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The Relationship Between Gene Polymorphism of Leptin and Leptin Receptor and Growth Hormone Deficiency

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Backgrounds: Growth hormone deficiency (GHD) is a major cause of congenital short stature. GHD patients have significantly decreased serum leptin levels, which are regulated by gene polymorphism of leptin and leptin receptor. This study thus investigated the relationship between gene polymorphism and susceptibility to GHD.





Material/Methods: A case-control study was performed using 180 GHD children in addition to 160 healthy controls. After the extraction of whole genomic DNA, the genotypes of leptin and leptin receptor gene *loci* were analyzed by sequencing for single-nucleotide polymorphism.

Results: The frequency distribution of all alleles identified in leptin gene (*loci* rs7799039) and leptin receptor gene (*loci* rs1137100 and rs1137101) fit Hardy-Weinberg equilibrium. There was a significant difference in allele frequency at *loci* rs7799039 or rs1137101, as individuals with heterozygous GA allele had lower (rs7799039) or higher (rs1137101) GHD risk. No significant difference in allele frequency was discovered at *loci* rs1137100 ($p > 0.05$), which was unrelated to GHD susceptibility.

Conclusions: Gene polymorphism of leptin (*loci* rs7799039) and leptin receptor (*loci* rs1137101) are correlated with GHD susceptibility.

MeSH Keywords: **Absorptiometry, Photon • Human Growth Hormone • Receptors, Leptin**

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Background

Growth hormone deficiency (GHD) causes significant retardation of growth and short stature in children, due to the insufficient secretion of growth hormone [1]. GHD is a major cause of congenital dwarfism, severely affecting infantile development. Both genetic and acquired factors, including hypothalamic-pituitary axis abnormal development and brain trauma or radiation, may cause decreased secretion levels of growth hormone in the pituitary, thus causing GHD. Studies have shown the close relationship between GH level and GHF occurrence [2] and the critical role of gene polymorphism of growth hormone and its receptor genes in GHD pathogenesis [3,4], in addition to the correlation between growth hormone effector insulin-like growth factor 1 (IGF-1) and occurrence of GHD [5,6].

Leptin is a peptide hormone secreted by adipocytes and encoded by the "fat gene" [7] and can increase body energy expenditure along with decreased energy intake by suppressing appetite [8], thus playing a crucial role in metabolism and body growth mediation. Early studies reported decreased levels of leptin when growth hormone levels are insufficient [9] and elevated growth hormone secretion by leptin intervention in mice [10], suggesting the close relationship between growth hormone and leptin. Therefore, it is possible that leptin may participate in GHD pathogenesis. Recent studies have identified significantly suppressed serum leptin levels in GHD patients, showing significant correlations with IGF-1 levels [11–14], further supporting the role of lower leptin levels in GHD. A genetic study found possible involvement of leptin and leptin receptor gene polymorphism in regulating leptin secretion level [15]. Based on these findings, we hypothesized that the gene polymorphism of leptin and leptin receptor genes might be related to GHD pathogenesis. To test this hypothesis, we performed a case-control study to genotype leptin and leptin receptor genes at 3 loci from GHD patients, in an attempt to elucidate the relation between gene polymorphism and GHD susceptibility.

Material and Methods

Patient information

A total of 180 GHD children (97 males and 83 females, ages 5–10 years old) who were diagnosed in our hospital (Zhangzhou Municipal Hospital Affiliated to Fujian Medical University) were recruited as the disease group, along with 160 age-matched healthy children (82 males and 78 females) as the control group. Inclusion criteria were: (1) body height was lower than average levels by more than 2 standard deviations (SD); (2) growth hormone peak levels were lower than 10 ng/mL in 2 consecutive growth hormone provocation tests (by arginine and L-dopa); (3) bone age was younger than actual age by more

Table 1. PCR primer sequence.

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

than 2 years; (4) body growth rate was lower than 5 cm per year; (5) at prepuberty stage (Tanner I stage, between 5 and 10 years old); (6) no thyroid dysfunction, bone developmental disorder, chronic disease or karyotype abnormalities. This study was pre-approved by the ethics committee of our hospital and we obtained written consent from all guardians of the children. Fasted venous blood samples (5 mL) were collected and kept in EDTA-treated tubes for further studies.

