pc	OR	p	pc
1–1	2.7 (5)	5.6 (9)	7.1 (27)	–	–	–	–	–	–	–	–
2–1	8.2 (15)	1.2 (2)	18.7 (71)	0.001	0.014	0.39	6.56E−10	8.53E−09	0.056	0.0024	0.031
3–2	0.1 (1)	1.2 (2)	0.3 (1)	–	–	–	–	–	–	–	–
4–1	0.0 (0)	0.6 (1)	1.6 (6)	–	–	–	–	–	–	–	–
4–3	4.4 (8)	4.3 (7)	8.2 (31)	–	–	–	–	–	–	–	–
4–4	41.8 (76)	22.8 (37)	12.1 (46)	1.47E−15	1.91E−14	5.21	0.0015	0.019	2.22	0.00019	0.0025
5–3	2.7 (5)	4.3 (7)	6.3 (24)	–	–	–	–	–	–	–	–
6–1	12.6 (23)	11.7 (19)	9.2 (35)	–	–	–	–	–	–	–	–
6–3	1.6 (3)	0.0 (0)	3.2 (12)	–	–	–	–	–	–	–	–
8–1	1.6 (3)	1.9 (3)	9.2 (35)	0.00047	0.0061	0.17	0.0014	0.019	0.19	–	–
8–3	1.1 (2)	3.7 (6)	3.7 (14)	–	–	–	–	–	–	–	–
8–4	1.1 (2)	0.6 (1)	4.0 (15)	–	–	–	–	–	–	–	–
9–3	19.8 (36)	39.5 (64)	15.0 (57)	–	–	–	3.57E−10	4.64E−09	3.86	–	–
Others	1.6 (3)	2.5 (4)	1.6 (6)	–	–	–	–	–	–	–	–

Data are % (n). Others contain a rare haplotype, whose total frequencies in all of patients and controls were less than three

Table 4 Combination of HLA-DR-DQ haplotype in type 1 diabetic and control subjects

	Fulminant (n=91)	Autoimmune (n=81)	Control (n=190)	Fulminant vs. control			Autoimmune vs. control			Fulminant vs. autoimmune	
				p	pc	OR	p	pc	OR	p	pc
4–4/4–4	17.6 (16)	6.2 (5)	1.6 (3)	5.74E−07	2.87E−06	13.3	–	–	–	0.034	NS
4–4/X	33.0 (43)	21.0 (17)	14.7 (28)	0.00041	0.0002	2.85	–	–	–	0.00041	0.0021
4–4/9–3	15.4 (14)	12.3 (10)	6.3 (12)	0.014	NS	1.29	–	–	–	–	–
9–3/9–3	3.3 (3)	22.2 (18)	2.1 (4)	–	–	–	2.08E−07	1.04E−06	13.3	0.00014	0.0007
9–3/X	15.4 (14)	22.2 (18)	19.5 (37)	–	–	–	–	–	–	–	–
X/X	15.4 (14)	16.0 (13)	55.8 (106)	–	–	–	–	–	–	–	–

Data are % (n)
NS Not significant, *X* neither DR4-DQ4 nor DR9-DQ3

diabetes compared with controls. The HLA-DR8 allele was less frequent in fulminant and autoimmune type 1 diabetes than in healthy controls [p=0.000059, pc=0.00052 (OR 0.25) and p=0.00023, pc=0.0021 (OR 0.32), respectively]. HLA-DR1 and -DR5 were also less frequent in fulminant type 1 diabetes than in healthy controls [p=0.017, pc>0.05 (OR 0.34) and p=0.0082, pc>0.05 (OR 0.29), respectively], but there were no significant differences between fulminant and autoimmune type 1 diabetes or between autoimmune diabetes and healthy controls. The differences between phenotypic and allele frequencies in HLA-DR3, -DR6 and -DR7 among the three groups were not significant.

Serological typing of HLA-DQ The allele frequency of HLA-DQ4 was 43.4% in fulminant diabetic patients, 23.5% in autoimmune diabetic patients, and 16.3% in healthy control subjects. HLA-DQ4 was significantly more frequent in fulminant diabetic patients [p=4.15E−12, pc=1.66E−11 (OR 3.91)] and in autoimmune type 1 diabetic patients [p=0.0497, pc>0.05 (OR 1.62)] than in healthy controls. There were significant differences in the frequency of HLA-DQ1 between fulminant or autoimmune diabetes and healthy controls [p=3.38E−06, pc=1.35E−05 (OR 0.40) and p=3.01E−07, pc=1.21E−06 (OR 0.35), respectively]. HLA-DQ3 occurred at a significantly greater frequency in autoimmune type 1 diabetes than in healthy controls [p=0.0014, pc=0.0056 (OR 1.83)], but not in fulminant type 1 diabetes compared with controls.

