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The Onset of Diabetes in Three out of Four Sisters: A Japanese Family with Type 1 Diabetes. A Case Report

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Abstract. Type 1A diabetes is an autoimmune disease characterized by the destruction of insulin-producing β-cells in the pancreas. The HLA-*DR* and -*DQ* genes are well established as being associated with increased risk for type 1 diabetes. Moreover, polymorphisms in *CTLA4* have been reported to be associated with susceptibility to type 1 diabetes and autoimmune thyroid disease (AITD). In both Caucasian and Japanese populations, the lifetime risk in siblings of type 1 diabetic probands is much higher than that in general populations. However, in Japan, where the prevalence of type 1 diabetes is less than one-tenth that of most Caucasian populations, it is rare for type 1 diabetes to develop in three or more siblings within a family. Here, we report a Japanese family in which type 1 diabetes occurred in three siblings amongst four sisters. Three probands of type 1 diabetes had the same combination of HLA haplotypes, *DRB1*0405-DQB1*0401/DRB1*0802-DQB1*0302*, which occurs significantly more often in type 1 diabetes patients than in control subjects in the Japanese population. With respect to the rs3087243 (+6230G>A) polymorphism of *CTLA4*, the first sister had type 1 diabetes and AITD and had the GG genotype, whereas the second and third sisters, who had type 1 diabetes without AITD, had the AG genotype. This is the first report of a family in which type 1A diabetes developed in three siblings. We performed genetic analysis of HLA-*DR*, -*DQ*, and *CTLA4* in all family members. Even in a country where the prevalence of type 1 diabetes is low, diabetic proband siblings should be monitored for the onset of type 1 diabetes.

Key words: Type 1 diabetes, Sibling, Japanese, HLA, *CTLA4*

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TYPE 1A diabetes is caused by autoimmune destruction of insulin-producing β-cells of the pancreas in genetically susceptible individuals. The major histocompatibility complex (MHC) is reported to account for 30–50% of the familial aggregation of type 1 diabetes [1]. The human leukocyte antigen (HLA)-*DR* and -*DQ* genes are well established as being associated with risk for type 1 diabetes. In the Japanese population, three haplotypes, *DRB1*0405-DQB1*0401*, *DRB1*0802-DQB1*0302*, and *DRB1*0901-DQB1*0303*, which are rare in Caucasian populations, confer susceptibility to type 1 diabetes [2, 3, 4]. In addition, several non-HLA loci have been identified as putative susceptibility genes by candidate gene approaches and/or genome-wide scanning. Among these is the gene for cytotoxic T-lymphocyte-associated-4 (*CTLA4*), which has been reported to be associated with increased susceptibility to type 1 diabetes and autoimmune thyroid disease (AITD) in Caucasian and Japanese populations [5, 6].

In Caucasian populations, the lifetime risk in siblings of type 1 diabetic probands has been reported to be much higher than that in the general population (6% versus 0.4%, λs 15), indicating that type 1 diabetes clusters in families [7]. In the Japanese population, although the prevalence of type 1 diabetes is very low and is less than one-tenth that in most Caucasian populations, studies with a large number of families with type 1 diabetic probands from different data sources have revealed much higher frequencies of type 1 dia-

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betes in siblings of diabetic probands than in the general population (1.3–3.8% versus 0.01–0.02%, λs >65) [3, 8, 9]. This indicates that type 1 diabetes clusters in families in Japan. Although the λs in the Japanese population is higher than that for Caucasian populations, the absolute number of multiplex families with type 1 diabetes is limited because of the very low incidence of type 1 diabetes in Japan. In particular, there are very few families in Japan in which type 1 diabetes has been diagnosed in three or more siblings. Here, we report a Japanese family with four sisters, in which three siblings developed type 1A diabetes and the fourth sibling was positive for islet-related autoantibodies, indicative of an ongoing type 1A process. In the present report, we determined the genotype of the HLA and *CTLA4* polymorphisms in the four siblings and in their parents to better understand the genetic basis for the familial clustering of type 1 diabetes in this family.

