PRELIMINARY REPORT

A Study of Human Growth Hormone and Insulin Gene Regions in Relation to Metabolic Control of Non–Insulin-Dependent Diabetes Mellitus

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The possible association of human growth hormone (hGH) and insulin (INS) gene regions with metabolic control in diabetes was investigated in 98 subjects with non–insulin-dependent diabetes mellitus (NIDDM); 54 control subjects from the same population were also studied. Two polymorphic restriction sites in the region of the hGH cluster (BGLIIA and BGLIIB) show significant association with both glycemic and hemoglobin A_{1c} (HbA_{1c}) levels. Mean values for plasma glucose and HbA_{1c} show a maximum in the BGLIIA *1/*1 genotype and a minimum in the BGLIIA *2/*2 genotype. Mean values for plasma glucose and HbA_{1c} show a maximum in the BGLIIB *1/*2 genotype. The BGLIIA*2/BGLIIB*1 haplotype shows a negative correlation with plasma glucose and HbA_{1c} levels. Since the two markers are located in the area surrounding the hGH-V locus, the expression of this gene in NIDDM warrants further investigation.

GENETIC VARIABILITY within the gene regions controlling glucose metabolism may have an important role in the clinical variability of diabetes. In diabetic pregnancy[1] and non–insulin-dependent diabetes mellitus (NIDDM),[2] we have recently shown an association between the plasma glucose level and the genotype of cytosolic low–molecular weight phosphotyrosin-phosphatase (cLMW PTPase-ACP1, chromosome 2), an enzyme involved in the signal transduction of the insulin pathway.[3] In consideration of its diabetogenic action, we have now analyzed in NIDDM subjects a set of polymorphic markers in the gene regions of the human growth hormone ([hGH] chromosome 17q22-q24) locus in relation to plasma glucose and glycosylated hemoglobin (HbA_{1c}) levels. The gene region for insulin ([INS] chromosome 11p15.5) was also studied.

SUBJECTS AND METHODS

Subjects

Ninety-eight NIDDM subjects from Penne, a small rural town in southeast Italy, were studied. The series includes males and females aged 34 to 89 years. No pregnant women are included in the present study. All subjects were previously diagnosed with NIDDM based on current standard criteria[4] and provided verbal consent for the use of their blood samples for the present study.

Plasma glucose and HbA_{1c} are the fasting values for determinations (in most cases, the mean value of 2 determinations) within the trimester preceding the collection of blood samples.

Fifty-four healthy newborn infants were studied in the same population. Analyses were performed on umbilical cord blood.

DNA Analysis

Genomic DNA extracted from peripheral blood samples was processed by conventional Southern blot analysis.[5] From each total genomic DNA, 8 μg was digested overnight according to the conditions specified by the supplier (Promega, Madison, WI). Following electrophoresis in 0.7% to 1.2% (wt/vol) agarose gel in Tris-acetate/EDTA buffer, DNA was blotted overnight onto Hybond-N nylon membrane (Amersham, Italy) and fixed by baking at 90°C. The filters were prehybridized and hybridized in a rotating oven (Techne [Cambridge, England] hybridizer HB-ID) at 65°C for 18 hours in 6× SSC 0.5% in sodium dodecyl sulfate (SDS) and 0.5% Denhardt solution plus 3×10^7 cpm of probe that was radiolabeled to a specific radioactivity greater than 10^{10} cpm/μg DNA by a nick-translation system (Promega) using [α^{32}P]dATP (Amersham). All filters were washed twice for 30 minutes at 65°C in 2× SSC plus 0.1% SDS and once for 30 minutes at 65°C in 0.1× SSC plus 0.1% SDS. The filters were exposed to x-ray films using an intensifying screen at −70°C.

Probes and Restriction Endonucleases

hGH gene region. The 4 polymorphisms examined using the probe C-H800[6] are detailed in Table 1, and the relative positions of the 4 polymorphic sites with respect to the genes in the hGH cluster are shown in Fig 1a.

INS gene region. In this region, we examined 1 variable number of tandem repeats polymorphism (VNTR)[7] and 3 restriction fragment length polymorphisms (RFLPs)[7-9] using 3 different probes (Table 1). Figure 1b shows the position of the 4 sites with respect to INS and insulin-like growth factor 2 (IGF2) genes.

