Brief Genetics Report

Nonsense Mutation of Islet-1 Gene (Q310X) Found in a Type 2 Diabetic Patient With a Strong Family History

Hiroko Shimomura, Tokio Sanke, Tadashi Hanabusa, Keiko Tsunoda, Hiroto Furuta, and Kishio Nanjo

Islet-1 (Isl-1) is one of the transcription factors that play an important role for the formation of the islet cells. We scanned the Isl-1 gene in 77 Japanese type 2 diabetic patients with a family history and found a heterozygous nonsense mutation (Q310X) in 1 diabetic patient. The mutation was not found in 180 nondiabetic subjects. This mutation is located in the putative transactivation domain and deletes 40 amino acids of the COOH-terminal lesion. The Q310X mutant exhibited a 50% reduction in activity compared with the wild-type when tested for stimulation of transcription of a human amylin promoter-linked luciferase reporter gene in βTC3 cells. The patient was a 49-year-old nonobese man who was diagnosed as having type 2 diabetes at 32 years of age and has been treated with sulfonylureas. The mutation was found in his mother, who has type 2 diabetes, and in his 14-year-old daughter, who has normal glucose tolerance but a relatively low insulin response. This is the first reported finding of Isl-1 gene mutation in type 2 diabetes. Although Isl-1 is not a common predisposing gene for Japanese type 2 diabetes, the mutation in this gene may be a rare cause of diabetes in isolated families. ***Diabetes*** **49:1597–1600, 2000**

Type 2 diabetes has been considered a multifactorial disorder. Although several gene mutations have been found in type 2 diabetic patients, the main susceptibility genes for this disease have not been found. Recently, mutations of the transcription factors, which were thought to play an important role in regulating the β-cell differentiation, have been reported to be responsible for early onset of type 2 diabetes in individuals with a strong family history (maturity-onset diabetes of the young) (1–4). Islet-1 (Isl-1) is one of the transcription factors with LIM and homeodomain expressed in the islet cells (5). In 1997, Isl-1–deficient mice were created to define the role of Isl-1 in the development of the pancreas (6). Dorsal pancreatic mesenchyme did not form in Isl-1 mutant embryos, and there was an associated failure of exocrine cell differentiation in the dorsal pancreas. A complete loss of differentiated islet cells and motor neuron deficiency were observed in those mice. These findings suggest that the Isl-1 is a candidate gene for type 2 diabetes. Thus, we scanned the Isl-1 gene for mutations in Japanese type 2 diabetic patients.

In this study, we examined 77 nonobese (BMI <30 kg/m^2) unrelated Japanese type 2 diabetic patients with a family history (either or both parents and more than one sibling) and 180 nondiabetic subjects without a family history of diabetes (age >60 years). Nondiabetic subjects were confirmed to have fasting plasma glucose <6.1 mmol/l and HbA_{1c} <6.0%. Using genomic DNA extracted from peripheral leukocytes from these subjects, the promoter region and all 6 exons of the Isl-1 gene were amplified by polymerase chain reaction (PCR) and were analyzed by the single-strand comformation polymorphism (SSCP) method. As a result, 3 kinds of aberrant bands were found. Direct nucleotide sequencing revealed a C to G substitution in the intron 2 (15-bp upstream from the splice acceptor site of exon 3), a P168P silent mutation in exon 4, and a Q310X nonsense mutation in exon 5, respectively. Allele frequencies of the former 2 variants were not significantly different in diabetic and nondiabetic groups (C to G change 11.9 vs. 7.0%; P168P 30.7 vs. 34.0%, diabetic vs. nondiabetic, respectively). The Q310X mutation was found only in a diabetic patient in the heterozygous state and was not found in the 180 nondiabetic subjects after using site-directed PCR combined with *Mfe*I restriction fragment length polymorphism (RFLP). From these results, although Isl-1 gene was not a common predisposing gene for Japanese type 2 diabetes, an interesting nonsense mutation was detected in a patient with type 2 diabetes with a family history.

The patient with Q310X was a 49-year-old nonobese (BMI 19.4 kg/m^2) Japanese man. He was diagnosed with type 2 diabetes at 32 years of age and has been treated with sulfonylureas (recently treated with glibenclamide 5 mg/day). He had a previous history of insulin treatment for a couple of weeks at the onset of the disease and at 42 years of age because of extremely poor metabolic control due to occupational stress (HbA_{1c} 9.8 and 10.2%, respectively). He had no chronic diabetic complications. The exocrine pancreatic function as estimated by the Pancreatic Function Diagnostic test (which indirectly

From the First Department of Medicine, Wakayama University of Medical Science, Wakayama, Japan.