Case Report

A 40-year-old woman, who was the eldest of four sisters, was referred to a hospital because of general malaise, thirst, polydipsia, polyuria, and body weight loss. Although she had no prior history of diabetes, she was diagnosed as hyperglycemic and she was admitted to our hospital in April 2008. Laboratory data on admission (Table 1) revealed diabetic ketosis, elevated HbA_{1c}, and she was positive for antibodies against glutamic acid decarboxylase (GAD), insulinoma-associated protein 2 (IA-2), thyroglobulin (Tg), and thyroid peroxidase (TPO). Based on these findings, we diagnosed her with type 1A diabetes and AITD, and we started her on insulin therapy.

Figure 1 shows the pedigree of the present family. The second and third sisters developed type 1A diabetes at 6 and 12 years old, respectively. Table 2A shows the results of urinary and serum C-peptide in each of the family members, including the parents. The urinary and serum C-peptide levels were markedly decreased in the three probands of type 1 diabetes. On the other hand, the insulin secretory capacities of the fourth sibling and of the parents were not reduced, based on the their postprandial serum C-peptide levels. Table 2A also shows the characteristics of the anti-islet and anti-thyroid antibodies in each family member. In the second and third siblings, frozen sera, which were obtained 10 years ago, were used to measure the antibodies because many years had passed after their onset of type 1 diabetes. The first and third siblings had high titers of anti-GAD and anti-IA-2 antibodies. Although the fourth sibling had no prior history of diabetes, anti-GAD and anti-IA-2 antibodies were present. Because of the presence of anti-Tg and anti-TPO antibodies in the first sibling, we determined them in all of the family members. In addition to the first sibling, the mother had high titers of anti-Tg and anti-TPO antibodies, but their thyroid functions were within the normal range.

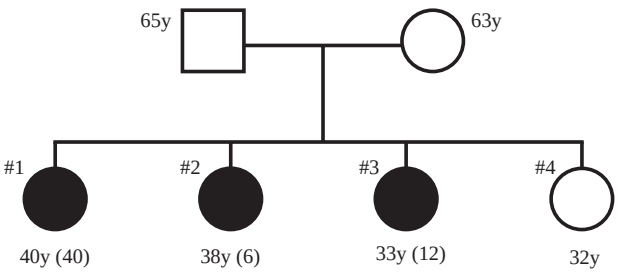

Fig. 1. Pedigree of the present family. The numbers in the brackets show the age at onset of type 1 diabetes. y = years old.

Table 1. Laboratory data for the first sibling on admission.

Postprandial plasma glucose	345 *mg/dL*	Postprandial serum C-peptide	0.4 *ng/mL*
HbA_{1C}	14.3*%*	Urinary C-peptide	8.0*μg/day*
3-OHBA	5819*μmol/L*	Urinary glucose	5+
ACAC	780*μmol/L*	Urinary ketone	4+
		Urinary protein	-
WBC	3600*/μL*		
Hb	14.3 *g/dL*	Anti-GAD antibody	433 *U/mL*
Plts	$25*10^4/\mu L$	Anti-IA-2 antibody	18 *U/mL*
Alb	4.2 *g/dL*	Anti-Tg antibody	5.2 *U/mL*
AST	58 *IU/L*	Anti-TPO antibody	50.0< *U/mL*
ALT	53 *IU/L*	TRAb	6.2*%*
AMY	48 *IU/L*	f-T_4	1.16 *ng/dL*
Na	134 *mEq/L*	f-T_3	1.4 *pg/mL*
K	3.9 *mEq/L*	TSH	1.65*μU/mL*
Cl	95 *mEq/L*	Arterial Blood Gas Analysis	
BUN	11 *mg/dL*	on room air	
Cre	0.36 *mg/dL*	pH	7.384
LDL-C	119 *mg/dL*	pCO_2	37.7 *mmHg*
TG	106 *mg/dL*	pO_2	102.3 *mmHg*
HDL-C	110 *mg/dL*	HCO_3^-	22 *mmol/L*
		B.E.	-2.6 *mmol/L*

TRAb = anti TSH receptor antibody. TSH = Thyrotropin. Anti-GAD, anti-IA-2, anti-Tg and anti-TPO antibodies were determined with a commercially available radioimmunoassay kit (Cosmic, Tokyo, Japan). TRAb was measured by a commercially available radioreceptor assay kit (Cosmic, Tokyo, Japan).

Table 2. Analytical parameters of the present family.
A) Urinary and serum C-peptide, plasma glucose, anti-islet antibodies, and anti-thyroid antibodies.

