

Clinical Vignette

Young Thai sisters with growth hormone insensitivity or Laron syndrome

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Background: Growth hormone insensitivity (GHI) or Laron syndrome can result from GH receptor (GHR) or postreceptor defects, such as in GH binding or transduction, or insulin-like growth factor 1 (IGF-1) synthesis. Multiple defects in GHI have been reported in cohorts from the Middle East, Ecuador, and the Mediterranean, but rarely reported from Southeast Asia.

Methods: Genomic DNA was isolated from peripheral blood leukocytes of young Thai sisters with severe short stature. Coding exons, including the intronic boundaries of the GHR were amplified from genomic DNA by PCR, and products were purified and sequenced. Serum GH, IGF-1, and IGF binding protein-3 were assayed immunometrically.

Results: We found an extreme GHI phenotype and a homozygous mutation in exon 7 of GHR.

Conclusions: This mutation can cause a new donor splice site and interfere with mRNA splicing. To our knowledge, these are first cases of Laron syndrome in Thais confirmed by genotyping.

Keywords: Growth hormone, growth hormone insensitivity, growth hormone receptor, insulin-like growth factor-1

In 1966, Laron et al. first described growth hormone insensitivity (GHI) in 3 children from a consanguineous Yemenite family who had extreme growth failure and a phenotype of hypopituitarism, but high concentrations of circulating GH [1]. This disorder, now known eponymously as Laron syndrome, was shown to be caused by a defect in the GH receptor (GHR) resulting in the inability of GH to bind to the GHR to produce insulin-like growth factor (IGF)-1 required for linear growth [2]. Therefore, IGF-1 deficiency associated with normal or increased GH levels are the cardinal biochemical features [2].

The subsequent era of molecular genetics has shown a number of molecular defects of the GH–IGF-1 axis to be responsible for GHI. These include genes coding for proteins that regulate GH binding, signal transduction and IGF-1 synthesis, transport, or action [2, 3]. The phenotypes and biochemical abnormalities associated with GHI can vary from

extreme to mild, and the genotype–phenotype correlations of some of the associated disorders remain unclear [4, 5]. GHI caused by mutation in the *GHR* is a rare condition and to our knowledge has not previously been reported in Thai people.

Case presentation

Clinical cases

Two sisters with severe short stature presented to the pediatric endocrine clinic, Faculty of Medicine, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Their parents are Thai, living in a Northeastern province of Thailand. They do not report a consanguineous marriage. Birth weight and height data are expressed below as standard deviation score (SDS) units, according to the appropriate Thai national standards. Their father's height was 165 cm (−0.87 SDS) and mother's height was 150 cm (−1.44 SDS) [6].

The older sister, aged 12.5 y, was born at term with a birth weight of 2,500 g (−1.5 SDS). Birth length was not recorded. Her parents noticed she was small compared to her peers from the age of 1 y.

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There was one reported episode of a febrile convulsion aged 2 y, but blood glucose was not determined. Apart from severe short stature, she was healthy, active, and attended a normal school. On physical examination, her height was 92 cm (−11.59 SDS), weight 12.9 kg (−3.76 SDS), her upper: lower ratio of body proportions was 1.0 (normal = 0.9) head circumference (HC) was 48 cm (−1.54 SDS).

She had mild frontal bossing, midfacial hypoplasia, a high-pitched voice, blue sclerae, breast development Tanner stage 2, and pubic hair Tanner stage 1. Valvular pulmonary stenosis and a secundum atrial septal defect was detected by echocardiography. Investigations showed normal thyroid function tests (T4 12.17 µg/dL, thyroid stimulating hormone (TSH) 2.86 mU/L), normal renal and liver function, and normal chromosome analysis (46, XX). Peak GH on provocative testing (clonidine) was high at 41.15 ng/mL. Baseline serum IGF-1 and IGF binding protein (IGFBP)-3 levels were 67.5 ng/mL (normal range: 143–693 ng/mL) and 608 ng/mL (normal range: 2700–8900 ng/mL) respectively and increased to 72.2 ng/mL and 1120 ng/mL, respectively after recombinant human GH (rhGH) 0.7 mg (54 µg/kg/day subcutaneously for 7 consecutive days). Bone age was 11.0 years by comparison with bones in the Greulich and Pyle atlas.

