

NeuroD/BETA2 Gene G→A Polymorphism May Affect Onset Pattern of Type 1 Diabetes in Japanese

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OBJECTIVE — The majority of type 1 diabetes is considered to be autoimmune with, for the most part, abrupt development. However, type 1 diabetes with slow onset, or the so-called slowly progressive type 1 diabetes or latent autoimmune diabetes in adults, has been recently recognized and is considered to be autoimmune-related. Although some investigators tried to explain the difference in onset pattern by the genetic background, including HLA type, it has not been established thus far. We hypothesized that the difference in onset pattern may relate to regeneration or differentiation of pancreatic β -cells, and we therefore focused on the NeuroD/BETA2 gene, which encodes a transcription factor for the insulin gene and β -cell differentiation.

RESEARCH DESIGN AND METHODS — We examined the NeuroD/BETA2 gene polymorphism in 105 Japanese type 1 diabetic patients and in 122 nondiabetic Japanese subjects in a case-control study, and we stratified the patients according to their onset pattern and islet-associated autoantibody positivity.

RESULTS — Regardless of the existence of islet-associated autoantibody, we found a significant difference in A allele frequency between type 1 diabetic patients with acute-onset type and control subjects. However, no difference was found between type 1 slow-onset diabetic patients and control subjects.

CONCLUSIONS — These results support our hypothesis that NeuroD/BETA2 may affect the ability of regeneration of β -cells, leading to a difference in the onset pattern and clinical course of type 1 diabetes.

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Type 1 diabetes is characterized by a destruction of pancreatic β -cells, thereby leading to insulin deficiency, and it is mostly considered to be a cell-mediated autoimmune disease (1). Antibodies against various islet antigens, such as GAD, appear in the serum of patients, and these antibodies are considered to reflect the autoimmune process in the pancreatic islets, which is termed insulinitis (2). In typical cases of type 1 diabetes, the onset is abrupt and insulin administration is required for survival soon after the onset. However, in the last decade, several studies demonstrated that type 1 diabetes is a heterogeneous disorder, and some cases are known to have a slow onset. These cases are initially classified as type 2 diabetes, and they gradually progress to an insulin-requiring state (3,4). Not only are antibodies to islet antigens detected, but also T-cell insulinitis was demonstrated in anti-GAD65 antibody (GADA)-positive diabetic patients with residual β -cell function or “slow-onset” type 1 diabetes (5). Although some investigators tried to reveal a genetic difference between “acute-onset” and “slow-onset” type 1 diabetes, they have examined only HLA type thus far (3,6). We hypothesized that the difference in onset pattern may relate to regeneration or differentiation of pancreatic β -cells. Among several differentiation factors for the pancreatic islets, NeuroD/BETA2 (β -cell E-box trans-activator 2) is known to act on the earliest islet precursors (7). Therefore, we examined the NeuroD/BETA2 gene in Japanese type 1 diabetes to elucidate the genetic difference between acute-onset and slow-onset type 1 diabetes.

RESEARCH DESIGN AND METHODS — We studied 105 unrelated Japanese type 1 diabetic patients (mean onset age 32.0 years, range 2–78) at Saitama Social Insurance Hospital and Keio University Hospital.

Diabetic patients who were proven to have none of the autoantibodies (GADA, anti-insulinoma-associated protein-2 [IA-2], and insulin autoantibody [IAA]) were designated as the autoantibody-negative group. Autoantibody positivity was defined as positive for having at least one GADA, IA-2 antibody, or IAA. No case was positive for IAA only, and five cases were positive for IA-2 antibody alone. No control subject was positive for these autoantibodies. The diagnosis of autoantibody-negative type 1 diabetes was made based on the criteria of the American Diabetes Association (ADA) for type 1 diabetes, with pancreatic β -cell destruction as the primary cause of the disorder and a tendency toward ketoacidosis (8). Patients who met these criteria formed

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Abbreviations: ADA, American Diabetes Association; GADA, anti-GAD65 antibody; IAA, insulin autoantibody; IA-2, anti-insulinoma-associated protein-2; VDR, vitamin D receptor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

group A ($n = 33$, mean onset age 26.3 years [range 2–62], mean period from diabetes onset to insulin treatment 1 month [range 0–6]). Group B consisted of patients with acute-onset type 1 diabetes with autoantibody who also met the ADA criteria for type 1 diabetes ($n = 48$, mean onset age 30.5 years [range 4–70], mean period from diabetes onset to insulin treatment 2 months [range 0–6]). Group C comprised patients with slow-onset type 1 diabetes ($n = 22$, mean onset age 45.2 years [range 25–78], mean period from diabetes onset to insulin treatment 71 months [range 17–216]) who met all of the following criteria: 1) originally diagnosed as type 2 diabetic and no sign of ketosis at diabetes onset; 2) proven autoantibody positivity; and 3) insulin treatment started ≥ 12 months after the diagnosis of diabetes. Insulin treatment was started when ketosis was proven or when at least two diabetologists considered that insulin treatment was necessary for survival. Two subjects in the period between onset and insulin requirement of 8 and 11 months, respectively, were excluded from the subpopulation study because these subjects were borderline between the group B and C categories.

