Islet β-Cell Function and Polymorphism in the 5′-Flanking Region of the Human Insulin Gene

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SUMMARY

The present study investigates the possible relationship between human β-cell secretory capacity and polymorphism in the 5′-flanking region of the human insulin gene. The glucose potentiation slope was measured in normal and non-insulin-dependent diabetic subjects (NIDDM). This slope, as reported previously (Ward, W. K., et al., Am. J. Physiol. 1984; 246:E405–11), is an index of the ability of hyperglycemia to potentiate the insulin response to arginine and as such is a measure of β-cell responsiveness to glucose. Restriction enzyme analysis using a human insulin gene probe was performed on leukocyte DNA isolated from the same individuals. We conclude that a 1.6 kb polymorphism in the 5′-flanking region of the human insulin gene in both normal and NIDDM subjects has no association with insulin secretory responses as defined here by the glucose potentiation slope. DIABETES 1985; 34:311–14.

A polymorphic region exists near the human insulin gene about 500 base pairs (bp) upstream from the transcription initiation site.[1,2] The region is composed of a family of repeating oligonucleotides,[3,4] and length variation may result from unequal crossing over of misaligned oligonucleotide repeats. It has been hypothesized that polymorphic alleles may be transcribed less efficiently than normal alleles in pancreatic β-cells based on the observation in a previous study of a significant association between a 1.6 kb larger allele and non-insulin-dependent diabetes mellitus (NIDDM).[5] In the present study, we attempted to elucidate whether abnormal islet β-cell function, as determined by measurements of insulin secretory responses in NIDDM, is associated with length polymorphism in the 5′-flanking region of the human insulin gene. The ability of hyperglycemia to potentiate insulin responses to nonglucose stimuli was measured in all subjects to calculate the glucose potentiation slope, an index of β-cell responsiveness to glucose.[6] The glucose potentiation slope is thought to be a measure of the β-cell capacity to synthesize and secrete insulin.[7]

Although it is unlikely that the polymorphic insert on the 11th chromosome has regulatory function for insulin release in NIDDM, given the observation that it is common in nondiabetic and type I diabetic populations (see DISCUSSION) we felt it important to formally rule this out. The present study compares the potentiation for both normal and NIDDM subjects with the size of insulin gene alleles.

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MATERIALS AND METHODS

Study protocol (Seattle). Studies were performed in the Special Studies Unit of the Seattle Veteran's Administration Hospital after written, informed consent was obtained. At 0800 h, after a 12-h fast, a 2-in. intravenous (i.v.) Teflon catheter was inserted into a superficial hand vein for blood sampling. In addition, an 8-in., 18.5-gauge Teflon catheter was inserted into a contralateral antecubital vein for administration of glucose and arginine. Catheters were kept open with slow infusions of 0.9% normal saline. During blood sampling, the hand used for blood sampling was kept in a wooden box that was thermostatically controlled at 60°C to "arterialize" the venous blood.[8] Subjects remained recumbent for the studies.

Normal subjects. Two fasting blood samples (4 ml) were obtained 45 and 50 min after catheter insertion for measurement of plasma glucose and insulin. A maximally stimulating dose of 10% arginine (5 g) was then delivered via the antecubital catheter over a period of 30 s, the end of which was designated as time zero. Samples for measurement of insulin and glucose were obtained 2, 3, 4, and 5 min after this and all other arginine pulses. After completion of