Haplotype analysis of HLA-DR-DQ HLA-DR-DQ analysis revealed that the allele frequency of DR4-DQ4 was 41.8% in fulminant type 1 diabetes, 22.8% in autoimmune diabetes and 12.1% in normal controls. This haplotype occurred at a significantly greater frequency in both fulminant and autoimmune type 1 diabetes compared with normal controls, as shown in Table 3. The frequency of DR4-DQ4 was also different between autoimmune and fulminant diabetes. DR2-DQ1 was extremely rare and observed in only 1.2% of autoimmune type 1 diabetes, but in 8.2% of fulminant type 1 diabetes and 18.7% of controls. The frequency of DR2-DQ1 was significantly different between the three groups. DR8-DQ1 occurred less frequently in both fulminant and autoimmune type 1 diabetes. Of note, DR9-DQ3 occurred at a significantly greater frequency in typical type 1A diabetes than in healthy controls, but not in fulminant diabetes.

In the combination analysis of the HLA-DR-DQ haplotype (Table 4), the frequency of DR4-DQ4 homozygotes was 17.6% in fulminant type 1 diabetes and was significantly higher than in healthy controls [1.6%, p=5.74E−07, pc=2.87E−06 (OR 13.3)], but not in autoimmune type 1 diabetes (6.2%). The frequency of the DR4-DQ4 heterozygotes was significantly higher only in fulminant diabetes compared with normal controls (OR 2.85). A significant difference in the frequency of DR9-DQ3 was observed only in homozygotes between autoimmune diabetes and normal controls or fulminant diabetes, as shown in Table 4.

Discussion

Type 1 diabetes is defined as the absolute loss of insulin-secreting beta cells in the pancreas. In this context, fulminant type 1 diabetes is a typical subtype of type 1 diabetes, because of low C-peptide response following glucagon load, and an absence of beta cells by histological examination [15, 17, 20]. However, this subtype exhibits clearly distinct clinical features from typical autoimmune type 1 (type 1A) diabetes. Islet-related autoantibodies, such as ICA or GADAb were seldom positive in fulminant diabetes. Markedly acute onset of diabetes, which was confirmed by near-normal HbA_1c levels against high plasma glucose concentration at the onset, is characteristic in fulminant type diabetes. Moreover, this subtype was not rare, and accounted for approximately 20% of Japanese type 1 diabetes. This suggests that type 1 diabetes is a heterogeneous disease and typical, autoantibody-positive type 1 (type 1A) diabetes is not exclusive, at least in Japan. In such circumstances, analysis of the detailed clinical phenotype and subclassification of the *IDDM*1 region, may provide further information of class II HLA in type 1 diabetes. However, there have been few previous reports examining this standpoint [6–9]. This nationwide multicentre study has clearly indicated susceptible class II HLA in fulminant type 1 diabetes, has re-evaluated susceptible and resistant class II HLA in typical autoimmune type 1 diabetes, and has found that they are different from that of

fulminant type 1 diabetes and previous reports of Japanese type 1 diabetes [6–9].

First, HLA-DR4-DQ4 was clearly more frequent in fulminant type 1 diabetes. Analysis of the allelic combination of HLA-DR-DQ has shown that homozygotes with DR4-DQ4 exhibit a greater effect than heterozygotes with DR4-DQ4 regarding predisposition to fulminant diabetes. These findings coincide with small pilot studies, which were performed independently of this study [21, 22]. These findings also suggest that HLA-DR4 and/or DQ4 could play an important role in the development of fulminant type 1 diabetes. In typical autoimmune type 1 diabetes, the role of class II HLA has been emphasised in the context of the antigen-presenting process [2, 23]. However, there is very little evidence regarding the molecular mechanisms of beta cell destruction in fulminant type 1 diabetes. Therefore, it is essential to elucidate how certain class II HLA can contribute towards the molecular mechanisms of beta cell destruction in fulminant type 1 diabetes.

