Low Frequency of the Large Insertion in the Human Insulin Gene in Japanese

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SUMMARY

We have studied the restriction fragment-length polymorphism in the 5′-flanking region of the human insulin gene in 47 nondiabetic Japanese subjects and in 52 subjects with non-insulin-dependent diabetes (NIDDM) to elucidate the ethnic variation of the genetic polymorphism and its relationship with NIDDM. Allelic frequencies in the nondiabetic subjects were 0.957 in class 1 (Bgl I fragments of 2800 ± 300 bp), 0 in class 2 (fragments of 3500 ± 300 bp), and 0.043 in class 3 (fragments of >3900 bp with a mean of 4500 bp). Corresponding frequencies in the NIDDM subjects were 0.962, 0, and 0.038, respectively. Four subjects with NIDDM who had the class 3 allele did not exhibit any particular clinical characteristics compared with the rest of the patients. Thus, the class 3 allele or the large insertion of the human insulin gene is much less frequent in Japanese than reported in other races, including Caucasians, and this class of allele is not associated with NIDDM in Japanese. Ethnic homogeneity is, thus, important in the analysis and interpretation of the genetic polymorphism. DIABETES 1986; 35:115–18.

Diabetes mellitus, especially non-insulin-dependent diabetes (NIDDM), is a syndrome with unknown, heterogeneous etiologies. Patients with NIDDM demonstrate a broad spectrum of defects in insulin secretion and/or in insulin sensitivity. Analysis of the human insulin gene may be one of various approaches to detect abnormalities in insulin biosynthesis or to elucidate the genetic factor(s) that may contribute to the pathogenesis of NIDDM. An example of such analysis is the identification of a point mutation in the coding region of the human insulin gene in the familial hyperinsulinemia-diabetes syndrome.[1,2] Recently, the length polymorphism of the restriction fragments of the human insulin gene was discovered,[3,4] and its association with NIDDM has been studied.[4–7] Tandemly repeated sequences composed of GC-rich, 14–15-bp oligonucleotides were identified to be inserted in the 5′-flanking region of the human insulin gene.[8] In the earlier studies, the large insertion of these repetitive sequencies was more frequently found in patients with NIDDM than in control subjects in Caucasians,[4–7] American Blacks,[6] and Pima Indians.[6] In the recent studies, however, the lack of association of the large insertion with NIDDM in American Blacks[9] and Pima Indians[10] has been noticed. Because of this discrepancy and the possible ethnic variations in the genetic polymorphism, we have studied the length polymorphism of the restriction fragments in the human insulin gene in Japanese and examined whether or not the large insertion is associated with NIDDM.

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MATERIALS AND METHODS

Subjects. Forty-seven unrelated, nondiabetic subjects without a family history of diabetes and with a mean age of 41.6 ± 9.2 yr were studied. Fifty-two unrelated patients with NIDDM were randomly selected from the Outpatient Clinic of the Third Department of Medicine of Shiga University of Medical Science. [The mean (±SD) of their age was 55.6 ± 12.7 yr and the mean age at the onset of diabetes was 47.8 ± 12.3 yr.] Eighteen patients had a family history of NIDDM in at least one of their parents or siblings. Eleven patients were treated with insulin, and the others with oral hypoglycemic agents and/or diet. These subjects live in the area of Otsu city, but their native places are widely distributed in the southern part of Japan.

Laboratory methods. Restriction endonucleases Bgl I, Bgl II, and Sac I were purchased from Bethesda Research Laboratories, Gaithersburg, Maryland; Toyobo Co., Osaka, Japan; and Boehringer-Mannheim GmbH-Biochemica, Mannheim, FRG, respectively. Genomic DNA was prepared from leukocytes from 20 ml of blood as described previously,[2,11] with a yield of approximately 10 μg/ml of blood. Ten micro-

