Growth Hormone Deficiency in Children: From Suspecting to Diagnosing

VARUNA VYAS, #ANIL KUMAR AND #VANDANA JAIN

From Department of Pediatrics,AIIMS, Jodhpur; and #Division of Pediatric Endocrinology, Department of Pediatrics, AIIMS, New Delhi; India.

Correspondence to: Dr Vandana Jain, Professor, Division of Pediatric Endocrinology, Department of Pediatrics, All India Institute of Medical Sciences, NewDelhi 110029. drvandanajain@gmail.com

Received: May 22, 2017; Initial Review: June 22, 2017; Accepted: September 07, 2017.

Isolated Growth hormone deficiency is an important and treatable cause of short stature. However, it is often difficult to diagnose the condition with certainty due to the lack of a single robust diagnostic test. Short children, other than those with the classical phenotype of immature chubby facies, truncal obesity and micropenis in boys, or those with history of cranial lesions with known association with hypopituitarism, should be evaluated for growth hormone deficiency only after excluding the other more common conditions. These children typically have height markedly below that expected for their midparental height with low height velocity and delayed bone age. Growth hormone levels should be checked by provocative testing, after ensuring that the child is euthyroid, and after priming with sex steroids if indicated. Low levels of Insulin-like growth factor 1 and Insulin-like growth factor binding protein 3 and pituitary abnormalities on neuroimaging provide important corroborative evidence to the diagnosis.

Keywords:*Diagnosis,Short stature,Height velocity, Hypopituitarism, IGF1.*

Short stature or poor growth is a common reason for referral to a pediatrician. For children in whom causes such as nutritional deficit, familial short stature, constitutional delay of growth and puberty, chronic systemic illness or malabsorption (including celiac disease), hypothyroidism, and in case of girls, Turner syndrome have been excluded, the deficiency of growth hormone needs to be considered.

Growth hormone (GH) is a polypeptide hormone secreted by the anterior pituitary gland and is the chief driver of statural growth during childhood. Human Growth Hormone (hGH) extracted from cadaver pituitary glands was first isolated in 1956, and by 1959 its clinical use in patients with presumed GH deficiency had started. In 1985, the use of hGH was abruptly halted amidst reports of death due to Creutzfeldt Jacob Disease (CJD) in some recipients [1]. Later in the same year, synthetic recombinant human growth hormone (rhGH) was approved by the United States Food and Drug Administration (USFDA) for clinical use. The indications for GH therapy were sequentially expanded to include chronic renal insufficiency in 1993, Turner syndrome in 1997, Prader Willi syndrome in 2000, small for gestational age in 2001, idiopathic short stature in 2003, and Noonan syndrome in 2007 [2].

However, the unlimited availability of rhGH increased the potential for its misuse; as an anabolic agent by athletes, as a 'supposed' anti-ageing agent, and in children who are not short by definition but falling short on parents' expectation of stature. Lack of a simple one point robust test for diagnosing GH deficiency, compounds this problem further [3]. Erroneous interpretation of a random GH level as low by a physician leaves room for unwarranted prescriptions.

We, herein, present a brief review of the physiology of growth hormone, and discuss the clinical, biochemical, radiological and genetic investigations for diagnosis of growth hormone deficiency in children, with special focus on avoidance of over-diagnosis of this condition.

PHYSIOLOGY OF GROWTH HORMONE

GH is secreted from the anterior pituitary in a pulsatile manner under hypothalamic regulation and other physiologic regulators. There are approximately 10 daily pulses of GH secretion of about 90 minutes duration in total, each separated by about 128 minutes. The level of GH in between the pulses is nearly undetectable. Therefore single isolated unprovoked GH measurement is of no value, and diagnosis of GH deficiency is made by drawing 4-5 blood samples at half-hourly intervals after administering a pharmacological stimulant [4].

Around 50% of the circulating GH is bound to Growth hormone binding protein (GHBP). GH acts on the liver, muscle and bone, and mediates the production and release of insulin-like growth factor (IGF) 1 and 2 from these organs. Stimulation of linear growth in children is chiefly mediated by IGF1. IGF2 is important for antenatal growth but its role postnatally is not clear. Hepatic IGF1 circulates

in blood almost completely bound to IGF binding proteins (IGFBPs), a group of sixstructurally related proteins that bind IGFs with high affinity. Of these, IGFBP3 binds 75% to 90% of the circulating IGF1. This complex is stabilized by Acid Labile Subunit (ALS), which increases the half life of IGF1 [5].