We have also shown that the frequency of HLA-DR2-DQ1 and DR8-DQ1 is less in fulminant type 1 diabetes. The DR2-DQ1 and DR8-DQ1 alleles were also less frequent in typical autoimmune type 1 diabetes in this study, but the protective effect of DR2-DQ1 was significantly less in fulminant diabetes than in type 1A diabetes. DR9-DQ3 was not more frequent in fulminant type 1 diabetes than in type 1A diabetes. These findings suggest a differential contribution of class II HLA in the mechanisms of beta cell damage between fulminant and type 1A diabetes. By contrast, non-susceptible or resistant HLA-A, a class I HLA, subtype was evident in fulminant type 1 diabetes.

Second, the present results indicated that patients with the HLA-DR9(-DQ3) allele, but not DR4 were susceptible to and those with the HLA-DR2 allele were quite resistant to typical autoimmune type 1 diabetes in Japanese. HLA-DR9 is generally encoded by *DRB1*0901-DQA1*0302-DQB1*0303* in Japanese and Caucasian populations [23, 24]. This haplotype has been demonstrated to confer a recessive, neutral or weak predisposing effect (OR 1.3–2.39) in previous reports in Japanese [6, 8, 9, 25]. However, the present study has clearly demonstrated that DR9-DQ3 exhibits a strong predisposing effect (OR 3.86) towards typical autoimmune type 1 diabetes in Japanese. This study also confirmed that patients who were homozygous for HLA-DR9 were susceptible to typical autoimmune type 1 diabetes, but that heterozygotes of this subtype were not, as shown by Kawabata et al. [25]. The cause of the predisposition of DR9 to type 1A diabetes in Japanese but not in Caucasians is unknown. One possible reason is that the HLA-DR9 haplotype is rare in a Caucasian population and another reason could be that the strong effect of the HLA-DR 3/4 heterozygosity, which is rare in Japanese, could impair the effect of HLA-DR9 in Caucasian populations [8]. The absence of a predisposing effect of HLA-DR4 in Japanese patients with typical autoimmune type 1 diabetes could be explained by the difference of DR-DQ haplotypes between Japanese and Caucasian populations. In Japan, HLA-DR4 is generally encoded by a haplotype of DR4-DQ4 (*DRB1*0405-DQA1*0302-DQB1*0401*), but by a haplotype of DR4-DQ3 (*DRB1*0401-DQA1*0301-DQB1*0302*) in Caucasians [23, 24]. It has been reported that *DRB1*0405-DQA1*0302-DQB1*0401* confers a strong predisposing effect towards type 1 diabetes in Japanese [6, 8, 9], but this study suggests that the effect of DR4 haplotype influences fulminant type 1 diabetes but not typical autoimmune type 1 diabetes. These findings suggested that the DR-DQ haplotype, not the DR subtype, plays an important role in the development of typical autoimmune type 1 diabetes. In Japanese, the absence of DR3 and DR4-DQ3 (*DQB1*0302*) could highlight the effect of the haplotype of DR9(-DQ3) in autoimmune diabetes. In contrast, HLA-DR2, which is encoded by *DRB1*1502-DQB1*0601* or *DRB1*1501-DQB1*0602*, was extremely resistant to typical autoimmune type 1 diabetes in Japanese, as shown previously in Caucasian populations. These findings suggest that Japanese typical autoimmune type 1 diabetes and the majority of Caucasian type 1 diabetes may be similar in the disease phenotype and also the contribution of class II HLA.

As described above, the present study has provided several new findings not only in the novel clinical entity of fulminant type 1 diabetes, but also in typical autoimmune type 1 (type 1A) diabetes, which has already been the subject of intensive investigations [3–9]. The current methodology, based on the intensive analysis of clinical phenotype and the subclassification of a disease, was effective in the analysis of a polygenic genetic factor in type 1 diabetes.

The distribution of HLA-A in this study was not different between fulminant and type 1A diabetes. These data are consistent with those of normal control subjects shown in the 11th International Histocompatibility Workshop [19].

In conclusion, the present results suggest that class II HLA contributes to the development of fulminant type 1 diabetes. Susceptibility and resistance of the class II HLA subtype to type 1 diabetes are distinct between fulminant and typical autoimmune type 1 diabetes.

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Duality of Interest

The authors declare that there is no current or past (within the last 12 months) interest in a relationship with a company/organisation that could financially benefit from the publication of the data in our manuscript.

Appendix

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