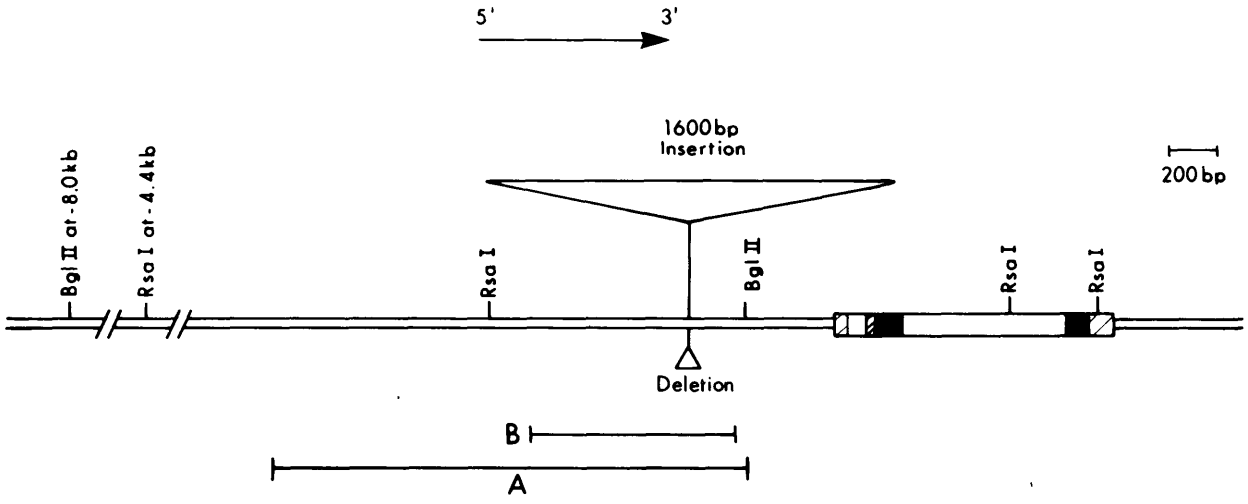

FIGURE 1. Restriction map of the insulin gene on chromosome 11. The diagram shows the location of DNA cleavage sites by restriction endonucleases Bgl II and Rsa I, and illustrates the predicted size of the insulin gene fragments detected when analyzed with ^{32}P-labeled probes A and B. The intron regions of the gene are represented by open boxes. The structural portion of the gene is represented by the solid boxes. Slashed boxes are transcribed but untranslated sections of the gene. Probes A and B are double-stranded, cloned human insulin gene probes and are described in the text.

the baseline arginine study, a variable rate infusion of 10% glucose in water was initiated through a peristaltic pump (Polystaltic, Haake-Buchler Instruments, Fort Lee, New Jersey) to raise and maintain the plasma glucose level at approximately 165 mg/dl. During the variable rate infusion, a blood sample was analyzed for glucose level every 7.5 min by use of a bedside glucose analyzer (Beckman Instruments, Palo Alto, California), and the infusion rate adjusted as previously described.[9] A repeat 5-g arginine pulse was given 45 min after beginning the glucose infusion and the appropriate samples for measurements of insulin and glucose were obtained. After completion of the second arginine study, the glucose infusion rate was raised and adjusted to achieve and maintain a glucose level of approximately 240 mg/dl. A third arginine pulse was administered 45 min after increasing the glucose infusion rate and samples for measurement of insulin and glucose were drawn as before.

NIDDM subjects. Before administration of arginine, diabetic patients underwent infusion of purified pork insulin (Actrapid, Novo Laboratories, 2.5 mU/kg/min) for 40–80 min to achieve a plasma glucose level of 90–130 mg/dl. A 60-min insulin washout period followed the insulin infusion. At this point, a protocol identical to that of normal subjects was followed. That is, acute insulin responses to arginine were measured at glucose levels of approximately 110, 165, and 240 mg/dl.

A 10-ml blood sample was drawn from each normal and diabetic subject into a heparin-containing tube. Leukocytes were isolated from this sample as previously described[5] and transported to St. Louis on dry ice for analysis of insulin gene polymorphism.

Calculations. Acute insulin responses to arginine are calculated as the mean of the 2-, 3-, 4-, and 5-min hormone levels minus the prestimulus level. As a measure of β-cell responsiveness to glucose, we calculated the slope of the least-squares regression line relating the acute insulin response to arginine to the prestimulus plasma glucose level. This measurement, termed the glucose potentiation slope, is an index of the ability of hyperglycemia to potentiate the insulin response to arginine. Since potentiation slopes are distributed in a log-normal fashion in normal subjects, we used log-transformed potentiation slopes for statistical comparisons. Body mass index (BMI) is defined as the weight (kg)/height (m^2).

Restriction endonuclease analysis (St. Louis). High-molecular-weight DNA was isolated from peripheral blood leukocytes, digested with restriction endonucleases, and subjected to DNA blot hybridization after electrophoresis.[2,5] This method involved lysis of cells from approximately 10 ml of peripheral blood, isolation of leukocyte nuclei by centrifugation, and DNA extraction with proteinase K and SDS. After ethanol precipitation and phenol extraction, 5 or 10 μg of DNA was digested with one of the following restriction endonucleases: Bgl II (1 U/μg DNA) or Rsa I (5 U/μg DNA). These enzymes cut the human insulin gene and surrounding area on chromosome 11 at the locations indicated schematically in Figure 1. Digested DNA was subjected to agarose gel electrophoresis on a 1–1.2% agarose gel and blotted onto nitrocellulose filters.[10] The filters were then hybridized with 2–10 × 10^6 cpm of ^{32}P-labeled[11] cloned human insulin gene fragments (probe A or B, as illustrated in Figure 1) for 24 h. Finally, the filters were exposed to x-ray film for 7 days and autoradiographs obtained.

