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Single nucleotide polymorphism (*D68D, T to C*) in the *syntaxin 1A* gene correlates to age at onset and insulin requirement in Type II diabetic patients

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Abstract

Aim/hypothesis. Syntaxin 1A is a candidate gene for Type II (non-insulin-dependent) diabetes mellitus, because it plays an important role in insulin secretion from the islet beta cells. We aimed to scan this gene for mutations or genetic markers that correlate with Type II diabetes.
Methods. We identified and characterized coding exons of the *syntaxin 1A* gene and scanned the newly identified 10 exons using direct sequencing.
Results. In the single nucleotide polymorphism (SNP) of exon 3 (*D68D, T* to *C*) among three newly identified SNPs, genotype frequency of the homozygote of *C* allele (*CC*) occurred more frequently in a Type II diabetic group than in a non-diabetic group (16.48 %, $n = 182$, vs 11.05 %, $n = 181$, $p = 0.0499$). Among the diabetic patients, age of onset in patients with *CC* genotype was lower than that in patients with the *TT* and *TC* genotypes [40.10 ± 1.50 years old (means ± SEM) vs 44.20 ± 0.58, $p = 0.005$]. Patients with the *CC* genotype had a higher frequency of insulin treatment (78.30 % vs 46.80 %, $p = 0.006$) with a duration equal to, or longer than, 10 years. Multiple regression analysis confirmed that the genotype was significantly and independently associated with age at onset and mode of treatment, respectively.
Conclusion/interpretation. These data indicate that the SNP in the *syntaxin 1A* gene (*D68D, T* to *C*) correlates to the age of onset and insulin requirements of Type II diabetic Japanese patients. [Diabetologia (2001) 44: 2092–2097]

Keywords Syntaxin 1A, SNP, Type II diabetes, genetics, age at onset, insulin requirement.

Type II (non-insulin-dependent) diabetes mellitus has been considered to be a polygene disorder [1]. Although several gene mutations have been found in the patients with Type II diabetes [2–8] including maturity onset diabetes of the young (MODY) [5, 9–12], the main susceptibility genes that are common to different ethnic backgrounds have not been identified. On the other hand, reduced insulin secretion is a pathophysiological characteristic observed in non-obese patients with Type II diabetes. Among proteins related to insulin secretion in the islet beta cells, we focused on syntaxin 1A, one of the SNAP receptor (SNARE) proteins [13], which is expressed in the islet beta cells [14, 15], because insulin secretion from islet cells has been reported to be inhibited by the anti-syntaxin 1 antibodies [16]. It has also been reported that expression of syntaxin 1A in the isolated islets of GK rats – a putative animal model for non-obese Type II diabetes – was reduced, and that restoration of its expression by the recombinant adenovirus-mediated gene transduction system improved the impaired insulin secretion [17]. These findings suggest that the syntaxin 1A plays a functional role in the process of insulin secretion and is a candidate gene for Type II diabetes. Thus, we identified and

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Abbreviations: SNAP, Soluble NSF attachment protein; GAD, glutamic acid decarboxylase; HSNP, single nucleotide polymorphism

characterized gene encoding syntaxin 1A and screened the gene for mutations in Japanese Type II diabetic patients.

Materials and methods

Characterization for coding exons of syntaxin 1A gene. Human *syntaxin 1A* gene was cloned from bacterial artificial chromosome (Genome System, St Louis, Mo., USA) by polymerase chain reaction (PCR) using primers designated with reference to the sequence of cDNA (GenBank ACC No. L37792). The positions of exon-intron junctions were determined by comparison with the sequence of cDNA [18] and the *GT-AG* rule.

