Class II HLA genotype in fulminant type 1 diabetes: A nationwide survey with reference to glutamic acid decarboxylase antibodies

Chiharu Tsutsumi[1], Akihisa Imagawa[2]*, Hiroshi Ikegami[3], Hideichi Makino[4], Tetsuro Kobayashi[5], Toshiaki Hanafusa[1] on behalf of the Japan Diabetes Society Committee on Type 1 Diabetes Mellitus Research[†]

ABSTRACT

Aims/Introduction: Fulminant type 1 diabetes is a subtype of type 1 diabetes characterized by a remarkably abrupt onset of insulin-deficient hyperglycemia within a few days. The aim of the present study was to clarify characteristic class II HLA genotypes in a large number of patients with fulminant type 1 diabetes to date.

Materials and Methods: We analyzed the HLA-*DRB1* and *DQB1* genotypes, and their haplotypes in 207 patients with fulminant type 1 diabetes and 325 control subjects in the Japanese population.

Results: The frequencies of the *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03* haplotypes were significantly higher, and those of the *DRB1*01:01-DQB1*05:01*, *DRB1*15:02-DQB1*06:01* and *DRB1*08:03-DQB1*06:01* haplotypes were significantly lower in patients with fulminant type 1 diabetes than in the control subjects. Combination analysis showed that the frequencies of homozygotes with *DRB1*04:05-DQB1*04:01* [odds ratio (OR) 7.0] and *DRB1*09:01-DQB1*03:03* (OR 9.5) were significantly higher in patients with fulminant type 1 diabetes. Within a limited portion of patients with fulminant type 1 diabetes with antibodies to glutamic acid decarboxylase (GADab; $n = 25$), the frequency of *DRB1*09:01-DQB1*03:03*, but not *DRB1*04:05-DQB1*04:01*, was significantly higher than in control subjects (44.0% vs 13.7%; $Pc < 0.05$, OR 5.0).

[Correction to last line of Results, added after online publication 29 July 2011: "OR 5.1" is changed to "OR 5.0".]

Conclusions: Our large-scale study showed the characteristic class II HLA genotypes in fulminant type 1 diabetes, and implicated that genetic contribution to disease susceptibility is distinct between GADab-positive and GADab-negative fulminant type 1 diabetes.

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KEY WORDS: Fulminant type 1 diabetes, HLA, Glutamic acid decarboxylase

INTRODUCTION

Fulminant type 1 diabetes is a novel subtype of type 1 diabetes identified in 2000[1–3]. It is defined as diabetes that results from the extremely rapid and almost entire destruction of pancreatic β-cells within a few days. The clinical characteristics of this subtype are different in many aspects from those of typical type 1A diabetes[3]. Although fulminant type 1 diabetes resembles the typical form of type 1 diabetes in that it is characterized by high plasma glucose levels accompanied by ketosis or ketoacidosis, it clearly differs by an extremely acute onset of diabetes, which is confirmed by nearly normal HbA_{1c} levels against high plasma glucose concentration, and virtually no C-peptide secretion at the onset of the disease, indicating that the process of pancreatic β-cell destruction is very rapid.

Fulminant type 1 diabetes is common in the Asian population; it accounts for approximately 20% of ketosis-onset type 1 diabetes in Japan[2,3] and 7% in Korea[4,5]. Furthermore, several cases have been reported from China[6], Taiwan[7], the Philippines[8], Malaysia[9] and France[10].

It is suggested that both genetic factors[11–13] and environmental factors, such as viral infection[14–19], contribute to the pathogenesis of this disease. In regard to genetic factors, it has been reported that class II HLA strongly confers susceptibility to the development of fulminant type 1 diabetes. In the analysis of the serological typing of class II HLA, we have shown that HLA-DR4-DQ4 was significantly more frequent in fulminant type 1 diabetes in Japan[12]. Several studies have so far reported the association of class II HLA genotype with fulminant type 1 diabetes[20–22]; however, the number of patients was limited in these reports as a result of the low incidence of type 1 diabetes in general, fulminant type 1 diabetes in particular, in the Japanese population.

[1]Department of Internal Medicine (I), Osaka Medical College, [2]Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, [3]Department of Endocrinology, Metabolism, and Diabetes, Kinki University School of Medicine, Osaka, [4]Shiraishi Hospital Diabetes Center, Ehime, and [5]Third Department of Internal Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

*Corresponding author. Akihisa Imagawa Tel.: +81-6-6879-3732 Fax: +81-6-6879-3739 E-mail address: aimagawa@endmet.med.osaka-u.ac.jp

[†]See Appendix for members of the research Committee of type 1 Diabetes, Japan Diabetes Society.

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The aim of the present study was thus to investigate the class II HLA genotypes and re-evaluate the contribution of the class II HLA to susceptibility and resistance to fulminant type 1 diabetes in a large number of patients.