ETIOLOGY OF GROWTH HORMONE DEFICIENCY

The major congenital and acquired causes of GH deficiency are summarized in ***Table* I** [6].

Suspecting Growth Hormone Deficiency

Short stature is defined as height less than -2 standard deviations (SD) for age-and sex- appropriate population norms. This implies that one in every 40 normal children is short. However, the estimated prevalence of GH deficiency is 1 in 4,000 to 1 in 10,000 [7], and hence, GH deficiency is a relatively rare cause of short stature. Before evaluating a short child for GHD, commoner causes such as physiological (familial short stature or constitutional delay of growth and puberty), hypothyroidism, small for gestational age (SGA), chronic systemic disease, celiac disease, Turner syndrome, or skeletal dysplasia need to be considered and appropriately ruled out [7].

Children with GHD have normal anthropometry at birth. Those with congenital hypopitutarism may have hypoglycemia, jaundice and micropenis in the neonatal period. The most common presentation is with complaint of poor growth in childhood. Typically, these children have immature facies, mid-facial hypoplasia, pot belly, low height velocity (<4 cm/ year during childhood) and delayed skeletal maturation.Presence of midline anomalies such as single central incisor may be a pointer to hypopituitarism. Acquired deficiency due to cranial lesions typically presents as slowing or even cessation of linear growth. This may be accompanied by other features such as polyuria and polydipsia (posterior pituitary involvement), visual impairment and headache.

As per the consensus guidelines of the Growth Hormone Research Society [7], investigation for GH deficiency should be considered only in children who fulfil at least one of the criteria listed in ***Box* 1**.

TABLE I ETIOLOGY OF GROWTH HORMONE DEFICIENCY

Congenital
Genetic
• Multiple pituitary hormone deficiencies: Mutations in *HESX1, LHX3, LHX4, SOX3, GLI2, PROP1, PITX2* and *PIT1* genes.
• Isolated GH deficiency: Mutations in *GH1, GHRH* and *GHRHR* genes
Congenital cranial malformations
• Holoprosencephaly, schizencephaly, septo optic dysplasia
• Syndromic: Pallister Hall syndrome, Rieger syndrome, Prader Willi syndrome
Acquired
Tumors
• Benign: Craniopharyngioma, arachnoid cyst, pituitary adenoma, Rathke's cleft cyst
• Malignant: Dysgerminoma, meningioma, glioma, metastatic Hodgkin's disease
Trauma: Surgical, skull fracture, birth injury
Inflammation: Histiocytosis, sarcoidosis, tuberculosis, meningitis, hemochromatosis, autoimmune hypophysitis,
Pituitary apoplexy
Irradiation

DIAGNOSIS OF GROWTH HORMONE DEFICIENCY

Provocative GH Testing

A period of fasting is required before all provocative GH testing protocols. It is imperative to ensure that all patients are euthyroid at the time of testing. A summary of the various protocols is given in ***Table* II [4]**. It is recommended that in children with suspected isolated GH deficiency, two provocative tests should be done either sequentially or on separate days, and the deficiency should be diagnosed only if the peak GH values in both the tests are below the diagnostic cut-off.

Puberty and its implications on GH testing

With the onset of puberty, there is a large increase in the concentration of circulating sex steroids, which augment

Box 1 CRITERIA FOR CONSIDERING INVESTIGATION FOR GH DEFICIENCY

- Height below -3 standard deviations (SD) for age- and sex-appropriate population norms
- Height more than 1.5 SD below midparental height
- Height below -2 SD and height velocity below -1 SD for age and sex, or a decrease in height SD of more than 0.5 over 1 yr in children aged >2 yr
- Children who are not short, but have height velocity below -2 SD over 1 yr, or below -1.5 SD sustained over 2 years (can occur in organic acquired GHD)
- Signs suggestive of intracranial lesion
- Features of multiple pituitary hormone deficiencies
- Neonatal symptoms and signs of growth hormone deficiency

