The polymorphism linked to the human insulin gene: its lack of association with either IDDM or NIDDM in Japanese

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Abstract. Polymorphism of 5' portion of the human insulin gene was examined in 188 unrelated Japanese subjects (49 normal, 71 with IDDM, and 68 with NIDDM) using restriction endonuclease analysis. Restriction fragments were classified according to the insertion size: Class 1 (600 base pairs), Class 2 (1300 base pairs), and Class 3 (2000 base pairs). We found a very high frequency of Class 1 alleles (96.8%) and a low frequency of both Class 2 (0.8%) and Class 3 alleles (2.4%) and that approximately 94% of the genotypes were Class 1/Class 1 homozygote. In addition, there was no correlation of allelic or genotypic frequency with NIDDM or IDDM. We conclude that length polymorphism of the human insulin gene cannot be a useful marker for diabetes in Japanese.

It has been suggested that both genetic and environmental factors contribute to susceptibility of diabetes mellitus. Viral infection, for example, often precedes the onset of insulin-dependent diabetes mellitus (IDDM) and obesity is a predisposing factor for non-insulin-dependent diabetes mellitus (NIDDM). As a genetic factor, HLA has been found to be strongly associated with IDDM. Recently, the complete nucleotide sequence of the human insulin gene has been determined (Bell et al. 1980a,b; Ullrich et al. 1980) and a polymorphic locus linked to the human insulin gene has been found (Bell et al. 1981). Several researchers have detected variant DNA sequences adjacent to the insulin gene using restriction endonuclease analysis and have suggested an association with diabetes mellitus. Rotwein et al. (1981) and Owerbach et al. (1982a,b) reported a significant association between the insertion of large alleles and NIDDM, the former studied a racially mixed group and the latter a Danish Caucasian group. In addition, Bell et al. (1984) and Hitman et al. (1985) showed that a polymorphic locus was associated with IDDM. A more recent cooperative group study between two research centers (San Francisco and St. Louis) has shown a lack of association between the classes of alleles at the polymorphic locus and diabetes in American blacks (Elbein et al. 1985). In the present study, we have examined the polymorphic regions upstream of the insulin gene in normal subjects and patients with IDDM and NIDDM in a racially homogenous group of Japanese living in the Kinki district of central Japan.
Material and Methods

One hundred eighty-eight individuals were selected. 49 non-diabetic subjects, 71 patients with IDDM and 68 patients with NIDDM. DNA (100–300 μg) was prepared from nucleated white blood cells obtained from 10 ml of heparinized peripheral venous blood. Five to ten μg of DNA was digested overnight with an excessive dose of restriction endonuclease Sac I (Toyobo, Osaka, Japan) or Bgl I (PL-Pharmacia). The digested DNA samples were applied to 1% agarose gel electrophoresis for 10–12 h. Separated fragments were transferred to nitrocellulose filters (Schleicher and Schell) by the method of Southern (1975) and hybridized for one day with the probe phns 96, a kind gift from Dr G. I. Bell, which included a 1430 bp insulin structural gene and was labelled with 32P by nick translation (Rigby et al. 1977) to specific activities 1.5–2 × 10⁸ cpm/μg. Filters were exposed to X-ray films with an intensifying screen at −70°C for 1–5 days. The details of the method were as previously reported by Bell et al. (1981). We have adopted the classification of restriction fragments introduced by Bell et al. (1984), namely Class 1 alleles include Bgl I fragments of 2800 ± 300 bp and Sac I fragments of 6000 ± 400 bp and 7200 bp, respectively. All fragments larger than Class 1 and smaller than Class 3 are classified into Class 2.

Results

Using endonuclease Sac I, various sizes of alleles were detected (Fig. 1); they consisted mainly of one large group, varying in size from 5.4 to 6.4 kb, with a peak value of 5.8 kb. The other small

Fig. 1.
An autoradiograph of Sac I-digested DNA sequences of 9 unrelated subjects. λ-DNA double-digested with EcoRI and Hind III (Toyobo, Osaka, Japan) was used as molecular weight markers. The data of the panel showed various length heterogeneity. Almost all bands but one were approximately 5.8 kb, varying from 5.6 to 6.0 kb (Class 1) and homozygous. In one lane, two bands were detectable of approximately 6.0 kb (Class 1) and 7.4 kb (Class 3). This length heterogeneity indicated insertions of various sizes in both alleles.

Table 1.
Allelic frequency (%) of Class 1, Class 2 and Class 3 insulin gene polymorphism in non-diabetic subjects and patients with IDDM and NIDDM.