MATERIALS AND METHODS

Subjects and Methods

We examined 207 patients with fulminant type 1 diabetes and 325 healthy control subjects in Japan. Among them, 152 patients with fulminant type 1 diabetes were registered with the committee of the Japan Diabetes Society, and data for the other 55 patients were collected from reports in the literature from June 2000 to March 2007.

Inclusion criteria for fulminant type 1 diabetes were: (i) ketosis or ketoacidosis within a week after the onset of hyperglycemic symptoms; (ii) urinary C-peptide excretion <10 μg/day or fasting serum C-peptide <0.3 ng/mL (0.10 nmol/L) or serum C-peptide <0.5 ng/mL (0.17 nmol/L) after glucagon injection or meal load soon after disease onset; and (iii) plasma glucose level ≥16.0 mmol/L (288 mg/dL) and HbA_{1c} <8.9% at the first visit[2]. Healthy control subjects had normal glucose tolerance as assessed by a 75 g oral glucose tolerance test, had no family history of diabetes, and resided in the Ehime and Osaka areas as described previously[23]. GAD antibodies (GADab) were positive in 25 patients and negative in 182 patients (Table 1). We also analyzed 15 patients with pregnancy-associated fulminant type 1 diabetes (PF), 51 female patients of child-bearing age (13–49 years) with fulminant type 1 diabetes that was not associated with pregnancy (NPF) and 70 female control subjects of child-bearing age.

The present study was approved by the ethics committee of the Japan Diabetes Society, and informed consent was obtained from all subjects. The detailed characteristics of these subjects are shown in Table 1.

The value for HbA_{1c} (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} (JDS) (%) + 0.4%, considering the relational expression of HbA_{1c} (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP)[24].

Typing of HLA-DR and -DQ

HLA-DRB1 and *-DQB1* were genotyped by the PCR sequence-specific primer and PCR sequence-specific oligonucleotide methods (Invitrogen, Carlsbad, CA, USA). The most probable *DRB1-DQB1* haplotypes were deduced from known linkage disequilibria.

Statistical Analysis

Clinical data of GADab-negative and -positive fulminant type 1 diabetes was analyzed by using chi-squared-test or Kruskal–Wallis test. Allele frequencies were estimated by direct counting. Genotypes, whose total frequencies in both total subjects with fulminant type 1 diabetes and control subjects were five or more than five, were listed in the present study. The significance of the difference in distribution of alleles between patients with fulminant type 1 diabetes and healthy control subjects was determined by a chi-squared-test. *P*-values were corrected by using the number of different alleles tested (denoted as *Pc*). Statistical significance was defined as $Pc < 0.05$.

RESULTS

Characteristics of GADab-Negative and -Positive Fulminant Type 1 Diabetes

GADab was detected in 25 (12.1%) of 207 patients with fulminant type 1 diabetes in the present study. Therefore, first of all, we compared detailed characteristics between GADab-negative and -positive fulminant type 1 diabetes (Table 1). There were no differences between the two groups in age, body mass index, mean HbA_{1c} level at onset and presence or absence of family history of type 1 or type 2 diabetes in first-degree relatives. One, but not another, allele of class II HLA haplotype was common between two patients (father and his son) with a family history of

Table 1 | Clinical characteristics of patients with fulminant type 1 diabetes

	Total	With GADab	Without GADab	Control
n	207	25 (12.1)	182 (87.9)	325
Sex (male/female)	118/89 (57.0)	20/5 (80.0)	98/84 (53.8)	202/123 (62.2)
Pregnancy (PF*/NPF†)	15/51 (22.7)	0/5 (0.0)	15/49 (23.4)	ND
Age at disease onset (years)	41 (0–87)	43 (0–75)	41 (1–87)	47 (25–78)
Body mass index (kg/m^2)	21.1 ± 3.2‡	20.9 ± 3.4§	21.2 ± 3.2¶	ND
Family history of type 1 diabetes	5/157 (3.1)	0/20 (0.0)	5/137 (3.5)	0/0 (0.0)
Family history of type 2 diabetes	11/151 (6.8)	2/18 (10.0)	9/133 (6.3)	0/0 (0.0)
Family history of unclassified diabetes	6/156 (3.7)	1/19 (5.0)	5/137 (3.5)	0/0 (0.0)
HbA_{1c} at disease onset (%)	6.6 ± 0.8	6.7 ± 0.7	6.6 ± 0.8	ND

GADab, antibodies to glutamic acid decarboxylase; ND, not determined.

Data are *n*, median (range), mean ± SD, (±), or *n* (%).

