β-Cell Dysfunction in Late-Onset Diabetic Subjects Carrying Homozygous Mutation in Transcription Factors NeuroD1 and Pax4

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Polymorphisms in β-cell transcription factor genes, Ala45Thr in the *NeuroD1* gene and Arg121Trp in the *Pax4* gene, have been reported. To clarify the role of these mutations in the pathogenesis of late-onset diabetes, we examined the insulin secretion and sensitivity in diabetic patients carrying the homozygous mutation in the *NeuroD1* gene or *Pax4* gene. We screened for the polymorphisms in *NeuroD1* and *Pax4* genes in 296 late-onset diabetic patients and 177 unrelated control subjects over 60 years of age. Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) followed by direct sequencing. Acute insulin secretion was evaluated using a 2-compartment model analysis of C-peptide kinetics after intravenous glucose load (CS1). Insulin sensitivity was estimated by the insulin-modified minimal model analysis (Si). Four diabetic patients carried the homozygous mutation (Thr/Thr) in the *NeuroD1* gene and 3 patients carried the homozygous mutation (Trp/Trp) in the *Pax4* gene, while both homozygous mutations were not detected in the control subjects. In patients A, B, C, and D with homozygous mutations in *NeuroD1*, CS1 (normal range, 6.8 to 18.5 ng/mL/min) was 0.508, 1.481, 1.223, and 1.584 ng/mL/min, respectively, and Si (normal range, 2.6 to 7.6 × 10^{-4}/min/[μU/mL]) was 0.727, 3.31, 3.79, and 0.00 × 10^{-4}/min/(μU/mL), respectively. In patients X, Y, and Z with homozygous mutation in *Pax4*, CS was 0.418, 0.208, and 1.279 ng/mL/min, respectively, and Si was 1.11, 2.88, and 0.00 × 10^{-4}/min/(μU/mL), respectively. Since acute insulin secretion in response to glucose was markedly impaired and insulin resistance was varied in the patients carrying the homozygous mutations in the *NeuroD1* and *Pax4* genes, the mutations are ones of the factors involved in the β-cell dysfunction and do not relate to the insulin resistance. These homozygous mutations appear to play a part in the pathogenesis of β-cell defect in about 2.5% of Japanese patients with late-onset diabetes.

GENETIC FACTORS play an important part in the pathogenesis of late-onset diabetes mellitus, a heterogeneous disorder characterized by defects in insulin secretion and insulin resistance. However, there has been little progress in defining the major genes. On the other hand, the autosomal-dominant subtype maturity-onset diabetes of the young (MODY) is caused by mutations in the genes coding the transcription factors, hepatocyte nuclear factor (HNF)-1α, HNF-4α, HNF-1β, and insulin promoter factor-1 (IPF-1).[1-4] These genes form crucial links in the cascade of transcription factors that control the appropriate expression of β-cell genes, such as insulin and GLUT2.[5]

Recently, it has been documented that several transcription factors of the homeodomain family are important for islet cell differentiation. Pax6 and IslI are required for normal formation of all islet cell types[6] and Pax4 is involved in the differentiation of β and δ cells.[7] Also, NeuroD1, a cell-type–specific (class B) member of the basic helix-loop-helix (bHLH) family of transcription factors, is involved in islet cell development and insulin gene transcription.[8] Neurogenin3, a second, related bHLH protein, is expressed in islet cell progenitors and functions as a pro-endocrine gene driving islet cell differentiation.[9]

Although β-cell defect and insulin resistance are interlinked, resulting in the development of late-onset diabetes, it seems reasonable that without deranged insulin secretion, hyperglycemia cannot develop: diabetes results from an inadequate mass of functional β cells.[10] Such inadequacy could result from lack of compensation to overcome the insulin resistance or an intrinsic β-cell defect. Since the transcription factors play a crucial role in the normal development and function of the β cell, mutations in the genes would confer a strong predisposition to the development of diabetes mellitus.