Probe A (pgHI 5′) is a double-stranded portion of the gene isolated from the recombinant plasmid pgHI 12.5, inserted into plasmid pBR327, and grown in *Escherichia coli* HB101.[5,12] It contains the 5′-flanking sequence from −168 to −2400 bp (base pairs). Probe B (double-stranded) was isolated from pgHI 5′ by Hinf I digestion of pgHI 5′, insertion into pBR327, and growth in *Escherichia coli* HB101. With radioactive probe A, the common-size insulin allele shows bands approximately 7.8 kb with Bgl II and approximately 3.2 kb and 2.35 kb with Rsa I. With radioactive probe B, the common-size allele shows similar patterns except that the 3.20 kb band from Rsa I digestion does not hybridize with probe B (see Figure 1).

TABLE 1
Polymorphism in the 5′-flanking region of the insulin gene

	Genotype (frequency)*			Allelic frequency	
	+/+	+/1.6	1.6/1.6	+	1.6
Nondiabetic	12 (0.46)	11 (0.42)	3 (0.12)	0.67	0.33
NIDDM	20 (0.61)	11 (0.33)	2 (0.06)	0.77	0.23

DNA was analyzed by Southern blot hybridization with ^{32}P-labeled insulin gene fragments as described in the text. + = Common-size allele, 1.6 = 1.6 kilobase pairs larger than the common-size allele. *3 × 2 Analysis, X^2 = 1.63 (uncorrected), df = 2, NS.

RESULTS

Insulin gene polymorphism. Peripheral leukocyte DNA from 26 nondiabetic and 33 non-insulin-dependent diabetic subjects was analyzed for insulin gene polymorphism in the 5′-flanking region. Three different size classes of alleles were identified, including the common-size allele (+ in Table 1), an allele that was 1600 bp larger than the common-size allele (1.6 in Table 1) and an allele 100–150 bp smaller than the common-size allele in three individuals (not shown). The genotypes observed and the genotypic frequencies in the nondiabetic and NIDDM subjects are shown in Table 1. There was no significant difference in the genotypic frequency or the allelic frequency of the 1.6 kb allele between the two groups.

Clinical characteristics and insulin secretory responses. The insulin secretory responses and some clinical characteristics of 26 nondiabetic subjects classified according to insulin gene polymorphic status are listed in Table 2. Three of the subjects (12%) were homozygous for the 1.6 kb allele. There was no significant difference in the age, body mass index, fasting plasma glucose or insulin, acute insulin response to arginine, or the slope of glucose potentiation among the three groups. Body mass index appeared to be greater in the +/1.6 and 1.6/1.6 subjects, but these differences were not significant.

The NIDDM subjects were older and more obese than the nondiabetic subjects. When classified according to insulin gene polymorphism, there was no significant difference in age, age at diagnosis, body mass index, fasting plasma glucose or insulin, acute insulin response to arginine, or slope of glucose potentiation among the 33 NIDDM.

DISCUSSION

In this study, the frequency of the insulin gene polymorphism did not differ between nondiabetic and NIDDM individuals. Fifty-six of the 59 individuals in the present study were Caucasian. In a larger series of Caucasians, the frequency of the 1.6 kb insert allele was 23% and not significantly different in nondiabetic and NIDDM subjects.[20] Similarly, in a group of 115 Pima Indians, the frequency of the 1.6 kb allele was 23% and not different in diabetic subjects.[21] Only 4 Pima individuals were homozygous for the 1.6 kb allele, and all 4 were diabetic. The 1.6 kb allele, especially when homozygous, may influence the severity of the disease in Pimas as indicated by the need for drug treatment. The relationship between NIDDM and insulin gene polymorphism has been investigated by others.[13–15] Owerbach and associates[14] found a positive association of the 1.6 kb allele with NIDDM in Danish Caucasians, while Bell et al.[13,15] found no association with either type of diabetes in Asians or American Blacks or Caucasians. In view of our more recent findings in Pimas and Caucasians, it appears that, with the possible exception of black subjects,[5] the 1.6 kb allele does not directly predispose to NIDDM.