GH secretion. In the immediate prepubertal period, discriminating between constitutional delay in growth and puberty (CDGP) and GHD is difficult. In a study in 84 children, it was observed that 61% of the children with Tanner stage I failed to achieve peak GH levels of >7 μg/L in response to pharmacological provocative testing, while all the children at stage IV/V were able to achieve peak GH >7μg/L. Administration of estrogen to prepubertal subjects resulted in an increase in the range of peak GH level from 1.9-20.3 μg/L to 7.2–40.5 μg/L [8]. In another study, 84 pre- or early-pubertal boys with short stature, height velocity <4 cm/year and failed GH provocative test were divided into two groups, one primed with low dose (62.5 mg/m^2) and the other, with conventional dose (125 mg/m^2) intramuscular testosterone. On retesting, 54% of the low dose group and 60% of the conventional dose group achieved peak GH values >10 μg/L [9].

Surveys of pediatric endocrinologists indicate that there is no standard practice for priming peripubertal children with sex steroids [10]. The Pediatric Endocrine Society in its recent guidelines on diagnosis and treatment of GH deficiency has recommended that prepubertal boys >11years and prepubertal girls >10 years of age should undergo priming, especially if their predicted adult height is within -2 SD of the reference population mean. Priming with sex steroids reduces the chances of misdiagnosis of children with constitutional delay of growth and puberty as GHD [11]. Estradiol valerate1-2 mg can be used for both boys and girls on each of the two evenings prior to the test. Alternatively, boys can be primed with intramuscular depot testosterone 50 to 100 mg 1 week prior to the test [11].

Peak GH during provocative testing: what is the cut-off for GH deficiency?

Conventionally peak GH values below 7 μg/L or 10 μg/L after provocative tests are considered as indicative of GH deficiency. However, these cut-offs are a matter of debate. In a study that evaluated the secretion of GH after stimulation by clonidine, insulin and arginine in 7- to -18 year-old children with normal stature, it was noted that the mean (SD) peak values for GH were higher in response to clonidine [21.0 (10.7) μg/L], compared to those in response to arginine[13.1 (6.1) μg/L] or insulin [14.2 (6.3) μg/L] [12]. It was also observed that the mean (SD) peak GH level after clonidine stimulation increased from 12.8 (5.1) μg/L in Tanner I girls to 35.5 (5.1) μg/L in Tanner IV/V girls and similarly, from 16.9 (6.7)μg/L in Tanner I boys to 26.5 (12.0) μg/L in Tanner IV/ V boys. In 1996, Ghigo,*et al.* [13] published a study that assessed the reliability of some of the provocative agents used in GH testing. Ideally, administration of these agents should be able to produce peak GH values above the conventional cut-offs in normal children. In 472 children, including those with short as well as normal stature, but with normal height velocity, normal IGF1 levels, and no delay in bone age, it was observed that using various provocative agents, 23% to 49% of these GH non-deficient subjects failed to achieve peak GH values above 10 μg/L, and 10–24% had peak GH <7 μg/L [13].

The Pediatric Endocrine society (PES) 2016 guidelines also caution against using provocative GH testing as the sole basis for diagnosing GH deficiency [11]. While patients with severe GH deficiency generally have very low values on provocative testing, the threshold that distinguishes partial GHD from normal is not clear. It is also important to remember that obese children have blunted GH peak in response to various stimuli [11]. Data from several big post-marketing surveys of GH therapy suggest that the most significant increase in height velocity and height standard deviations are seen in those children who had a peak GH value <5μg/L at diagnosis [14-16].

Situations when provocative GH testing is not needed for diagnosing GH deficiency

In short children with low height velocity, who have a known hypothalamic-pituitary defect, such as a major congenital malformation, history of cranial tumor or irradiation, and deficiency of at least one more pituitary hormone, provocative GH testing is not needed to diagnose GHD. Similarly, a newborn with hypoglycemia, who has a GH level <5 μg/L in a critical blood sample and has the classical triad of hypoplastic anterior pituitary, ectopic posterior pituitary and abnormal stalk on neuroimaging; and/or deficiency of at least one other pituitary hormone can be diagnosed as having GH deficiency [11].