Polymorphisms in β-cell transcription factor genes, Ala45Thr in the *NeuroD1* gene and Arg121Trp in the *Pax4* gene, have been reported.[11-13] We have found homozygous mutations, Thr/Thr in the *NeuroD1* gene and Trp/Trp in the *Pax4* gene, in Japanese late-onset diabetic subjects. To clarify the role of these mutations in the pathogenesis of diabetes, we examined insulin secretion and sensitivity in patients carrying these homozygous mutations, using a 2-compartment model analysis of C-peptide kinetics after intravenous glucose load[14,15] and an insulin-modified minimal model analysis.[16,17]

MATERIALS AND METHODS

Subjects

Late-onset diabetic subjects were recruited at the Diabetes Centre, Kasori Hospital, Chiba, Japan, and control subjects were recruited from the Kasori Hospital and Misaki Clinic, Funabashi, Japan. All of the subjects enrolled in this study were ethnic Japanese. Diabetes mellitus was diagnosed according to the 1985 World Health Organization (WHO) criteria.[18] Control subjects had to meet all of the following 3 criteria: (1) age greater than 60 years, (2) hemoglobin A_{1c} (HbA_{1c}) less than 5.7% (normal range, 4.3% to 5.7%), and (3) no family history of type 2 diabetes. Before participation, the purpose and risk of the study were explained, and written informed consent was obtained from all participants. The protocol was approved by the BML ethics committee.

We screened for the polymorphisms in *NeuroD1* and *Pax4* genes in 296 patients with late-onset diabetes, aged 61.2 ± 12.5 years with an age at clinical onset of diabetes of 50.6 ± 3.6 years, and in 177 unrelated control subjects, aged 70.1 ± 7.8 years. The characteristics of the study subjects are listed in Table 1. We found 4 patients carrying homozygous mutations: Thr/Thr in the *NeuroD1* gene and 3 patients carrying Trp/Trp in the *Pax4* gene in 296 late-onset diabetic subjects.

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Submitted October 26, 2001; accepted March 3, 2002.

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0026-0495/02/5109-0013$35.00/0
doi:10.1053/meta.2002.34707

Table 1. Clinical Features of Study Subjects

	Late-Onset Diabetic Subjects	Nondiabetic Subjects
No. (male/female)	296 (199/97)	177 (87/90)
Age (yr)	61.2 ± 12.5	70.1 ± 7.8
Age at diagnosis (yr)	50.6 ± 13.6	
Body mass index (kg/m^2)	24.8 ± 3.8	22.6 ± 2.7
Treatment		
Diet	88	
Oral hypoglycemic agent	122	
Insulin	86	

NOTE. Values are mean ± SEM.

These patients were investigated as to the first-phase insulin secretion and insulin action, using a 2-compartment model analysis of C-peptide kinetics after intravenous glucose load and the insulin-modified minimal model analysis, described elsewhere.

Detection of Ala45Thr Variant in the NeuroD1 Gene and Arg121Trp Variant in Pax4 Genes

Genomic DNA was extracted from the peripheral blood using a QIAamp DNA Blood Mini Kit (QIAGEN, Tokyo, Japan). The *NeuroD1* Ala45Thr variant results in a loss of *MwoI* (New England Biolabs, Beverly, MA) site and, therefore, subjects were screened for these substitutions by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). PCR was carried out using the forward primer 5'-GGAAGCTGAAGGCGTATCTGGC-3' (nt 4035-4056, GenBank accession no. AB018693), and the reverse primer 5'-GGGGGCATGTCCTGGTTCTGC-3' (nt 4740-4720). PCR products were digested with *MwoI*. Also, *Pax4* Arg121Trp variant results in a loss of *AciI* (New England Biolabs) site and, therefore, subjects were screened for these substitutions by PCR-RFLP. PCR was carried out using the forward primer 5'-GGGTTGTTGTGAGGGTGATCCAA-3' (nt 29898-29920 GenBank accession number AC000359), and the reverse primer 5'-TAGGTGGAGACAGATGGGAAAAAG-3' (nt 30158-30181). PCR products were digested with *AciI*. The fragments were separated on a 3% agarose gel and made visable by ethidium bromide staining. Some of the PCR products were directly sequenced using an ABI Prism BigDye Terminator Cycle sequencing kit (PE Biosystems, Foster City, CA) and an automated sequencer (ABI 377) to confirm the results of PCR-RFLP.