	Urinary C-peptide (μg/day)	Postprandial serum C-peptide (ng/mL)	Postprandial plasma glucose (mg/dL)	GAD (U/mL)	IA-2 (U/mL)	IAA (%)	TgAb (U/mL)	TPOAb (U/mL)	TRAb (%)
Father	n.d.	3.56	117	<0.3	<0.4	0.4	<0.3	<0.3	0
Mother	n.d.	2.85	127	<0.3	<0.4	<0.4	1.3	30.4	4.4
#1	8.0	0.4	345	433	18	n.d.	5.2	50<	6.2
#2	2.9	<0.03	189	<0.3	<0.4	n.d.	<0.3	<0.3	0
#3	1.1	0.05	383	3.5	0.5	n.d.	<0.3	<0.3	0
#4	n.d.	2.48	95	6.8	4.9	<0.4	<0.3	<0.3	0

B) HLA-*DRB1* and -*DQB1* haplotypes, and *CTLA4* polymorphisms.

	DRB1-DQB1	*CTLA4*	
		rs3087243 (+6230G>A)	rs231775 (+49G>A)
Father	*DRB1*0405-DQB1*0401/ DRB1*0405-DQB1*0401*	GG	GG
Mother	*DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*	AG	AA
#1	*DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*	GG	AG
#2	*DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*	AG	AG
#3	*DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*	AG	AG
#4	*DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*	GG	AG

Serum C-peptide and plasma glucose were measured in the postprandial state. GAD = anti glutamic acid decarboxylase antibody. IA-2 = anti insulinoma-associated protein 2 antibody. IAA = anti insulin autoantibody. TgAb = anti thyroglobulin antibody. TPOAb = anti thyroid peroxidase antibody. TRAb = anti TSH receptor antibody. n.d. = not determined.
Anti-GAD, anti-IA-2, anti-Tg and anti-TPO antibodies were determined with a commercially available radioimmunoassay kit (Cosmic, Tokyo, Japan). Anti-IAA antibody was measured by a sensitive radioimmunoassay kit (Yamasashoyu Co. Ltd., Chiba, Japan. Normal range; <0.4%). TRAb was determined with a commercially available radioreceptor assay kit (Cosmic, Tokyo, Japan).

After obtaining written informed consent, we performed genetic analysis in all of the subjects. Table 2B shows the HLA-*DRB1* and -*DQB1* haplotypes, and *CTLA4* polymorphisms in the present family. Three probands of type 1 diabetes had the same combination of HLA haplotypes, namely *DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*. Furthermore, the same combinations of HLA haplotypes were detected in the fourth sibling and in the mother who had no prior history of diabetes. The father had *DRB1*0405-DQB1*0401/ DRB1*0405- DQB1*0401*. Two single nucleotide polymorphisms in the *CTLA4* gene, rs3087243 (+6230G>A) and rs231775 (+49G>A), have been reported to be associated with type 1 diabetes and AITD [6, 10]. In the analysis of the rs3087243 polymorphism of *CTLA4*, the father, and the first and fourth siblings had the GG genotype. On the other hand, the mother, and the second and third siblings had the AG genotype. With respect to the *CTLA4* rs231775 polymorphism, the father and mother had the GG and AA genotype, respectively, and all four siblings had the AG genotype.

Discussion

Large families with type 1 diabetes arising from a common ancestor have been described for Bedouin Arabs [11] and in the Netherlands [12]. The diabetes susceptibility locus, *IDDM17*, was mapped by genetic linkage analysis in a Bedouin Arab family, in which 19 family members across three generations had type 1 diabetes [11]. In addition to the HLA locus, evidence for type 1 diabetes loci was observed on chromosome 8q24 and on chromosome 17q24 in an analyses of 43 subjects in the Netherlands that could be traced back

to a common ancestor within 15 generations [12]. In a study of 767 multiplex Caucasian families, which showed evidence of linkage of seven regions of the genome to type 1 diabetes, 51 families had three affected siblings, one had four affected siblings, and two with five affected siblings [13]. However, to our knowledge, no case reports have described the development of type 1 diabetes in three or more siblings or performed genetic analysis in Japanese subjects. In this report, we have described a Japanese family in which type 1A diabetes occurred in three siblings and ongoing islet autoimmunity was present in the fourth sibling of the four sisters.