Genomic DNA extraction

We used a whole genomic DNA purification kit (Applied Biosystem, USA) to extract DNA from peripheral blood cells following the manual's instructions. Purity and concentration of DNA products were analyzed by spectrophotometer and agarose gel electrophoresis.

Single-nucleotide polymorphism (SNP) of leptin and leptin receptor genes

Both target genes were first amplified by PCR method using a test kit (Takara, Japan) using the parameters: pre-denaturing at 94°C for 2 min, followed by 40 cycles each of 94°C denaturing for 2 min, 63°C annealing for 30 s and 72°C elongation for 60 s. The reaction was repeated for 40 cycles using specifically designed primers (Table 1). PCR products were purified by agarose gel electrophoresis, extracted, and sequenced.

Statistical analysis

The SPSS 16.0 software package was used to process all collected data, of which measurement data are represented as mean±standard deviation (SD). Hardy-Weinberg equilibrium was tested by chi-square method. For between-group comparisons, the t-test was used. Correlation analysis was performed using logistic regression analysis to calculate genotype frequency.

Table 2. General information of patients and control individuals.

Parameter	Control	Disease	Statistics value	P value
N	160	180		
Sex				
Male	82	97	0.237	0.627
Female	78	83		
Residential area				
Rural	79	91	0.047	0.828
Urban	81	89		
Age (years)	8.15±1.91	8.05±1.78	0.499	0.995
Bone age (years)	7.92±1.60	6.89±2.06	5.37	<0.001
Height (cm)	133.45±11.42	115.18±13.80	13.34	<0.001
Weight (kg)	32.44±7.07	28.60±6.06	5.39	<0.001
BMI (Kg/m ²)	18.64±5.31	16.21±6.14	3.90	<0.001

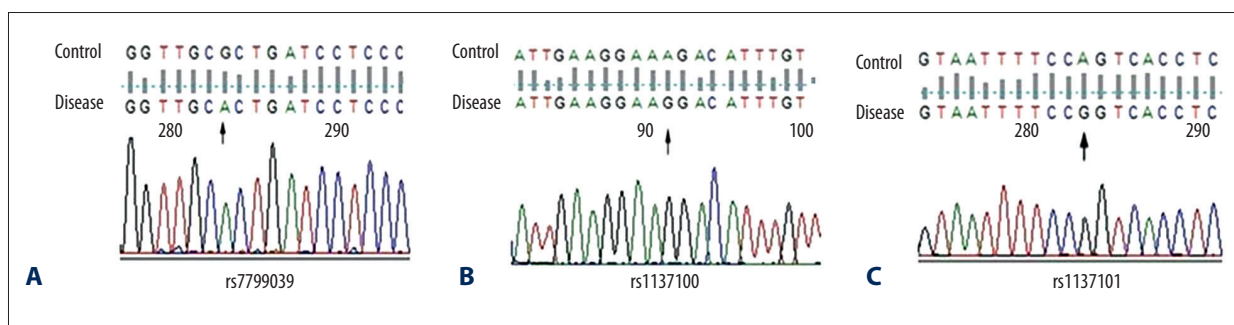


Figure 1. Nucleotide sequences of gene loci. Three loci, rs7799039 (A), rs1137100 (B), and rs1137101 (C) refer to genotype AA, GG, and GG, respectively.

Table 3. Allele frequency of leptin and leptin receptor genes.

Group	SNP	Allele Gene	Genotype			Allele		χ^2	P
			AA N (%)	AB N (%)	BB N (%)	A N (%)	B N (%)		
Control	rs7799039	A/G	81 (50.6)	66 (41.2)	13 (8.1)	228 (71.3)	92 (28.8)	0.01	0.93
	rs1137100	G/A	118 (73.8)	38 (23.8)	4 (2.5)	274 (85.6)	46 (14.4)	0.20	0.66
	rs1137101	G/A	136 (85.0)	21 (13.1)	3 (1.9)	293 (91.6)	27 (8.4)	3.63	0.06
GHD	rs7799039	A/G	116 (64.4)	56 (31.1)	8 (4.4)	288 (80.0)	72 (20.0)	0.14	0.71
	rs1137100	G/A	136 (75.6)	41 (22.8)	3 (1.7)	313 (86.9)	47 (13.1)	0.00	0.96
	rs1137101	G/A	138 (76.7)	36 (20.0)	6 (3.3)	312 (86.7)	48 (13.3)	3.26	0.07

A/B in rs7799039, rs1137100, rs1137101 indicates A/G, G/A, G/A, respectively. χ^2 and P value denote Hardy-Weinberg equilibrium results.