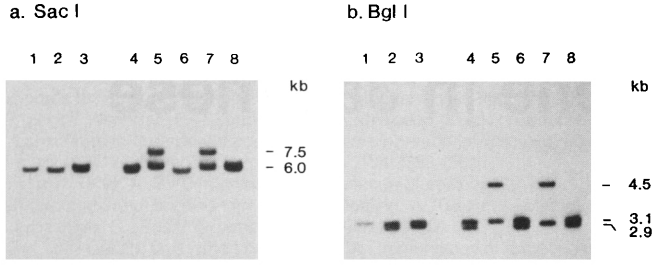

FIGURE 1. An autoradiogram of the hybridization of Sac I (A) or Bgl I (B)-digested genomic DNA from nondiabetic (1–3) and diabetic (4–8) subjects with ^{32}P-labeled cloned human insulin gene.

grams of genomic DNA was digested with 30 U of a restriction endonuclease at 37°C for 14 h under the conditions suggested by the manufacturers. After the incubation, 10 U of enzyme was added and the digestion was continued for an additional 4 h to ensure its completion. The digested DNA was separated on a 1.0% agarose gel and transferred to a nitrocellulose filter by the method of Southern.[12] The filter was then hybridized with 2×10^6 cpm of Bgl I-Hinc II fragment of the cloned human insulin gene[2] (pJF101, gift from Dr. D. Steiner, University of Chicago) labeled with ^{32}P by nick translation, washed, and exposed to x-ray film as previously described.[2] Lambda DNA digested with Hind III was used as a molecular-weight standard.

Plasma glucose, triglyceride, and cholesterol were measured by the enzyme method using glucose-oxidase, glycerol-3-phosphate-oxidase, and cholesterol-oxidase, respectively. Hemoglobin A_1 (HbA_1) was measured by the microcolumn method. Serum insulin was determined by radioimmunoassay. Retinopathy was diagnosed by an ophthalmologist and nephropathy by persistent proteinuria. Cerebral vascular disease and coronary artery disease were diagnosed by history, CT scan, and ECG.

RESULTS

An example of an autoradiogram of the hybridization of Sac I- or Bgl I-digested genomic DNA with ^{32}P-labeled cloned human insulin gene is shown in Figure 1. The polymorphic loci were classified according to the report of Bell et al.,[9] in which Bgl I fragments of 2800 ± 300 bp indicate a class 1 allele; fragments of 3500 ± 300 bp, a class 2 allele; and fragments of >3900 bp and with a mean of 4500 bp, a class 3 allele. As shown in Table 1, the allelic frequencies in the nondiabetic subjects were 0.957 in class 1, 0 in class 2, and 0.043 in class 3; the corresponding frequencies in the patients with NIDDM were 0.962, 0, and 0.038, respectively. Thus, nonclass 1 alleles were very rare in both nondiabetic and diabetic Japanese subjects. All subjects with the class 3 allele demonstrated the genotype of class 1/3, and the homozygote of the class 3 allele was not found in the population studied. There was no significant difference in either genotypic or allelic frequencies between the nondiabetic subjects and the subjects with NIDDM. The distribution of the sizes of the insulin gene fragments produced by Bgl I digestion is illustrated as a histogram in Figure 2. The distribution of the fragments within the size of the class 1 allele is similar to that reported in Caucasians.[3] With statistical analysis, NIDDM was not associated with any size of the fragments.

To examine whether patients with the class 3 allele exhibit any particular clinical characteristics different from patients with the genotype of class 1/1, various clinical parameters were compared between them. The results are listed in Table 2. Hemoglobin A_1 levels at the time of diagnosis of diabetes were significantly higher in patients with the class 3 allele. However, four patients with the class 3 allele could not be distinguished from the rest of the patients by any other clinical parameters.