IGF1 and IGFBP3

The production of IGF1 and IGFBP3 is dependent on GH. More than 75% of circulating IGF1 is bound to IGFBP3 and this complex has a half life of 16 hours [17]. The two, hence, seem to be very convenient molecules that can be measured for a functional bioassay of GH. However, these also have their own limitations; the foremost being that their levels may be affected by nutritional status, age, thyroid function, degree of sexual maturation, genetic factors and liver function [18]. IGFBP3 is less affected by these factors as compared to IGF1. There is no single cut-off for IGF1 and IGFBP3, and values have to be compared to age, genderand pubertyspecific normative reference data [19-21].

In a retrospective analysis in 33 children with GH

TABLE II PROTOCOLS FOR PROVOCATIVE TESTING OF GROWTH HORMONE SECRETION

Pharmacologic stimulus (route) Dose	*Mechanism*	*Time of sampling & GH peak*	*Remarks*
Insulin (i.v.) 0.05- 0.1 unit/kg	Induces hypoglycemia and GH secretion occurs as a counter regulatory mechanism	0, 30, 60 90, 120 minutes, GH peak at 30-60 minutes	There is significant risk of hypoglycemia* and has to be performed under strict supervision.
Clonidine (p.o.) 5 µg/kg (maximum 250 µg)	α2 adrenergic agonist, increases GHRH release and inhibits somatostatin release.	0, 30, 60, 90 minutes GH peak at 60 minutes	Hypotension, and drowsiness may occur during the test. Water should be allowed freely. Low chances of a false positive result.
Glucagon (s.c. / i.m.) 0.03mg/kg (maximum 1mg)	Causes hyperglycemia which further leads to insulin secretion and then GH secretion	0, 1,2,2.5 and 3 hrs GH peak at 2-3 hrs	
Levodopa (p.o.) 125 mg for wt < 13.5 kg; 250 mg for wt 13.5- 31.5 kg; 500 mg for wt >31.5 kg	Stimulates GHRH release Usually done in combination with Arginine stimulation test	0, 30, 60, 90 and 120 minutes GH peak at 45 minutes	Levodopa is only available as levodopa – carbidopa combination. The combination with lowest carbidopa: levodopa (1:10) ratio should be used.Can cause vomiting and headache.
Arginine (i.v.) 0.5mg/kg (max 40 mg) diluted in NS and infused over 30 minutes	Inhibits somatostatin release	0, 30, 60, 90, and 120 miutes. GH peak at 60 minutes	Not available in India.
Growth hormone releasing hormone (i.v.)1 or 2mg/kg i.v. bolus	Acts directly on the pituitary to cause GH secretion hence a quicker onset of action	0, 15, 30, 45 and 60 minutes GH peak at 15-30 minutes	Not available in India. May be associated with flushing.

*i.v.-intravenous;wt-weight; NS – Normal Saline; p.o.- per oral; s.c.- subcutaneous; i.m.- intramuscular; *GH provocative testing with insulin needs constant supervision as hypoglycemia can lead to seizures and even death. The blood glucose levels should fall below 40 mg/dl, or to 50% of the previous levels. The peak GH secretion usually occurs 20 minutes after the glucose nadir, which is usually seen at 20-30 minutes. It should not be done in children younger than 3 years, those with history of seizures, and those with suspected adrenal insufficiency due to risk of hypoglycemia. Glucose solution and hydrocortisone injection should be kept ready. This test can also be used to assess adrenal insufficiency by measuring serum cortisol in the 0, 60 and 90 minute samples.*

deficiency (diagnosed on the basis of short stature, delayed bone age, two failed GH provocative tests, hypothalamo-pituitary anomalies on MRI, height catch-up on GH therapy, and a repeat GH provocative test value <10 µg/L after completion of linear growth) and 56 children with idiopathic short stature, a cut-off value of <10 µg/L to define GH deficiency using provocative tests was 100% sensitive and 57% specific. Decreasing the cut-off to 7 µg/L changed the sensitivity and specificity to 66% and 78%, respectively. Low IGF1 had a sensitivity of 73% and specificity of 95%, low IGFBP3 had a sensitivity of 30% and specificity of 98%, andlow height velocity had a sensitivity of 82% and specificity of 43%. Combination of height velocity and IGF1 had a sensitivity of 95% and specificity of 96% [22].In another similar study, IGFBP3 and IGF1 were evaluated in patients with GH deficiency and idiopathic short stature. It was observed that for diagnosing GHD, low IGF1 (<5th centile) had a sensitivity of 69%. The specificity was 91% in children aged <11 years but only 53% in children aged >11 years.The specificity of low IGFBP3 was 100% for diagnosis of GHD, but sensitivity was less than 50% [23].

