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Clinical features and growth hormone receptor gene mutations of patients with Laron syndrome from a Chinese family

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Abstract: Laron syndrome is an autosomal recessive disorder caused by defects of growth hormone receptor (*GHR*) gene. It is characterized by severe postnatal growth retardation and characteristic facial features as well as high circulating levels of growth hormone (GH) and low levels of insulin-like growth factor I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3). This report described the clinical features and *GHR* gene mutations in 2 siblings with Laron syndrome in a Chinese family. Their heights and weights were in the normal range at birth, but the growth was retarded after birth. When they presented to the clinic, the heights of the boy (8 years old) and his sister (11 years old) were 80.0 cm (−8.2 SDS) and 96.6 cm (−6.8 SDS) respectively. They had typical appearance features of Laron syndrome such as short stature and obesity, with protruding forehead, saddle nose, large eyes, sparse and thin silky hair and high-pitched voice. They had higher basal serum GH levels and lower serum levels of IGF-I, IGFBP-3 and growth hormone binding protein(GHBP) than normal controls. The peak serum GH level after colonidine and insulin stimulations in the boy was over 350 ng/mL. After one-year rhGH treatment, the boy's height increased from 80.0 cm to 83.3 cm. The gene mutation analysis revealed that two patients had same homozygous mutation of S65H (TCA →CCA) in exon 4, which is a novel gene mutation. It was concluded that a definite diagnosis of Laron syndrome can be made based on characteristic appearance features and serum levels of GH, IGF-I, IGFBP-3 and GHBP. The S65H mutation might be the cause of Laron syndrome in the two patients. [**Chin J Contemp Pediatr, 2007, 9 (4): 335 – 338**]

Key words: Laron syndrome; Growth hormone receptor gene; Gene mutation; Polymerase chain reaction

一个 Laron 综合征家系患者临床特点和生长激素受体基因突变分析

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[**摘　要**] Laron 综合征是一种常染色体隐性遗传病,生长激素受体(*GHR*)基因缺陷是导致 Laron 综合征的主要病因。Laron 综合征主要临床特征为生后严重的生长落后伴特殊面容,血生化特点为高生长激素(GH)、低胰岛素样生长因子-I (IGF-I) 和低胰岛素样生长因子结合蛋白-3 (IGFBP-3)。该研究报道一家系 2 例 Laron 综合征患者的临床特点及 *GHR* 基因突变。这两个病人为同胞姐弟。弟弟 8 岁,身高 80.0 cm (-8.2 SDS),姐姐 11 岁,身高 96.6 cm (-6.8 SDS)。他们出生体重和身长无特殊,自生后出现生长落后,身高明显落后于同龄正常儿童,并均呈现了典型 Laron 综合征外貌特征:身材矮、肥胖、前额突出、大眼睛、塌鼻梁、头发稀软。这两个病人空腹血清 GH 值均明显高于正常儿童,空腹血清 IGF-I 明显低于同年龄同性别正常儿童,血浆 IGFBP-3 和生长激素结合蛋白(GHBP)低于检测线。其中 1 例(8 岁男孩)胰岛素和可乐定刺激后 GH 峰值大于 350 ng/mL,给予重组人生长激素治疗 1 年,身高由治疗前的 80.0 cm 增加至 83.3 cm。*GHR* 基因序列测定结果显示 2 例患者均存在外显子 4 上第 65 位氨基酸的纯合突变 S65H(TCA → CCA),为新发现的突变。Laron 综合征患者存在特殊的面貌特征,结合血 GH、IGF-I、IGFBP-3 和 GHBP 测定可以明确诊断。*GHR* 基因外显子 4 上 S65H 突变可能是这两位 Laron 综合征患者的致病原因。 [**中国当代儿科杂志,2007,9(4):335 – 338**]