C-Peptide Secretion Rate and Minimal Model Analysis

First-phase insulin secretion was estimated using the method of 2-compartment model analysis of C-peptide kinetics after intravenous glucose administration.[14,15] Insulin sensitivity was estimated using insulin-modified minimal model parameters,[16,17] performed as described elsewhere.[15,17] In brief, testing began at 9 AM after an overnight fast. Medication with an oral hypoglycemic agent or insulin was stopped in the morning. An intravenous glucose tolerance test was performed as follows: 25 g dextrose was administered into an antecubital vein ipsilateral to the sampling vein over 60 seconds. Regular human insulin (0.05U/kg; Humalin R, Eli Lilly, Japan K.K., Kobe, Japan) dissolved in 5 mL of 0.9% normal saline was infused over 30 seconds at time 20 minutes. Blood was withdrawn 5 minutes before and just prior to the injection, and 2, 3, 5, 7, 10, 15, 20, 22, 23, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, and 150 minutes after the start of the injection for the determination of plasma glucose, serum C-peptide reactivity (CPR), and immunoreactive insulin (IRI) concentrations. The C-peptide secretion rate (CSR) was calculated using a computer program which we had developed according to the report of Eaton et al.[14,15] The first-phase CSR (CS1) determined by the sum of the CSR levels from 0 to 5 minutes is 6.8 to 18.5 ng/mL/min in subjects with normal glucose tolerance and without diabetic patients in their family (Fig 1).[15,17] Insulin sensitivity index (Si) was calculated by the minimal model software program which we developed according to the algorithm described by Bergman et al.[16,17] Si is 2.6 to 7.6 × 10^{-4}/min/(μU/mL) in subjects with normal glucose tolerance and without diabetic patients in their family (Fig 1).[17]

Statistical Analysis

Data are means ± SEM. The statistical significance of the differences in mean values and frequencies was determined by Student's *t* test and simple chi-squared test, respectively.

RESULTS

Allele frequencies of Thr45 in the *NeuroD1* gene and Trp121 in the *Pax4* gene were 0.098 and 0.048, respectively, in diabetic subjects, and 0.062 and 0.023 in control subjects, respectively. Simple chi-square test showed that allele frequencies of the genes did not statistically differ between the 2 groups, although the frequencies tended to be higher in diabetic patients than in control subjects (Thr45 in *NeuroD1* gene: $\chi^2 = 3.6728$, $P = .0553$; Trp121 in *Pax4* gene: $\chi^2 = 3.2918$, $P = .0696$). The homozygous mutation (Thr/Thr) in the *NeuroD1* gene was observed in 4 diabetic patients (0.013) and the distributions of genotypes Ala/Ala and Ala/Thr were 0.818 and 0.169 in diabetic patients, respectively, and of Ala/Ala, Ala/Thr, and Thr/Thr were 0.876, 0.124, and 0.000 in control subjects, respectively. The homozygous mutation (Trp/Trp) in the *Pax4* gene was found in 3 patients (0.010) and the distributions of genotypes Arg/Arg and Arg/Trp were 0.919 and 0.071 in diabetic

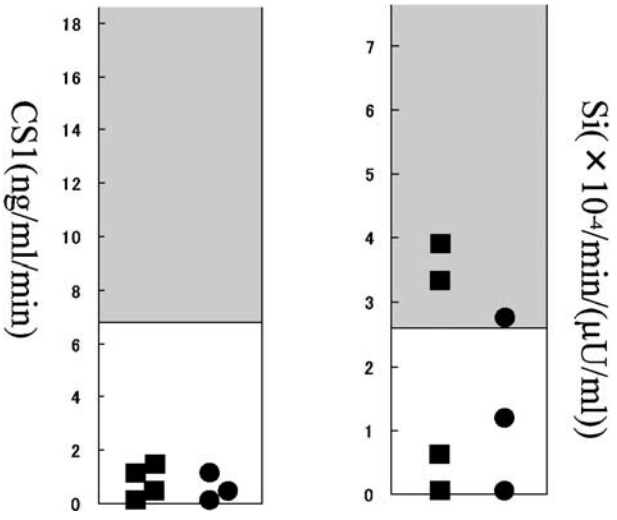

Fig 1. Analysis of acute insulin secretion ability and insulin sensitivity using the method of 2-compartment model analysis of C-peptide kinetics (left) and the insulin-modified minimal model parameters (right) in 4 patients carrying the Thr45 homozygote in the *NeuroD1* gene (■) and 3 patients carrying the Trp121 homozygote in the *Pax4* gene (●). The shaded area in each panel represents the normal range in the first-phase C-peptide secretion rate (CS1) determined by the sum of the C-peptide secretion rate from 0 to 5 minutes: 6.8 to 18.5 ng/mL/min; and insulin sensitivity index (Si): 2.6 to 7.6 × 10^{-4}/min/(μU/mL) in subjects with normal glucose tolerance and without diabetic patients in their families.[15,17]