*Pregnancy-associated fulminant type 1 diabetes; †Female patients of child-bearing age (13–49 years) with fulminant type 1 diabetes not associated with pregnancy; ‡except seven children; §Except two children; ¶Except five children.

fulminant type 1 diabetes. GADab was measured by radioimmunoassay[1], except for one patient in whom GADab was measured by radioligand binding assay[25]. There were no differences in sensitivity and specificity between the two assays. GADab was determined within a week after the onset of diabetes, except for two patients in each hospital. GADab was negative in one patient measured 6 months after the onset and positive in another patient measured 16 years after the onset. The median level of GADab was 3.0 U/mL (range 1.5–20.0 U/mL). In 78% of GADab-positive patients, the titer was <10 U/mL at the onset of disease and GADab became negative within 2 years during the follow up. Of 25 GADab-positive patients with fulminant type 1 diabetes, IA-2ab was negative in 16 patients and not measured in the other nine patients. In GADab-positive patients with fulminant type 1 diabetes, median duration of hyperglycemic symptoms was 4 days (range 0–11 days); median HbA_{1c} level was 6.7% (range 5.6–8.3%) despite very high plasma glucose levels (median 700, range 313–1944 mg/dL), showing the similarity in the clinical features, except the positivity of GADab, between GADab-positive and GADab-negative fulminant type 1 diabetes.

Table 2 | *DRB1* and *DQB1* alleles in patients with fulminant type 1 diabetes and control subjects

		Fulminant			Control (n = 650†)	Total vs control		GADab(+) vs control		GADab(–) vs control	
		Total (n = 414†)	GADab(+) (n = 50†)	GADab(–) (n = 364†)							
		n (%)	n (%)	n (%)	n (%)	Pc	OR	Pc	OR	Pc	OR
DRB1	**01:01*	9 (2.2)	0 (0.0)	9 (2.5)	50 (7.7)	2.8×10^{-3}	0.27	NS		0.015	0.30
	**04:01*	6 (1.4)	1 (2.0)	5 (1.4)	5 (0.8)	NS		NS		NS	
	**04:03*	6 (1.4)	1 (2.0)	5 (1.4)	21 (3.2)	NS		NS		NS	
	**04:05*	135 (32.6)	11 (22.0)	124 (34.0)	92 (14.2)	1.7×10^{-11}	2.9	NS		2.4×10^{-12}	3.1
	**04:06*	3 (0.7)	1 (2.0)	2 (0.5)	23 (3.5)	NS		NS		NS	
	**04:07*	1 (0.2)	0 (0.0)	1 (0.3)	5 (0.8)	NS		NS		NS	
	**04:10*	13 (3.1)	0 (0.0)	13 (3.6)	9 (1.4)	NS		NS		NS	
	**08:02*	14 (3.4)	2 (4.0)	12 (3.3)	30 (4.6)	NS		NS		NS	
	**08:03*	13 (3.1)	1 (2.0)	12 (3.3)	58 (8.9)	5.0×10^{-3}	0.33	NS		0.015	0.35
	**09:01*	106 (25.6)	22 (44.0)	84 (23.1)	90 (13.8)	3.1×10^{-5}	2.1	4.6×10^{-7}	4.9	4.1×10^{-3}	1.9
	**10:01*	1 (0.2)	0 (0.0)	1 (0.3)	9 (1.4)	NS		NS		NS	
	**11:01*	3 (0.7)	0 (0.0)	3 (0.8)	13 (2.0)	NS		NS		NS	
	**12:01*	7 (1.7)	1 (2.0)	6 (1.6)	27 (4.2)	NS		NS		NS	
	**12:02*	5 (1.2)	2 (4.0)	3 (0.8)	9 (1.4)	NS		NS		NS	
	**13:02*	23 (5.6)	1 (2.0)	22 (6.0)	26 (4.0)	NS		NS		NS	
	**14:01*	7 (1.7)	1 (2.0)	6 (1.6)	23 (3.5)	NS		NS		NS	
	**14:03*	1 (0.2)	0 (0.0)	1 (0.3)	6 (0.9)	NS		NS		NS	
	**14:05*	3 (0.7)	0 (0.0)	3 (0.8)	12 (1.8)	NS		NS		NS	
	**14:06*	3 (0.7)	0 (0.0)	3 (0.8)	7 (1.1)	NS		NS		NS	
	**15:01*	22 (5.3)	3 (6.0)	19 (5.2)	45 (6.9)	NS		NS		NS	
	**15:02*	16 (3.9)	0 (0.0)	16 (4.4)	73 (11.2)	5.1×10^{-4}	0.32	NS		4.9×10^{-3}	0.36
	**16:02*	8 (1.9)	2 (4.0)	6 (1.6)	6 (0.9)	NS		NS		NS	
	Others	9 (2.2)	1 (2.0)	8 (2.2)	11 (1.6)						
DQB1	**03:01*	18 (4.3)	3 (6.0)	15 (4.1)	62 (9.5)	0.019	0.43	NS		0.020	0.41
	**03:02*	21 (5.1)	2 (4.0)	19 (5.2)	67 (10.3)	0.028	0.46	NS		NS	
	**03:03*	109 (26.3)	22 (44.0)	87 (23.9)	97 (14.9)	4.9×10^{-5}	2.0	1.5×10^{-6}	4.5	4.1×10^{-3}	1.8
	**04:01*	133 (32.1)	11 (22.0)	122 (33.5)	91 (14.0)	1.7×10^{-11}	2.9	NS		2.8×10^{-12}	3.1
	**04:02*	22 (5.3)	2 (4.0)	20 (5.5)	27 (4.2)	NS		NS		NS	
	**05:01*	11 (2.7)	0 (0.0)	11 (3.0)	59 (9.1)	4.2×10^{-4}	0.27	NS		2.9×10^{-3}	0.31
	**05:02*	11 (2.7)	3 (6.0)	8 (2.2)	19 (2.9)	NS		NS		NS	
	**05:03*	8 (1.9)	1 (2.0)	7 (1.9)	23 (3.5)	NS		NS		NS	
	**06:01*	30 (7.2)	1 (2.0)	29 (8.0)	132 (20.3)	8.1×10^{-8}	0.31	0.030	0.08	2.7×10^{-6}	0.34
	**06:02*	21 (5.1)	3 (6.0)	18 (4.9)	44 (6.8)	NS		NS		NS	
	**06:04*	20 (4.8)	1 (2.0)	19 (5.2)	26 (4.0)	NS		NS		NS	
	Others	10 (2.4)	1 (2.0)	9 (2.5)	4 (0.6)						