<table>
<thead>
<tr>
<th></th>
<th>Class 1</th>
<th>Class 2</th>
<th>Class 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic</td>
<td>97.0 (95)</td>
<td>1.0 (1)</td>
<td>2.0 (2)</td>
</tr>
<tr>
<td>IDDM</td>
<td>95.8 (136)</td>
<td>1.4 (2)</td>
<td>2.8 (4)</td>
</tr>
<tr>
<td>NIDDM</td>
<td>97.8 (133)</td>
<td>0</td>
<td>2.2 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>96.8 (364)</td>
<td>0.8 (3)</td>
<td>2.4 (9)</td>
</tr>
</tbody>
</table>

The percentage of each allelic frequency is indicated. The number within parenthesis is the sum of alleles. The χ²-test was used to compare frequencies found in non-diabetic, IDDM and NIDDM. There was no statistical difference among them.
group was made up of two sub-groups, one varying from 6.6 to 6.8 kb, the other from 7.2 to 7.6 kb. With another endonuclease, Bgl I, the same distribution of fragments was obtained. We classified these fragments into Class 1, 2, and 3, as designated by Bell et al. (1984). We determined allelic and genotypic frequencies based upon the classification of diabetes mellitus (Tables 1 and 2). More than 95% of alleles examined in the present study were assigned to Class 1. There was no statistically significant difference among normal subjects, patients with IDDM, or patients with NIDDM. Regarding genotypic frequencies, approximately 94% belong to Class 1/Class 1, and no statistically significant difference was found among the three groups of subjects. Only two individuals had Class 2 alleles (less than 1%); one was homozygote, the other heterozygote. Class 3 alleles were noted in only 4.8% of the subjects examined and none of these were homozygote. Genotypic frequencies containing Class 3 were observed in approximately 4% of the non-diabetic controls as well as in the diabetic patients.

Discussion

Bell et al. (1981, 1982) have found polymorphism due to the insertion of tandem repeats of 14–15 nucleotides in the potentially critical control region of the human insulin gene. It is important to clarify the possible association of these insertions and diabetes mellitus. Initially, researchers at three institutions studied this length polymorphism and reported an association with NIDDM or IDDM. Rotwein et al. (1981) reported the association of long length polymorphism (Class 3) with NIDDM in a racially mixed group. Owerbach et al. (1982a,b) also reported the same association in a Danish Caucasian group. Bell et al. (1984) compared the classes of insertions in several different racial groups and found an association of Class 1 homozygote with IDDM in Caucasians. A recent study by Hitman et al. (1985) confirmed this association in British Caucasians.

In the present study, we have analyzed restriction fragment length polymorphism in 188 unrelated Japanese individuals comprised of only one race and obtained results quite different from some previous reports. The combined data (Bell et al. 1984) of San Francisco, St. Louis, and Copenhagen showed that about 34% of the genotypes were heterozygote and about 23% of the alleles were Class 2 and 3 in Caucasians. The cooperative study (Elbein et al. 1985) of American blacks between San Francisco and St. Louis showed that 35% of the genotypes were Class 1 homozygote. In the Japanese of our study, Class 1 homozygote was of strikingly high frequency (94.2%), and both Class 2 and 3 alleles were very rare. Additionally, there was no significant difference in genotypic or allelic frequencies between non-diabetic subjects and diabetic patients in the Japanese of our study. Although we analyzed a relatively great number of IDDM patients and IDDM is much less common in Japan than in America or Europe, we found no statistical difference between non-diabetic subjects and IDDM patients. The recent study of American blacks (Elbein et al. 1985) also showed a lack of associa-

**Table 2.**

Genotypic frequency (%) of Class 1, Class 2 and Class 3 insulin gene polymorphism in non-diabetic subjects and patients with IDDM and NIDDM.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>1/1</th>
<th>1/2</th>
<th>1/3</th>
<th>2/2</th>
<th>2/3</th>
<th>3/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-diabetic</td>
<td>93.9 (95)</td>
<td>2.0 (1)</td>
<td>4.1 (2)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>IDDM</td>
<td>93.0 (66)</td>
<td>0</td>
<td>5.6 (4)</td>
<td>1.4 (1)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>NIDDM</td>
<td>95.6 (65)</td>
<td>0</td>
<td>4.4 (3)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Total | 94.2 (177) | 0.5 (1) | 4.8 (9) | 0.5 (1) | 0     |       |

The percentage of each genotypic frequency is indicated. The number within parenthesis is the sum of individuals. The χ²-test was used to compare frequencies found in non-diabetic, IDDM and NIDDM. There was no statistical difference among them.
tion of the polymorphic locus and diabetes. In conclusion, the 5'-length polymorphism linked to the human insulin gene can not presently be used as a marker of diabetes mellitus in persons of Japanese ancestry.

Acknowledgments

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References


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