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The Absence of Any Obvious Association of the Human Glucagon-Like Peptide-1 (GLP-1) Receptor Gene Simple Tandem Repeat Polymorphism with NIDDM

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Summary. **Glucagon-like peptide-1 (GLP-1) has a potent glucose-dependent insulin secretory effect, and is thought to play an important role in regulating blood glucose levels as an "Incretin". In the early stage of non-insulin-dependent diabetes mellitus (NIDDM), impaired glucose-regulated insulin secretion in the rapid phase is a common feature. This feature is regarded to be partly due to defects in the biological actions of GLP-1, which are mediated by the GLP-1 receptor. Thus, defects in the islet B cell GLP-1 receptor gene may contribute to NIDDM. To test this hypothesis, we examined the association of the reported simple tandem repeat polymorphism (STRP) in GLP-1 receptor gene with NIDDM in Japanese. Genomic DNAs were extracted from peripheral blood leukocytes obtained from 24 independent NIDDM patients as well as 31 independent normal control subjects. The DNA fragments containing the reported STRP region were amplified by PCR. The sizes of the amplified DNA fragments varied between 108 to 122 bp (corresponding to 17 to 24 CA repeats); accordingly 8 different alleles were observed. The allelic frequencies did not differ between the Japanese NIDDM patients and the control subjects. These results indicate the absence of any obvious association of GLP-1 receptor gene STRP with NIDDM in Japanese.**

Key words—glucagon-like peptide-1 (GLP-1) receptor, simple tandem repeat polymorphism (STRP), NIDDM.

INTRODUCTION

Non-insulin-dependent diabetes mellitus (NIDDM) is a disorder of glucose homeostasis characterized by hyperglycemia, peripheral insulin resistance, increased hepatic glucose efflux, and diminished glucose-dependent secretion of insulin from the pancreatic beta-cell.[1-3] The pathogenesis of the disorder is apparently multi factorial. Many genetic factors are involved in the pathogenesis, but only a few of them are sufficient to cause NIDDM by itself. One example of such a kind of genetic factor is MELAS, or a defect in mitochondrial DNA.[4] Most factors are not sufifcient to cause NIDDM by itself, but they can cause the disorder when several of them coexist. The glucokinase gene appears to be such a kind of gene.[5-8] The glucagon-like-peptide-1 receptor gene, namely GLP-1R gene, is also a candidate gene for causing NIDDM, since it plays an important role in glucose metabolism.

Glucose is absorbed in the small intestine during meals and after oral glucose administration, and tGLP-1, a peptide derived from proglucagon, is secreted from L cell in the small intestine upon glucose absorption.[9] Glucose stimulates insulin secretion from the pancreatic beta-cell, and this glucose effect is enhanced by tGLP-1.[10-12] This effect of tGLP-1 to enhance insulin secretion is apparently mediated through glucagon-like peptide-1 (GLP-1) receptor. Accordingly, mutations in the GLP-1 receptor gene could contribute to the impaired beta-cell function and development of the disorder.

To assess the possible role of the GLP-1 receptor gene in determining the genetic susceptibility to NIDDM in Japanese, association studies of the gene with NIDDM have been done. Stoffel et al. reported a simple tandem repeat polymorphism (STRP) in GLP-1 receptor gene.[13] Later, Tanizawa et al. reported three STRP in the gene and named them GLP-1R-CA1, CA2 and CA3.[14] One of them, GLP-1R-CA3 is

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the same that Stoffel et al. reported. In this report, we compared the allelic frequencies of the GLP-1R-CA3 between NIDDM patients and non-diabetic control subjects in Japanese. These results reported here indicated no obvious association between NIDDM patients and non-diabetic control subjects in Japanese.

MATERIALS AND METHODS

DNA extraction

Genomic DNAs were extracted from peripheral blood leukocytes from 24 Japanese NIDDM patients (15 men and 9 women: age range 26-70 years) and 31 non-diabetic control subjects (13 men and 18 women: age range 24-41 years) by the method reported by Sambrook et al..[15] The genomic DNAs extracted were used as templates for PCR amplification.