Table 2. Clinical Features of Late-Onset Diabetic Subjects Carrying the Homozygous Mutation in the *NeuroD1* Gene

	Patient			
	A	B	C	D
Age (yr)	65	67	63	61
Sex	Male	Male	Male	Male
Age at diagnosis (yr)	61	64	59	53
Family history		Mother, brothers		Grandfather, son
Body mass index (kg/m^2)	22.9	24.1	25.8	29.1
HbA_{1c} (%)	6.2	5.5	7.7	6.5
F-IRI (μU/mL)	4	6	8	8
F-CPR (ng/mL)	0.7	1.9	1.5	2.5
αGAD Ab (U/mL)	<1.3	<1.3	<1.3	<1.3
IA-2 Ab (U/mL)	<1.0	<1.0	<1.0	<1.0
Treatment	OHA	Diet	Insulin	Insulin

Abbreviations: F-IRI, fasting immunoreactive insulin; F-CPR, fasting C-peptide reactivity; αGAD Ab, antiglutamic acid decarboxylase antibody; IA-2, tyrosine phosphatase–like protein antibody; OHA, oral hypoglycemic agent.

patients, respectively, and of Arg/Arg, Arg/Trp and Trp/Trp were 0.955, 0.045, and 0.000 in control subjects, respectively. The homozygous mutations were not detected in the control subjects.

Patients carrying homozygous mutation in the *NeuroD1* or *Pax4* gene were diagnosed with diabetes at over 50 years of age with the exception of case X (Tables 2 and 3). Four of 7 patients had diabetic relatives in their families. In case Z, the sisters were diagnosed with diabetes at over 65 years of age and the son at 40 years of age. Fasting CPR levels were approximately 1 ng/mL among the patients with the exception of case X, suggesting that these patients did not suffer from severe insulin deficiency. Autoantibodies against β cells, antiglutamic acid decarboxylase antibody (αGAD Ab), and tyrosine phosphatase-like proteins (IA-2 antibody) were negative in 6 patients and αGAD Ab was positive in case Z. Four patients were treated with insulin injection, 2 patients with oral hypoglycemic agent, and only 1 patient by diet regimen.

In these 7 patients, the first-phase insulin secretion in response to glucose was estimated using the method of 2-compartment model analysis of C-peptide kinetics, and insulin sensitivity was estimated by the insulin-modified minimal model parameters. In patients A, B, C, and D with homozygous mutations in the *NeuroD1* gene, CS1 was 0.508, 1.481, 1.223 and 1.584 ng/mL/min, respectively, and Si was 0.727, 3.31, 3.79 and 0.00 $\times$ 10^{-4}/min/(μU/L), respectively (Fig 1). In patients X, Y, and Z with homozygous mutations in the *Pax4* gene, CS1 was 0.418, 0.208, and 1.279 ng/mL/min, respectively, and Si was 1.11, 2.88, and 0.00 $\times$ 10^{-4}/min/(μU/mL), respectively.

Table 3. Clinical Features of Late-Onset Diabetic Subjects Carrying the Homozygous Mutation in the *Pax4* Gene

	Patient		
	X	Y	Z
Age (yr)	53	79	73
Sex	Male	Male	Female
Age at diagnosis (yr)	37	71	71
Family history	Mother, uncle		Mother, sisters, son
Body mass index (kg/m^2)	21.5	22.8	20.3
HbA_{1c} (%)	7.6	7.1	6.2
F-IRI (μU/mL)	7	6	5
F-CPR (ng/mL)	0.1	1.1	1.3
αGAD Ab (U/mL)	<1.3	<1.3	17.1
IA-2 Ab (U/mL)	<1.0	<1.0	<1.0
Treatment	Insulin	Insulin	OHA

