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The correlation of leptin/leptin receptor gene polymorphism and insulin-like growth factor-1 and their impact on childhood growth hormone deficiency

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Abstract. – OBJECTIVE: Growth hormone deficiency (GHD) is the most common cause for childhood dwarfism. Currently, the significance of insulin-like growth factor-1 (IGF-1) in diagnosis of GHD is still debatable. Due to the possible correlation between leptin (LEP) and GHD pathogenesis, this study investigated the gene polymorphism of LEP and its receptor (LEPR) genes, along with serum IGF-1 and LEP levels in GHD patients. This study attempted to illustrate the correlation between gene polymorphism and GHD pathogenesis.

PATIENTS AND METHODS: A case-control study was performed using 180 GHD children in addition to 160 healthy controls. PCR-DNA sequencing method was employed for genotyping various polymorphism loci of LEP and LEPR genes in both GHD and healthy individuals. Serum IGF-1 and LEP levels were also determined.

RESULTS: Results revealed a statistically significant difference between the levels of IGF-1 and LEP in the serum samples collected from patients in the GHD and the control groups. Both IGF-1 and LEP levels were found to be correlated with polymorphism at rs7799039 loci of LEP gene, in which GG and GA genotypes carriers had higher serum IGF-1 levels when compared to AA genotype carriers.

CONCLUSIONS: GHD pathogenesis is well correlated with the LEP and IGF-1 levels in the both of which were mediated by the gene polymorphism at rs7799039 loci of LEP gene.

Key Words:
Growth hormone deficiency, Leptin receptor gene, Insulin-like growth factor-1, Gene polymorphism.

Introduction

Growth hormone deficiency (GHD) is mainly caused by the insufficient secretion of growth hormone (GH), thus leading to growth retard and childhood dwarfism[1]. Classical diagnosis is based on the GH stimulation test[2,3], which is an invasive and expensive assay and may have certain risks. Therefore, it is very important to find new biomarkers for the early diagnosis of GHD. Insulin-like growth factor-1 (IGF-1) is one of the most significant stimulating factors that is synthesized and secreted by hepatocytes[4] and plays an important role in mediating cell proliferation, mitosis and apoptosis. Previous studies revealed that lower level of IGF-1 could cause GH insensitivity and the feedback regulation of GH secretion by IGF-1and proved IGF-1 to be an important GH effector[5,6]. In GHD patients with insufficient IGF-1, the application of IGF-1 had satisfactory treatment effects[7]. Other studies suggested that the level of serum IGF-1 can be used as a clinical index for GHD occurrence[8,9], but there are scholars[10,11] who are not convinced about the reliability of this index. Consequently, the clinical significance of serum IGF-1 as an index in diagnosis of GHD still needs further investigation. In an attempt to provide further evidence to support the significance of IGF-1 in the diagnosis of GHD, we developed a case-control study in which the level of IGF-1 in serum was quantified to elucidate its relationship with GHD pathogenesis. We know Leptin (LEP) plays an important mediating role in body metabolism and growth via inhibiting appetite and de-

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creasing energy intake[12]. Certain metabolic disorders such as obesity were showed to be closely related to LEP level[13]. A prior study[14] showed that GHD patients with depressed GH secretion had low levels of serum LEP. LEP also showed to alleviate the reduced secretion of GH in fasted mice[15], suggesting a link between GH and LEP. Thus, it is possible that LEP may be related to the pathogenesis of GHD. This study illustrated the correlation between serum LEP and GHD occurrence via the serum assay of LEP in GHD patients. It provided clues for finding early biomarkers of GHD diagnosis.

A previous study[16] showed that the altered serum IGF-1 level in GHD patients, but with unclear mechanisms. The level of LEP is largely affected by gene polymorphism of LEP and its receptor (LEPR) genes[6,17-20], both of which are also related to the occurrence of GHD as previously reported[21,22]. Therefore, the polymorphism of LEP and LEPR genes may mediate the pathogenesis of GHD via regulating LEP level, which exerts certain regulatory effects on IGF-1 level[23]. In an attempt to elucidate the effect of gene polymorphism on serum IGF-1 and LEP levels in GHD patients, we analyzed the correlation between LEP and LEPR genes polymorphism and IGF-1/LEP level in the serum.