GADab, antibodies to glutamic acid decarboxylase; NS, not significant.
Pc-, *P*-values corrected for number of different alleles tested (×22 for *DRB1*and ×11 for *DQB1*).
†Allele number.

Frequencies of Alleles of HLA-DRB1 and DQB1

As shown in Table 2, the allele frequencies of *DRB1*04:05*, *DRB1*09:01*, *DQB1*04:01* and *DQB1*03:03* were significantly higher, and those of *DRB1*01:01*, *DRB1*08:03*, *DRB1*15:02*, *DQB1*03:01*, *DQB1*03:02*, *DQB1*05:01* and *DQB1*06:01* were significantly lower in total subjects with fulminant type 1 diabetes than in control subjects.

Similarly, the allele frequencies of *DRB1*04:05*, *DRB1*09:01*, *DQB1*04:01* and *DQB1*03:03* were significantly higher, and those of *DRB1*01:01*, *DRB1*08:03*, *DRB1*15:02*, *DQB1*03:01*, *DQB1*05:01* and *DQB1*06:01* were significantly lower in GADab-negative patients with fulminant type 1 diabetes than in control subjects.

In contrast, the allele frequencies of *DRB1*09:01* and *DQB1*03:03* were significantly higher, and that of *DQB1*06:01* was significantly lower in GADab-positive patients with fulminant type 1 diabetes than in control subjects (Table 2).

The frequencies of *DRB1*09:01* and *DQB1*03:03* were significantly higher in GADab-positive patients than in GADab-negative patients with fulminant type 1 diabetes (44.0 vs 23.1%, $Pc = 0.033$ and 44.0 vs 23.9%, $Pc = 0.027$, respectively).

Frequencies of the Genotypes of DRB1-DQB1 Haplotypes

As shown in Table 3, *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03* are significantly more frequent in total subjects with fulminant type 1 diabetes than in controls. *DRB1*15:02-DQB1*06:01*, but not *DRB1*15:01-DQB1*06:02*, was significantly less frequent in these patients than in control subjects. Furthermore, *DRB1*01:01-DQB1*05:01* and *DRB1*08:03-DQB1*06:01* were significantly less frequent in these patients than in controls.

Similarly, the frequencies of *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03* were significantly higher and those of *DRB1*01:01-DQB1*05:01*, *DRB1*15:02-DQB1*06:01* and *DRB1*08:03-DQB1*06:01* were significantly lower in

Table 3 | *DRB1-DQB1* haplotypes in patients with fulminant type 1 diabetes and control subjects