[**关　键　词**] Laron 综合征;生长激素受体基因;基因突变;聚合酶链式反应

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Laron syndrome, also known as growth hormone insensitivity syndrome, is a rare congenital disorder due to defects of growth hormone receptor (*GHR*) (gene bank number: 600649)[1]. It is clinically characterized by severe postnatal growth failure and very low serum levels of insulin-like growth factor-I (IGF-I) despite increased secretion of growth hormone(GH). The adult stature in the larger Ecuadorian cohort has been reported to be 95-124 cm in females and 106-141 cm in males [2]. *GHR* gene was located at 5p12, and consists of 10 exons. The 3-10 exons encode the mature *GHR* protein [3]. More than 70 mutations related to Laron syndrome have already been discovered, but there were few reports about this syndrome in China [4, 5].

This report described two patients presenting with a typical Laron syndrome in a Chinese family carrying a novel missense in exon 4 of the *GHR* gene.

Subjects and methods

Clinical materials

Patients The two patients with Laron syndrome were siblings from a Chinese family. Their heights and weights were in the normal range at birth. The growth velocity declined obviously after birth. When they presented to the clinic, the heights of the boy (8 years old) and his sister (11 years old) were 80.0 cm (-8.2 SDS) and 96.6 cm (-6.8 SDS) and the bone age was 5 and 8 years according to Greulich & Pyle, respectively. They had typical appearance features of Laron syndrome such as short stature and obesity, with protruding forehead, saddle nose, large eyes, sparse and thin silky hair and high-pitched voice. There was no family history of growth retardation. Their parents' heights were in the normal range of Chinese adults (father: 172 cm; mother: 152 cm).

Endocrine examinations

Serum GH and IGF-1 concentrations were measured by enzyme immunoassay (Syntron Bioresearch Inc and Diagnostic Systems Laboratories Inc, respectively). Serum levels of growth hormone binding protein (GHBP) and insulin-like growth factor binding protein-3 (IGFBP)-3 were measured using radioimmunoassay. The boy's serum GH level was 319.1 ng/mL at baseline and more than 350 ng/mL after colonidine and insulin stimulations. The IGF-I level of the boy was 34.0 ng/mL. The girl's serum GH level was 24.43 ng/mL and the IGF-I level was 54.0 ng/mL at baseline. Because of the family history, typical appearance features and abnormal serum GH and IGF-I levels, the GH stimulation test was waived for the girl. The IGFBP-3 and GHBP levels in the two patients were below the detecting limits. They had normal thyroid and liver functions and normal fasting blood glucose and insulin levels.

GHR mutation analysis

Genomic DNA extraction

Peripheral blood white cells were isolated by conventional method. Protenase K was added to digest the cells, and RNase was then added to inactive the RNA. The genomic DNA was extracted by phenol/chloroform/isoamylol method and precipitated by dehydrated alcohol. The final samples were re-suspended in pH 8.0 TE buffer. The genomic DNA was stored at -20℃ before PCR amplification.

PCR amplification

The primers were designed and synthesized by Beijing AuGCT Company. The primers and the annealing temperature are shown in Table 1. PCR reactions were run in a thermocycler (Biometra) with 0.5 U Taq DNA polymerase, 20 pmol of 2 primers, 100-200 ng of genomic DNA, in a final volume of 50 μL of 1 × Taq polymerase buffer, 1.5 mM $MgCl_2$. PCR was conducted as following: pre-denaturation at 94℃ for 3 minutes → denaturation at 94℃ for 1 minute → annealing for 30 seconds →extending at 72℃ for 3 minutes. After 30 circles, it was extended at 72℃ for 3 minutes. The amplification products were purified and directly sequenced using ECQ880 automated DNA sequencer (Beckman).