Address correspondence and reprint requests to Tokio Sanke, MD, PhD, First Department of Medicine, Wakayama University of Medical Science, 811-1 Kimi-idera, Wakayama 641-8509, Japan. E-mail: sanke-t@wakayama-med.ac.jp.

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Isl-1, Islet-1; PABA, para-amino benzoic acid; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-strand conformation polymorphism.

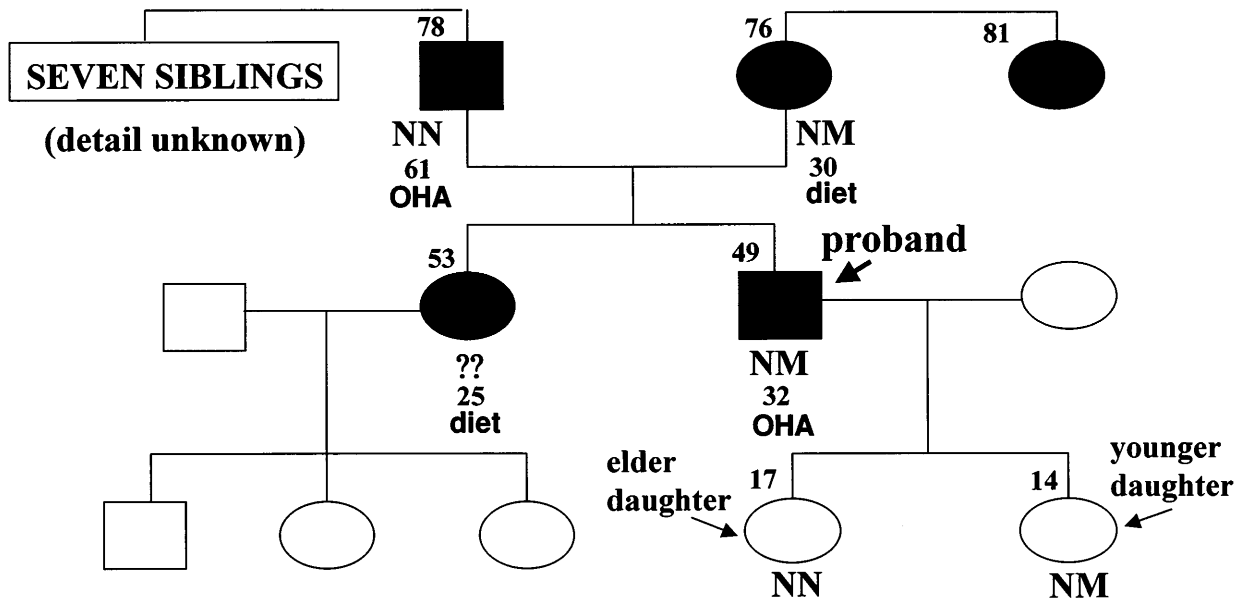

FIG. 1. The pedigree of the family with Q310X mutation in the Isl-1 gene. Type 2 diabetic patients are represented by ● and ■, and nondiabetic subjects are represented by ○ and □. The present age is shown above each symbol. The genotype, the age at diagnosis of diabetes, and the mode of treatment for diabetes are indicated below the symbol. M, mutant allele; N, normal allele; OHA, oral hypoglycemic agent; ?, not tested.

reflects chymotripsin activity in the pancreatic juice by measuring the recovered para-amino benzoic acid [PABA]) in urine after oral load of *N*-benzoil-L tyrosyl-PABA), was slightly decreased (68.1; normal range, >70%). However, the patient's pancreas seemed to be morphologically normal according to a computed tomography scan. His median nerve conduction velocity was in the normal range. The family tree of the patient is shown in Fig. 1. The mutation was found in his 76-year-old mother who had type 2 diabetes that was treated with diet alone (age at onset, ~30 years; HbA_{1c} level, 7.1%) and in his 14-year-old daughter. The 14-year-old daughter with the mutation revealed a relatively high plasma glucose response with a relatively low insulin response during 75-g oral glucose tolerance test compared with the elder daughter without the mutation, although their glucose responses were both within normal range according to World Health Organization criteria (Table 1). The patient's 78-year-old father, who carried no mutation, also had type 2 diabetes (age at onset, 61 years) that was treated with an oral hypoglycemic agent. The patient's sister (age at onset, ~25 years; treated with diet) and his maternal aunt also had type 2 diabetes. However, they did not accept blood sampling for a DNA study and laboratory analysis.

