Differences in the Contribution of the *CTLA4* Gene to Susceptibility to Fulminant and Type 1A Diabetes in Japanese Patients

Eiji Kawasaki, md[1]
Akihisa Imagawa, md[2]
Hideichi Makino, md[3]
Miho Uga[1]
Norio Abiru, md[4]
Toshiaki Hanafusa, md[2]
Yasuko Uchigata, md[5]
Katsumi Eguchi, md[5]

OBJECTIVE — To examine the contribution of the *CTLA4* gene in the susceptibility to fulminant type 1 diabetes and compare it with classic type 1A diabetes.

RESEARCH DESIGN AND METHODS — We genotyped the +49G>A and CT60G>A variants of the *CTLA4* gene in fulminant type 1 diabetic patients ($n = 55$), classic type 1A diabetic patients ($n = 91$), and healthy control subjects ($n = 369$). We also assessed serum levels of the soluble form of CTLA4 (sCTLA4).

RESULTS — The +49GG and CT60GG genotypes were associated with type 1A diabetes ($P < 0.001$). In contrast, the CT60AA genotype, but not the +49G>A variation, was associated with fulminant type 1 diabetes ($P < 0.05$), especially in patients carrying HLA-*DR4* ($P < 0.01$). Serum levels of sCTLA4 were significantly decreased in patients with fulminant type 1 diabetes ($P < 0.05$).

CONCLUSIONS — These results suggest that *CTLA4* CT60 affects the genetic susceptibility to fulminant type 1 diabetes. Furthermore, the contribution of *CTLA4* to disease susceptibility is distinct between fulminant type 1 diabetes and classic type 1A diabetes.

Diabetes Care **31**:1608–1610, 2008

Fulminant type 1 diabetes is a subtype of type 1 diabetes characterized by extremely rapid onset that can be classified as type 1B diabetes (1). Although frequent flu-like symptoms prior to onset suggest the contribution of virus infection in the etiology of fulminant type 1 diabetes, both environmental and genetic factors are largely unknown. Susceptibility to classic type 1A diabetes is determined by multiple genes within the HLA region and non-HLA genes, including *INS-VNTR*, *CTLA4*, and *PTPN22* (2). Among them, *CTLA4* is associated with autoimmunity, cancer, allergy, and infectious disease. In the *CTLA4* region, a number of variants, such as +49G>A and CT60, have shown type 1 diabetes association (3). Although the association between class II HLA and fulminant type 1 diabetes has been reported (4), the contribution of the non-HLA genes to the susceptibility to fulminant type 1 diabetes has not been investigated. In this study, we examined the genetic contribution of the *CTLA4* gene to fulminant type 1 diabetes compared with classic type 1A diabetes.

RESEARCH DESIGN AND METHODS

— We examined 55 patients with fulminant type 1 diabetes (49% female, median age at onset 35.0 years), 91 patients with classic type 1A diabetes (57% female, median age at onset 17.0 years), and 369 healthy control subjects. Diagnostic criteria for fulminant type 1 diabetes are described elsewhere (1). The criteria for the recruitment of type 1A diabetic patients were presence of diabetic ketosis at onset, duration of hyperglycemic symptoms <3 months prior to initiation of insulin therapy, and positive for at least one of the anti-islet autoantibodies. This study was approved by the appropriate ethics committees, and informed consent was obtained from all subjects.

Genotyping of two single nucleotide polymorphisms in the *CTLA4* gene, +49G>A (rs231775) and CT60 (rs3087243), was performed as reported previously (5). Serum concentration of sCTLA4 was measured by enzyme-linked immunosorbent assay using human soluble CTLA4 (sCTLA4) kit (MedSystems Diagnostics, Vienna, Austria), according to the manufacturer's protocol. Sera from type 1 diabetic patients were obtained at disease onset and stored at −20°C until use.

The significance of differences in the distribution of genotypes between case and control subjects was determined by χ^2 test or Fisher's exact probability test. Comparisons of the sCTLA4 levels were made by ANOVA with phenotypic group alone and ANOVA with phenotypic group and *CTLA4* genotype. $P < 0.05$ was considered to be statistically significant.