Table 1 The primers used for *GHR* and the annealing temperature

Exons	Upper primers	Lower primers	Products(bp)	Annealing temp. (℃)
E2	5′AATGGTCTGCTTTTAATTGCTG3′	5′TTTCAAAACACTGAGGGTGGA3′	174	53.4
E3	5′GACTAGATGGTTTTGCCTTCCTC3′	5′CATGGAAAATATCATTCGAAGGA3′	147	52.6
E4	5′CTCACCTGATTTCATGCCTTG3′	5′ATCCATGGAGAGGAAAATCAGA3′	212	53.8
E5	5′ACCCTTCATTTTAGGAACACTCA3′	5′TCAAATGAAACAAAAACCTGTGA3′	215	51.4
E6	5′CTAATGCTCTGTTGAATTGCACA3′	5′AGTCAAAGTGTAAGGTGTAGCAACA3′	234	52.7
E7	5′CTGTAGTGTTCATTGGCATTGAG3′	5′TGACAAAAGCCAGGTTAGCTACT3′	283	52.1
E8	5′GAAACTGTGCTTCAACTAGTCGT3′	5′TGGCAAGGTCTAACACAACTG3′	211	50.7
E9	5′TGTAGCTTTTAAGATGTCAAAACCA3′	5′CATATGACAGGAGTCTTCAGGTGT3′	182	50.1

Gene mutation analysis

All the sequences of the two patients were compared with normal *GHR* sequences. To confirm the mutations, every sequence with mutation was amplified and sequenced twice.

Results

Treatment and follow-up

The boy with Laron syndrome received rhGH (0.1 IU/kg · d) treatment for one year. His height increased from 80.0 cm to 83.3 cm. Then rhGH was substituted with clonidine (100 mg/d) and stanozol (1 mg/d). After 6 months of therapy, the boy's height increased to 84.7 cm. The girl with Laron syndrome was administered with clonidine (100 mg/d) and stanozol (1 mg/d). After 6 months of therapy, her height increased from 96.6 cm to 100.4 cm.

Gene mutation analysis

The gene mutation analysis revealed that the two patients had a homozygous mutation of S65H (TCA→CCA), which was a novel mutation. This mutation was located in exon 4, correspoding to the extracellular part of the protein (Figure 1). This mutation might impair the *GHR* dimerization or activation of the cytokine factor.

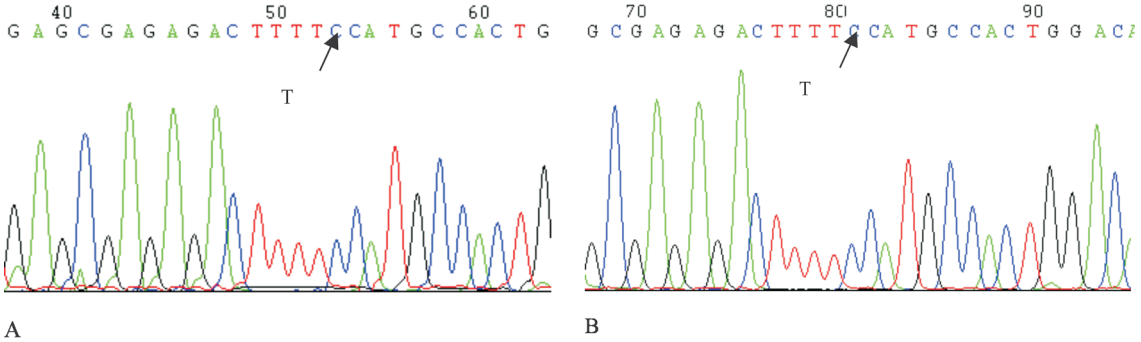

Figure 1 The gene mutations in exon 4 of *GHR* of the boy (A) and the girl (B) with Laron syndrome. The T base changed to C, which made the 65 amino acid of *GHR* changed from S to H.