DRB1-DQB1	Fulminant			Control ($n = 650$†)	Total vs control		GADab(+) vs control		GADab(−) vs control	
	Total ($n = 414$†)	GADab(+) ($n = 50$†)	GADab(−) ($n = 364$†)							
	n (%)	*n* (%)	*n* (%)	*n* (%)	*Pc*	OR	*Pc*	OR	*Pc*	OR
**01:01-*05:01*	9 (2.2)	0 (0.0)	9 (2.5)	50 (7.7)	3.1×10^{-3}	0.27	NS	0.12	0.016	0.30
**04:01-*03:01*	3 (0.7)	1 (2.0)	2 (0.5)	5 (0.7)	NS		NS		NS	
**04:03-*03:02*	6 (1.4)	1 (2.0)	5 (1.4)	22 (3.2)	NS		NS		NS	
**04:05-*04:01*	135 (32.6)	11 (22.0)	124 (34.1)	92 (14.2)	2.0×10^{-11}	2.9	NS	1.7	2.7×10^{-12}	3.1
**04:06-*03:02*	3 (0.7)	1 (2.0)	2 (0.5)	23 (3.5)	NS		NS		NS	
**04:07-*03:02*	1 (0.2)	0 (0.0)	1 (0.3)	5 (0.7)	NS		NS		NS	
**04:10-*04:02*	13 (3.1)	0 (0.0)	13 (3.6)	9 (1.3)	NS		NS		NS	
**08:02-*03:02*	6 (1.4)	0 (0.0)	6 (1.6)	15 (2.2)	NS		NS		NS	
**08:02-*04:02*	8 (1.9)	2 (4.0)	6 (1.6)	16 (2.3)	NS		NS		NS	
**08:03-*06:01*	13 (3.1)	1 (2.0)	12 (3.3)	58 (8.9)	5.7×10^{-3}	0.33	NS	0.21	0.017	0.35
**09:01-*03:03*	105 (25.4)	22 (44.0)	83 (22.8)	89 (13.7)	3.8×10^{-5}	2.1	3.9×10^{-7}	5.0	5.2×10^{-3}	1.9
**10:01-*05:01*	1 (0.2)	0 (0.0)	1 (0.3)	10 (1.5)	NS		NS		NS	
**11:01-*03:01*	1 (0.2)	0 (0.0)	1 (0.3)	13 (1.9)	NS		NS		NS	
**12:01-*03:01*	4 (1.0)	1 (2.0)	3 (0.8)	21 (3.1)	NS		NS		NS	
**12:01-*03:03*	2 (0.5)	0 (0.0)	2 (0.5)	5 (0.7)	NS		NS		NS	
**12:02-*03:01*	4 (1.0)	1 (2.0)	3 (0.8)	9 (1.3)	NS		NS		NS	
**13:02-*06:04*	19 (4.6)	1 (2.0)	18 (4.9)	23 (3.5)	NS		NS		NS	
**14:01-*05:02*	2 (0.5)	0 (0.0)	2 (0.5)	13 (1.9)	NS		NS		NS	
**14:01-*05:03*	5 (1.2)	1 (2.0)	4 (1.1)	13 (1.9)	NS		NS		NS	
**14:03-*03:01*	1 (0.2)	0 (0.0)	1 (0.3)	6 (0.9)	NS		NS		NS	
**14:05-*05:03*	3 (0.7)	0 (0.0)	3 (0.8)	13 (1.9)	NS		NS		NS	
**14:06-*03:01*	3 (0.7)	0 (0.0)	3 (0.8)	7 (1.0)	NS		NS		NS	
**15:01-*06:02*	20 (4.8)	3 (6.0)	17 (4.7)	43 (6.6)	NS		NS		NS	
**15:02-*06:01*	16 (3.9)	0 (0.0)	16 (4.4)	79 (11.2)	9.5×10^{-5}	0.29	NS	0.07	1.2×10^{-3}	0.33
**16:02-*05:02*	7 (1.7)	2 (4.0)	5 (1.4)	6 (0.9)	NS		NS		NS	
Others	24 (5.8)	2 (4.0)	22 (6.0)	20 (2.9)						

GADab, antibodies to glutamic acid decarboxylase; NS, not significant.
Pc-, *P*-values corrected for number of different haplotypes tested (×25).
†Allele number.

GADab-negative patients with fulminant type 1 diabetes than in control subjects.

In contrast, only *DRB1*09:01-DQB1*03:03* was significantly more frequent in GADab-positive patients with fulminant type 1 diabetes than in controls. The frequency of *DRB1*09:01-DQB1*03:03* was significantly higher (44.0 vs 22.8%, *Pc* = 0.031) in GADab-positive patients than in GADab-negative patients with fulminant type 1 diabetes.

Comparison between DRB1*04:05-DQB1*04:01 and DRB1*09:01-DQB1*03:03 Haplotypes

To clarify the difference in the genetic contribution of the two major HLA haplotypes, *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03*, to fulminant type 1 diabetes, we analyzed the frequencies of homozygotes and heterozygotes with *DRB1*04:05-DQB1*04:01* and/or *DRB1*09:01-DQB1*03:03* in patients with this form of diabetes and control subjects. As shown in Table 4, homozygotes with both *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03* were significantly more frequent in total subjects of fulminant type 1 diabetes than in control subjects. Heterozygotes with *DRB1*04:05-DQB1*04:01*, but not *DRB1*09:01-DQB1*03:03*, were also significantly more frequent in these patients than in control subjects.