In 1995, human full-length Isl-1 cDNA was isolated and the Isl-1 gene was screened in 75 French Caucasian type 2 diabetic patients with PCR/SSCP (7). Although 3 variants were identified, none altered the amino acid sequence. Thus, this article is the first report of Isl-1 nonsense mutation found in human beings. The recent observation that human, hamster, and rat Isl-1 proteins are identical (7) indicates that this protein has been conserved throughout 75 million years of evolution and therefore suggests an important role for this protein in human tissue. Three puta-

TABLE 1
Plasma glucose and insulin responses during a 75-g oral glucose tolerance test in proband and 2 daughters

	Time (min)					
	Pre OGTT	30	60	90	120	Σ Δ value*
Proband						
Plasma glucose (mmol/l)	6.6	12.8	15.8	12.4	11.5	26.1
Plasma insulin (pmol/l)	35	97	167	118	146	388
Younger daughter with mutation						
Plasma glucose (mmol/l)	4.6	7.6	4.7	6.8	7.6	8.3
Plasma insulin (pmol/l)	35	340	125	257	125	707
Elder daughter without mutation						
Plasma glucose (mmol/l)	4.8	6.8	6.4	5.3	5.9	5.2
Plasma insulin (pmol/l)	28	160	486	313	264	1,111

*Total sum of glucose or insulin values above basal. OGTT, oral glucose tolerance test.

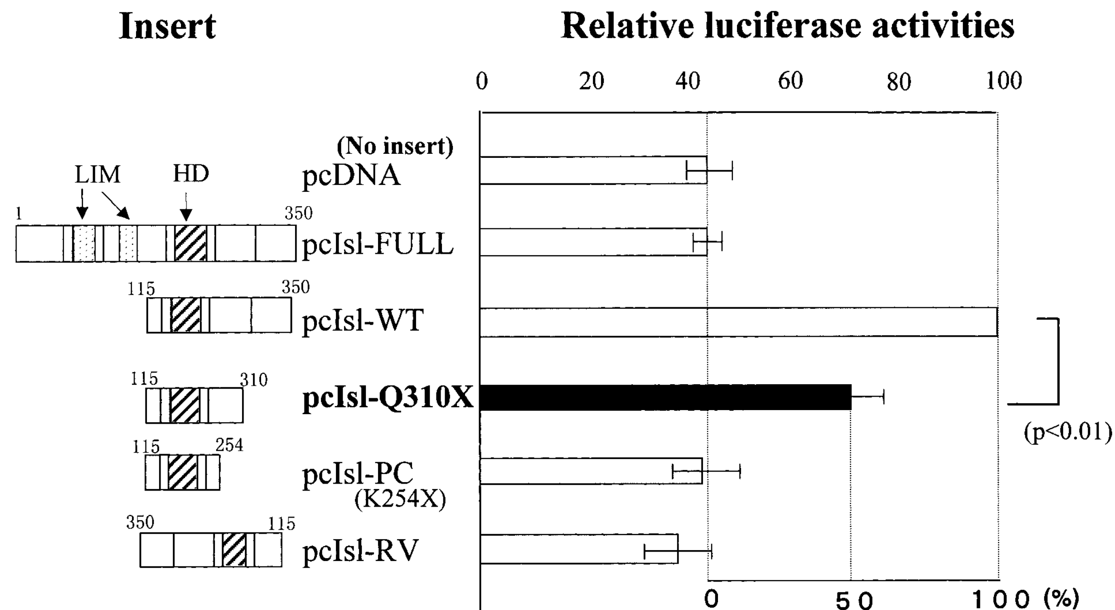

FIG. 2. Luciferase activities of human amylin-luciferase plasmids in the βTC3 cells. The luciferase activities relative to psIsl-WT are shown. The constructions of Isl-1 are schematically shown on the left side. The amino acid number of the human Isl-1 is shown above each box. LIM, LIM domain; HD, homeodomain.

tive regulatory regions have been noted in Isl-1, including 2 LIM domains, a homeodomain, and a glutamine-rich transcription activation domain (5). The Q310X mutation is located in the putative transcription activation domain and deletes 40 amino acids of the COOH-terminal region.

