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PRELIMINARY REPORT

BGLIIA-BGLIIB Haplotype of Growth Hormone Cluster Is Associated With Glucose Intolerance in Non–Insulin-Dependent Diabetes Mellitus and With Growth Hormone Deficit in Growth Retardation

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We studied 101 growth-retarded children from the population of Ancona (Italy). Plasma growth hormone (GH) levels at the end of insulin and clonidine tests were considered for classification of children into 3 categories according to severity of GH deficit: total deficit of GH (TD), partial deficit (PD, and familiar short stature (FSS; no deficit of GH). The BGLIIA*2/BGLIIB*1 haplotype of GH cluster that was previously found to be negatively associated with severe glucose intolerance in non–insulin-dependent diabetes mellitus (NIDDM) is negatively associated with GH deficit in growth-retarded children. The hypothesis that intrauterine growth retardation and glucose intolerance in adult life could be phenotypes of the same underlying genotype has been recently put forward. The present observation suggests that genes influencing both growth and glucose tolerance are encoded in the GH cluster.

IT HAS BEEN SUGGESTED that intrauterine growth retardation and glucose intolerance in adult life could be phenotypes of the same underlying genotype.[1] We have recently reported a negative association between the BGLIIA*2/BGLIIB*1 haplotype of the growth hormone (GH) genomic area and severe glucose intolerance in non–insulin-dependent diabetes mellitus (NIDDM).[2] Here, we show that BGLIIA*2/BGLIIB*1 haplotype is negatively associated with GH deficit in growth-retarded children, suggesting that the area including BGLIIA and BGLIIB encodes genes that influence both growth and glucose tolerance.

SUBJECTS AND METHODS

Subjects

A total of 101 growth-retarded children in the population of Ancona, Ialy, have been studied, along with their parents. The GH value at the end of insulin and clonidine tests were considered for classification of children into 3 categories: total deficit (TD), partial deficit (PD), and familiar short stature (FSS). For insulin testing the cut-off points were 4 and 8 ng/L, and for clonidine testing 6 and 10 ng/L, respectively. Both test results had to be below the cut-off point in order to be included in the lower class. Based on the results of GH stimulation tests with insulin and clonidine, 23 children were classified as FSS, 54 as PD, and 24 as TD.

The mean age (and standard deviation) at diagnosis was 11.3 years (2.6) for FSS, 10.3 years (3.3) for PD, and 9.9 years (4.7) for TD. The mean deviation from the mean stature was −2.11 (0.73) for FSS, −2.03 (0.96) for PD, and −2.00 (0.86) for TD. The male/female sex ratio was 2.0 for FSS, 2.41 for PD, and 1.44 for TD. The overall sex ratio was 2.05 ($P < .01$). The mean maternal statures were 152.9 (4.4), 154.3 (5.9), and 154.5 (7.1) for FSS, PD, and TD, respectively. Paternal statures for the 3 categories were 164.3 (4.7), 168.6 (6.4), and 168.5 (6.9), respectively.

With the exception of 2 subjects (1 TD and 1 PD) who showed an associated deficit of thyrotropin (TSH), all cases had isolated GH deficiency. No other evident cause of growth retardation such as malabsorption was present. No sign of sellar area lesion was detectable.

Herein, we report new statistical analyses on NIDDM data.[2] No case of growth retardation was recorded among these subjects. Data on normal newborns from an Italian population living in the same geographic region[2] are also provided; however, these data were not used

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

	RFLP	Probe	Restriction	Size (kb)
hGH gene cluster	BGLIIA	C-H800	BGLII	13.0/10.5
(17q22-q24)	BGLIIB	C-H800	BGLII	8.1/3.0

Abbreviation: RFLP, restriction fragment length polymorphism.

for statistical analyses and are reported in order to depict a qualitative pattern of relationships.

DNA Analysis

Genomic DNA extracted from peripheral blood samples was processed by conventional Southern blot analysis. 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) 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 6x SSC (NaCl and Na citrate 6 mol/L) 0.5% in sodium dodecyl sulfate (SDS) and 0.5% Denhardt solution plus 3x 107 cpm of probe that was radiolabeled to a specific radioactivity greater than 1010 cpm/μg DNA by a nick-translation system (Promega) using [^{32}P]deoxy adenosine phosphate (dATP) (Amersham). All filters were washed twice for 30 minutes at 65°C in 2x SSC plus 0.1% SDS and once for 30 minutes at 65°C in 0.1x SSC plus 0.1% SDS. The filters were exposed to x-ray films using an intensifying screen at −70°C.