DISCUSSION

We have observed 4 type 2 diabetic patients carrying the homozygous mutation in the *NeuroD1* gene, and 2 late-onset type 2 diabetic patients and 1 type 1A diabetic patient carrying the homozygous mutation in the *Pax4* gene, when screening for these polymorphisms in 296 Japanese late-onset diabetic patients and 177 nondiabetic subjects. Since it has been reported that the frequency of the Thr45 allele in the *NeuroD1* gene was relatively high in both diabetic and nondiabetic subjects,[11,12] the homozygote Thr45Thr had been speculated to be detected in diabetic patients. In this study, the homozygote was actually detected in 4 diabetic patients and was not found in the control subjects. Dupont et al reported that the *NeuroD1* variant Ala45Thr was not associated with type 2 diabetes and some normoglycemic spouses used as the control from the families of type 2 diabetic patients had the homozygote, although their ages were not given.[12] Because age of the diabetic probands was reported to be 55 $\pm$ 13 years, the age of the spouses may be younger than that of our control subjects, 70.1 $\pm$ 7.8 years. The discrepancy of age as well as ethnicity in studied subjects may have caused the different results. Late-onset diabetes may develop in the future among homozygous carriers of normoglycemic spouses. Similarly, the homozygote in the *Pax4* gene was detected in 3 diabetic subjects and was not found in the control subjects. It was recently reported that 1 patient carrying the homozygote was detected among 200 Japanese type 2 diabetic subjects.[13] It is speculated that 0.5% to 1.0% of Japanese late-onset diabetic patients carry this homozygote in the *Pax4* gene.

All patients carrying the homozygote in the *NeuroD1* gene were diagnosed with diabetes at over 50 years of age, suggest-

ing that the mutation might be related to late-onset diabetes, in contrast to MODY genes.[1-4] They were negative for αGAD Ab and IA-2 Ab and did not suffer from severe insulin deficiency. Thus, we thought that the patients carrying the homozygote had late-onset type 2 diabetes rather than type 1 diabetes, although it was recently reported that the frequency of the Thr allele in the *NeuroD1* gene was higher in type 1 diabetic subjects than in nondiabetic control subjects.[19] Clinical features in the patients carrying the homozygote in the *Pax4* gene were somewhat complex. Case X was diagnosed with diabetes at a relatively young age and cases Y and Z at an old age. Case X had the heterozygous Thr45 in the *NeuroD1* gene in addition to the homozygous Trp121 in the *Pax4* gene, and had marked impairment of acute insulin secretion in response to glucose and severe insulin deficiency. The mutations in the 2 transcription factors might have induced the relatively earlier and marked impairment of β-cell function. Since case Z was positive for αGAD Ab and negative of IA-2 Ab, the differentiation between type 1 and type 2 diabetes could not be established. It is suggested that her diabetes had been slowly progressing along with slow destruction of β cells by autoimmune processes[20] or a defect in maturation of the cell. In case Z, there were 3 generations of diabetes, suggestive of autosomal-dominant inheritance. However, the sisters were diagnosed with diabetes at 65 years of age and the son at 40 years, so this could not be a MODY family.

Estimation by 2-compartment model analysis of C-peptide kinetics after intravenous glucose administration revealed that the first-phase insulin secretion in response to glucose was markedly impaired in all patients carrying the homozygote in the *NeuroD1* gene or the *Pax4* gene. Using this method, we have observed that the first-phase insulin secretion is reduced to varying degrees, from the mild impairment to a marked defect, in Japanese late-onset diabetes.[15,17] Thus, the severe defect in the first-phase insulin secretion in all patients carrying the homozygote strongly suggests that the homozygotes are related to β-cell dysfunction. Impairment of acute insulin secretion during intravenous glucose tolerance test is characteristic of definitive diabetes,[21] and the β-cell dysfunction in late-onset diabetes might be caused by many undefined factors. We hypothesize that the homozygous mutations in the *NeuroD1* and *Pax4* genes are one of many factors involved in β-cell dysfunction in late-onset diabetes. In contrast, insulin sensitivity was varied among the patients carrying the homozygote in the *NeuroD1* gene, and also among the patient carrying the homozygote in the *Pax4* gene, suggesting that the insulin resistance in these patients was caused by a factor(s) other than the mutations in *NeuroD1* and *Pax4*, such as genetic and/or environmental factors. Hyperglycemia could cause a secondary decrease in insulin sensitivity, although there was not any correlation between Si and HbA1c level examined during the study for CS1 and Si. From these findings, we conclude that the homozygous mutations in the *NeuroD1* and *Pax4* genes are involved in the pathogenesis in about 2.5% of Japanese late-onset diabetic patients, and that the mutations confer a predisposition to the inability of β cells to overcome the insulin resistance or an intrinsic β-cell defect, resulting in an inadequate mass of functional β cells along with aging[10] and, thus, development of diabetes in old age.