PCR primers

PCR primers used to amplify the genomic region containing the STRP, GLP-1R-3A, were as follows:

GR-1, 5′ GATGGGTTTGGGGAGGGAAAG 3′
GR-2, 5′ GCCAATCCAGGTGGGAGAGAC 3′

Analysis of the STRP by PCR

PCR reactions were performed in 10 μl volumes using about 100 ng of genomic DNA, 2.5 units of rTaq polymerase (Takara, Otsu, Japan), 1 μM of each primer (GR-1 and GR-2), 200 μM each of dATP, dCTP, dTTP, dGTP, and 1 X Buffer supplied by the vender. Products were amplified in the Gene Amp 9600 (Perkin Elmer Cetus) using 30 cycles under the following conditions: denaturing at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min. The final extension step was for 5 min. Primers GR-1 was end-labeled by ^{32}P prior to the amplification. Thus, one strand of the amplified DNA fragment was labeled by ^{32}P. The products were resolved on 5% Long Ranger (AT Biochem, Malvern, USA) gel containing 7 M urea. The separated PCR products on the gel were visualized by autoradiography and subsequently analyzed. The sizes of the PCR products were determined by comparison of the co-migration ratio of the PCR products with a known nucleotide sequence ladder loaded on the same gel.

Statistics

Allelic and genotypic frequencies for each group were determined from the observed data. Frequency differences were tested by Fisher's exact probability test.

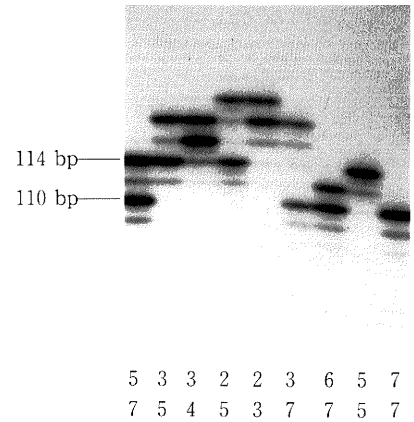

Fig 1. Illustrative autoradiogram of the STRP, GLP-1R-CA3. The alleles of each individual are indicated at the bottom. The sizes of the PCR-amplified repeat regions determined are indicated on the left.

Table 1. Characterization of STRP in human GLP-1R (GLP-1R-CA3) in Japanese

Allele	bp	NIDDM	Control
1	122	0 (0.0)	1 (1.6)
2	120	10 (20.8)	12 (19.4)
3	118	12 (25.0)	11 (17.7)
4	116	4 (8.3)	9 (14.5)
5	114	8 (16.7)	5 (8.1)
6	112	1 (2.1)	4 (6.5)
7	110	12 (25.0)	14 (22.6)
8	108	1 (2.1)	6 (9.7)
	Total	48 (100.0)	62 (100.0)
Heterozygosity (%)		87.5	83.8

Date are n (%). P values were greater than 0.10 for any alleles observed.

RESULTS

An example of an autoradiogram obtained in this study is shown in Fig. 1. The alleles of the STRP, GLP-1R-CA3, found were named from 1 to 8 in order of size. The sizes of alleles 5 and 7 were 114 and 110 bp, respectively, and so corresponded to the repeat numbers of 20 and 18, respectively. As shown in this figure, this STRP showed a very heterogeneous pattern also in Japanese.

Table 1 summarizes the results. Eight alleles ranging from 108 to 122 bp in length were observed. The

frequencies of each allele of the STRP varied between the Japanese NIDDM patients and the non-diabetic control subjects. However, none of them was significantly different when Fisher's exact probability test was applied. The highest p value calculated was 0.134 for allele 8.

DISCUSSION

Three STRP (GLP-1R-CA1, CA2 and CA3) in the GLP-1 receptor gene region have been reported so far.[13,14] The allelic frequencies of these STRPs have been also reported, and the heterozygosity of two of them (GLP-1R-CA1 and CA3) appear to be very high. Stoffel et al. have reported the allelic frequency of the STRP, GLP-1R-CA3 in Caucasian, African-American and Japanese subjects.[13] Tanizawa et al. have also reported the allelic frequencies of the STRPs, GLP-1R-CA1 and CA3, in Caucasian and African-American subjects.[14] They also compared the allelic frequencies of the STRPs, GLP-1R-CA1 and CA3, between African-American NIDDM patients and control subjects, and found no difference.[14]

We compared the allelic frequencies of the STRP, GLP-1R-CA3, between Japanese NIDDM patients and control non-diabetic subjects, and no difference was found. It therefore can be said that the STRP is not obviously associated with NIDDM in the Japanese population. Our result reported here is in agreement with that of Tanizawa et al. reported in their association study based on an African-American population.[14] Our study indicates that mutations in or near the GLP-1 receptor gene are unlikely to be the major cause of the inherited predisposition to NIDDM in Japanese, and so may be only a minor risk factor. Nevertheless, an extensive study with an increased number of subjects, and the accurate discrimination of subjects genetically predisposed to NIDDM from non-diabetic control subjects are needed before pronouncing a more definitive conclusion.

