Rare human leukocyte antigen genotype in two siblings with type 1 diabetes in a Japanese family clustered with type 1 diabetes

Yujiro Ina1, Yumiko Kawabata2, Ryuichi Sakamoto1, Naotaka Sekiguchi1, Hiroshi Ikegami2*

1Department of Diabetes and Endocrinology, Saiseikai Fukuoka General Hospital, Fukuoka, and 2Department of Endocrinology, Metabolism and Diabetes, Kindai University Faculty of Medicine, Osaka, Japan

Keywords
Genetic susceptibility, Human leukocyte antigen haplotype, Type 1 diabetes

*Correspondence
Hiroshi Ikegami
Tel.: +81-72-366-0221
Fax: +81-72-366-2095
E-mail address: ikegami@med.kindai.ac.jp

J Diabetes Investig 2017; 8: 762–765
doi: 10.1111/jdi.12628

INTRODUCTION
Type 1 diabetes is an autoimmune disease caused by destruction of insulin-producing β-cells of the pancreas in genetically susceptible individuals1. Type 1 diabetes clusters in families, not only in Western countries2, but also in Japan, as evidenced by the much higher frequency in siblings of a type 1 diabetic proband than in the general population3,4. However, because of the very low prevalence of type 1 diabetes in the Japanese population, there are very few families in Japan in which type 1 diabetes has been diagnosed in three or more members. Identification and studies on the genetic background of such families are expected to provide important information on susceptibility genes for type 1 diabetes, which cannot be identified in case-control association studies.

In the present study, we report a Japanese family in which three members developed type 1 diabetes. We studied the genotype of the human leukocyte antigen (HLA), a well-known susceptibility gene for type 1 diabetes, to better understand the genetic basis for the familial clustering of type 1 diabetes.

MATERIALS AND METHODS
The pedigree of the family is shown in Figure 1. The proband (case 1) was a 36-year-old man. He presented thirst, polyuria and weight loss at the age of 11 years. When he was admitted to a hospital 2 weeks after the initial symptoms, his blood glucose was approximately 800 mg/dL, and his glycated hemoglobin (HbA1c) was approximately 12%. He was diagnosed as having acute-onset type 1 diabetes, and insulin therapy was started immediately and continued thereafter. He is currently being treated with multiple injection of insulin. His laboratory data is shown in Table 1. His serum C-peptide immunoreactivity was below the detection limit (<0.01 ng/mL).

The 40-year-old older sister (case 2) of case 1 was referred to a hospital because of excessive fatigue since the age of 38 years. Her blood glucose and HbA1c levels were 129 mg/dL and 6.5%, respectively. She was diagnosed as having diabetes. Although she was suspected to have slowly progressive type 1 diabetes because anti-glutamic acid decarboxylase (GAD) antibody (Ab) was positive (12.2 U/mL, assessed by radioimmunoassay, cut-off value <1.5 U/mL), she was observed without medication, because her insulin secretory
capacity was not reduced (serum C-peptide immunoreactivity was 1.43 ng/mL). One year later, the patient developed thirst and polyuria, and she was again referred to a hospital. Her blood glucose and HbA1c levels were 238 mg/dL and 8.4%, respectively (Table 1). She was positive for anti-GAD Ab, and was diagnosed as having slowly progressive type 1 diabetes. She was immediately treated with intensive insulin therapy.

Their 61-year-old mother (case 3) had been receiving treatment with oral antidiabetic drugs since she was diagnosed as diabetes 10 years earlier. When her daughter (case 2) was suspected to have slowly progressive type 1 diabetes, she became anxious and was admitted to a hospital for an examination. Her anti-GAD Ab was positive (4.7 U/mL), and she was diagnosed as having slowly progressive type 1 diabetes and insulin therapy was started.

Anti-GAD, anti-insulinoma-associated protein-2 (IA-2), anti-thyroglobulin (Tg) and anti-thyroid peroxidase (TPO) Abs were determined with radioimmunoassay (Cosmic, Tokyo, Japan). Anti-zinc transporter 8 (ZnT8) Ab were assayed by enzyme-linked immunosorbent assay (Cosmic). Thyrotropin receptor antibody was measured by radioreceptor assay (Cosmic). HLA was genotyped by the polymerase chain reaction sequence-based typing method. The most probable haplotypes were deduced according to known linkage disequilibria in the Japanese population, and were confirmed by the comparison with the genotypes of other members of the family.