We did not examine molecular mechanisms of the mutations in β-cell dysfunction in vitro. A previous report demonstrated that the Thr45 mutation in the *NeuroD1* gene had normal transcription activity on human insulin promoter in vitro.[22] Since the mutation is not located in the bHLH domain and the tx/p300 interaction domain, but rather in the N-terminus,[8,23] the effect of the variant on function might not be noted in an in vitro experiment. Also, the Trp121 mutation is not located in the paired domain or the homeodomain in the *Pax4* gene.[7,24] The charged R group of arginine has a key role in stabilization of salt bonds. Mutation of Arg to a Trp residue containing an aromatic ring would affect the stabilization of the Pax4 molecule, possibly resulting in a reduction in transcription activity, as well as in the case of a mutation in the *CD38* gene observed previously.[25] It was recently reported that homozygous mutations of 2 MODY genes, glucokinase and IPF-1, cause severe diabetes at birth.[26,27] Homozygous mutations of the glucokinase gene result in a complete deficiency of this enzyme and lead to permanent neonatal diabetes.[26] Homozygous mutations in IPF-1 result in pancreatic agenesis and permanent neonatal diabetes.[27] In contrast to these cases, our patients with homozygous mutations on the *NeuroD1* and *Pax4* genes had late-onset type 2 diabetes, possibly because of the mild effect of the homozygous mutations on transcription activity. Recently, Thomas et al demonstrated that β cells regulated the transcription to maintain IPF-1 levels within a narrow range, and that impaired IPF-1 expression diminished glucose tolerance over 18 months of age in mice, suggesting that a slight reduction of IPF-1 levels induces β-cell dysfunction with aging.[28]The homozygous mutations in *NeuroD1* and *Pax4* appear to contribute to the slow progression of the β-cell dysfunction along with aging via a slight reduction of transcription activity. Alternatively, the Thr45 allele in the *NeuroD1* gene or the Trp121 allele in the *Pax4* gene could be in linked disequilibrium with an unidentified functional mutation in each gene or with gene(s) closely located in each gene.

ACKNOWLEDGMENT

We thank the staffs at Kasori Hospital, Dr N. Kuribayashi and the staffs at the Misaki Clinc, Funabashi, Japan, and M. Nagaoka and the staffs at Hoken Kagaku Co, Chiba Japan for technical assistance

REFERENCES

1. Yamagata K, Oda N, Kaisaki PJ, et al: Mutations in the hepatic nuclear factor 1 alpha gene in maturity-onset diabetes of the young (MODY3). Nature 384:455-458, 1996

2. Yamagata K, Furuta H, Oda N, et al: Mutations in the hepatocyte nuclear factor 4 alpha gene in maturity-onset diabetes of the young (MODY1). Nature 384:458-460, 1996

3. Horikawa Y, Iwasaki N, Hara M, et al: Mutation in hepatocyte nuclear factor-1β (TCF2) associated with MODY. Nat Genet 17:384-385, 1997

4. Stoffers DA, Ferrer J, Clarke WL, et al: Early-onset type-II diabetes mellitus (MODY4) linked to IPF. Nat Genet 17:138-139, 1997

5. Ahlgren U, Jonsson J, Jonsson L, et al: Beta-cell-specific inactivation of the mouse Ipf1/Pdx1 gene results in loss of the beta-cell phenotype and maturity onset diabetes. Genes Dev 12:1763-1768, 1998

6. Sander M, Neubuser A, Kalamaras J, et al: Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. Genes Dev 11:1662-1673, 1997

7. Sosa-Pineda B, Choudhury K, Torres M, et al: The Pax4 gene is essential for differentiation of insulin-producing beta cells in the mammalian pancreas. Nature 386:399-402, 1997