The younger sister, aged 3 years, was born at term by cesarean section because of breech presentation with a birth weight of 2,700 g (−1.0 SDS). Birth length was not recorded. She has had normal developmental milestones and no history of hypoglycemia. On physical examination, her height was 70.5 cm (−6.94 SDS), weight 7 kg (−3.83 SDS), her upper: lower ratio of body proportions was 1.3 (normal = 1.3), HC 45 cm (−2.69 SDS). She had mild frontal bossing, midfacial hypoplasia, a high-pitched voice, and blue sclerae. Investigations showed normal thyroid function tests (free T4 1.38 ng/dL, TSH 3.96 mU/L), normal renal and liver function, and normal chromosome analysis (46, XX). Peak GH on provocation testing (L-dopa) was very high 108.44 ng/mL. Serum IGF-1 was undetectable (<1.0 ng/mL; normal range 49–289) and increased to 1.76 ng/mL after rhGH 0.4 mg (57 µg/kg/day subcutaneously for 7 consecutive days). However, serum IGFBP-3 remained unchanged; 527 ng/mL (normal range: 900–4300 ng/mL) before and 523 ng/mL after rhGH injection. Bone age was 1–2 years by comparison with bones in the Greulich and Pyle atlas.

This manuscript was assessed by the Institution Review Board of the faculty of Medicine, Chulalongkorn University, and the study exempted from full board review (Certificate of Exemption No. 032/2016; IRB No. 643/59). Both parents of the patients provided their written informed consent for the publication of the case reports, and the older patient provided her informed assent.

Methods

Genetic analysis

Genetic analysis was performed in the molecular laboratory, Centre for Endocrinology, William Harvey Research Institute, London, United Kingdom. Genomic DNA was isolated from peripheral blood leukocytes using a QIAGEN DNeasy kit. Coding exons, including the intronic boundaries of the *GHR* were amplified from the genomic DNA by polymerase chain reaction (PCR; primer sequences are available on request). PCR products were visualized on a 1% agarose gel. The products were purified and directly sequenced on an automated DNA sequencer (ABI 3700) in accordance with the manufacturer's instructions.

Hormone assays

Serum GH, IGF-1, and IGFBP-3 levels were measured using solid-phase, enzyme-labeled chemiluminescent immunometric assays (Immulite, Siemens).

Results

The clinical manifestations and hormonal investigations in our patients were consistent with the diagnosis of GHI syndrome, i.e., extremely short stature phenotypes associated with high concentrations of circulating GH with low IGF-1 and IGFBP-3 levels. Additionally, the IGF-1 generation test failed to induce a significant increase in serum IGF-1 and IGFBP-3 confirming GH resistance. The diagnosis was also strongly suggested by a previously described scoring system [7]. Molecular genotype analysis of DNA from both sisters revealed a homozygous mutation in exon 7 of the *GHR*. This mutation at position hg19 chr5 g.[42711311C>T] (= c.723C>T, p.G223G old nomenclature; p.G241G new nomenclature) does not change the genetic code, but is predicted to create a new donor splice site and therefore interfere with mRNA splicing (HGMD number CS971752) [13].

Discussion

Laron et al. first described 3 children with phenotypic features of GH deficiency associated with high serum GH levels and no response to exogenous GH treatment [1]. Laron syndrome is an autosomal recessive condition, which usually presents with severe postnatal growth failure. The clinical features of patients with GHI are similar to those with severe GH deficiency including severe growth retardation, typical craniofacial appearance, and hypoglycemic episodes because of insensitivity to GH [4, 5, 7]. Biochemical investigations showed GH resistance, with high concentrations of circulating GH associated with low levels of IGF-1 and IGFBP-3. GH-binding protein levels are low when the mutation is identified in the extracellular domain of the GH receptor. As such, exogenous GH administration does not increase serum IGF-1 and IGFBP-3 levels significantly.