RESULTS

The status of autoantibodies against islets and the thyroid gland are shown in Table 1. Case 1 was negative for islet-related Abs (anti-GAD Ab: 0.3 U/mL, anti-IA-2 Ab: 0.1 U/mL, anti-ZnT8 Ab: <10.0 U/mL), which were tested 25 years after the onset of the disease. Case 2 was positive for anti-GAD Ab (15.0 U/mL) and anti-ZnT8 Ab (15.7 U/mL), but negative for anti-IA-2 Ab. Case 3 and the father were positive for anti-GAD Ab (4.7 and 2.6 U/mL, respectively), but negative for anti-IA-2 Ab and anti-ZnT8 Ab. Case 2 was positive for anti-Tg Ab, and the mother (case 3) was positive for anti-TPO Ab and anti-Tg Ab. The data of the older brother in this family is not available, because we could not obtain his agreement.

After obtaining written informed consent, we studied the HLA genotypes in this family (Table 2). The proband (case 1) and his older sister (case 2) had the same combination of HLA class II haplotypes, DRB1*08:02-DQB1*03:02/DRB1*08:02.

Table 1 | Laboratory data of the family members

| Normal range | Proband | Sister | Mother | Father (unaffected) |
|--------------|---------|--------|--------|---------------------|
| Age at onset (years) | 11 | 39 | 52 | – |
| Age at the examination (years) | 36 | 40 | 61 | 71 |
| Blood glucose (mg/dL) | 187 | 238 | 137 | 96 |
| HbA1c (%) | 7.3 | 8.4 | 8.2 | 5.9 |
| Serum C-peptide (ng/mL) | <0.01 | 2.1 | 1.3 | 2.1 |
| Anti-GAD Ab (U/mL) | <1.5 U/mL | 0.3 | 15.0 | 4.7 |
| Anti-IA-2 Ab (U/mL) | <0.4 U/mL | 0.1 | 0.0 | 0.0 |
| Anti-ZnT8 Ab (U/mL) | <15.0 U/mL | <10.0 | 15.7 | <10.0 |
| FT4 (ng/mL) | 1.32 | 1.40 | 1.11 | ND |
| TSH (µIU/mL) | 14.0 | 1.00 | 1.49 | ND |
| TPO Ab (U/mL) | <16.0 U/mL | 14.0 | 10.0 | 48.0 |
| Tg Ab (U/mL) | <280 U/mL | 17.0 | 54.0 | 65.0 |
| TR Ab (IU/L) | <1.0 IU/L | <1.0 | <1.0 | <1.0 |

Anti-glutamic acid decarboxylase (GAD), anti-insulinoma-associated protein-2 (IA-2), anti-thyroglobulin (Tg) and anti-thyroid peroxidase (TPO) antibodies (Ab) were determined with radioimmunoassay (Cosmic, Tokyo, Japan). Anti-zinc transporter 8 (ZnT8) antibody was determined with enzyme-linked immunosorbent assay (Cosmic, Tokyo, Japan). Thyrotropin receptor antibody was measured by radioreceptor assay (Cosmic, Tokyo, Japan). FT4, free thyroxin; HbA1c, glycated hemoglobin; ND, not done; TR, thyrotropin receptor; TSH, thyroid-stimulating hormone.
SHORT REPORT

Table 2 | Genotypes of DRB1, DQB1, A, B, C, and genotypic combination of class II DRB1-DQB1 and class I A-B-C haplotypes of the family members