Results

General information of patients

No significant difference in ages was identified between the 2 groups (8.15±1.91 vs. 8.05±1.78, in years). Other parameters, such as sex and areas of residence also had no significant difference using chi-square test ($p>0.05$), suggesting that comparable groups were utilized. Bone ages were 7.92±1.60 and 6.89±2.06 for control and disease group (in years), respectively. Therefore, GHD patients had significantly younger bone age compared to those in the control group ($p<0.001$ by t-test). Such developmental retardation also occurred for height, body weight, and body-mass index (BMI) in GHD patients (Table 2).

Allele frequency of SNPs

We found that the allele frequencies of all 3 SNP *loci* (rs7799039, rs1137100, and rs1137101) fit Hardy-Weinberg equilibrium ($p>0.05$), suggesting the homogeneity of subjects recruited in this study (Figure 1, Table 3). Three *loci* in Figure 1 refer to genotype AA, GG, and GG. In the control group of *loci* rs7799039, allele A and G were 228 (71.3%) and 92 (28.8%), respectively, while in the GHD group they were 288 (80.0%) and 72 (20.0%), respectively. In the control group of *loci* rs1137100 allele G and A were 274 (85.6%) and 46 (14.4%), respectively, while in the GHD group they were 313 (86.9%) and 47 (13.1%), respectively. In the control group of *loci* rs1137101, allele G and A were 293 (91.6%) and 27 (8.4%), respectively, while in the GHD group they were 312 (86.7%) and 48 (13.3%), respectively. We then used the chi-square test to compared both genotype and allele frequency at 3 SNP *loci* between disease and control individuals. Results (Table 3) showed significant difference in genotype frequencies (AA, AG, and GG) and allele frequencies (A and G) in leptin gene *loci* rs779039 ($\chi^2=7.08$ and 7.09 , $p<0.05$ in both cases). Leptin receptor gene *loci* rs1137100 showed no significant difference in either genotype (AA, AG, and GG) or allele (A and G) frequency ($\chi^2=0.36$ and 0.25 , $p>0.05$ in both cases). Another *loci* rs1137101 of the leptin receptor gene, however, showed no significant difference of genotype (AA, AG, and GG) frequency ($\chi^2=0.36$, $p>0.05$) but did show a significant difference in allele (A and G) frequency ($\chi^2=4.14$, $p<0.05$).

Gene polymorphism and GHD susceptibility

Unconditional logistic regression analysis was used to elucidate the relationship between gene polymorphisms of leptin/leptin receptor genes and GHD risks. As shown in Table 4, people with GA phenotype at *loci* rs7799039 of the leptin gene had significantly lower risk of having GHD when compared with people having AA genotype at the same *loci* (OR=0.59, 95% CI=0.38~0.93, $p<0.05$). At this locus, G allele brought about only 62% of GHD risk compared to A allele (95% CI=0.44~0.88,

Table 4. Gene polymorphism and GHD susceptibility.

<i>Loci</i> (genotype & allele)	OR (95%CI)	P value
rs7799039		
GA/AA	0.59 (0.38–0.93)	0.022*
GG/AA	0.42 (0.17–1.06)	0.065
GA+GG/AA	0.56 (0.37–0.88)	0.009*
G/A	0.62 (0.44–0.88)	0.007*
rs1137100		
GA/GG	0.92 (0.56–1.51)	0.739
AA/GG	0.69 (0.15–3.14)	0.629
GA+AA/GG	0.90 (0.56–1.45)	0.664
A/G	0.89 (0.58–1.37)	0.602
rs1137101		
GA/GG	1.82 (1.01–3.26)	0.046*
AA/GG	2.13 (0.52–8.71)	0.293
GA+AA/GG	1.86 (1.07–3.24)	0.026*
A/G	1.80 (1.09–2.95)	0.020*

For the purpose of quantitative analysis, we denoted AA=1, GA=2 and GG=3 at *loci* rs7799039, and made GG=1, GA=2 and AA=3 for *loci* rs1137100 and rs1137101. * $p<0.05$.