DISCUSSION

The present study of the length polymorphism of restriction fragments in the 5′-flanking region of the human insulin gene in the nondiabetic Japanese population revealed a low frequency (only 0.043) of the class 3 allele, which corresponds to the U-allele[5] or the allele with a 1.6-kb insertion.[6] The class 2 allele was not found, and all of the subjects with the class 3 allele had the genotype of class 1/3. Since our study did not cover the whole area of Japan, the data might not represent all of Japan. However, the large insertion, or the class 3 allele, is infrequent at least in the southern part of the country. The frequencies of the class 3 allele in Caucasians have been reported to be in the range of 0.18–0.33 (Table 3), which is much higher than the frequencies in the Japanese population studied in this report. Bell et al. studied the ethnic variation of the polymorphism and reported that the class 1 allele was the major allele in Asians, including Chinese, Japanese, and Koreans.[9] However, the numbers they studied were too small to be compared with Caucasians and it is necessary to separate these three races, since they have a different genetic background, including HLA system.[13,14] As shown in Table 3, among the subjects of different races who have been studied in sufficient numbers, the frequency of the class 3 allele is the lowest in the Japanese population described in this communication.

The frequency of the class 3 allele in NIDDM was almost equal to that in the nondiabetic subjects, indicating that the large insertion in the 5′-flanking region of the human insulin gene is not associated with NIDDM in Japanese. Although heterogeneity could exist within the class 1 allele in both

TABLE 1
Allelic and genotypic frequencies of the polymorphic loci

	Allelic frequencies			Genotypic frequencies	
	Class 1	Class 2	Class 3	Class 1/1	Class 1/3
Nondiabetic subjects (N = 47)	0.957	0	0.043	0.915	0.085
NIDDM subjects (N = 52)	0.962	0	0.038	0.923	0.077

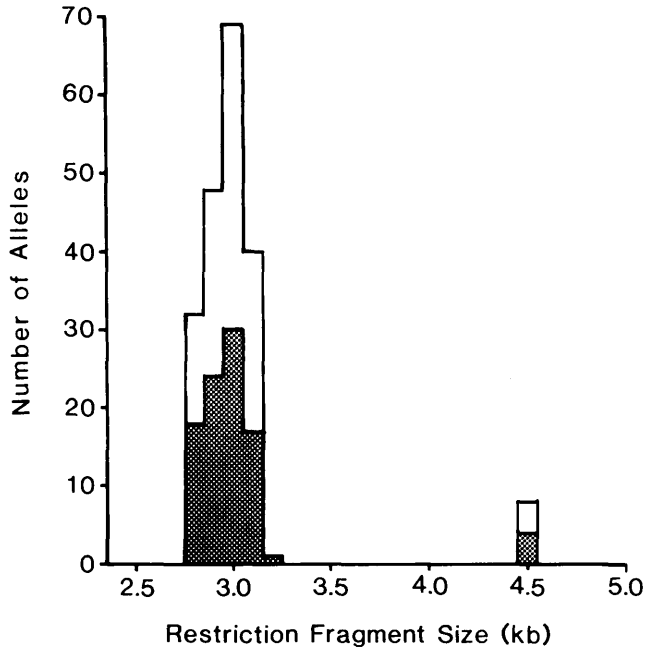

FIGURE 2. Histogram of the sizes of the insulin gene fragments generated by Bgl I digestion in nondiabetic (■) and diabetic (□) subjects.

nondiabetic and diabetic subjects (Figure 1), the distribution of the sizes of the insulin gene fragments within the class 1 allele generated by Bgl I digestion (Figure 2) was similar to that in Caucasians[3] and NIDDM was not associated with any size of the fragments. Furthermore, the association of the class 3 allele with atherosclerosis[15] and/or hypertriglyceridemia[16] was not found in the present study (Table 2).

The association of the large insertion in the 5′-flanking region of the human insulin gene with NIDDM is still controversial. In earlier studies, the association was found in Caucasians, American Blacks, and Pima Indians in some laboratories.[4-7] In Pima Indians, however, Knowler et al. have recently reported that the class 3 allele plays no role in the genesis of NIDDM but may have an influence on the severity of the disease, as indicated by need for drug treatment.[10] The regulatory role of the large insertion in glycemic control, as indicated by high HbA_1 levels, has been reported as well.[17] Indeed, the patients with the class 3 allele in the present study showed higher HbA_1 levels at the time of diagnosis of diabetes than the patients with the genotype of class 1/1, and either insulin or oral hypoglycemic agents were required for their treatment. However, the number of patients with the class 3 allele was too small to confirm the conclusion of Knowler et al. In American Blacks, Bell et al. did not find any association of the class 3 allele with NIDDM. The lack of association in American Blacks was confirmed by a recent cooperative study between two centers (San Francisco and St. Lous).[18] Thus, large numbers of other races should be studied to clarify this genetic association.