RESULTS

— The +49G>A variant was associated with classic type 1A diabetes but not with fulminant type 1 diabetes (Table 1). In contrast, the contribution of CT60 to disease is distinct from that of +49G>A. The frequency of the CT60AA genotype in fulminant type 1 diabetic patients was significantly higher than in control subjects ($P = 0.021$) and type 1A diabetic patients ($P = 0.031$). CT60GG was associated with type 1A diabetes ($P = 0.008$). Because of the strong association of HLA-*DR4* in both patient groups (1), the effect of *CTLA4* on type 1 diabetes susceptibility relative to HLA-*DR4* was also examined. Among *DR4*-

From the [1]Department of Metabolism/Diabetes and Clinical Nutrition, Nagasaki University Hospital of Medicine and Dentistry, Nagasaki, Japan; the [2]First Department of Internal Medicine, Osaka Medical College, Osaka, Japan; the [3]Department of Laboratory Medicine, Ehime University School of Medicine, Ehime, Japan; the [4]Department of Endocrinology and Metabolism, Unit of Translational Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; and the [5]Diabetes Center, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.
Corresponding author: Eiji Kawasaki, eijikawa@nagasaki-u.ac.jp.
Received 7 February 2008 and accepted 20 April 2008.
Published ahead of print at http://care.diabetesjournals.org on 28 April 2008. DOI: 10.2337/dc08-0280.

Table 1—*CTLA4* polymorphisms in fulminant type 1 diabetic patients, type 1A diabetic patients, and healthy control subjects

				P value			OR (95% CI)		
	Fulminant	Type 1A	Control	Fulminant vs. control	Type 1A vs. control	Fulminant vs. type 1A	Fulminant vs. control	Type 1A vs. control	Fulminant vs. type 1A
+49 G>A									
n	55	91	369						
AA	13 (23.6)	7 (7.7)	61 (16.5)						
AG	23 (41.9)	36 (39.6)	186 (50.4)						
GG	19 (34.5)	48 (53.7)	122 (33.1)	NS	0.0013	0.012			
GG	19 (34.5)	48 (53.7)	122 (33.1)	NS	0.0005	0.033		2.26 (1.41–3.60)	0.47 (0.24–0.94)
AA + AG	36 (65.5)	43 (46.3)	247 (66.9)						
CT60 G>A									
n	55	91	369						
AA	8 (14.5)	4 (4.4)	22 (6.0)						
AG	26 (47.3)	28 (30.8)	165 (44.7)						
GG	21 (38.2)	59 (64.8)	182 (49.3)	0.043	0.029	0.0036			
GG	21 (38.2)	59 (64.8)	182 (49.3)	NS	0.008	0.0017		1.89 (1.18–3.05)	0.33 (0.17–0.67)
AA + AG	34 (61.8)	32 (35.2)	187 (50.7)						
AA	8 (14.5)	4 (4.4)	22 (6.0)	0.021	NS	0.031	2.68 (1.13–6.37)		3.70 (1.06–12.9)
AG + GG	47 (85.5)	87 (95.6)	347 (94.0)						
DR4 (+)									
n	42	52	121						
+49GG	14 (33.3)	27 (51.9)	38 (31.4)	NS	0.011	NS		2.35 (1.21–4.59)	
CT60AA	8 (19.0)	2 (3.8)	6 (5.0)	0.005	NS	0.018	4.51 (1.46–13.9)		5.88 (1.18–29.4)
DR4 (−)									
n	13	36	183						
+49GG	5 (38.5)	19 (52.8)	61 (33.3)	NS	0.026	NS		2.24 (1.08–4.61)	
CT60AA	0 (0.0)	3 (8.3)	16 (8.7)	NS	NS	NS			

Data are *n* (%). The interaction between *CTLA4* polymorphisms and HLA-*DR4* was assessed by a χ^2 test with a 2 × 2 contingency table (+49 GG vs. AG + AA or CT60 AA vs. AG + GG) in *DR4*-positive or -negative patients and the corresponding control subjects.

positive individuals, the frequency of the CT60AA genotype was significantly increased in patients with fulminant type 1 diabetes ($P = 0.005$). However, stratification of patients by the presence or absence of HLA-*DR4* did not affect the association between the +49GG genotype and type 1A diabetes (Table 1).

It has been reported that the CT60G allele might be associated with lower production of sCTLA4 mRNA (3). We therefore determined serum sCTLA4 levels. The mean sCTLA4 levels in fulminant type 1 diabetic patients (0.56 ± 0.24 ng/ml [mean ± SD], $n = 36$) was significantly lower than those in type 1A diabetic patients (0.94 ± 0.87 ng/ml, $n = 45$) and control subjects (0.89 ± 0.76 ng/ml, $n = 23$) ($P = 0.043$). A mixed-model ANOVA using phenotypic group (fulminant type 1 diabetes, type 1A diabetes, and control) and CT60 genotype (GG and GA+AA) as factorial fixed effects revealed no differences in sCTLA4 levels between CT60 genotypes ($P = 0.76$) or phenotype/genotype interactions ($P = 0.40$).