REFERENCES

1) Defronzo RA: The triumvirate: β-cell, muscle, liver: a collusion responsible for NIDDM. *Diabetes* **37:** 667-687, 1988.
2) Porte DJr: β-cell in type II diabetes mellitus. *Diabetes* **40:** 166-180, 1991.
3) Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR: Role of glucose and insulin resistance in development of type 2 diabetes mellitus:results of a 25-year follow-up study. *Lancet* **340:** 925-926, 1992.
4) van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, Sandkuijl LA, de Vijlder MF, Struyvenberg PAA, van de Kamp JJP, Maassen JA: Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. *Nature Genet* **1:** 368-371, 1992.
5) Hattersley AT, Turner RC, Permutt MA, Tanizawa Y, Chiu KC, Patel P, Watkins P, Wainscoat JS: Type 2 diabetes in linked to the glucokinase gene in a large pedigree. *Lancet* **339:** 1307-1310, 1992.
6) Froguel PH, Vaxillaire M, Sun F, Velho G, Zouali H, Butel MO, Lesage S, Vionnet N, Clement K, Fougerousse F, Tanizawa Y, Weissenbach J, Bechmann JS, Lathrop GM, Passa PH, Permutt MA, Cohen D: The glucokinase locus on chromosome 7p is closely linked to early onset non-insulin-dependent diabetes mellitus. *Nature* **356:** 162-164, 1992.
7) Katagiri H, Asano T, Ishihara H, Inukai K, Anai M, Miyazaki J, Tsukuda K, Kikuchi M, Yazaki Y, Oka Y: Nonsense mutation of glucokinase gene in late-onset non-insulin-dependent diabetes-mellitus. *Lancet* **340:** 1316-1317, 1992.
8) Sakura H, Eto K, Kadowaki H, Simokawa K, Ueno H, Koda N, Fukushima Y, Akanuma Y, Yazaki Y, Kadowaki T: Structure of the human glucokinase gene and identification of a missence mutation in a Japanese patient. *J Clin Endocrinol Metab* **75:** 1571-1573, 1992.
9) Sugiyama K, Manaka H, Kato T, Yamatani K, Tominaga M, Sasaki H: Stimulation of truncated glucagon-like peptide-1 release from the isolated perfused canine ileum by glucose absorption. *Digestion* **55:** 24-28, 1994.
10) Gutniak M, Orshov C, Holst JJ, Ahren B, Efendic S: Antidiabetogenic effect of glucagon-like peptide-1 (7-36) amide in normal subjects and patients with diabetes mellitus. *N Engl J Med* **326:** 1316-1322, 1992.
11) Nathan MD, Schreiber E, Fogel H, Mojsov S, Habener JF: Insulinotropic action of glucagon-like peptide-1 (7-37) in diabetic and nondiabetic subjects. *Diabetes Care* **15:** 270-276, 1992.
12) Holz GGIV, Kuhtreiber WM, Habener JF: Pancreatic beta-cells are rendered glucose-competent by the insulinotropic hormone glucagon-like peptide-1 (7-37). *Nature* **361:** 362-365, 1993.
13) Stoffel M, Espinosa IIIR, Le Beau MM, Bell GI: Human glucagon-like peptide-1 receptor gene: Localization to chromosome band 6p21 by fluorescence *in situ* hybridization and linkage of a highly polymorphic simple tandem repeat DNA polymorphism to other markers on chromosome 6. *Diabetes* **42:** 1215-1218, 1993.
14) Tanizawa Y, Riggs AC, Elbein SC, Whelan A, Donis-Keller H, Permutt MA: Human glucagon-like peptide-1 receptor gene in NIDDM; Identification and use of simple sequence repeat polymorphisms in genetic analysis. *Diabetes* **43:** 752-757, 1994.
15) Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: a laboratory manual (2nd ed) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989.