Similarly, both homozygotes and heterozygotes with *DRB1*04:05-DQB1*04:01* were significantly more frequent in GADab-negative patients with fulminant type 1 diabetes than in control subjects. Homozygotes, but not heterozygotes, with *DRB1*09:01-DQB1*03:03* were present significantly more frequently in GADab-negative patients than in control subjects.

In contrast, both homozygotes and heterozygotes with *DRB1*09:01-DQB1*03:03* were significantly more frequent in GADab-positive patients with fulminant type 1 diabetes than in control subjects. Furthermore, neither homozygotes nor heterozygotes with *DRB1*04:05-DQB1*04:01* were associated with GADab-positive patients with fulminant type 1 diabetes.

When analyzed by using a 2 × 3 contingency table (homozygote, heterozygote and null of *DRB1*04:05-DQB1*04:01* or *DRB1*09:01-DQB1*03:03* between GADab-positive and GADab-negative patients; Table 4), there was a significant difference in the frequency of *DRB1*09:01-DQB1*03:03* ($P = 0.0093$), but not in the frequency of *DRB1*04:05-DQB1*04:01* ($P = 0.29$), between GADab-positive and GADab-negative patients.

To further investigate the disease susceptibility and protection provided by HLA haplotypes in fulminant type 1 diabetes, we examined the genotypic combinations classified as high-frequency haplotypes (*DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03*) and low-frequency haplotypes (*DRB1*01:01-DQB1*05:01*, *DRB1*08:03-DQB1*06:01* and *DRB1*15:02-DQB1*06:01*) in patients with fulminant type 1 diabetes and in control subjects. As shown in Table 5, none of low-frequency haplotypes, such as *DRB1*01:01-DQB1*05:01*, *DRB1*08:03-DQB1*06:01* and *DRB1*15:02-DQB1*06:01*, conferred protection to fulminant type 1 diabetes in combination with high-frequency haplotypes, such as *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03*, although the number of patients was small.

Frequencies of the Genotypes of DRB1-DQB1 Haplotypes in Pregnancy

*DRB1*04:05-DQB1*04:01* was found to be significantly more frequent in the NPF group than in control subjects, whereas *DRB1*09:01-DQB1*03:03* was not significantly more frequent in either PF or NPF group compared with the controls (Table S1).

Homozygotes with *DRB1*04:05-DQB1*04:01* were significantly more frequent in the NPF group than in control subjects (Table S2). The frequency of homozygotes with *DRB1*04:05-DQB1*04:01* tended to be lower in the PF group than in the NPF group, but there was no significant difference between the groups. In contrast, neither homozygotes nor heterozygotes with *DRB1*09:01-DQB1*03:03* were associated with either the PF or NPF groups compared with the controls.

Table 4 | Combination of HLA-*DRB1-DQB1* haplotype in patients with fulminant type 1 diabetes and control subjects

DRB1-DQB1/DRB1-DQB1	Fulminant			Control ($n = 325$)	Total vs control		GADab(+) vs control		GADab(–) vs control	
	Total ($n = 207$)	GADab(+) ($n = 25$)	GADab(–) ($n = 182$)							
	n (%)	*n* (%)	*n* (%)	*n* (%)	*Pc*	OR	*Pc*	OR	*Pc*	OR
**04:05-*04:01/*04:05-*04:01*	31 (15.0)	2 (8.0)	29 (15.9)	8 (2.5)	2.0×10^{-7}	7.0	NS	3.4	6.6×10^{-8}	7.5
**04:05-*04:01/X*	73 (35.3)	7 (28.0)	66 (36.3)	76 (23.4)	8.8×10^{-3}	1.8	NS	1.3	5.8×10^{-3}	1.9
X/X	103 (49.8)	16 (64.0)	87 (47.8)	241 (74.2)	2.9×10^{-8}	0.35	NS	0.62	7.8×10^{-9}	0.32
**09:01-*03:03 *09:01-*03:03*	22 (10.6)	5 (20.0)	17 (9.3)	4 (1.2)	8.0×10^{-6}	9.5	1.3×10^{-6}	20.1	9.4×10^{-5}	8.3
**09:01-*03:03/Y*	61 (29.5)	12 (48.0)	49 (26.9)	81 (24.9)	NS	1.3	0.035	2.8	NS	1.1
Y/Y	124 (59.9)	8 (32.0)	116 (63.7)	240 (73.8)	2.2×10^{-3}	0.53	2.8×10^{-5}	0.17	NS	0.62

NS, not significant; GADab, antibodies to glutamic acid decarboxylase.

Pc-, *P*-values corrected for number of different haplotypes tested. *X* does not contain *DRB1*04:05-DQB1*04:01*. *Y* does not contain *DRB1*09:01-DQB1*03:03*.