8. Naya F, Stellrecht C, Tsai MJ: Tissue-specific regulation of the insulin gene by a novel basic helix-loop-helix transcription factor. Genes Dev 9:1009-1019, 1995

9. Schwitzgebel VM, Scheel DW, Conners JR, et al: Expression of neurogenin3 reveals an islet cell precursor population in the pancreas. Development 127:3533-3542, 2000

10. Weir SB: β-cell turnover: Its assessment and implications. Diabetes 50:S20-S24, 2001 (suppl 1)

11. Iwata I, Nagafuchi S, Nakashima H, et al: Association of polymorphism in the NeuroD/Beta2 gene with type 1 diabetes in the Japanese. Diabetes 48:416-419, 1999

12. Dupont S, Vionnet N, Chevre JC, et al; No evidence of linkage or diabetes-associated mutations in the transcription factors BETA2/NEUROD1 and PAX4 in type II diabetes in France. Diabetologia 42:480-484, 1999

13. Shimajiri Y, Sanke T, Furuta H, et al: A missense mutation of the Pax4 gene in Japanese type 2 diabetic subjects. Diabetes 49:A202, 2000 (suppl 1, abstr)

14. Eaton RP, Allen RC, Schade DS: Prehepatic insulin production in man: Kinetic analysis using peripheral connecting peptide behavior. J Clin Endocrinol Metab 51:520-528, 1980

15. Kanatsuka A, Makino H, Sakurada M, et al: First-phase insulin response to glucose in nonobese or obese subjects with glucose intolerance: Analysis by C-peptide secretion rate. Metabolism 37:878-884, 1988

16. Bergman RN, Ider YZ, Bowden CR: Quantitative estimation of insulin sensitivity. Am J Physiol 236:E667-E677, 1979

17. Tokuyama Y, Sakurai K, Yagui K, et al: Pathophysiological phenotypes of Japanese subjects with varying degrees of glucose tolerance: Using the combination of C-peptide secretion rate and minimal model analysis. Metabolism 50:812-818, 2001

18. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20:1183-1197, 1997

19. Yamada S, Hirose H, Motohashi Y, et al: NeuroD/BETA2 gene G-A polymorphism may affect onset pattern of type 1 diabetes in Japanese. Diabetes Care 24:1438-1441,2001

20. Kobayasi T, Nakanishi K, Okubo M, et al: GAD antibodies seldom disappear in slowly progressive IDDM. Diabetes Care 19:1031, 1996

21. Brunzell JD, Robertson RP, Lerner RT, et al: Relationships between fasting plasma glucose levels and insulin secretion during intravenous glucose tolerance test. J Clin Endocrinol Metab 42:222-229, 1976

22. Hansen L, Jensen JN, Urioste S: NeuroD/BETA2 gene variability and diabetes. No association to late-onset type 2 diabetes but an A45 allele may represent a susceptibility marker for type 1 diabetes among Danes. Diabetes 49:876-878, 2000

23. Malecki MT, Jhala US, Antonellis A, et al: Mutations in NEUROD1 are associated with the development of type2 diabetes mellitus. Nat Genet 23:323-328, 1999

24. Tokuyama Y, Yagui K, Sakurai K, et al; Molecular cloning of rat Pax4: Identification of four isoforms in rat insulinoma cells. Biochem Biophys Res Commun 248:153-156, 1998

25. Yagui K, Shimada F, Mimura M, et al: A missense mutation in the CD38 gene, a novel factor for insulin secretion: Association with type II diabetes mellitus in Japanese subjects and evidence of abnormal function when expressed in vitro. Diabetologia 41:1024-1028, 1998

26. Njolstad PR, Sovik O, Cuesta-Munoz A, et al: Neonatal diabetes mellitus due to complete glucokinase deficiency. N Engl J Med 344: 1588-1592, 2001

27. Stoffers DA, Zinkin NT, Stanojevic V, et al: Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene coding sequence. Nat Genet 15:106-110, 1997

28. Thomas MK, Devon ON, Lee JH, et al: Development of diabetes mellitus in aging transgenic mice following suppression of pancreatic homeoprotein IDX-1. J Clin Invest 108:319-329, 2001