Patients and Methods

Patients

From May 2012 to November 2014, a total of 180 childhood GHD patients in two hospitals (Fujian Provincial Hospital and Zhangzhou Municipal Hospital Affiliated to Fujian Medical University) were recruited in the GHD group, in which there were 97 males and 83 females, with an average age at 8.05 years old. Meanwhile, 160 age-/sex-matched healthy children were selected in the control group, which had 82 males and 78 females, with an average age at 8.15 years old.

The inclusion criteria of GHD: (1) Body height was shorter than the average value in the relevant age group by more than two folds of standard deviations (SD); (2) Peak GH concentration was lower than 10 ng/mL in two GH stimulation tests (by arginine and L-DOPA); (3) Bone age was younger than actual age by more than 2 years; (4) Growth speed was less than 5 cm per year; (5) Aging between 4 to 10 years old (Tanner I stage); (6) No thyroid dysfunction, bone malformation, chronic disease or abnormal chromosome karyotype. This study was reviewed and approved by the Ethical Committee in our hospital and obtained written consents form all participating families.

Gene Polymorphism Examination

Fasted venous blood samples (5 mL) were collected from all participants in EDTA-containing anti-coagulant tubes. Genomic DNA was extracted by DNA extraction kit (Applied Biosystem, Foster City, CA, USA). Single nucleotide polymorphism (SNP) loci of LEP and LEPR genes were genotyped by PCR-DNA sequencing approach. The target gene fragment was amplified by PCR kit (Takara, Otsu, Shiga, Japan) in a 10 μl system including DNA sample, PCR buffer and specific primers (Table I). The reaction condition included a 94°C per-denature for 2 min, followed by 40 repeating cycles each including 94°C denaturing for 30 sec, 63°C annealing for 30 sec and 72°C elongation for 60 sec. PCR products were then purified by agarose gel electrophoresis, enzymatic digestion and sequencing.

Serum Assays for IGF-1 and LEP

We used an automatic chemiluminescence immunity analyzer (CHIA) (Depu, China) for detecting serum IGF-1 level following the manual instruction. Serum LEP level was further determined by enzyme-linked immunosorbent assay (ELISA) using the test kit (Rapid Bio, San Jose, CA, USA) following the manual instruction.

Table I. Primers used for PCR.

SNP loci		Primer sequence (5′-> 3′)
LEP rs7799039	Forward	CCACA GACGG ACTGG AAAG
	Reverse	CTGCC ACGGC TCCAG CCGAT C
LEPR rs1137100	Forward	TTTCC ACTGT TGCTT TCGGA
	Reverse	GACAT CTATT TCATA CAGGT AT
LEPR rs1137101	Forward	ACCCT TTAAG CTGGG TGTCC
	Reverse	AGCTA GCAAA TATTT TTGTA

Statistical Analysis

SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. For between-group-comparison, we used chi-square test while measurement data were compared by *t*-test. Analysis of variance (ANOVA) was used to make comparisons among three or more groups. Comparison between groups was conducted using one-way ANOVA test followed by post hoc test (LSD). Statistical significance was defined when $p < 0.05$.

Results

Serum IGF-1/LEP Level and Their Correlation with GHD Pathogenesis

CHIA and ELISA methods were employed to quantify serum IGF-1 and LEP levels, respectively, in all patients. The IGF-1 level in GHD group was about 97.50 ± 36.69 μg/L, which was significantly lower than the control group ($t = 12.56$, $p < 0.001$). Serum LEP level showed consistent patterns, as serum LEP levels in GHD patients was only 3.89 ± 1.44 μg/L in average, which was significantly lower than the control group ($t = 3.5$, $p < 0.001$) (Table II).

Table II. Serum IGF-1 and LEP levels.

Group	N	IGF-1 (μg/L)	LEP (μg/L)
GHD	180	97.50 ± 36.69	3.89 ± 1.44
Control	160	164.96 ± 58.51	4.42 ± 1.34
t-value		12.56	3.50
p-value		< 0.001	< 0.001

Gene Polymorphism of LEP and LEPR Genes and its Relation with Serum IGF-1

To further illustrate the correlation between gene polymorphism of LEP and LEPR genes and serum IGF-1 concentration, we compared the serum IGF-1 levels between different genotypes at various SNP loci. As shown in Table III and Figure 1, serum IGF-1 levels showed a significant difference in each of the three genotype carriers ($p < 0.01$). After further sequencing, both GG and GA genotype carriers had significantly higher serum IGF-1 levels when compared to AA genotype carrier ($p < 0.05$). To rule out any interference including sex and age, covariance analysis was performed using serum IGF-1 levels as the dependent variable and age/sex as the covariance. The result showed a significant difference of serum IGF-1 among all individuals with different genotypes ($F = 104.90$, $p < 0.01$; $F = 91.16$, $p < 0.001$). Other gene loci, such as LEPR rs1137100 and rs1137101, had no direct relationship with serum IGF-1 level.