To assess the mutant Isl-1 function, we compared the transcription activity of wild-type with mutant Isl-1 using the luciferase reporter system in βTC3-cells. As shown in Fig. 2, pcIsl-FULL, pcISl-PC(K254X), and pcIsl-RV showed complete loss of luciferase activity, whereas the wild-type (pcIsl-WT) increased the activity and the mutant (pcIsl-Q310X) exhibited ~50% reduction in activity compared with the wild-type when the activity of the vehicle (pcDNA) was taken into consideration. These findings suggest that the Q310X mutation may have some effect on the development or differentiation of the β-cells in this patient.

Because the patient's father (age of onset 61 years) without the mutation also had type 2 diabetes, it was not clear whether this mutation caused diabetes in this family or not. However, 2 of the 3 people carrying the mutation had type 2 diabetes with relatively early onset, except for 1 young girl 14 years of age, who had relatively high glucose and relatively low insulin responsiveness compared with her sister who was without the mutation. Because type 2 diabetes is considered a polygene disease, it is possible that this mutation may cause mild diabetes on its own, as observed in this patient's mother. It may also make diabetes more severe when it combines with other susceptibility factors for diabetes from his father.

In conclusion, this is the first report of Isl-1 gene nonsense mutation identified in a type 2 diabetic patient with a strong family history. Although Isl-1 is not a common predisposing gene for type 2 diabetes in Japanese people, the mutation in this gene may be a rare cause of diabetes in isolated families, and future genetic studies will be required to confirm that isl-1 mutation causes diabetes in humans.

RESEARCH DESIGN AND METHODS

PCR/SSCP analysis for screening mutations. Primers for PCR and sequencing were designed as previously described by Riggs et al. (8), except for exon 5 (5′-ATTTGCTCATTAACATGTTG-3′ and 5′-AGTTAAACCAGAGTC TCCTA-3′). SSCP was performed at 3 different temperature conditions (9–22°C) using ALFred autosequencer (Pharmacia, Uppsala, Sweden).

PCR/RFLP for screening the Q310X in nondiabetic subjects. Primers for site-directed PCR/RFLP to screen the Q310X mutation in exon 5 were designed to create an *Mfe*I restriction site in the wild-type sequence (sense 5′-ATTTGCTCATTA ACATGTTG-3′ and reverse 5′-CTTCCATCTGGGAGCTGACACTTACCAATT-3′).

Luciferase assay. For construction of a reporter vector, a human amylin gene (191-bp fragment of promoter region followed by 458-bp fragment including whole exon 1, whole intron 1, and a part of exon 2) was subcloned into a pGL2-basic plasmid (Promega, Madison, WI), because it was already reported that Isl-1 activated amylin gene transcription (9). Isl-1 cDNA was obtained from a human pancreas cDNA library (Clontech, Palo Alto, CA) by PCR using Pfu polymerase (Stratagene, La Jolla, CA). The expression vectors were constructed using pcDNA3.1(+) (Invitrogen, the Netherlands) subcloned by each full-length wild-type cDNA (pcIsl-FULL), short wild-type DNA (pcIsl-WT), and short wild-type cDNA in the reversed position (pcIsl-RV), and short mutant cDNA (pcIsl-Q310X). Short cDNA lacked 133 amino acids of the NH_2-terminal 133 amino acids, which includes 2 LIM domains because it was reported that LIM domains of the Isl-1 decreased the transcription activity in vitro (10). In addition to these plasmids, we generated a nonsense mutation (K254X) for positive control (pcIsl-PC[K254X]). The reporter plasmid (20 μg) and expression vector (5 μg) were cotransfected to βTC3 cells (1×10^7) by electropolation. The cells were cultured for 48 h and harvested, and then we measured the luciferase activity. The transfection efficiencies were normalized with renilla luciferase activity cotransfected in each experiment. The experiments were repeated 6 times, and the results were shown as means ± SE. The expression levels of the wild-type Isl-1 and the Q310X mutant forms were confirmed to be similar by SDS-PAGE and Western immunoblot assay using anti-Xpress antibody (Invitrogen) in extracts of the cells used for the luciferase assay.

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