Statistical Analysis

Statistical analysis was performed using SPSS programs.[10] Three-way contingency table analyses were performed according to the method of Sokal and Rohlf.[11] Haplotype frequencies are maximum-likelihood estimates (program MENDEI, Department of Biostatistics, University of Michigan, Ann Harbor, MI).

RESULTS

Among the 8 loci tested, BGLIIA and BGLIIB show an association with plasma glucose and HbA_{1c} levels.

Table 2 shows the mean values for plasma glucose and HbA_{1c} in relation to BGLIIA and BGLIIB genotypes. For BGLIIA, there is a strong allelic effect, with maximum plasma glucose and HbA_{1c} levels in subjects with the *1/*1 genotype and minimum levels in subjects with the *2/*2 genotype. The association is significant after Bonferroni correction for multiple comparisons. For BGLIIB, the maximum plasma glucose and HbA_{1c} is observed in *1/*2 heterozygous subjects and the

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Table 1. Polymorphic Sites Studied in the hGH and INS Gene Regions: Probes, Restriction Enzymes, and Restriction Fragment Sizes

Gene Region	VNTR	RFLP	Probe	Restriction Enzyme	Size (kb)
hGH (17q22-q24)		MSPIA	C-H800	MSPI	4.6/3.6
		MSPIB	C-H800	MSPI	3.9/3.3
		BGLIIA	C-H800	BGLII	13.0/10.5
		BGLIIB	C-H800	BGLII	8.1/3.0
INS (11p15.5)					
INS	PVUII		pHINS310	PVUII	0.6/1.5/2.4
IGF2		BAMHIA	phins311	BAMHI	2.2/1.2
		BAMHIB	phins311	BAMHI	18.0/17.0*
		APAI	phigf2-11	APAI	3.6/2.3-1.3

*Personal communication from Graeme I. Bell, January 1991.

associations are much weaker compared with those observed with BGLIIA.

Sex, age, age at onset, duration of disease, body mass index, and treatment with insulin have shown no significant association with BGLIIA. However, the pattern of the relationship between plasma glucose and BGLIIA is significantly influenced by the age at onset ($P < .005$) and by treatment with insulin ($P < .025$). In fact, the relationship between plasma glucose and BGLIIA is much more evident in subjects with an early onset of disease and in those treated with insulin, suggesting that variability within the BGLIIA area may have a relatively stronger effect on clinical metabolic control in the more severe forms of NIDDM.

Table 3 shows the distribution of BGLIIA/BGLIIB haplotypes in NIDDM according to plasma glucose and HbA_{1c} levels. The distribution of haplotypes in normal controls is also reported. Haplotype distribution in NIDDM is associated with both plasma glucose and HbA_{1c} levels. The frequency of the BGLIIA*2/BGLIIB*1 haplotype in subjects with poor diabetic control is less than half of that observed in subjects with satisfactory diabetic control and in normal control subjects.

DISCUSSION

The present study suggests that the structural organization of the hGH genomic area may have an important role in plasma glucose control in NIDDM subjects. Since the area between BGLIIA and BGLIIB includes the hGH-V locus, the hypothesis that the BGLIIA/BGLIIB haplotype is a marker of the regulatory sequences of this locus appears suggestive.

Table 2. Glycemic and HbA_{1c} Levels in Relation to RFLPs BGLIIA and BGLIIB

RFLP	Glycemia (mg/dL) Mean ± SE	No.	HbA_{1c} (%) Mean ± SE	No.
BGLIIA				
*1/*1	151.6 ± 7.9	40	8.13 ± 0.27	40
*1/*2	129.1 ± 4.4	52	7.28 ± 0.17	49
*2/*2	101.2 ± 4.5	6	6.44 ± 0.39	5
Variance analysis	$P = .0033$ ($P^* = .026$)		$P = .0057$ ($P^* = .046$)	
Linearity	$P = .0008$ ($P^* = .0064$)		$P = .0013$ ($P^* = .01$)	
Deviation from linearity	NS		NS	
BGLIIB				
*1/*1	124.8 ± 5.7	34	7.11 ± 0.24	31
*1/*2	146.5 ± 7.3	47	7.93 ± 0.24	46
*2/*2	126.9 ± 9.6	11	7.19 ± 0.32	11
Variance analysis	$P = .0603$ (P^* = NS)		$P = .043$ (P^* = NS)	
Linearity	NS		NS	
Deviation from linearity	$P = .0339$ (P^* = NS)		$P = .0227$ (P^* = NS)	

*Significance after Bonferroni correction for multiple comparisons (8 comparisons).

