Identification of a variant associated with early-onset diabetes in the intron of the insulin gene with exome sequencing

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INTRODUCTION
In 1979, Tager et al.1 reported a patient with hyperinsulinemia and diabetes, and the subsequent genetic analysis identified a heterozygous missense mutation in the INS gene2. Our group also reported a Japanese family with the similar clinical phenotype as a result of the INS gene mutation3. The glucose-lowering effect of exogenous insulin was normal in the family, and the insulin receptor binding activity of abnormal insulin was reduced in vitro3. In contrast, Stoy et al.4 reported that INS gene mutations were also the cause of neonatal diabetes as a result of impaired insulin secretion. All mutations reported were heterozygous missense mutations, and were located in critical regions of insulin for normal protein folding and progression in the secretory pathway. Furthermore, expression of abnormal insulin induced severe endoplasmic reticulum stress and β-cell apoptosis5, as had been described in the Akita mouse6.

Whole-exome sequencing (WES) is a new technology. Here, we used it to explore the gene responsible for early-onset diabetes as a result of impaired insulin secretion in a family, and identified a heterozygous intronic mutation in the INS gene.

METHODS
Participants
The proband was born at the 40th week of gestation with birthweight 3,300 g (81.8 percentile). She was diagnosed with diabetes on regular health checkups at the age of 3 years, and has been treated with insulin from diagnosis. She was aged 43 years (body mass index [BMI] 24.0 kg/m2) at the time of study, and was treated with multiple daily insulin injections (0.48 IU/kg/day). Her elder daughter was born at the 37th week of gestation with birthweight 2,760 g (73.6 percentile). She developed symptoms of thirst, polydipsia and polyuria at the age of 12 months. Hyperglycemia (fasting plasma glucose 230 mg/dL [12.7 mmol/mL], glycated hemoglobin 15.0%) and low serum C-peptide level (0.9 ng/mL [298 pmol/L]) were detected, and insulin therapy was started. She was aged 14 years (BMI 20.5 kg/m2) at the time of study, and was treated with an insulin pump (1.18 IU/kg/day). The younger daughter of the proband was born at the 38th week of gestation with birthweight 2,805 g (62.4 percentile). She was found

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to have hypoglycemia (40 mg/dL [2.2 mmol/L]) with normoinsulinemia (serum immunoreactive insulin 4.8 μU/mL [34.4 pmol/L]) for 3 days after birth, and her hypoglycemia was naturally improved. Asymptomatic hyperglycemia (fasting plasma glucose 160 mg/dL [8.8 mmol/mL], glycated hemoglobin 7.7%) was observed at the age of 14 months during a random investigation of blood glucose. Her serum immunoreactive insulin level was low (1.8 μU/mL [12.9 pmol/L]) at the time of diagnosis, and insulin therapy was started. She was aged 9 years (BMI 16.5 kg/m²) at the time of study, and was treated with an insulin pump.

In the present study, sequencing was carried out to achieve the average read depth of 100 × for target regions of WES, and we obtained data with the depth of 114 ×. At first, we checked the result for 26 genes known to cause mature onset diabetes of the young and/or neonatal diabetes. The list and each average read depth of the 26 genes are shown in Table S1. We found a heterozygous c.188-31G>A mutation (reference sequence: NM_000207.2) in intron 2 of the INS gene, which was registered in the databases as rs797045623. The average read depth for the INS gene was 30 ×, which was lowest among the 26 genes, and the read depth on the region in where the c.188-31G>A mutation was located was 15 ×. The mutation was then validated by Sanger sequencing and further investigated in the family group. The mutation was also identified in the proband’s two daughters with diabetes, but not in her son without diabetes. The mutation was also not identified in her husband, who had late-onset diabetes and was treated with oral hypoglycemic agents. Furthermore, the mutation was not identified in her parents without diabetes, suggesting that the mutation might be a de novo mutation in the proband (Figure 1).

The substitution was located 31 bp proximal to exon 3 in intron 2 of the INS gene (Figure 2). It was predicted to create an ectopic splice site by in silico analysis (http://www.cbs.dtu.dk/services/NetGene2/) leading to insert 29 nucleotides of intron 2 as an exonic sequence in the transcript. The insertion altered the reading frame, and the new stop codon was located at 19 amino acids downstream from the original stop codon. Furthermore, the mutation had been reported to be associated with early-onset diabetes in White people (Table 1).7–9 For these reasons, we concluded that the heterozygous c.188-31G>A mutation was the pathogenic mutation in the present family.

**DISCUSSION**
This is the first report for the pedigree with diabetes as a result of the c.188-31G>A mutation in Asian people. The
clinical phenotype of diabetes observed in the present patients was impaired insulin secretion, the same as in the previous reports. The mutation was initially identified in patients with permanent neonatal diabetes. In this report, the abnormal transcript predicted was detected with reverse transcription polymerase chain reaction in messenger ribonucleic acids of patients’ lymphoblastoid cells established by Epstein–Barr virus transformation. Furthermore, they analyzed the three-dimensional structure of the mutant protein with computer modeling and predicted that the mutant protein would fail to fold properly in the endoplasmic reticulum, and concluded that the abnormal insulin could induce pancreatic β-cell dysfunction and apoptosis as a result of the endoplasmic reticulum stress.

A majority of patients were diagnosed with diabetes before 12 months-of-age as a result of the heterozygous missense mutations in the INS gene. In an initial report, patients with the c.188-31G>A mutation were diagnosed at 1 month after birth. However, the ages at diagnosis observed in another two families reported were older than that of the initial report and were similar to the present patients (Table 1), suggesting that other genetic and environmental factors might modulate the age at onset of diabetes.

Although the present results would help in understanding the role of the c.188-31G>A INS gene mutation in developing diabetes, the present study had several weaknesses. WES cannot detect relatively large structural variations, such as an exon deletion. Furthermore, we checked the result of WES for only the 26 genes. It is possible that other structural variations or non-synonymous mutations have also contributed to the phenotype of diabetes in the present family.

Because patients with the ABCG8, KCNJ111 or HNF1A gene abnormalities respond to sulfonylureas and do not require insulin therapy in many cases, the genetic diagnosis is useful for the therapeutic decision in patients with early-onset diabetes. The cost for WES is rapidly decreasing, so the method could be more widely used for genetic testing of monogenic diabetes in the near future.

Table 1 | Summary of families with the c.188-31G>A mutation in the insulin gene

| Family | Country   | Sex | Relationship | Zygosity | Age at diagnosis | Treatment          | References |
|--------|-----------|-----|--------------|----------|-----------------|--------------------|------------|
| 1      | Spain     | M   | Father       | Hetero   | 1 month         | INS                | [7]        |
|        |           | F   | Proband      | Hetero   | 1 month         | INS                |            |
| 2      | USA       | M   | Proband      | Hetero   | 10 months       | INS                | [8]        |
| 3      | Czech Republic | F   | Mother       | Hetero   | 6 years         | OHA → INS         | [9]        |
| 4      | Japan     | F   | Proband      | Hetero   | 3 years         | INS                |            |
|        |           | F   | Daughter     | Hetero   | 12 months       | INS                |            |
|        |           | F   | Daughter     | Hetero   | 14 months       | INS                |            |

† The patient was treated with oral hypoglycemic agents (OHA) for 1 year. ‡ The diabetes was transiently resolved from 18 months, but appeared at the age of 3 years. F, female; INS, insulin; M, male.
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DISCLOSURE
The authors declare no conflict of interest.

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | The list of 26 genes known to cause mature onset diabetes of the young and/or neonatal diabetes and the average read depth at each locus.