Subjects and mutation analysis. We enrolled 182 unrelated Japanese Type II diabetic patients without severe obesity (body mass index less than 30 kg/m^2) (Table 1) and 181 non-diabetic subjects without a family history of diabetes mellitus. Type II diabetic patients with early onset (under 25 years of age) and late onset (over 56 years of age) were excluded. All patients had had diabetes for longer than 3 years. Patients whose insulin treatment started within 3 years after onset were excluded. Anti-GAD antibodies were measured using radioimmunoassay kit based on recombinant human GAD65 (Cosmic corporation, Tokyo, Japan) in all patients treated with insulin or sulfonylureas and the patients carrying the antibodies were excluded. Non-diabetic subjects were over 60 years of age and without severe obesity and were confirmed to have fasting plasma glucose of less than 6.1 mmol/l and HbA_{1c} less than 5.8%. Informed consent was obtained from all participants. The study was approved by the Ethical Committee of Wakayama University of Medical Science and was in accordance with the principle of the Declaration of Helsinki. Genomic DNA was extracted from peripheral leukocyte. All newly identified 10 coding exons of the *syntaxin 1A* gene were amplified by PCR using the primers shown (Table 2), and were directly sequenced by dideoxy chain termination method using ABI Prism 310 (PE Biosystem Japan, Tokyo, Japan).

In the analysis of clinical characteristics, the age at onset refers to the age of diagnosis of diabetes mellitus.

Statistical analysis. Data is shown as means ± SEM. Categorical variables were compared by chi-square analysis or Fisher's extraction test. Differences between continuous variables were evaluated by Student's *t* test. Multiple regression analysis was used to find out the independent association between genotype status (*CC* vs *CT* + *TT*) of the SNP (*D68D*) in *syntaxin 1A* gene and clinical characteristics [gender, age at onset, BMI, duration of the disease, and mode of treatment (insulin treatment)]. A *p* value of less than 0.05 was considered to be statistically significant.

Table 1. Clinical characteristics of diabetic patients (n = 182)

		(min–max)
Age (years)	60.88 ± 1.09	(34–85)
Sex (male/female)	100/82	
BMI (kg/m^2)	23.43 ± 0.67	(17.66–29.96)
Age of onset (years)	43.52 ± 1.06	(26–55)
Duration (years)	17.36 ± 1.87	(4–40)
Frequency of insulin-treated patients	80/182 (44.0%)	
Period[a] (years)	13.33 ± 1.49	(4–37)

[a] Period from onset to the start of the insulin therapy in 80 patients treated with insulin

Results

Structure of the gene and mutation screening. The *syntaxin 1A* gene spread around 10 kb and composed of at least 10 exons (Fig. 1, Table 3). Mutation screening showed that there were three single nucleotide polymorphisms (SNPs); silent mutation [*D68D*, *GAT* to *GAC* (*T* to *C*)] in the exon 3, *T* to *C* at 52 bp down stream from the 3' end of the exon 7, and *G* to *A* at 93 bp upstream from the 5' end of exon 8. Genotype frequencies of these SNPs were the statistically consistent in Hardy-Weinberg equilibrium. The allele frequencies of these SNPs were almost same between patients with Type II diabetes and patients without diabetes. However, in the SNP of exon 3 (*D68D*, *T* to *C*), genotype frequency of the homozygote of C allele (*CC*) was higher in the diabetic group than in the non-diabetic group, and the difference showed marginal significance [*TT*, *TC* and *CC* were 41.76%, 41.76% and 16.48% in Type II diabetic group (n = 182) and 34.8%, 54.12% and 11.05% in the non-diabetic group (n = 181), respectively (p = 0.0499)].