The Relationship of Gene Polymorphism of LEP and LEPR Genes with LEP Level

Using one-way ANOVA to compared serum LEP levels across different SNP loci and genotypes, we found the correlation between serum LEP and rs7799039 loci polymorphism of LEP gene. As shown in Table IV and Figure 2, a sig-

Table III. Serum IGF-1 variation.

SNP loci	GHD group			Control		
	IGF-1 level	F-value	*p*-value	IGF-1 level	F-value	*p*-value
rs7799039						
AA	78.13 ± 22.64	92.23	< 0.001	122.98 ± 37.31	106.49	< 0.001
GA	131.51 ± 32.22[a]			200.22 ± 39.38[a]		
GG	140.24 ± 16.61[a]			247.48 ± 39.58[a,b]		
rs1137100						
GG	96.21 ± 36.84	0.41	0.664	166.38 ± 58.80	0.51	0.602
GA	101.05 ± 37.08			158.40 ± 57.46		
AA	108.77 ± 28.61			185.18 ± 68.73		
rs1137101						
GG	100.17 ± 36.89	1.64	0.197	163.18 ± 57.85	0.52	0.598
GA	87.95 ± 34.68			172.99 ± 63.38		
AA	93.19 ± 39.89			189.27 ± 65.83		

Note: [a], $p < 0.05$ when compared to AA carriers; [b], $p < 0.05$ compared to GA carriers.

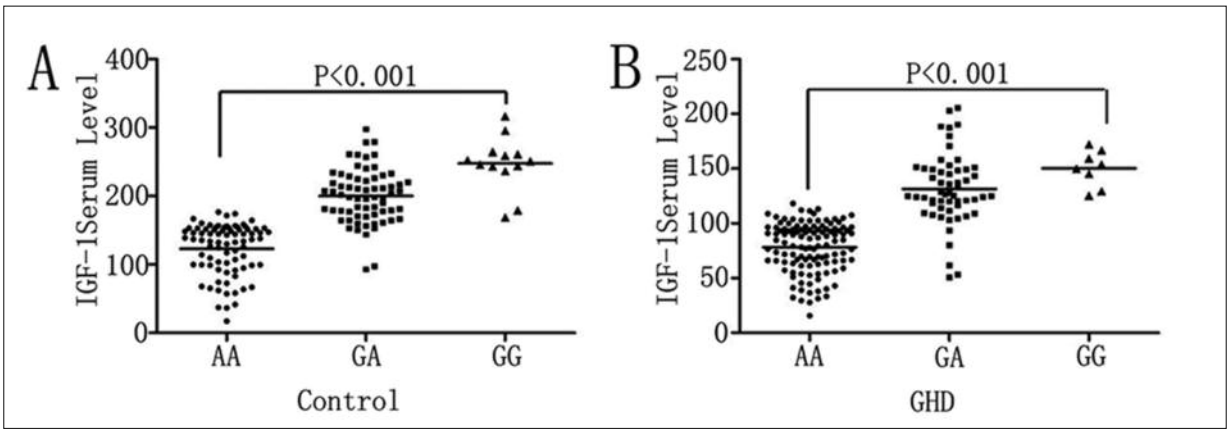

Figure 1. Serum IGF1 level in people with different genotype at loci rs7799039 of LEP gene in both control ***(A)*** and GHD group ***(B)***.

nificant difference in serum LEP levels existed across three different genotypes (AA, GA and GG) at rs7799039 locus ($p < 0.001$). Further between-group-comparisons showed higher serum LEP levels in GG or GA carriers compared to those with AA genotype ($p < 0.05$ in both cases). Further co-variance analysis removed the possible interference from sex or age and obtained consistent results ($p < 0.05$). No correlation existed between serum IGF-1 and LEPR gene polymorphism at loci rs1137100 or rs1137101.