$p<0.05$). SNP at *loci* rs1137100 in the leptin receptor gene had no significant relationship with GHD incidence. *Loci* rs1137101, however, was related to GHD incidence, as heterozygous GA people had significantly elevated GHD risks when compared to GG ones (OR=1.82, 95% CI=1.01~3.26, $p<0.05$). Moreover, people with A allele at this locus had a 1.80-fold increased disease risks compared to those having G allele (95% CI=1.09~2.95, $p<0.05$).

Discussion

Growth hormone-IGF-1 is an important hormonal axis underlying infantile growth and has received abundant research attention; therefore, there are many studies investigating the relationship among growth hormone, IGF-1, and GHD pathogenesis [13–15]. Previous studies have established the important role of leptin in mediating energy metabolism and body growth [16], in addition to its correlation with secreted levels of IGF-1 and growth hormone, as supported by suppression of leptin expression after IGF-1 injection and potentiated leptin mRNA levels in adipocytes after growth hormone treatment [17]. A study of leptin level in GHD patients showed

significantly depressed secretion [18]. The expression level of leptin was affected by gene polymorphism [19–21], suggesting the correlation between GHD pathogenesis and gene polymorphism of leptin and leptin receptor genes.

This study revealed lower GHD risk in heterozygous (G/A) at rs7799039 *loci*, which was located at –2,548 bp upstream of the start codon of the leptin gene. A previous study, however, found no significant difference in genotype frequency at this locus between GHD patients and healthy controls [18]. This inconsistency can be attributed to different research objectives and sample sizes. The abovementioned study, however, found that GHD patients had differential SNP patterns at rs7799039 locus of leptin gene and this was correlated with leptin levels [18]. Such a relationship has also been supported by evidence from thyroid cancer patients [12]. These reports, plus our results, suggest the potency of rs7799039 SNP in affecting GHD risks via regulating leptin level, although more *in vitro* and *in vivo* experiments are required for substantiation.

No significant correlation was detected between rs1137100 locus of leptin receptor gene and GHD susceptibility, consistent

with previous report [22,23]. The Q223R polymorphism, also named rs1137101 SNP, is one of the most common SNP *loci* of leptin receptor gene. This study found significantly elevated disease risk of GHD in people with heterozygous (GA) genotype at this locus, suggesting it is a potential risk factor. Previous studies are consistent with our results by showing higher genotype frequency of GA/AA in GHD patients at the same *loci* [23], although OR calculation was not performed. In another study, gene polymorphism at *loci* rs1137101 was found to be unrelated with leptin level [12]. Therefore, the mechanism underlying GA genotype at rs1137101 for elevating GHD risk requires further exploration.

Conclusions

This study for the first time reports a correlation between gene polymorphisms of leptin and leptin receptor genes and GHD susceptibility, suggesting the potency of GA at locus rs7799039 and at rs1137101 as risk factors for GHD, although their use as novel biomarkers for population screening requires substantiation in larger-sample groups.

References:

- Mazzanti L, Tamburrino T, Bergamaschi R et al: Developmental syndromes: growth hormone deficiency and treatment. *Endocr Dev*, 2009, 14: 114–34
- Beuschlein F, Hancke K, Petrick M. et al. Growth hormone receptor mRNA expression in non-functioning and somatotroph pituitary adenomas: implications for growth hormone substitution therapy? *Exp Clin Endocrinol Diabetes*, 2005, 113(4): 214–18
- Giordano M, Godi M, Mellone S et al: A functional common polymorphism in the vitamin D-responsive element of the GH1 promoter contributes to isolated growth hormone deficiency. *J Clin Endocrinol Metab*, 2008, 93(3): 1005–12
- Buzi F, Mella P, Pilotta A et al: Growth hormone receptor polymorphisms. *Endocr Dev*, 2007; 11: 28–35
- Federico G, Street ME, Maghnie M et al: Assessment of serum IGF-I concentrations in the diagnosis of isolated childhood-onset GH deficiency: a proposal of the Italian Society for Pediatric Endocrinology and Diabetes (SIEDP/ISPED). *J Endocrinol Invest*, 2006; 29(8): 732–37
- Wassenaar MJ, Dekkers OM, Pereira AM et al: Impact of the exon 3-deleted growth hormone (GH) receptor polymorphism on baseline height and the growth response to recombinant human GH therapy in GH-deficient (GHD) and non-GHD children with short stature: a systematic review and meta-analysis. *J Clin Endocrinol Metab*, 2009; 94(10): 3721–30
- Zhang Y, Proenca R, Maffei M et al: Positional cloning of the mouse obese gene and its human homologue. *Nature*, 1994; 372(6505): 425–32
- Haynes WG: Role of leptin in obesity-related hypertension. *Exp Physiol*, 2005; 90(5): 683–88
- Florkowski CM, Collier GR, Zimmet PZ et al: Low-dose growth hormone replacement lowers plasma leptin and fat stores without affecting body mass index in adults with growth hormone deficiency. *Clin Endocrinol (Oxf)*, 1996; 45(6): 769–73
- Gregoire Nyomba BL, Johnson M, Berard L, Murphy LJ: Relationship between serum leptin and the insulin-like growth factor-I system in humans. *Metabolism*, 1999; 48(7): 840–44
- Zotter H, Kerbl R, Gallistl S et al: Leptin responses to insulin administration in children with short stature. *Metabolism*, 2005; 54(7): 862–65
- Marcello MA, Calixto AR, de Almeida JF et al: Polymorphism in LEP and LEPR may modify leptin levels and represent risk factors for thyroid cancer. *Int J Endocrinol*, 2015; 2015: 173218
- van der Klaauw AA, van der Straaten T, Baak-Pablo R et al: Influence of the d3-growth hormone (GH) receptor isoform on short-term and long-term treatment response to GH replacement in GH-deficient adults. *J Clin Endocrinol Metab*, 2008; 93(7): 2828–34
- Rosenbloom AL: Insulin-like growth factor-I (rhIGF-I) therapy of short stature. *J Pediatr Endocrinol Metab*, 2008; 21(4): 301–15
- Federico G, Street ME, Maghnie M et al: Assessment of serum IGF-I concentrations in the diagnosis of isolated childhood-onset GH deficiency: a proposal of the Italian Society for Pediatric Endocrinology and Diabetes (SIEDP/ISPED). *J Endocrinol Invest*, 2006; 29(8): 732–37
- Dagogo-Jack S, Franklin SC, Vijayan A et al: Recombinant human insulin-like growth factor-I (IGF-I) therapy decreases plasma leptin concentration in patients with chronic renal insufficiency. *Int J Obes Relat Metab Disord*, 1998; 22(11): 1110–15
- Houseknecht KL, Portocarrero CP, Ji S et al: Growth hormone regulates leptin gene expression in bovine adipose tissue: correlation with adipose IGF-1 expression. *J Endocrinol*, 2000; 164(1): 51–57
- Su PH, Yang SF, Yu JS et al: Study of the leptin levels and its gene polymorphisms in patients with idiopathic short stature and growth hormone deficiency. *Endocrine*, 2012; 42(1): 196–204
- Ben Ali S, Kallel A, Sediri Y et al: LEPR p.Q223R Polymorphism influences plasma leptin levels and body mass index in Tunisian obese patients. *Arch Med Res*, 2009; 40(3): 186–90
- Furusawa T, Naka I, Yamauchi T et al: The Q223R polymorphism in LEPR is associated with obesity in Pacific Islanders. *Hum Genet*, 2010; 127(3): 287–94
- Ben Ali S, Sediri Y, Kallel A et al: The G3057A LEPR polymorphism is associated with obesity in Tunisian women. *Nutr Metab Cardiovasc Dis*, 2011; 21(8): 591–96
- Su PH, Yang SF, Yu JS et al: A polymorphism in the leptin receptor gene at position 223 is associated with growth hormone replacement therapy responsiveness in idiopathic short stature and growth hormone deficiency patients. *Eur J Med Genet*, 2012; 55(12): 682–87
- Su PH, Chen JY, Yu JS et al: Leptin expression and leptin receptor gene polymorphisms in growth hormone deficiency patients. *Hum Genet*, 2011; 129(4): 455–62