A total of 122 unrelated nondiabetic Japanese subjects with no history of autoimmune disease were selected as the normal control subjects. This study was approved by the institutional review board, and written informed consent was obtained from all subjects.

Autoantibody measurement

Screening for GADA was performed using a recombinant human GAD kit (RSR, Cardiff, U.K.); positive was defined as a value above the mean + 3 SD of that in healthy subjects (an index >1.3 U/ml). Screening for IA-2 antibody (an index >0.010) and IAA (>50 nU/ml) was performed as previously described (9,10).

Genetic analyses

The G→A transition polymorphism in codon 45 of the NeuroD/BETA2 gene, leading to an Ala/Thr substitution in the NH₂-terminus, was determined by polymerase chain reaction–restriction fragment-length polymorphism method (11). HLA typing was performed by the hybridization protection assay as previously described (6).

Table 1—NeuroD/BETA2 G→A polymorphism in type 1 diabetic patients and control subjects

	Diabetic patients ($n = 105$)	Control subjects ($n = 122$)
Genotype frequencies*		
AA	11 (10.5)	4 (3.3)
AG	46 (43.8)	33 (27.0)
GG	48 (45.7)	85 (69.7)
Allele frequencies		
A	68 (32.4)	41 (16.8)
G	142 (67.6)	203 (83.2)

Data are n (%). For genotype frequencies $P = 0.0004$; for allele frequencies $P = 0.0001$. *Statistical analysis was made between “AA plus AG” and GG.

Statistical analyses

The frequencies of various alleles among the groups were compared by Fisher's exact test. To perform Fisher's exact test, numbers of AA and AG genotypes were combined as shown in the tables. Bonferroni's correction for multiple comparisons was performed where appropriate.

RESULTS— First, we compared NeuroD/BETA2 genotype and allele frequencies between the overall type 1 diabetes and control groups and found a significant difference between type 1 diabetic and control subjects ($P = 0.0004$ for genotype and $P = 0.0001$ for allele frequencies) (Table 1).

Moreover, when comparing NeuroD/

BETA2 genotype and allele frequencies between each subgroup of type 1 diabetic and control subjects, we found a significant difference between group A ($P = 0.0009$ for genotype frequencies, $P = 0.0002$ for allele frequencies) or B ($P = 0.0009$ for genotype frequencies, $P = 0.0007$ for allele frequencies) and the control group (patients with acute-onset type 1 diabetes with or without autoantibody and control subjects), whereas no significant difference was found between group C and the control group (slow-onset type 1 diabetic and control subjects) (Table 2).

One might argue that the patients in this study differed in the age at onset of diabetes. We compared genotype and allele frequencies of the NeuroD/BETA2 gene between patients with onset age over and below the mean onset age in each of the subgroups. In all of the subgroups, no significant difference was found regardless of the onset age. Moreover, we also compared genotype and allele frequencies of the NeuroD/BETA2 gene between control subjects over and below the mean age, and no significant difference was found.

In the Japanese population, HLA DR4 and DR9 are considered to be major susceptibility genes for type 1 diabetes (6), and Kobayashi et al. (3) reported that the frequencies of HLA A24 and Bw54 were different between acute-onset and slow-onset type. Therefore, we examined the HLA types of all the type 1 diabetic sub-

Table 2—NeuroD/BETA2 G→A polymorphism in patients with type 1 diabetes, analyzed according to clinical classification

	Group A	Group B	Group C	Control subjects
n	33	48	22	122
Autoantibody	Negative	Positive	Positive	
Onset	Acute onset	Acute onset	Slow onset	
Onset age* (years)	26.3 (2–62)	30.5 (4–70)	45.2 (25–78)	
Genotype frequencies†				
AA	5 (15.2)	5 (10.4)	1 (4.5)	4 (3.3)
AG	16 (48.5)	23 (47.9)	6 (27.3)	33 (27.0)
GG	12 (36.4)	20 (41.7)	15 (68.2)	85 (69.7)
P (vs. control subjects)	0.0009	0.0009	NS (>0.9999)	
Allele frequencies				
A	26 (39.4)	33 (34.4)	8 (18.2)	41 (16.8)
G	40 (60.6)	63 (65.6)	36 (81.8)	203 (83.2)
P (vs. control subjects)	0.0002	0.0007	NS (0.8284)	

Data are median (range) and n (%) unless otherwise indicated. *Significant differences on Mann-Whitney U test were found between A and C ($P < 0.0001$) and B and C ($P = 0.0005$); †statistical analysis was made between “AA plus AG” and GG.

jects ($n = 105$) who participated in this study. There was no significant difference regarding genotype and allele frequencies of the NeuroD/BETA2 gene between the HLA A24-positive and A24-negative groups. Regarding HLA Bw54, DR4 and DR9, as well as HLA A24, no significant difference between the HLA positive and negative groups was found.