Table 2. Primers used to amplify the exons of the human *syntaxin 1A* gene

Exon	Forward primer	Reverse primer	Product (bp)
1	5'-GTCGCGCATGCGGGGCTCAC-3'	5'-CGGCCACCATCCTGGCCGCG-3'	190
2	5'-AGTGCTGTACCCTGGGCACC-3'	5'-GAGCACTGAACCTGGCTCAG-3'	241
3	5'-CTACTCTGGGCCATCTCTG-3'	5'-CAGAGGTCCCGTGAGGCCTC-3'	263
4	5'-GGCTGAGCCTGCACATCAG-3'	5'-GAGGAAGCAGGCCTAGAATGC-3'	195
5	5'-GGTTATGACTTAGCAGCCAC3-'	5'-CGCCCATCCTAGACTCCGTG-3'	133
6	5'-CACCTGAGTCTCTCTGGGTCG-3'	5'-CTTAACACAGCAGCCGCCTCAG-3'	267
7	5'-CAATGCTGCTGCTGAACTC-3'	5'-CGCTGACATTTATGTGACC-3'	312
8a	5'-AAGGCTGCGGCTTGGGAG-3'	5'-GAGCATGGCCATGTCCATG-3'	260
8b	5'-CTCGAAGCAGGCTCTGAGC-3'	5'-GTAACCTGAGAACTTGGTC-3'	238
9	5'-GTCTCTAGCCTCAGGGTGC-3'	5'-GCATGGCCTTGGGCAGGGC-3'	241
10	5'-GACTGTCCAGTCCTCAGCC-3'	5'-CATGGCAGAGAAGGGAGCATG-3'	310

Numbers of exons are tentative

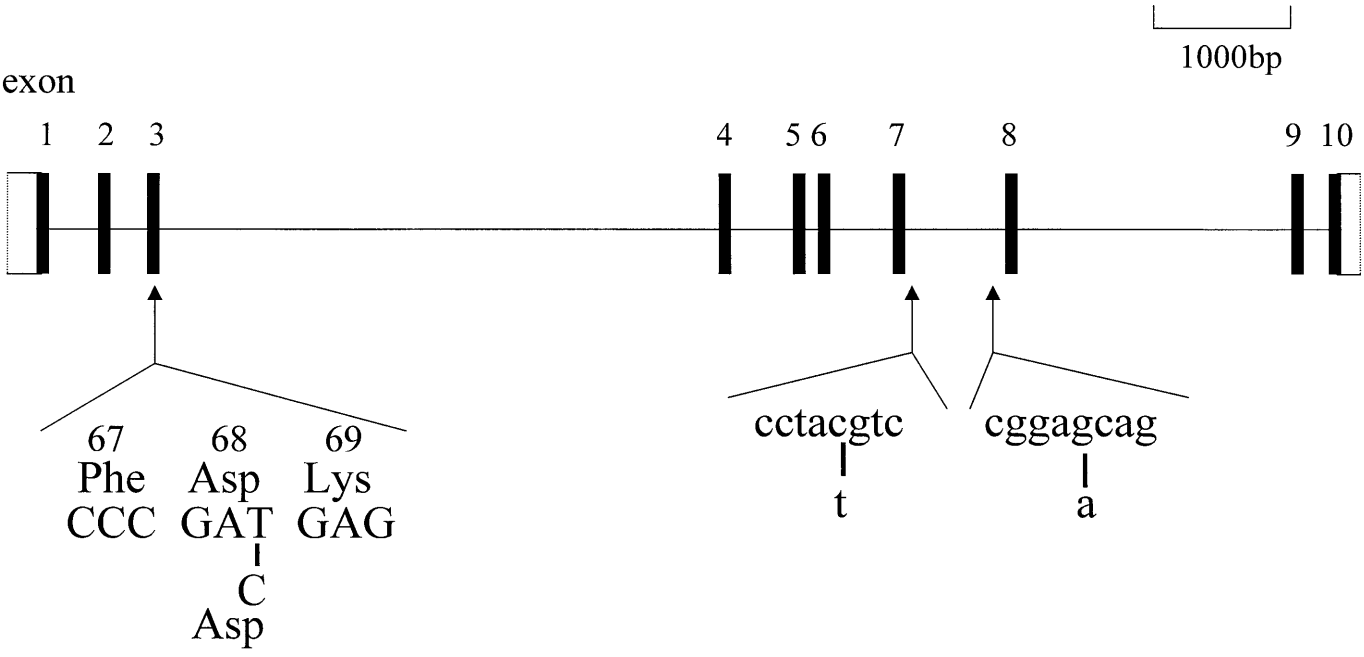

Fig. 1. Structure of the human *syntaxin 1A* gene and localization of the SNPs

SNP (D68D, T to C) and clinical parameters in Type II diabetic patients. Diabetic patients were divided into two subgroups according to the genotype of the SNP in *D68D*, *CC* and other genotypes (*TT* and *TC*), and clinical parameters were compared between these subgroups (Table 4). The age of onset in the patients with the *CC* genotype was significantly lower than in the patients with the other genotypes. This influenced the marked difference of age between the two subgroups because duration of diabetes in each subgroup was almost same. Multiple regression analysis confirmed that the *CC* genotype was strongly and independently associated with the onset age (Table 5).