These 2 Thai sisters had clinical features and hormonal profiles consistent with a GHI disorder. A clinical scoring system has been devised to identify patients with suspected GHI, which includes height standard deviation score (SDS), basal GH level, basal IGF-1 level, IGF-1 response to an IGF-1 generation test, and GH-binding protein (GHBP) level [7]. In a series of 27 cases of GHI from 8 European countries and Australia [7], median height of the patients was –6.1 SDS and weight was –3.2 SDS. Additionally, 33% of these patients had evidence of hypoglycemia. Birth weight and birth length were normal or slightly below average (median birth weight –0.72 SDS, range 1.75 to –3.92), suggestive of a possible prenatal effect of GH resistance. The parents of the young sisters reported here, had heights below average for Thai growth standards [6]. This has previously been reported in the parents of patients with GHI secondary to *GHR* mutations [5]. Blue sclera are a clinical feature of Laron syndrome, initially described in the original report of the Ecuadorean cohort in 1990 [8]. *GHR* spans 87 kilobases and consists of 9 coding exons [9]. The GHR protein consists of a large extracellular domain involved in GH binding and GHR dimerization, a single transmembrane domain that anchors the receptor to the cell surface, and an intracellular domain involved in GH signaling. The circulating GH-binding protein corresponds to the extracellular domain of GHR. Numerous GHR defects have been reported worldwide and are associated with a range of phenotypes [10]. A milder phenotype, which may not have the cardinal features of Laron syndrome, is

reported in patients with dominant negative *GHR* mutations and the intronic pseudoexon *GHR* mutations compared with those with missense and nonsense *GHR* mutations who have more severe growth failure [5].

Additionally, a group of patients with GHI have a positive correlation between height SDS and IGF-1 SDS and IGFBP-3 SDS [10]. Guevera-Aguirre et al. originally described the correlation of height SDS with serum IGF-1 in adults with Laron syndrome and with IGFBP-3 in adults and children [4]. Some *GHR* defects are more common in certain ethnic groups such as the homozygous *GHR* splice site mutation known as E180 in the Ecuadorean GHI population [11] and exon 3, 5, and 6 deletion in Oriental Jewish families [9, 12]. The same exon 7 (723 C>T) *GHR* mutation carried by these 2 Thai sisters was previously identified in a Spanish patient with Laron syndrome [13]. Although this mutation does not change the genetic code, the mutated sequence creates a putative cryptic donor splice site [ggtgagt], resulting in the deletion of 63 nucleotides from the mRNA, and 21 amino acids in the carboxy-terminal end of the extracellular domain of the GHR. This defect can be compared to the mutation identified in Ecuadorean patients with Laron syndrome [11] and another exon 7 splice site mutation that results in the elimination of 21 amino acids from the extracellular domain of the GHR reported in a Bahamian genetic isolate of Anglo-Saxon origin [14]. Consistent with our patients, the Bahamian patients also had severe short stature (height SDS range –3.7 to –6.3) and clinical features suggestive of LS including the typical craniofacial appearance and truncal obesity.

In conclusion, we report 2 Thai sisters who have a homozygous mutation of the GHR gene. To our knowledge, this is a first report of Laron syndrome in Thais confirmed by genotyping.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SW assessed patients, performed hormone investigations, reviewed literature, and drafted the manuscript; OP assessed patients, performed initial investigations, and referred patients; LS performed the genetics laboratory work; LAM and MOS advised on the molecular genetic data; HLS performed the

molecular study; OP, LS, LAM, MOS, and HLS critically revised the manuscript. All authors approved the final version submitted for publication and take full responsibility for all statements made in the manuscript.

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