Table 5 | Genotypic combination of *DRB1-DQB1* haplotype in patients with fulminant type 1 diabetes and control subjects

Allele frequency High/Low *DRB1-DQB1/ DRB1-DQB1*	Fulminant			Control (n = 325)	Total vs control		GADab(+) vs control		GADab(−) vs control	
	Total (n = 207)	GADab(+) (n = 25)	GADab(−) (n = 182)							
	n (%)	n (%)	n (%)	n (%)	P	OR	P	OR	P	OR
**04:05-*04:01/*										
**01:01-*05:01*	2 (1.0)	0 (0.0)	2 (1.1)	10 (2.9)	NS	0.31	NS	0.59	NS	0.35
**08:03-*06:01*	4 (1.9)	0 (0.0)	4 (2.2)	4 (1.2)	NS	1.6	NS	1.4	NS	1.8
**15:02-*06:01*	5 (2.4)	0 (0.0)	5 (2.7)	9 (2.6)	NS	0.87	NS	0.65	NS	0.99
**09:01-*03:03/*										
**01:01-*05:01*	1 (0.5)	0 (0.0)	1 (0.5)	7 (2.0)	NS	0.22	NS	0.83	NS	0.25
**08:03-*06:01*	4 (1.9)	0 (0.0)	4 (2.2)	7 (2.0)	NS	0.90	NS	0.83	NS	1.0
**15:02-*06:01*	2 (1.0)	0 (0.0)	2 (1.1)	15 (4.6)	0.037	0.20	NS	0.39	NS	0.23

GADab, antibodies to glutamic acid decarboxylase; NS, not significant.

DISCUSSION

The two important findings obtained from the present study were as follows: (i) the contribution of HLA genes to fulminant type 1 diabetes was clearly shown in a large-scale study; and (ii) the contribution of HLA genes to fulminant type 1 diabetes was different between GADab-positive and GADab-negative patients.

First, the present large-scale study has clarified the contribution of HLA genes to fulminant type 1 diabetes. We have reconfirmed that *DRB1*04:05-DQB1*04:01*, but not *DRB1*04:10-DQB1*04:02*, which also encodes DR4-DQ4, confers a strong predisposition to fulminant type 1 diabetes. Analysis of the combination of the HLA-*DRB1-DQB1* haplotype has shown that both homozygotes and heterozygotes with *DRB1*04:05-DQB1*04:01* show a strong effect regarding predisposition to fulminant type 1 diabetes (OR 7.0 and 1.8, respectively), as shown in a previous nationwide multicenter study[12,13]. [Correction to previous sentence, added after online publication 29 July 2011: "OR 6.4 and 1.9" is changed to "OR 7.0 and 1.8".] These findings suggest that *DRB1*04:05-DQB1*04:01* plays an important role in the development of fulminant type 1 diabetes.

We have also shown that the *DRB1*01:01-DQB1*05:01*, *DRB1*08:03-DQB1*06:01* and *DRB1*15:02-DQB1*06:01* haplotypes are negatively associated with fulminant type 1 diabetes. It is well known that both haplotypes of *DRB1*15:02-DQB1*06:01* and *DRB1*15:01-DQB1*06:02* encode DR2-DQ1[26]. In a previous study, we analyzed the serological subtype of HLA-DR-DQ and showed that the frequency of DR2-DQ1 was significantly lower in fulminant type 1 diabetes than in the control[12]. The present study has shown that *DRB1*15:02-DQB1*06:01*, but not *DRB1*15:01-DQB1*06:02*, which encode DR2-DQ1, was negatively associated with fulminant type 1 diabetes. Regarding the combination analysis, in the Japanese population, protective haplotypes, such as *DRB1*15:01-DQB1*06:02* and *DRB1*15:02-DQB1*06:01*, provide strong protection against type 1A diabetes regardless of the presence of susceptible haplotypes, such as *DRB1*09:01-DQB1*03:03* and *DRB1*04:05-DQB1*04:01*[13,26–31]. However, no such protective effect was observed in fulminant type 1 diabetes. This might show that protective haplotypes are not superior to susceptible haplotypes in fulminant type 1 diabetes.

*DRB1*09:01-DQB1*03:03*, in addition to *DRB1*04:05-DQB1*04:01*, haplotype was positively associated with fulminant type 1 diabetes. Recently, we have reported the differences in the contribution of HLA to genetic susceptibility to three subtypes of Japanese type 1 diabetes, acute-onset, fulminant and slowly-progressive, and that *DRB1*04:05-DQB1*04:01*, but not *DRB1*09:01-DQB1*03:03*, was associated with fulminant type 1 diabetes[13]. However, *DRB1*09:01-DQB1*03:03* was also high in frequency in the present study. We have two hypotheses to explain this discrepancy. One is that the maximum number of samples in the present study enabled us to re-evaluate the association of class II HLA genotype with fulminant type 1 diabetes. Another is the high frequency of *DRB1*09:01-DQB1*03:03* haplotype in GADab-positive patients with fulminant type 1 diabetes included in the present study. *DRB1*09:01-DQB1*03:03* conferred strong susceptibility to GADab-positive fulminant type 1 diabetes (OR 5.0). In addition, it has been reported that *DRB1*09:01-DQB1*03:03*, but not *DRB1*04:05-DQB1*04:01*, confers strong susceptibility to the disease development in pregnancy-associated fulminant type 1 diabetes in Japanese[32]. A similar trend was also observed in the present study, although the difference was not significant.