Patients with the *CC* genotype had a trend of higher frequency of insulin treatment than patients with the other genotypes in all diabetic patients and a significance was obtained when diabetic patients who had diabetes for 10 years or longer were analysed (Table 4). Although there was no significant association between genotype status of the SNP and insulin therapy when evaluated by multiple regression analysis using all patients, a significant association was obtained when patients who had had diabetes for 10 years or more were analysed (Table 5).

The number of insulin-treated patients in each subgroup was compared every 5 years after onset of the disease (Fig. 2). Patients with the *CC* genotype had insulin treatment more frequently than patients with the other genotypes at every point and showed significance at 10 years [39.1 % (9/23) vs 16.1 % (20/124), $p = 0.011$] and 15 years [57.1 % (8/14) vs 29.1 % (25/86), $p = 0.038$]. Multiple regression analysis confirmed the independent association between the genotype and insulin treatment at 10 and at 15 years after the onset of diabetes [at 5 years : $n = 179$,

Table 3. Exon-intron organization of the human *syntaxin 1A* gene

Exon No.	Exon size (bp)	Sequence at exon-intron junction	Intron size (kb)	Amino acid Interrupted
		5'splice donor 3'splice acceptor of next exon		
1	> 30	CGCACGgtgagt. .. .. .gcacagGCCAAG	0.4	Thr 10
2	78	GAGCAGgtggga. .. .. .ccgcagGTGGAG	0.3	Gln 36
3	100	ATGAGAgtgagt. .. .. ..ccccagAGACGA	4.0	Lys 70
4	75	TAAAGAgtgagt. .. .. . ccccagGCATCG	0.5	Ser 95
5	74	ACACAGgtgcgg. .. .. .ccccagCACTC C	0.1	Gln 119
6	109	AGATCAgtgagc. .. .. .ttgcagCCGGCA	0.4	Thr 156
7	74	TCTGGGgtgagt. .. .. .ccccagATCATC	0.7	Gly 180
8	138	AGCCAGgtgagt. .. .. .ccgcagGGAGAG	2.0	Gln 226
9	111	CGCCGGgtcagt. .. .. .atgcagAAGAAA	0.3	Arg 263
10	> 400			

The sites at which introns interrupt the mRNA and protein sequences are indicated. Exon sequences are in capital letters and intron sequences are in lowercase letters. The sizes of introns were estimated by restriction mapping. Numbers of exons are tentative.

Table 4. Comparison of clinical characteristics according to the SNP (*D68D*, *T* to *C*) of the human *syntaxin 1A* gene in Type II diabetic patients

	All patients (n = 182)			Patients with duration of 10 years or more (n = 147)		
	CC (n = 30)	*TT* + *TC* (n = 152)	p value	*CC* (n = 23)	*TT* + *TC* (n = 124)	p value
Age (years)	57.30 ± 1.69	61.34 ± 0.74	0.019	59.61 ± 1.81	63.42 ± 0.77	0.053
Sex (male/female)	16/14	85/70	0.846	12/11	67/57	0.870
BMI (kg/m^2)	23.26 ± 0.50	23.54 ± 0.25	0.709	22.63 ± 0.58	23.31 ± 0.29	0.294
Age of onset (years)	40.10 ± 1.50	44.25 ± 0.58	0.005	39.04 ± 1.76	43.64 ± 60.64	0.007
Duration (years)	17.20 ± 1.81	17.09 ± 0.70	0.915	20.57 ± 1.84	19.78 ± 0.69	0.660
Frequency of insulin-treated patients	18/30 (60.00 %)	62/155 (40.00 %)	0.052	18/23 (78.26 %)	58/124 (46.77 %)	0.006
Insulin-treated patients	(n = 18)	(n = 62)	(n = 18)	(n = 58)		
Period[a] (years)	12.78 ± 1.92	13.48 ± 0.86	0.711	12.78 ± 1.92	14.09 ± 0.86	0.489
Daily dosage of insulin (Unit/kg/day)	0.34 ± 0.03	0.43 ± 0.02	0.016	0.34 ± 0.03	0.43 ± 0.02	0.022

