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Case Report

Mutation screening of *INS* and *KCNJ11* genes in Taiwanese children with type 1B diabetic onset before the age of 5 years

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Type 1 diabetes (T1D) is caused by β-cell destruction, usually leading to absolute insulin deficiency. T1D is a heterogeneous disease and is divided into two subtypes according to the presence or absence of pancreatic autoantibodies: type 1A (immune mediated) and type 1B (idiopathic). Genes such as *KCNJ11* or *INS*, which play key roles in β-cell function, provide some insight into the pathogenesis of type 1B diabetes. In this study, we screened 110 Taiwanese children (61 males and 49 females) with T1D onset before the age of 5 years for mutations of *INS* and *KCNJ11*. We identified one missense heterozygous mutation in *KCNJ11* (c.989A>G, p.Y330C) and no *INS* mutations among 28 probands. This is the first study to screen patients with autoantibody-negative T1D diagnosed before the age of 5 years for *INS* and *KCNJ11* mutations in Taiwan. Although *KCNJ11* mutations are always reported in patients with permanent neonatal diabetes, this gene mutation can be detected after 6 months of age. Further studies in other patients with type 1B diabetes and their families are required to elucidate the contributions of the *KCNJ11* mutation to the T1D phenotype.

Type 1 diabetes (T1D) is caused by β-cell destruction, usually leading to absolute insulin deficiency.[1] T1D is a heterogeneous disease in which clinical presentation and disease progression may vary considerably.[1] Patients with T1D require insulin injection to prevent diabetic ketoacidosis, coma, and death. The etiology is heterogeneous and is divided into two subtypes according to the presence or absence of pancreatic autoantibodies such as GAD-Ab, IA-2 Ab, IAA, and ZnT8 Ab. These types are type 1A (immune mediated) and type 1B (idiopathic). The genes for susceptibility to type 1A diabetes are HLA class II genes on chromosome 6 and several other genes, including *INS-VNTR*, *CTLA4*, *PTPN22*, and *IL2RA/CD2*.[2]

KCNJ11 and *INS*, which play key roles in β-cell function, provide some insight into the pathogenesis of type 1B

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diabetes. *KCNJ11* encodes for the inward rectifier Kir6.2 subunit of the ATP sensitive potassium (K_{ATP}) channel and plays a critical role in regulating insulin secretion of the β-cells.[3] Mutations in the *KCNJ11* gene are responsible for approximately one-third to half of all cases of permanent neonatal diabetes mellitus (PNDM).[4] Most patients with *KCNJ11* PNDM showed satisfactory response to sulfonylurea (SU) treatment, and successful transfer from insulin to SU has been accomplished for both adults and children, including infants.[5]

The human insulin gene contains three exons. Exon 2 encodes the signal peptide, the B chain, and part of the C-peptide, whereas exon 3 encodes the remainder of the C-peptide and the A chain. Both dominant and recessive mutations in *INS* can result in PNDM. Moritani et al. (2012) reported five children who received diagnoses of heterozygous missense variants C31Y, R89C, C96R, and C109F in *INS* between the ages of 0.2 months and 4.9 years.[2]

At present, genetic testing for monogenic diabetes plays an increasingly important role in clinics, because it may influence diabetes treatment, explain pleiotropic features, and define the prognosis in the patient and their family members. The aim of this study was to identify potential causal mutations for monogenic forms of diabetes in young children with autoantibody-negative T1D. In this study, we screened 110 Taiwanese children (61 males and 49 females) with T1D onset before the age of 5 years for *INS* and *KCNJ11* mutations. To conduct this study, we 1) classified type 1A and type 1B diabetes by measuring serum GAD-Ab and IA-2 Ab; 2) performed mutation screening for *INS* and *KCNJ11* in children with type 1B diabetes; and 3) studied the correlation between the genotype and phenotype of patients with *INS* and *KCNJ11* mutations in Taiwan.

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital (101-5152A3). After obtaining informed consent, we recruited 110 unrelated Taiwanese individuals (61 males and 49 females) with T1D onset before the age of 5 years between August 2013 and December 2015. The diagnosis of T1D was assessed according to the criteria of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.[1] We measured serum GAD-Ab (CIS Bio International, France) and IA-2 Ab (CIS Bio International, France) using commercial radioimmunoassay (RIA) kits. From these patients, we selected 28 young children (16 males and 12 females) who were negative for both GAD-Ab and IA-2 Ab.