| Genes | Proband Case 1 | Sister Case 2 | Mother Case 3 | Father (unaffected) |
|-------|----------------|--------------|---------------|--------------------|
| DRB1  | *08:02*08:02   | *08:02*08:02 | *08:02*13:02  | *08:02*15:01       |
| DQB1  | *03:02*03:02   | *03:02*03:02 | *03:02*06:04  | *03:02*06:02       |
| A     | *02:01*26:01   | *02:01*26:01 | *26:01*33:03  | *02:01*24:02       |
| B     | *35:01*35:01   | *35:01*35:01 | *35:01*44:03  | *15:03*35:01       |
| C     | *03:03*08:01   | *03:03*08:01 | *03:03*14:03  | *03:03*08:01       |
| Haplotypes | DRB1*08:02-DQB1*03:02/ | DRB1*08:02-DQB1*03:02/ | DRB1*08:02-DQB1*03:02/ | DRB1*08:02-DQB1*03:02/ |
| A-B-C | A*02:01-B*35:01-C*08:01/A*26:01-B*35:01-C*03:03 | A*02:01-B*35:01-C*08:01/A*26:01-B*35:01-C*03:03 | A*26:01-B*35:01-C*03:03/A*33:03-B*44:03-C*14:03 | A*24:02-B*15:07-C*03:03 |

Haplotypes were deduced according to known linkage disequilibria in the Japanese population, and were confirmed by comparison with the genotypes of other members of the family.

DQB1*03:02. The father had DRB1*08:02-DQB1*03:02/DRB1*15:01-DQB1*06:02. The mother had DRB1*08:02-DQB1*03:02/DRB1*13:02-DQB1*06:04. The proband (case 1) and his older sister (case 2) had the same combination of HLA class 1 haplotypes, A*02:01-B*35:01-C*08:01/A*26:01-B*35:01-C*03:03. The father had A*02:01-B*35:01-C*08:01/A*24:02-B*15:07-C*03:03. The mother had A*26:01-B*35:01-C*03:03/A*33:03-B*44:03-C*14:03.

DISCUSSION

Although the incidence and prevalence of type 1 diabetes are much lower in Japanese than in Caucasian populations, frequencies of type 1 diabetes in siblings of a type 1 diabetic proband in the Japanese population is comparable with those in Caucasian populations. The ratio of frequencies in siblings and the general population, termed λs, is often used to express the degree of familial clustering of a disease and is estimated to be over 65 for type 1 diabetes in the Japanese population, showing strong clustering of type 1 diabetes in siblings of a type 1 diabetic proband. Studies on the genetic basis of clustering of type 1 diabetes in such families in the Japanese population are therefore expected to provide novel information on susceptibility to type 1 diabetes. Very low frequencies of type 1 diabetes in Japan, however, makes it difficult to identify such families. In particular, families with more than three patients with type 1 diabetes are extremely rare in Japan. In the present study, we identified a family with three members having type 1 diabetes and studied the HLA genotypes of this family.

The proband and his older sister had the same combination of HLA haplotypes, DRB1*08:02-DQB1*03:02. DRB1*08:02-DQB1*03:02 is specific to the Japanese population, and has been reported to confer strong susceptibility to type 1 diabetes among the Japanese. The frequency, however, of DRB1*08:02-DQB1*03:02 is very low in the general population (1.0–2.2%) and, and therefore, the DRB1*08:02-DQB1*03:02 homozygote is very rare, with an estimated frequency of 0.01–0.04% in Japan. In fact, none of the participants in a nation-wide study (396 controls and 545 cases) carried out by Japan Diabetes Society had DRB1*08:02-DQB1*03:02. Even in type 1 diabetes, the frequency of the DRB1*08:02-DQB1*03:02 homozygote is expected to be less than 0.5% based on the frequencies of the DRB1*08:02-DQB1*03:02 haplotype reported in type 1 diabetes (6.8–7.1%). To the best of our knowledge, the present study represents the first cases of DRB1*08:02-DQB1*03:02 homozygotes.

In addition to two siblings with type 1 diabetes, who were homozygotes for DRB1*08:02-DQB1*03:02 haplotype, their mother and father also had DRB1*08:02-DQB1*03:02 in combination with different HLA haplotypes. Their mother, who developed slowly progressive type 1 diabetes, had DRB1*13:02-DQB1*06:04, and the unaffected father had DRB1*15:01-DQB1*06:02. DRB1*13:02-DQB1*06:04 has been reported to be neutral or weakly susceptible for type 1 diabetes, whereas DRB1*15:01-DQB1*06:02 is a well-known protective haplotype for type 1 diabetes, suggesting the contribution of the second HLA haplotype in combination with DRB1*08:02-DQB1*03:02 to susceptibility or resistance to type 1 diabetes in this family.