Second, the present study has clarified that the contribution of HLA genes to fulminant type 1 diabetes was different between GADab-positive and GADab-negative patients despite the similar clinical status. In the present large-scale study, the majority of fulminant type 1 diabetes, GADab-negative patients, was characterized by the predominance of *DRB1*04:05-DQB1*04:01* both in homozygous and heterozygous states. In contrast, *DRB1*09:01-DQB1*03:03*, but not *DRB1*04:05-DQB1*04:01*, was predominant in GADab-positive patients with fulminant type 1 diabetes. In addition, the protective effect of the *DRB1*15:02-DQB1*06:01* haplotype tended to be stronger in GADab-positive (0.0%, OR 0.07) than in GADab-negative

fulminant type 1 diabetes (4.4%, OR 0.33). In contrast, it is well known that the *DRB1*09:01-DQB1*03:03* haplotype is frequent in GADab-positive or typical autoimmune diabetic patients in Japan[13,27,28,31]. Kawabata *et al.* showed that the *DRB1*09:01-DQB1*03:03* haplotype confers much stronger susceptibility to Japanese typical autoimmune type 1 diabetes when present in a homozygous state and that the *DRB1*09:01-DQB1*03:03* haplotype predisposes in a recessive fashion. *DRB1*15:02-DQB1*06:01* also shows strong protection to classical type 1A diabetes[30]. High frequency of *DRB1*09:01-DQB1*03:03* homozygous state was also observed in GADab-positive fulminant type 1 diabetes in the present study (OR 20.1). Taken together, these findings suggest the similarity in underlying genetic backgrounds between classical autoimmune type 1 diabetes and GADab-positive fulminant type 1 diabetes, but not GADab-negative fulminant type 1 diabetes.

In conclusion, the present large-scale study showed the characteristic class II HLA genotypes in fulminant type 1 diabetes. The present study also implied that genetic contribution to disease susceptibility is distinct between GADab-positive and GADab-negative fulminant type 1 diabetes. Consequently, this disorder might be heterogeneous, as reflected by class II HLA and GADab, and further divided into at least two subtypes.

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APPENDIX

Other members of the Japan Diabetes Society Committee on Type 1 Diabetes Mellitus Research: Takuya Awata (Division of Endocrinology and Diabetes, Department of Medicine, Saitama Medical University); Yasuko Uchigata (Diabetes Center, Tokyo Women's Medical University School of Medicine); Minoru Okubo (Department of Endocrinology and Metabolism, Toranomon Hospital); Haruhiko Osawa (Department of Molecular and Genetic Medicine, Ehime University Graduate School of Medicine); Hiroshi Kajio (Department of Endocrinology and Metabolism, National Center for Global Health and Medicine); Hisashi Kamoi (Department of Medicine, Nagaoka Red Cross Hospital); Eiji Kawasaki (Department of Metabolism/Diabetes and Clinical Nutrition, Nagasaki University Hospital); Yumiko Kawabata (Department of Endocrinology, Metabolism and Diabetes, Kinki University School of Medicine); Jo Sato (Department of Diabetes and Metabolism, Iwate Medical University); Akira Shimada (Department of Internal Medicine, Saiseikai Central Hospital); Ikki Shimizu (Department of Internal Medicine, Ehime Prefectural Imabari Hospital); Kazuma Takahashi (Division of Diabetes and Metabolism, Department of Internal Medicine, Iwate Medical University School of Medicine); Shoichiro Tanaka (Third Department of Internal Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi); Masao Nagata (Department of Internal Medicine, Kakogawa Municipal Hospital); Koji Nakanishi (Department of Internal Medicine, Fuji Toranomon Orthopaedic Hospital); Sumie Fujii (Department of Internal Medicine, Ishikawa Prefectural Central Hospital); Taro Maruyama (Department of Internal Medicine, Saitama Social Insurance Hospital); Junnosuke Miura (Diabetes Center, Tokyo Women's Medical University School of Medicine); Satoshi Murao (Diabetes Center, Takamatsu Hospital).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 | *DRB1-DQB1* haplotypes in female patients with PF and NPF and in control subjects
Table S2 | Combination of HLA-*DRB1-DQB1* haplotype in female patients with PF and NPF and in control subjects

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