Probes and Restriction Endonucleases

The 2 polymorphisms examined using the probe C-H8006 are detailed in Table 1, and the relative positions of the 2 polymorphic sites with respect to the genes in the hGH cluster are shown in Fig 1.

Statistical Analyses

Analyses were performed using SPSS programs.[3] Haplotype frequencies are maximum-likelihood estimates (program Mendel, Department of Biostatistics, University of Michigan, Ann Harbor, MI).

RESULTS

Table 2 shows the BGLIIA*2/BGLIIB*1 haplotype distribution in the 3 classes of growth retardation. Data on normal newborns from an Italian popolation[2] living in the same geographic area are also shown. The BGLIIA*2/BGLIIB*1 haplotype is negatively associated with GH deficiency in both offspring and their mothers. There is a negative correlation between frequency of *2/*1 haplotype and severity of GH

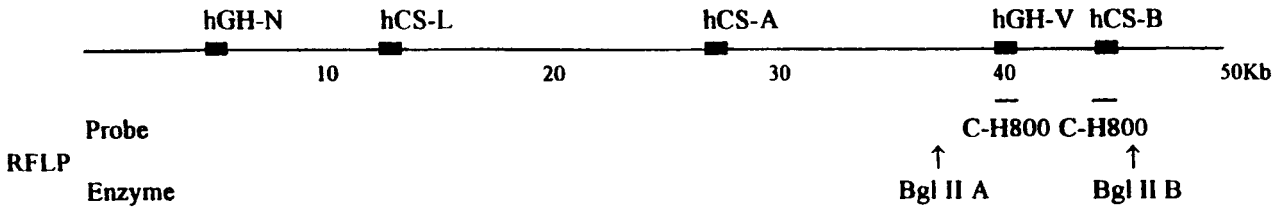

Fig 1. Map of the hGH gene cluster showing the locations of the restriction sites examined. RFLP, restriction fragment length polymorphism.

Table 2. BGLIIA*2/BGLIIB*1 Percent Haplotype Distribution in Children With Growth Retardation

	Growth Retardation								
	Offspring			Mothers			Fathers		
	%	SE	No.	%	SE	No.	%	SE	No.
FSS	43.4	7.1	48	36.1	6.7	52	34.6	6.6	52
PD	22.2	4.2	102	28.3	4.7	94	21.3	4.5	86
TD	17.1	6.0	44	10.1	4.7	46	34.8	7.0	46
Significance of difference (*P*)									
FSS *v* PD *v* TD		<.025			<.02			NS	
FSS *v* (PD + TD)		<.005							
	NIDDM								
	%	SE	No.						
Severe glucose intolerance	16.7	5.8	42						
Mild glucose intolerance	37.9	4.1	140						
Significance of difference (*P*)									
Severe *v* mild		≈.01							
	Control								
	%	SE	No.						
	40.3	4.8	108						

NOTE. Data on NIDDM and controls[2] are also reported for comparison.
Abbreviation: NS, not significant.

Table 3. Correlation Between Glycemic Level and BGLIIA*2/BGLIIA*1 Haplotype in NIDDM

Plasma Glucose Level (mg/dL)	Haplotype Frequency	SE	No.
≤105	0.4048	0.0757	21
>105 ≤114	0.3948	0.0793	19
>114 ≤136	0.3437	0.0839	16
>136 ≤168	0.3437	0.0839	16
>168	0.1389	0.0576	19
	$Rs = 0.95$		
	$P < .02$		

deficiency. As shown in Table 3, a correlation analysis between glycemic level and frequency of the BGLIIA*2/BGLIIB*1 haplotype performed on our NIDDM data[2] revealed a negative correlation between the 2 variables.

The possible association of the joint BGLIIA-BGLIIB haplotype with glucose intolerance in NIDDM and with GH deficit in growth retardation is shown in Table 4. The proportion of genotypes carrying both BGLIIA*2 and BGLIIB*1 is very low both in NIDDM subjects with severe glucose intolerance and in growth-retarded children with severe deficit of GH.

Table 5 shows the result of GH stimulation tests by insulin and clonidine in relation to the 2 classes of BGLIIA/BGLIIB genotype. Genotypes carrying both BGLIIA*2 and BGLIIB*1 alleles perform better than other genotypes when stimulated by clonidine. No differences are produced by stimulation by insulin. Children with TD have been excluded from this analysis since the increase of GH during the test was minimal.

Table 6 shows the distribution of the 2 classes of joint BGLIIA/BGLIIB genotypes in relation to severity of disease separately in males and females. In growth-retarded children the association between GH deficit and BGLIIA/BGLIIB genotype depends on sex and it is statistically signifiant in females only. In NIDDM also the association between high glycemic level and BGCIIA/BGCIIB genotype depends on sex and it is statistically significant in females only.