In conclusion, low levels of IGF1 and IGFBP3 are reasonably specific for diagnosis of GHD, especially in children younger than 10-11 years of age, but their sensitivity is low, so that normal values are not sufficient to exclude GH deficiency. If height velocity is combined with the laboratory parameters, the sensitivity and specificity of the diagnosis improve.

Neuroimaging

As per Growth Hormone Research Society Consensus

Guidelines, in patients with confirmed isolated GH deficiency or Multiple Pituitary Hormone Deficiency, magnetic resonance imaging (MRI) with 2 mm slices should be done to note the height/volume of anterior pituitary, position of posterior pituitary and anatomy of the stalk. The abnormalities include pituitary hypoplasia (gland height <-2SD for age), ectopic posterior pituitary and stalk abnormalities [7].

In a recent study in 68 children diagnosed with GH deficiency before 4 years of age, MRI abnormalities were noted in 84% patients with isolated GH deficiency, of which 49% had only isolated pituitary hypoplasia; while in patients with multiple hormone deficiencies100% had complex defects [24].

Genetic Studies

Genetic mutations are identified in a relatively small number of patients with GH deficiency. However, a genetic diagnosis should be established where feasible in children with congenital GH deficiency. It helps in planning follow-up and appropriate evaluation of other family members.GH deficiency can occur due to mutations in transcription factors genes involved in the development of the pituitary gland (summarized in ***Web Table* I**), which typically are associated with multiple pituitary hormone deficiencies; or due to mutations in genes encoding growth hormone (*GH1*), growth hormone releasing hormone (*GHRH*) or its receptor (*GHRHR*), which lead to isolated GH deficiency [25-27].

In a study by Desai, *et al.* [28] in 97 patients with isolated GHD, *GH1* gene deletion was noted in 17%, while *GHRHR* gene mutations were present in 35%. In recent studies from Delhi, among 51 patients with multiple pituitary hormone deficiency, 6% had mutations in *PROP1* gene and 14% in *POU1F1* gene [29]; while among 116 patients with isolated GH deficiency, mutations in *GH1* and *GHRHR* genes were observed in 7% and 21% patients, respectively [30].

BOX 2 KEY MESSAGES RELATED TO DIAGNOSIS OF GROWTH HORMONE DEFICIENCY

- Growth hormone deficiency is a relatively rare cause of short stature, and commoner causes should be ruled out before testing.
- Children with growth hormone deficiency typically have height more than 2 SD below the population norm, low height velocity, delayed bone age, immature facies, midfacial hypoplasia and trunkal obesity.
- Provocative testing lacks specificity. Depending on the provocative agent, between 10-50% of normally growing children can have peak growth hormone values below 10 µg/L. Hence, these tests should be undertaken only in those children where the pre-test probability is already high, such as those fulfilling at least one of the criteria listed in ***Box* 1**.
- Prior to provocative testing, ensure a period of fasting, euthyroid status and appropriate sex steroid priming.
- Low levels of IGF 1 and IGFBP3, especially in combination with low height velocity are important pointers to growth hormone deficiency.
- Presence of structural anomalies of the pituitary strengthens the diagnosis, and MRI is recommended in all patients diagnosed with growth hormone deficiency.
- Genetic testing should be done in children with congenital growth hormone deficiency, where feasible.

CONCLUSION

The key messages related to diagnosis of GH deficiency in children are summarized in ***Box* 2.**

Contributors: The review was conceptualized by VJ. All thethree authors contributed to literature search and writing the review.
Funding: None*; Competing interests:* None