A rare multiplex family with type 1 diabetes, in which three out of four sisters developed type 1 diabetes, has previously been reported in Japan. In that family, all three sisters with type 1 diabetes had the same combination of HLA haplotypes, one of which was DRB1*08:02-DQB1*03:02, and the other was DRB1*04:05-DQB1*04:01, which confers susceptibility to type 1 diabetes in the Japanese population. These data together with the data in the present study suggest the DRB1*08:02-DQB1*03:02 haplotype as a key factor for familial clustering of type 1 diabetes in Japanese people.

Cases 1 and 2 had the same HLA genotypes – the DRB1*08:02-DQB1*03:02 homozygote – but they developed different subtypes of type 1 diabetes, acute-onset in case 1 and slowly progressive in case 2. Environmental factors or genes besides the HLA might be the reason for the differences. They both received their mother’s breast milk and were raised in very similar situations. The analysis of other genes and further studies are needed to understand the mechanism of type 1 diabetes in this family.
studies are warranted to better understand the reasons for subtype differences between the two siblings.

In summary, we identified a rare Japanese family in which two siblings and a mother developed type 1 diabetes. Two siblings shared the same combination of HLA haplotype, DRB1*08:02-DQB1*03:02/ DRB1*08:02-DQB1*03:02. These data together with a previous report on a family in which three out of four siblings developed type 1 diabetes and all had the DRB1*08:02-DQB1*03:02 haplotype suggests DRB1*08:02-DQB1*03:02 as one of the contributing factors for familial clustering of type 1 diabetes in Japanese people.

ACKNOWLEDGMENTS
The present study was supported by grants-in-aid for scientific research from the Japan Society for the Promotion of Science (15K09404 to HI, 26461348 to YK); a grant from the Ministry of Health, Labor and Welfare (H28-Jyunkanto-ippan-006 to HI); and a grant from the Japan Agency for Medical Research and Development (16768653 to HI).

DISCLOSURE
The authors declare no conflict of interest.

REFERENCES
1. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001; 358: 221–229.
2. Rish N. Assessing the role of HLA-linked and unlinked determinants of disease. Am J Hum Genet 1987; 40: 1–14.
3. Ikegami H, Kawabata Y, Nosu S, et al. Genetics of type 1 diabetes in Asian and Caucasian populations. Diabetes Res Clin Pract 2007; 77(Suppl. 1): S116–S121.
4. Ikegami H, Ogihara T. Genetics of insulin-dependent diabetes mellitus. Endocr J 1996; 43: 605–613.
5. Karvonen M, Viik-Kajander M, Moltchanova E, et al. Incidence of childhood type 1 diabetes worldwide. Diabetes Mondiale (DiaMond) Project Group. Diabetes Care 2000; 23: 1516–1526.
6. Kawabata Y, Ikegami H, Awata T, et al. Differential association of HLA with three subtypes of type 1 diabetes: fulminant, slowly progressive and acute-onset. Diabetologia 2009; 52: 2513–2521.
7. Kawabata Y, Ikegami H, Kawaguchi Y, et al. Asian-specific HLA haplotypes reveal heterogeneity of the contribution of HLA-DR and -DQ haplotypes to susceptibility to type 1 diabetes. Diabetes 2002; 51: 545–551.
8. Awata T, Kuzuya T, Matsuda A, et al. Genetic analysis of HLA class II alleles and susceptibility to type 1 (insulin-dependent) diabetes mellitus in Japanese subjects. Diabetologia 1992; 35: 419–424.
9. Japanese Society for Histocompatibility and Immunogenetics. Available from: http://jshi.umin.ac.jp/database/hpl_drdq.txt Accessed December 25, 2016.
10. Saito S, Ota S, Yamada E, et al. Allele frequencies and haplotypic associations defined by allelic DNA typing at HLA class I and class II loci in the Japanese population. Tissue Antigen 2000; 56: 522–529.
11. Allele Frequency Net Database. Available from: http://www.allelefrequencies.net/1ala6003a.asp Accessed December 25, 2016.
12. Kishi A, Kawabata Y, Ugi S, et al. The onset of diabetes in three out of four sisters: a Japanese family with type 1 diabetes. A case report. Endocrine J 2009; 56: 767–772.