[a]Period from onset to the start of the insulin therapy

Table 5. Multiple regression analysis for genotype status of the SNP (*D68D*, *T* to *C*) of the human *syntaxin 1A* gene in Type II diabetic patients

	All patients (n = 182)		Patients with duration of 10 years or more (n = 147)	
Modulator	SRC	p value	SRC	p value
Sex	0.029	0.692	0.023	0.780
BMI	–0.062	0.409	–0.125	0.127
Age of onset	–0.229	0.005	–0.231	0.010
Duration	–0.152	0.105	–0.110	0.213
Insulin treatment	0.131	0.072	0.207	0.015

SRC, Standardized regression coefficient

SRC = 0.090, p = 0.240; at 10 years : n = 147, SRC = 0.218, p = 0.010; at 15 years : n = 100, SRC = 0.259, p = 0.013; at 20 years : n = 65, SRC = 0.218, p = 0.090].

In insulin-treated patients, the time from the onset of diabetes to the start of the insulin therapy was shorter in patients with the *CC* genotype, but not significantly, than in patients with the other genotype (Table 4). By contrast, the daily dosage of injected insulin in patients with the *CC* genotype was significantly lower than of that in the others (Table 4).

Discussion

We found that the *Syntaxin 1A* gene consists of 10 coding exons spanning 10 kb, a finding different from a previous study reporting (GenBank Acc No. U87310, U87312, U87314, and U87315) 7 coding exons for the gene. These new exons and their flanking introns have no serious mutations that might affect the function as far as we know. Three SNPs existed in this region. Among these, the frequency of the *CC* genotype in the SNP of *D68D* (*T* to *C*) was significantly higher in Type II diabetic patients than in non-diabetic subjects although it showed marginal significance. The *CC* genotype also influenced the clinical factors of Type II diabetic patients such as age of onset of the disease and the number of insulin-treated patients. Thus, although *syntaxin 1A* might not be a main susceptibility gene for Type II diabetes, the *CC* genotype of the SNP, *D68D* is one of a predisposing marker for Type II diabetes.

Our findings that patients carrying the *CC* genotype had a higher frequency of insulin treatment (Table 4 and 5) indicate that the SNP is one of genetic markers affecting the insulin requirement in Type II diabetic patients. However, the power of this SNP might be weak and reveals itself only in patients who have had diabetes for a long time, because the significant association was obtained when patients with a short duration of diabetes (less than 10 years) were excluded (Table 4 and 5). Consistent with these results, significant differences were obtained at 10 and 15 years when we compared the frequency of insulin treatment in patients with the *CC* genotype and patients with other genotypes chronologically (every 5 years) (Fig. 2). In general, Type II diabetic patients

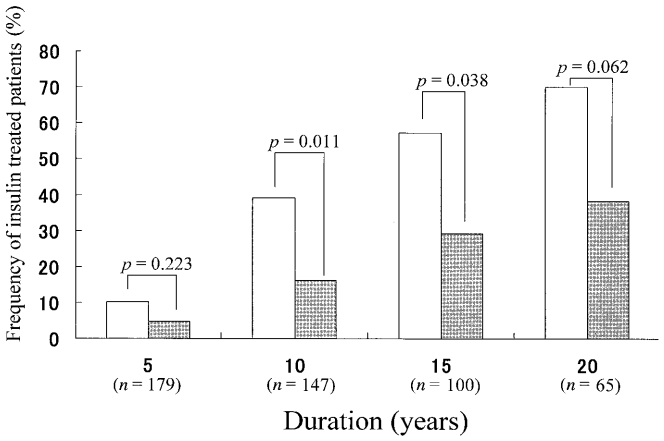

Fig. 2. Frequency of insulin-treated patients at every 5 years after onset of diabetes depending on genotype status (□, *CC* vs ■, *TC* + *TT*) of the SNP of the *syntaxin 1A* gene (*D68D*) of Type II diabetic patients