Genomic DNA was extracted from peripheral blood samples of the patients by using a standard protocol. For each patient, two *INS* and one *KCNJ11* coding exons were sequenced using the primers listed in a previous report.[2] The PCR products were then purified using a High Pure PCR Product Purification Kit (Roche Molecular Biochemicals, Mannheim, Germany) and directly sequenced using a cycle sequencing method.

We identified one missense heterozygous mutation in *KCNJ11* (c.989A>G, p.Y330C) and no mutations in the *INS* gene among 28 probands (Fig. 1). The following clinical findings were observed in the patient with type 1B diabetes caused by *KCNJ11* mutation.

An 11-year-4-month-old girl had received a diagnosis of T1D at the age of 2 years and 10 months. She was born to a healthy G1P1 mother after an uneventful and 41 weeks of pregnancy. She was delivered through normal spontaneous delivery and weighed 3340 g. She had no family history of diabetes mellitus. She presented with polyuria, weight loss, and constipation for more than two weeks. Laboratory data at diagnosis showed hyperglycemia (740 mg/dL), high HbA1c (12.7%), low serum C-peptide (0.16 nmol/L), normal vein blood gas (pH 7.372, PCO_2 41.4 mmHg, HCO3 23.5 mm/L, SBE −1.8 mm/L), and negative blood and urine ketone. Serum anti-insulin, anti-IA2, anti-GAD, anti-TPO, and antithyroglobulin autoantibodies were all negative. T1D without ketoacidosis was diagnosed and insulin therapy with 0.57 U/day of NPH subcutaneously before breakfast and dinner was started immediately. On insulin therapy, her growth was appropriate for age. She had normal psychomotor development and no history of seizure. The most recent insulin dose was 0.76 U/kg/day of NPH and RI injection subcutaneously before breakfast and dinner. At the age of 11.25 years, genetic testing confirmed the Y330C mutation. A glucose tolerance test showed very low peak C-peptide (0.07 nmol/L). Glibenclamide was started at 0.1 mg/kg/day and the dose increased according to the switching protocol described previously.[5] Subcutaneous insulin therapy was stopped on day 4, when the dose of glibenclamide was increased to 0.6 mg/kg/day. The C-peptide concentration and HbA1c was 0.4 nmol/L and 7.8% on the day 78, when the glibenclamide dose was 0.9 mg/kg/day, respectively. Continuous glucose monitoring (CGM) data revealed the improvement of glucose levels and variability for this patient before and after glibenclamide treatment.

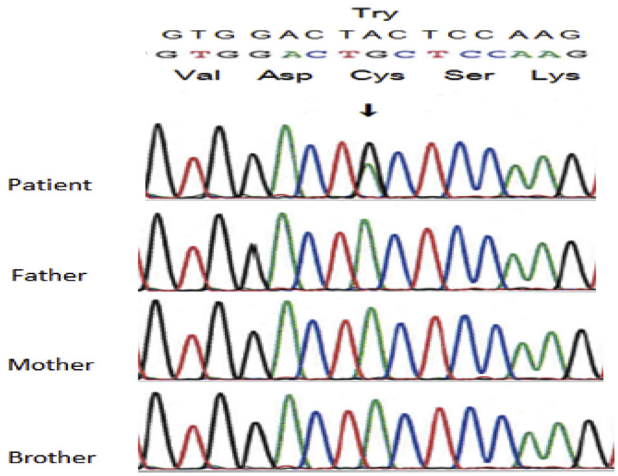

Figure 1 Mutation analysis of *KCNJ11* in a girl with diabetes with an onset age of 2 years and 10 months. A novel missense mutation (TAC to TGC, tryptophan to cysteine, codon 989) was observed in the patient, without the presence of mutation in her father, mother, or brother.

Discussion

In this study, we screened for *INS* and *KCNJ11* mutations in 28 unrelated Taiwanese children with GAD-Ab and IA2-Ab negative T1D diagnosed before the age of 5 years. We focused on *INS* and *KCNJ11* as the candidate monogenic genes. One heterozygous mutation (c.989A>G, p.Y330C)