Table 4. Severe Glucose Intolerance in NIDDM and GH Deficit in Growth Retardation in Relation to Joint BGLIIA/BGLIIB Genotype

		% of Genotypes Carrying Both BGLIIA*2 and BGLIIB*1 Alleles	Total No.
NIDDM			
Blood glucose ≤ 168 mg/dL		66.6%	72
Blood glucose > 168 mg/dL		31.6%	19
OR	4.33		
χ^2 test of independence		$P < .01$	
Growth retardation			
FSS		66.6%	24
DP		39.2%	51
DT		31.8%	22
OR (DP *v* FSS)	3.10		
OR (DT *v* FSS)	4.28		
χ^2 test of independence			
FSS *v* (DP + DT)		$P \sim .01$	

Abbreviation: OR, odds ratio.

Table 5. Increase of Plasma GH After Stimulation With Insulin (difference between 0′ and 60′) and After Stimulation With Cloridine (difference between 0′ and 120′)

	Genotypes Carrying BGLIIA*2 and BGLB*2	Other Genotypes	Significance of Difference (*P*)
Clonidine	Increase of GH (μg/L) after 120′ from stimulation		
FSS			
Mean	8.75	1.20	
SE	1.67	2.67	.023
n	16	7	
PD			
Mean	5.41	3.47	
SE	1.13	0.71	.140
n	20	27	
		Cumulative probability	<.025
Insulin	Increase of GH (μg/L) after 60′ from stimulation		
FSS			
Mean	8.14	7.24	
SE	1.25	3.39	NS
n	15	5	
PD			
Mean	2.77	2.71	
SE	0.45	0.53	NS
n	19	30	
		Cumulative probability	NS

DISCUSSION

Only a relatively low proportion of subjects with deficit of GH show GH gene alteration,[4,5] suggesting that the majority of GH deficiency (>80% in Caucasians) are of multifactorial origin. It is likely that the categorical classification in FSS, PD, and TD, although currently used in clinical practice, does not correctly reflect such origins. This may in part account for the poor reproducibility of clinical categorical definitions.[6,7] However, we believe that the 3 categories reflect an order that corresponds to GH deficit. Although based on semiquantitative valuation of GH response, such ordering seems adequate to the purpose of the present study.

Among the genetic factors contributing to the aetiology of growth retardation, control regions in the GH cluster are some of the most important candidates.[8] Polymorphic sites in the GH area have been mainly studied in relation to the evolutionary problems of the GH cluster rather than in relation to their possible importance as markers of functional variability of transcribed genes and therefore to their possible clinical relevance. It is possible that the BGLIIA*2/BGLIIB*1 haplotype is a marker of an extended haplotype influencing transcription and/or splicing of hGH-N (human growth hormone gene), which may be found to be important for growth during extrauterine life. The area between BGLIIA and BGLIIB includes the hGH-V (placenta human growth hormone) locus, which is

Table 6. Effect of Sex on the Association Between Clinical Severity and BGLIIA/BGLIIB Joint Genotype

	Females		Males	
	Genotypes Carrying BGLIIA*2 and BGLIIB*1	Other Genotypes	Genotypes Carrying BGLIIA*2 and BGLIIB*1	Other Genotypes
NIDDM				
Proportion of patients with high glycemic values	15%	50%	7%	17%
Total no.	26	18	28	18
χ^2 test of independence	$P \sim .01$		$P = NS$	
Growth retardation				
Proportion of children with GH deficit	47%	100%	73%	79%
Total no.	17	16	26	38
χ^2 test of independence	$P < .001$		$P = NS$	

active during intrauterine life. However, placentally expressed hGH-V has a spectrum of metabolic activity comparable to pituitary hGH-N,[9] and recently it has been shown that both pituitary and placental hGH transcripts are expressed in human peripheral blood mononuclear cells.[10] The parallel analysis of 2 independent samples suggests that the GH genomic area encodes a haplotype that protects from severe glucose intolerance and growth retardation. Clearly, the structure of the GH cluster deserves a more detailed analysis to elucidate its possible role in glucose intolerance, intrauterine growth, and growth retardation during extrauterine life.

The differences between males and females point to probable hormonal influences on the expression of BGLIIA*2/BGLIIB*1 haplotype and its subsequent effects on GH deficiency in growth-retarded children and on glucose tolerance in NIDDM. It is also important to note the strong association of the maternal genotype with GH deficiency in offspring, especially with the most severe form of GH deficiency, while such association is lacking with the paternal genotype. At present it cannot be excluded that the observed effects on GH deficiency are primarily due to maternal genotype.

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