do not require insulin treatment, especially in the early stages. However, insulin treatment is required with much high frequency in the Type II diabetic patients with a long duration of the disease. This is based on the results reported by UKPDS [19] and in our previous findings that of 188 Type II diabetic patients who had been treated for more than 20 years in our outpatient clinic (duration : 26.0 ± 0.4 years, onset age : 41.0 ± 0.7 years), frequencies of insulin-treated patients at 10 years and 20 years after onset were 21.5 and 53.2 %, respectively. These frequencies are almost consistent with present findings (Fig. 2). Thus, the effect of the genetic factor of the *CC* genotype on the insulin requirement could be explained with the natural course at the early stage (at 5 years) and at the late stage (at 20 years).

In insulin-treated patients, the period of years from the onset to the start of insulin therapy was not significantly different between patients with the *CC* genotype and patients with the others (Table 4). However, patients with a short duration and a long duration were excluded and the analysis was limited to the 54 insulin-treated patients with duration from 10 to 25 years, when a significant difference of the term was obtained (*CC*: $n = 14$, 9.50 ± 1.09 years vs $TT + TC$: $n = 40$, 12.25 ± 0.76 years, $p = 0.023$). These results suggest that the *CC* genotype might be a genetic marker for the exhaustion of the islet beta cells. A significantly lower daily dosage of injected insulin for patients with the *CC* genotype, despite the same levels of BMI (Table 4), points to the genetic uniformity of the patients carrying the *CC* genotype.

Recently, a SNP associated with Type II diabetic patients in Mexican Americans and a Northern European population was reported by the method of positional cloning [20]. It has been suggested that the SNP (UCSNP43) is located in the intron of *calpain-10* gene and that SNP could affect the expression of the *calpain-10* [20]. The precise mechanism for the association between the *T* to *C* SNP in *D68D* and insulin requirements in Type II diabetes is not known at present. One reason for the association could be linkage disequilibrium between this SNP and a variant in the promoter region that influences the expression of the *syntaxin 1A*. The promoter region was not, however, cloned in this study because the expression of *syntaxin 1A* in the islet beta cells has been reported to affect the insulin secretion in GK rats [17]. It is also possible that this SNP is due to a disequilibrium of linkage with another gene located near the *syntaxin 1A* gene.

Genetic analysis for susceptibility genes or markers for pathogenesis of Type II diabetes is important from the point of view of intervention [21–25]. It would also be informative to clarify the genetic marker of requirements of insulin treatment that contribute to the clinical course in the Type II diabetes. From such a point of view, the SNP (*D68D*) in the *syntaxin 1A* gene could be a useful marker for predicting insulin treatment in Type II diabetic patients.

In conclusion, the SNP (*D68D*, *T* to *C*) in the *syntaxin 1A* gene correlated to onset age in Type II diabetic patients in Japanese and also to insulin requirement in the patients who had had diabetes for a longer period of time (more than 10 years).