Bell et al. recently reported that the class 1 allele might be associated with the insulin-dependent form of diabetes (IDDM).[9] Although we did not investigate patients with IDDM in this study, it might be difficult to correlate the class 1 allele with IDDM in Japanese, since the frequency of the class 1 allele is so high in nondiabetic Japanese subjects, at least

TABLE 2
Clinical characteristics of the diabetic patients (mean ± SD)

	Class 1/1		Class 1/3	
Age at time of study (yr)	56.4 ± 12.7	(48)	46.5 ± 11.2	(4)
Age at onset* (yr)	48.4 ± 12.2	(48)	41.0 ± 13.3	(4)
Body mass index (kg/m²)	25.1 ± 4.2	(48)	25.4 ± 1.2	(4)
FPG at time of study (mg/dl)	143 ± 46	(48)	141 ± 12	(4)
FPG at onset (mg/dl)	190 ± 57	(43)	227 ± 40	(4)
HbA_1 at time of study (%)	8.1 ± 2.1	(48)	8.7 ± 1.0	(4)
HbA_1 at onset (%)	10.0 ± 1.8	(35)	13.0 ± 1.9†	(3)
TG at time of study (mg/dl)	122 ± 64	(48)	122 ± 32	(4)
TG at onset (mg/dl)	142 ± 75	(40)	130 ± 30	(4)
Cho. at time of study (mg/dl)	203 ± 41	(48)	193 ± 33	(4)
Cho. at onset (mg/dl)	220 ± 41	(40)	228 ± 30	(4)
Insulin during 75-g OGTT (μU/ml)				
0 min	11.0 ± 5.0	(35)	11.3 ± 6.2	(3)
60 min	28.9 ± 17.7	(35)	31.9 ± 16.8	(3)
120 min	34.0 ± 22.4	(35)	50.7 ± 19.7	(3)
Retinopathy (%)	33	(48)	50	(4)
Nephropathy (%)	25	(48)	50	(4)
Cerebral and/or coronary vascular disease (%)	8	(48)	0	(4)
Treatment (%)				
Diet	35	(48)	0	(4)
Oral hypoglycemic	44	(48)	75	(4)
Insulin	21	(48)	25	(4)

*The time when diabetes was diagnosed.
FPG, fasting plasma glucose; TG, triglyceride; and Cho., cholesterol. The number of patients is given in parentheses. Some of the laboratory data at the diagnosis of diabetes were not available, as some of the patients were referred to our clinic a considerable time after the beginning of treatment.
†$P < 0.05$ versus class 1/1.

TABLE 3
Reported frequencies of the class 3 allele in nondiabetic subjects

Group	Reference	N	Frequency
Caucasians	5	56	0.26
	6	33	0.182
	7	44	0.33
	9	83	0.32
American Blacks	6	28	0.214
	9	32	0.27
	18	77	0.29
Pima Indians	6	26	0.192
	10	38	0.23
Micronesians	19	60	0.192
Polynesians	19	46	0.326
Melanesians	19	36	0.111
Asians (Chinese, Japanese, and Korean)	9	17	0.029
Japanese	present study	47	0.043

in the southern part of the country as was studied in this report.

The analysis of the human insulin gene is important in the etiologic considerations of diabetes. In fact, a point mutation of the human insulin gene resulting in the particular form of the familial hyperinsulinemia-diabetes syndrome has been discovered.[1,2] The discovery of the polymorphism of the human insulin gene is an important finding, but the functional role of the insertion in the 5′-flanking region is still obscure. The present study indicates that ethnic differences should be considered in the analysis and interpretation of the genetic polymorphism.

ACKNOWLEDGMENT

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