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 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/m²)            | 18.64±5.31 | 16.21±6.14 | 3.90          | <0.001  |

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

| Group | SNP         | Allele Gene | AA N (%) | AB N (%) | BB N (%) | Statistics value | P value | χ² | P |
|-------|-------------|-------------|----------|----------|----------|------------------|---------|----|---|
| 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 (80.9) | 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. χ² 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 rs7799039 (χ²=7.08 and 7.09, p<0.05 in both cases). Leptin receptor gene loci rs7799039, and made GG=1, GA=2 and AA=3 for loci rs7799039, 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=1.01~2.95, 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).

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, 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 potentiation of 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 rs1137101 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.

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