Although it has been shown that placentally expressed hGH-V has a spectrum of metabolic activity comparable to pituitary hGH-N,[12] hGH-V has always been considered as a gene expressed exclusively in placental tissues. Only recently has it been shown that both pituitary and placental hGH transcripts are expressed in human peripheral blood mononuclear cells.[13] In light of these new observations, the suggestion of a possible involvement of this gene in the clinical manifestations of NIDDM seems worthy of further investigation. The study of hGH-V expression in the tissues of NIDDM subjects may be valuable.

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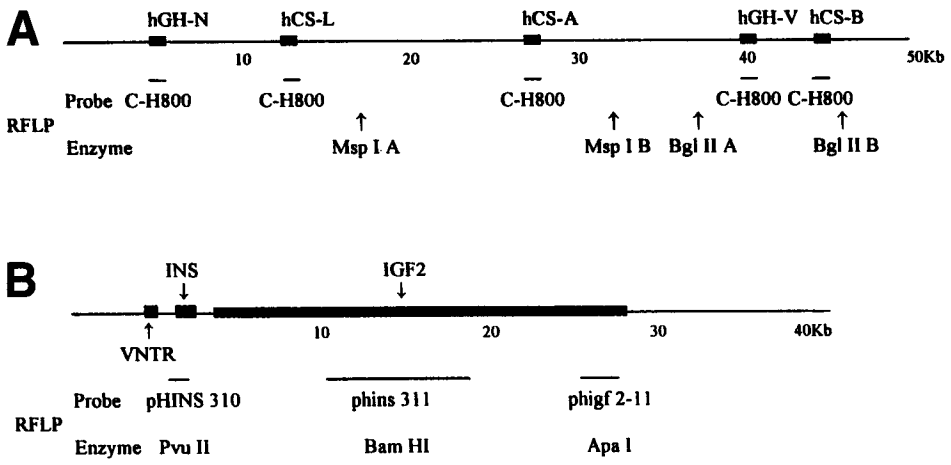

Fig 1. Map of the gene regions examined. (A) hGH gene cluster showing the locations of the 4 restriction sites. (B) Human INS/IGF2 gene region showing the position of the VNTR and the approximate locations of the 3 RFLPs.

Table 3. BGLIIA/BGLIIB Haplotype Distribution in Relation to Glycemic and HbA_{1c} Levels

Group	BGLIIA/BGLIIB Haplotype *1/*1	*1/*2	*2/*1	*2/*2	No. of Haplotypes	Comparison for Medium-Low *v* High
NIDDM						
Plasma glucose						
Medium-low (<160)						
Frequency	0.271	0.350	0.379	0.01	140	
SE	0.038	0.040	0.041	0.00		
High (>160)						
Frequency	0.357	0.476	0.167	0.01	42	$P = .037$ (*2/*1 *v* others, $P = .018$)
SE	0.074	0.077	0.058	0.00		
HbA_{1c}						
Medium-low (<8.8%)						
Frequency	0.269	0.373	0.358	0.01	134	
SE	0.038	0.042	0.041	0.00		
High (>8.8%)						
Frequency	0.375	0.450	0.175	0.01	40	$P = .085$ (*2/*1 *v* others, $P = .046$)
SE	0.077	0.078	0.060	0.00		
Controls						
Frequency	0.217	0.357	0.403	0.023	108	
SE	0.040	0.047	0.048	0.016		
Comparison between controls and NIDDM					All haplotypes	*2/*1 *v* others
Controls *v* NIDDM with medium-low plasma glucose					NS	NS
Controls *v* NIDDM with high plasma glucose					$P = .037$	$P = .009$

NOTE. Cutoff point for classification as medium-low and high plasma glucose and HbA_{1c} is, respectively, 160 mg/dL and 8.8% of total Hb.

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