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References

1. Permutt MA (1991) Use of DNA polymorphism for genetic analysis of non-insulin dependent diabetes mellitus. Baillieres Clin Endocrinol Metab 5: 495–526
2. Steiner DF, Tager HS, Chan SJ, Nanjo K, Sanke T, Rubenstein AH (1990) Lessons learned from molecular biology of insulin-gene mutations. Diabetes Care 13: 600–609
3. Nanjo K, Sanke T, Miyano M et al. (1986) Diabetes due to secretion of a structurally abnormal insulin (Insulin Wakayama) Clinical and functional and characteristics of [Leu^{A3}] Insulin. J Clin Invest 77: 514–519
4. Taylor SI (1992) Lilly Lecture: Molecular mechanisms of insulin resistance: Lessons from patients with mutations in the insulin-receptor gene. Diabetes 41: 1473–1490
5. Bell GI, Pilkis SJ, Polonsky KS (1996) Glucokinase mutations, insulin secretion, and diabetes mellitus. Annu Rev Physiol 58: 171–186
6. Ballinger SW, Shoffner JM, Hedaya EV et al. (1992) Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. Nat Genet 1: 11–15
7. van den Ouwenland JM, Lemkes HH, Ruitenbeek W et al. (1992) Mutation in mitochondrial tRNA$^{LEU(UUR)}$ genes in a large pedigree with maternally transmitted type 2 diabetes mellitus and deafness. Nat Genet 1: 368–371
8. Sakagashira S, Sanke T, Hanabusa T et al. (1996) Missense mutation of amylin gene (S20G) in Japanese NIDDM patients. Diabetes 45: 1279–1281

9. Yamagata K, Oda N, Kaisaki PJ et al. (1996) Mutations in the hepatocyte nuclear factor-4 a gene in maturity-onset diabetes of the young (MODY1). Nature 384: 458–460
10. Yamagata K, Furuta H, Oda N et al. (1996) Mutations in the hepatocyte nuclear factor-1 a gene in maturity-onset diabetes of the young (MODY3). Nature 384: 455–458
11. Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF (1997) Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF1 gene cording sequence. Nat Genet 15: 106–110
12. Horikawa Y, Iwasaki N, Hara M et al. (1997) Mutation hepatocyte nuclear factor-1β gene (TCF2) associated with maturity-onset diabetes of the young. Nat Genet 17: 384–385
13. Söllner T, Whiteheart SW, Brunner M et al. (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature 362: 318–324
14. Jacobsson G, Bean AJ, Scheller RH et al. (1994) Identification of synaptic proteins and their isoform mRNAs in compartments of pancreatic endocrine cells. Proc Natl Acad Sci USA 91: 12487–12491
15. Nagamatsu S, Fujiwara T, Nakamichi Y et al. (1996) Expression and functional role of syntaxin 1/HPC-1 in pancreaticβ-cells. J Biol Chem 271: 1160–1165
16. Martin F, Moya F, Gutierrez LM, Reig JA, Soria B (1995) Role of syntaxin in mouse pancreatic beta cells. Diabetologia 38: 860–863
17. Nagamatsu S, Nakamachi Y, Yamamura C et al. (1999) Decreased expression of t-SNARE, syntaxin 1, and SNAP-25 in pancreaticβ-cells is involved in impaired insulin secretion from diabetic GK rats. Restoration of decreased t-SNARE proteins improves impaired insulin secretion. Diabetes 48: 2367–2373
18. Inoue A, Obata K, Akagawa K (1992) Cloning and sequence analysis of cDNA for a neuronal cell membrane antigen, HPC-1. J Biol Chem 267: 10613–10619
19. UK Prospective Diabetes Study (UKPDS) Group (1998) Intensive blood-glucose control with sulfonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352: 837–853
20. Horikawa Y, Oda N, Cox NJ et al. (2000) Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. Nat Genet 26: 163–175
21. Nicolae DL, Concannon P, Hanis C, Bell GI, Kong A (1999) Loci on chromosomes 2 (NIDDM 1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. Nat Genet 21: 213–215
22. Hanis CL, Boerwinkle E, Chakraborty R et al. (1996) A genome-wide search for human non-insulin dependent diabetes genes reveals a major susceptibility locus on chromosome 2. Nat Genet 13: 161–166
23. Mahtani MM, Widen E, Lehto M et al. (1997) Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. Nat Genet 14: 90–94
24. Valle T, Tuomilehto J, Bergman RN et al. (1998) Mapping genes for NIDDM. Design of the Finland-United States Investigation of NIDDM genetics (FUSION) Study. Diabetes Care 21: 949–958
25. Ghosh S, Watanabe RM, Hauser ER et al. (1999) Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. Proc Natl Acad Sci USA 96: 2198–2203