Population studies have shown that HLA associations may vary depending on the geographic and ethnic origin. In Caucasian populations, predisposition to type 1 diabetes is mostly associated with the *DRB1*03-DQB1*0201* and/or *DRB1*04-DQB1*0302* haplotypes [14, 15]. Meanwhile, in Japanese populations, the *DRB1*03-DQB1*0201* haplotype is absent or very rare, and *DRB1*04-DQB1*0302* is not associated with type 1 diabetes because of differences in *DRB1*04* subtypes. Instead, three alternative haplotypes, *DRB1*0405-DQB1*0401*, *DRB1*0802-DQB1*0302*, and *DRB1*0901- DQB1*0303*, which are rare in Caucasian populations, confer susceptibility to type 1 diabetes [2, 3, 4]. Moreover, in a Japanese study, the frequencies of the *DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302* and *DRB1*0901-DQB1*0303/ DRB1*0901-DQB1*0303* genotypes were significantly higher in subjects with type 1 diabetes than in control subjects [2, 4]. In the present case, three siblings with type 1 diabetes had the same combination of high-risk HLA haplotypes, namely *DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*, suggesting an association between the onset of type 1 diabetes and HLA-linked susceptibility in the three probands.

In the present three probands, type 1 diabetes occurred in the first sibling at the age of forty, whereas the second and third siblings were diagnosed with type 1 diabetes under the age of 20 years. Several environmental agents have been reported as triggers for type 1 diabetes. However, all of the probands received their mother's breast milk and were raised in very similar situations. On the other hand, previous studies have suggested that the HLA class I region contributes to the susceptibility to and the age-at-onset of type 1 diabetes [16, 17, 18]. Indeed, the presence of HLA-*A*2402* was associated with age-at-onset of type 1 diabetes in an analysis of Caucasian sibling pairs [16]. Furthermore, the presence of HLA-A24 was shown to accelerate β-cell destruction in type 1 diabetes, and to confer susceptibility to type 1 diabetes in Japanese subjects [19, 20]. Thus, the analysis of the HLA class I gene and further studies are warranted to better understand the reasons for the difference of age-at-onset in the three probands.

The fourth sibling had a high titer of autoantibodies against GAD and IA-2, and had *DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*, which was the same genotypic combination as her three siblings with type 1 diabetes. In a Caucasian study, the presence of two or more anti-islet antibodies was reported to be highly predictive of the development of type 1 diabetes among first-degree relatives of type 1 diabetic patients [21]. Additionally, the risk for islet autoimmunity was found to be dramatically increased in siblings who shared both HLA haplotypes with their diabetic proband sibling compared with siblings who did not share both HLA haplotypes with their diabetic proband sibling [22]. Therefore, we speculate that this sibling is at very high risk of developing type 1 diabetes, and we need to continue to follow-up this subject with care.

Autoantibodies against GAD and IA-2 were not present in the second sibling. Type 1 diabetes occurs in genetically susceptible subjects and is probably triggered by one or more environmental agents, such as viral infections. The second sibling had rubella virus infection at the age of 6 years. Two months later, she complained of severe thirst and started insulin therapy to treat hyperglycemia. In addition, her urinary C-peptide excretion was very low. Furthermore, she had the HLA haplotypes *DRB1*0405-DQB1*0401/ DRB1*0802-DQB1*0302*. Based on these findings, we diagnosed the second sibling with type 1A diabetes, despite the absence of anti-GAD and anti-IA-2 antibodies.

CTLA4 has been reported to be associated with susceptibility to type 1 diabetes and AITD in Caucasian and Japanese populations. With respect to the *CTLA4* rs3087243 polymorphism, the frequency of the GG genotype was significantly higher in patients with type 1 diabetes and AITD than in control subjects in a Japanese population [6]. In the present family, only the first sibling of the three probands had both type 1 diabetes and AITD, and she had the GG genotype in

rs3087243 of *CTLA4*.

To our knowledge, our present report is the first to describe the development of type 1A diabetes in three siblings and to perform genetic analysis of HLA-*DR*, *-DQ*, and *CTLA4* in Japanese subjects. Even in a country where the prevalence of type 1 diabetes is very low, the onset of type 1 diabetes should be considered and anti-islet antibodies should be determined in diabetic proband siblings. Further studies on the genetic background of such families may lead to the identification of rare variants that confer susceptibility to type 1 diabetes.

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