Discussion

Laron syndrome was first described in 1966 as "genetic pituitary dwarfism with high serum concentrations of growth hormone". More than 300 cases have been reported through the world, mostly from the Mediterranean, mid-eastern region and Ecuador[6]. This syndrome is an autosomal recessive inherited disease. The alleles of patients' parents are heterozygotes and they had no abnormal clinical features. Patients with Laron syndrome had characteristic clinical features. They had severe short stature of -4 to -10 SDS below the mean normal height, acromicria, protruding forehead, saddle nose and very high-pitched voice. Their hair was sparse, thin, silky and easy to pluck. The onset of teething was delayed in most of patients. The patients were relatively obese due to the underdevelopment of bones and muscles leading to relatively more adipose tissues[7]. Basal overnight fasting serum GH level was high and peak level of GH after stimulation was more than 200-300 ng/mL. Serum level of IGF-I was very low, even undetectable. Serum IGFBP-3 level was low, but IGFBP-1 level was elevated. IGFBP-2 level was normal or increased. GH treatment is ineffective, and the only effective treatment is IGF-1 replacement therapy.

The underlying mechanism is a defect in *GHR* gene, which induces to an absence of responsiveness to GH. *GHR* gene spans 300 kb pairs including 10 exons and the *GHR* protein is a 620-residue, single membrane-spanning protein that belongs to the large family of cytokine receptors. Exon 1 is the non-encoding region; exon 2 encodes the signal peptide, and exons 3-7 encode the extracellular part of *GHR*. Exons 8 and 9 encode the trans-membrane and cytoplasmatic domain, respectively. Parts of *GHR* corresponding to its extracellular domain are cleaved and present in the circulation as GHBP. GHBP can bind with GH and prolong the half-life of GH. GH signaling is initiated by sequential binding of two distinct sites of the GH molecule to two *GHR* monomers, which is followed by receptor dimerization and activation of JAK-STAT pathway. The mutations of *GHR* gene can not only influence the function of the two proteins, but also lead to the physiological function changes of GH. To date, more than 70 mutations of the *GHR* have been identified in Laron syndrome patients. The majority of *GHR* gene mutation was located in exons 3-7, which influences the binding of GH to *GHR* and generation of

GHBP [8-10]. There are also some mutations belonging to the trans-membrane and cytoplasmatic domain, which might result in impaired intracellular signal transduction or dimerization of *GHR*, and the patients who carry these mutations have normal or increased serum GHBP levels [11]. Among all the mutations, most of them were point mutations [12]; even there were also reports on patients with base deletion or insertion leading to the frame-shift mutation [6, 13].

In this report, we described 2 patients in a Chinese family with typical features of Laron syndrome suffering from a novel missense mutation (S65H) in exon 4. The height SDSs in the boy and the girl were -8.2 and -6.8, respectively. The basal GH levels in the two patients were very high, and the peak GH level of the boy was more than 350 ng/mL, which suggested that two siblings had GH insensitivity or GH resistance. This was confirmed by low serum IGF-I levels and undetectable serum IGFBP-3 and GHBP. Low GHBP levels suggested that the gene mutation was belonged to the extracellular part of the protein. Furthermore, the *GHR* gene mutation analysis showed that the two patients had same point mutation (S65H) in exon 4. This missense mutation made the 65th amino acid of *GHR* change from serine to proline, which might result in the structure alteration of the extracellular domain of *GHR*, thereby affecting GH to bind with *GHR* or the generation of GHBP. Some research has shown that even one single amino acid substitution in the extracellular domain of the *GHR* can prevent the ligand binding to the *GHR* and the generation of GHBP [7]. In this report, high basal and peak level of GH after stimulation, low serum GHBP levels and ineffective GH therapy might result from the S65H mutation of *GHR*.

The only effective treatment is replacement therapy with IGF-I. IGF-I administration can suppress not only the IGFBPs including IGFBP-3, but also the GH and serum insulin, preventing hypoglycemia and stabilizing blood glucose levels [14]. Unfortunately, only a small number of patients can receive the therapy due to lmited drugs. In this study, a 6-month stanozol treatment increased the height for the two patients, but the long-term curative effect of stanozol was not observed.

In conclusion, this report described a novel homozygous missense mutation, S65H, in the *GHR* gene of two Chinese patients with Laron syndrome. A definite diagnosis of Laron syndrome can be made based on characterictic appearance features and blood biochemical changes. The S65H mutation in exon 4 of *GHR* gene might be the cause of Laron syndrome in the two patients.

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