Discussion

As the most common reason for childhood dwarfism, GHD requires an early diagnosis which can largely benefit the treatment efficacy. GH stimulation test, however, may not be accepted easily due to its high cost as well as its side effects. Serum IGF-1 level is still controversial for its use in GHD diagnosis. As serum LEP level has potential relationship with the occurrence of GHD and may be affected by gene polymorphisms of LEP and LEPR gene, this study tested both serum LEP/IGF-1 levels and LEP/LEPR gene polymorphisms in a case-control scenario of GHD patients, in an attempt to elucidate the relationship between LEP/IGF-1 level and gene polymorphisms, thus providing evidences for the pathogenesis of GHD and its early biomarkers.

Our results showed significantly lower levels of IGF-1 or LEP in the serum of patients in the

Table IV. Serum LEP levels across different genotypes of various SNP loci.

SNP loci	GHD group			Control		
	IGF-1 level	F-value	*p*-value	IGF-1 level	F-value	*p*-value
rs7799039						
AA	3.12 ± 0.92	98.89	< 0.001	3.47 ± 0.80	105.87	< 0.001
GA	5.24 ± 1.16[a]			5.20 ± 0.93[a]		
GG	5.60 ± 0.58[b]			6.36 ± 1.06[a,b]		
rs1137100						
GG	3.84 ± 1.44	0.45	0.638	4.46 ± 1.34	0.79	0.456
GA	4.03 ± 1.44			4.25 ± 1.31		
AA	4.38 ± 1.15			5.04 ± 1.71		
rs1137101						
GG	4.00 ± 1.44	1.80	0.168	4.38 ± 1.32	0.46	0.631
GA	3.50 ± 1.36			4.61 ± 1.49		
AA	3.72 ± 1.67			4.90 ± 1.38		

Note: [a], $p < 0.05$ when compared to AA carriers; [b], $p < 0.05$ compared to GA carriers.

GHD group when compared to the control group. This observation suggested the possible link between IGF-1/LEP levels and GHD pathogenesis. Previous investigations[24,25] also revealed a depressed IGF-1 level in the serum of GHD patients. Nevertheless, other researches[11] showed no significant difference in the level of serum IGF-1 between the GHD patients and healthy people. This inconsistency may be explained by the variation of age or sample size across different studies. Therefore, further comprehensive studies are required for elucidating the true value of IGF-1 as the diagnostic index of GHD. As a critical factor regulating energy metabolism and body growth, LEP has been observed regarding its correlation with GDH pathogenesis. This study revealed a significant decrease in the level of serum LEP in GHD patients, suggesting the close link between serum LEP abnormality and GHD occurrence. This was consistent with results obtained from previous studies[26,27]. Nevertheless, there are other reports that did not find elevated[24] or unchanged[28] levels of LEP in the serum of GHD patients. Such inconsistency can be justified as the latter two studies investigated adult patients, while the former two and the one reported here recruited children GHD patients. It is likely that LEP levels have different patterns in children GHD cases.

This work also revealed the correlation between LEP/IGF-1 serum levels and gene polymorphism at the rs7799039 locus of LEP gene, suggesting the modulation of serum LEP/IGF-1 levels by gene polymorphism. A previous study[27] also found consistent findings as suppressed serum LEP level in GHD patients with AA genotype at rs7799039 loci when compared to those with GG/AG genotype at the same loci. Meanwhile, they also found a significant difference of genotype distribution patterns at rs7799030 loci across the GHD and the control groups[27]. Our research obtained consistent results showing differential genotype distribution between the control and the GHD groups. These results showed that gene polymorphism at rs7799039 of LEP gene could affect GHD pathogenesis via its modulation on serum LEP levels. Also, the IGF-1 level was closely correlated with LEP level as was seen in patients with renal dysfunctions who were treated by IGF-1[29]. IGF-1 has been shown to be regulated by LEP[23], suggesting a feedback loop between LEP and IGF-1. This may explain the regulation of IGF-1 by LEP gene polymorphism.

Conclusions

This paper provided further clues for the application of serum IGF-1 and LEP levels in GHD diagnosis. We also, for the first time, showed the modulation of both LEP and IGF-1 serum levels by LEP gene polymorphism at rs7799039 loci, thus providing more evidence for GHD pathogenesis.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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