CONCLUSIONS — NeuroD/BETA2 encodes a transcription factor for the insulin gene (12) and neurogenic differentiation factor (13,14). Hyperglycemia and ketonuria are observed in knock-out mice for this gene, and they die within several days, although their nervous system appears to develop normally (15). Therefore, this gene is considered to be a candidate susceptibility gene for type 1 diabetes. It is known that there is a G→A transition polymorphism in codon 45 of the NeuroD/BETA2 gene, leading to an Ala/Thr substitution in the NH₂-terminus, although the functional difference between the G and A alleles is unknown.

Previously, several studies showed no association between this gene and type 1 diabetes (16,17), whereas one study showed a significant association (11). Therefore, the role of this gene in type 1 diabetes is controversial and inconclusive. In this study, we demonstrated a significant association between this gene and acute-onset type 1 diabetes with or without autoantibody. Recently, acute-onset type 1 diabetes without islet-associated autoantibodies was reported by Imagawa et al. (18) as type 1B (“idiopathic” type 1 diabetes) based on the histological findings of the pancreas. In their report, they termed this type of diabetes “nonautoimmune” type 1 diabetes because they found no insulinitis. Although more discussion and accumulation of cases are necessary to reach a conclusion, Tanaka et al. (19) did find insulinitis in this type of diabetes, suggesting that this group may consist of subjects with both autoimmune and non-autoimmune type 1 diabetes. Although group A in this study (acute-onset type 1 diabetes without autoantibody) may not be a homogeneous population, and we must establish a suitable marker of autoimmunity other than autoantibody measurement to detect autoimmune-related type in this group, we recently observed that there seems to be a difference between acute-onset type 1 diabetes without autoantibody and acute-onset type 1

diabetes with autoantibody regarding genotype frequencies and allele frequencies of the vitamin D receptor (VDR) gene, which may be related to T-helper 1 function according to the data of animal models. The VDR gene is considered to be more relevant to autoimmunity (Y.M., S.Y., T.Y., T.M., A.K., H.H., K.M., A.S., T.S., T. Fukazawa, M. Niino, unpublished observations). Taken together, we assume that the NeuroD/BETA2 gene may affect the clinical onset pattern of type 1 diabetes, and this effect may not be related to the existence of autoimmunity.

In previous studies, Owerbach et al. (16) and Dupont et al. (17) showed no association between this gene and type 1 diabetes. Dupont et al. (17) suggested an ethnic difference between Caucasians and Japanese because no AA homozygote was found in a previous study in Japanese (11). However, in our study, we did find AA homozygotes in both type 1 diabetes and control subjects (10.5 and 3.3%, respectively) in the Japanese population. Moreover, the onset age was different between other studies and ours, with a maximum age of 18 years in the Owerbach et al. (16) study, a mean age of 12.9 years in the Dupont et al. (17) study, and a mean age of 32.0 years in our study. The onset pattern was not assessed in other studies. Therefore, we propose that the discrepancy in several reports regarding this gene in type 1 diabetes may not be simply explained by the difference between Caucasians and Japanese and that at least onset age and onset pattern should be matched to reach a conclusion.

In another study, Hansen et al. (20) suggested the possibility that an unknown gene other than NeuroD/BETA2 is the actual responsible gene. In chromosome 2q31-35, however, Owerbach et al. (16) found no evidence that candidate genes, such as HOXD8, IGFBP5, and CTLA-4, are associated with susceptibility to type 1 diabetes, and we also found no association between the CTLA-4 gene and type 1 diabetes (21). Therefore, we think that NeuroD/BETA2 is the actual responsible gene.

We speculate that NeuroD/BETA2 may affect the regeneration or differentiation of pancreatic β -cells, thereby leading to a difference in the onset pattern and clinical course of type 1 diabetes, although a future histological study to assess regeneration of the pancreas may be necessary to reach a conclusion. We be-

lieve that this is the first report to show a genetic difference between acute-onset and slow-onset type 1 diabetes in relation to a non-MHC gene and that these results will contribute to the prediction of the onset and a better understanding of the pathophysiology of type 1 diabetes.

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