CONCLUSIONS — CTLA4, which delivers inhibitory signals to T-cell activation, is expressed on the surface of activated T-cells and regulatory T-cells, and the lack of CTLA4 results in uncontrolled T-cell–mediated lymphoproliferative disease (6). Furthermore, CTLA4 also has a significant biological role in attenuating T-cell responses in the context of an inflammatory environment, such as infection with a pathogen (7). We showed that *CTLA4* is associated with an increased risk of fulminant type 1 diabetes and that its contribution is distinct from classic type 1A diabetes. As reported previously (5,8), a significant association between classic type 1A diabetes and +49GG and CT60GG genotype was also found in the present study. However, the CT60AA genotype contributes to the susceptibility to fulminant type 1 diabetes. Moreover, it is implicated that HLA-*DR4* influences the association of fulminant type 1 diabetes with the CT60AA genotype.

In this study, we also revealed that serum sCTLA4 level in fulminant type 1 diabetic patients were significantly lower than those in type 1A diabetic patients and control subjects. Although it remains unknown how sCTLA4 regulates T-cell activation, recombinant sCTLA4 inhibits T-cell proliferation in vitro. Furthermore, sCTLA4 is constitutively expressed in nonstimulated T-cells, and its expression is downregulated after T-cell activation (9). Therefore, the decreased levels of sCTLA4 might indicate a lower potential of T-cell inhibition in fulminant type 1 diabetes, which might be caused by functional defects leading to reduced production of sCTLA4 or ongoing activation of the immune system eventually leading to decreased levels of sCTLA4.

In conclusion, the present study implicates that *CTLA4* confers susceptibility to fulminant type 1 diabetes. Furthermore, the different contributions of *CTLA4* to susceptibility to fulminant and classic type 1A diabetes indicate that the underlying immune process–primed β-cell injury might be distinct between these subtypes of type 1 diabetes.

Acknowledgments — This study was partly supported by a grant from the Ministry of Education, Culture, Science, Sports and Technology of Japan.

We thank Shinobu Mitsui for excellent technical assistance.

References

1. Kawasaki E, Matsuura N, Eguchi K: Type 1 in Japan. *Diabetologia* 49:828–836, 2006
2. Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 447: 661–678, 2007
3. Vaidya B, Pearce S: The emerging role of the CTLA-4 gene in autoimmune endocrinopathies. *Eur J Endocrinol* 150:619–626, 2004
4. Imagawa A, Hanafusa T, Uchigata Y, Kanatsuka A, Kawasaki E, Kobayashi T, Shimada A, Shimizu I, Maruyama T, Makino H: Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes mellitus. *Diabetologia* 48: 294–300, 2005
5. Ikegami H, Awata T, Kawasaki E, Kobayashi T, Maruyama T, Nakanishi K, Shimada A, Amemiya S, Kawabata Y, Kurihara S, Tanaka S, Kanazawa Y, Mochizuki M, Ogihara T: The association of CTLA4 polymorphism with type 1 diabetes is concentrated in patients complicated with autoimmune thyroid disease: a multicenter collaborative study in Japan. *J Clin Endocrinol Metab* 91:1087–1092, 2006
6. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW: Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270:985–988, 1995
7. McCoy K, Camberis M, Gros GL: Protective immunity to nematode infection is induced by CTLA-4 blockade. *J Exp Med* 186:183–187, 1997
8. Abe T, Yamaguchi Y, Takino H, Fujita N, Yamauchi DM, Ozaki M, Yamakawa K, Sera Y, Sakamaki H, Uotani S, Kawasaki E, Awata T, Yamasaki H, Eguchi K: CTLA4 gene polymorphism contributes to the mode of onset of diabetes with antiglutamic acid decarboxylase antibody in Japanese patients: genetic analysis of diabetic patients with antiglutamic acid decarboxylase antibody. *Diabet Med* 18: 726–731, 2001
9. Oaks MK, Hallett KM, Penwell RT, Stauber EC, Warren SJ, Tector AJ: A native soluble form of CTLA-4. *Cell Immunol* 201:144–153, 2000