In the present study, no differences in fasting insulin or glucose concentration, acute insulin response to arginine, or glucose potentiation of the insulin response to arginine could be detected according to insulin gene polymorphic status in nondiabetic and diabetic subjects. Among nondiabetic individuals, there was a trend toward larger body mass index in the polymorphic individuals. These differences were not significant. The number examined in the current study was small. No correlation between body mass index and insulin gene polymorphism was found in the larger population of Pima Indians. However, both nondiabetic and diabetic Pimas, regardless of polymorphic status, were obese. Whether there is a significant association between insulin gene polymorphism and obesity in other racial groups is unknown. Two other studies have suggested that insulin gene polymorphism may be more closely associated with disorders other than diabetes. Mandrup-Poulsen et al.[16] reported that the 1.6 kb allele was 2.5 times more common in

TABLE 2
Clinical characteristics of nondiabetic and NIDDM subjects by insulin gene polymorphic status

	Nondiabetic			NIDDM		
	+/+ (N = 12)	+/1.6 (N = 11)	1.6/1.6 (N = 3)	+/+ (N = 20)	+/1.6 (N = 11)	1.6/1.6 (N = 2)
Age (yr)	39.0 ± 5.0	40.4 ± 4.6	47.7 ± 2.7	59.5 ± 2.1	54.7 ± 2.7	60 ± 3.0
Age at diagnosis (yr)	—	—	—	50.7 ± 3.7	49.0 ± 2.7	54.5 ± 5.5
Slope	2.35 ± 0.62	2.46 ± 0.37	2.23 ± 0.65	0.268 ± 0.060	0.205 ± 0.079	0.20 ± 0.19
Fasting plasma glucose (mg/dl)	96.8 ± 2.2	94.7 ± 1.9	100.7 ± 5.9	209.4 ± 15.9	240.4 ± 19.7	299 ± 42
Fasting plasma insulin (μU/ml)	10.1 ± 1.3	12.4 ± 1.3	12.7 ± 2.3	17.5 ± 2.3	20.1 ± 4.7	15 ± 3
AIR arg (μU/ml)	61.8 ± 10.7	101.0 ± 29.8	37.7 ± 5.0	46.4 ± 7.5	44.9 ± 10.8	39 ± 10
BMI (kg/m^2)	24.56 ± 1.04	26.28 ± 1.34	27.3 ± 1.6	29.8 ± 1.5	31.9 ± 1.6	29.7 ± 0.8

As described in the text, + = common size allele, 1.6 = 1.6 kb larger allele, AIR arg = acute insulin response to arginine, BMI = body mass index. Where N ≥ 3, x = mean ± SEM. Where N = 2, x = mean ± range.

patients with atherosclerosis compared with controls, independent of diabetes. Jowett et al.[17] showed an increased number of 1.6 kb polymorphic alleles in subjects with hypertriglyceridemia and NIDDM compared with those with NIDDM alone. These associations should be interpreted with caution until the results can be verified by larger sample sizes.

In conclusion, these studies show no association between any particular 5′-flanking insulin gene polymorphic class and insulin secretory responses. This observation is important because it suggests that the presence of a 1.6 kb larger allele does not result in detectable differences in insulin secretion. It does not rule out the possibility, however, that certain polymorphic classes of insulin alleles are linked to specific defects leading to impaired secretion. Kazazian and associates[18,19] have shown that polymorphic restriction sites around the human β-globin gene on chromosome 11 occur in patterns. A particular pattern of restriction sites within the DNA region studied is defined as a haplotype, so that a single base mutation at a restriction enzyme cutting site will alter the haplotype. Furthermore, certain haplotypes defined by restriction sites around the β-globin gene appear to be associated with β-thalessemia. Specific haplotypes for the human β-globin gene occur with equal frequency in normal and thalessemic subjects, so population studies are not informative. Linkage analysis in families, however, has been very valuable in prenatal diagnosis of β-thalessemia. We have identified restriction site polymorphisms that occur in the 5′-flanking region of the human insulin gene both in the presence and absence of a length polymorphism. It remains to be determined through family studies whether any of these polymorphic haplotypes are associated with specific defects leading to insulin deficiency and diabetes.

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