REFERENCES

1. Tattersall R. A history of growth hormone. Horm Res. 1996;46:236-47.
2. Ayyar VS. History of growth hormone therapy. Indian J Endocrinol Metab. 2011;15:S162-S165.
3. Guyda HJ. Growth hormone testing and the short child. Pediatr Res. 2000;48:579-80.
4. Carrillo AA,Bao Y. Hormonal dynamic tests and genetic tests used in pediatric endocrinology. *In*: LifshitzF, editor. Pediatric Endocrinology, 5thed. New York: Informa Healthcare; 2007.p.737-67.
5. Domené HM, Bengolea SV, Martínez AS, Ropelato MG, Pennisi P, Scaglia P, *et al*. Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. N Engl J Med. 2004;350:570-7.
6. Rosenbloom AL, Connor EL. Hypopituitarism and other disorders of the growth hormone–insulin-like growth factor-I axis. *In*: LifshitzF, editor. Pediatric Endocrinology. 5thed. New York: Informa Healthcare; 2007.p.65-99.
7. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and Adolescence: Summary statement of the GH research society. J Clin Endocrinol Metab. 2000;85:3990-3.
8. Marin GA, Domene HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB Jr. The effects of estrogen priming and puberty on the growth hormone response to

standardized treadmill exercise and arginine-insulin in normal girls and boys. J Clin Endocrinol Metab. 1994;79: 537-41.
9. Gonc EN, Yordam N, Kandemir N, Alikasifoglu A. Comparison of stimulated growth hormone levels in primed versus unprimed provocative tests. The effect of various testosterone doses on growth hormone levels. Horm Res. 2001;56:32–7
10. Murray PG, Dattani MT, Clayton PE. Controversies in the diagnosis and management of growth hormone deficiency in childhood and adolescence. Arch Dis Child. 2016;101:96-100.
11. Grimberg A, DiVall SA, Polychronakos C, Allen DB, Cohen LE, Quintos JB, *et al*. Guidelines for growth hormone and insulin-like growth factor-I treatment in children and adolescents: Growth hormone deficiency, idiopathic short stature, and primary insulin-like growth factor-I deficiency. Horm Res Paediatr. 2016;86:361-97.
12. Zadik Z, Chalew SA, Kowarski A. Assessment of growth hormone secretion in normal stature children using 24-hour integrated concentration of GH and pharmacological stimulation. J Clin Endocrinol Metab. 1990;71:932–6.
13. Ghigo E, Bellone J, Aimaretti G, Bellone S, Loche S, CappaM,*et al*. Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. J ClinEndocrinolMetab. 1996;81: 3323-7.
14. Ranke MB, Lindberg A, Chatelain P, Wilton P, Cutfield W, Albertsson-WiklandK, *et al*. Derivation and validation of a mathematical model for predicting the response to exogenous recombinant human growth hormone (GH) in prepubertal children with idiopathic GH deficiency. J Clin Endocrinol Metab. 1999;84:1174-83.
15. Bright GM, Julius JR, Lima J, Blethen SL. Growth hormone stimulation test results as predictors of recombinant human growth hormone treatment outcomes: preliminary analysis of the national cooperative growth study database. Pediatrics. 1999;104:1028-31.
16. Fujieda K, Hanew K, Hirano T, Igarashi Y, Nishi Y, TachibanaK, *et al*. Growth response to growth hormone therapy in patients with different degrees of growth hormone deficiency. Endocr J. 1996;43:S19-S25.
17. Clemmons DR. Role of insulin-like growth factor-I in diagnosis and management of acromegaly. Endocr Pract. 2004;10:362-71.
18. Cianfarani S, Liguori A, Boemi S, Maghnie M, Iughetti L, WasniewskaM,*et al*. Inaccuracy of insulin-like growth factor (IGF) binding protein (IGFBP)-3 assessment in the diagnosis of growth hormone (GH) deficiency from childhood to young adulthood: association to low GH dependency of IGF-II and presence of circulating IGFBP-3 18-kilodalton fragment. J Clin Endocrinol Metab. 2005;90: 6028-34.
19. Dehiya RK, Deepa B, Chhaya K, Desai MP. Insulin like growth factor-I, insulin like growth factor binding protein-3 and acid labile subunit levels in healthy children and adolescents residing in Mumbai suburbs. Indian Pediatr. 2000;37:990-7.
20. Lofqvist C, Andersson E, Gelander L, Rosberg S, Blum WF, Wikland KA. Reference values for IGF-I throughout childhood and adolescence: Amodel that accounts simultaneously for the effect of gender, age, and puberty. J Clin Endocrinol Metab. 2001;86:5870-6.
21. Lofqvist C, Andersson E, Gelander L, Rosberg S, Hulthen L, Blum WF, *et al*. Reference values for insulin-like growth factor-binding protein-3 (IGFBP-3) and the ratio of insulin-like growth factor-I to IGFBP-3 throughout childhood and adolescence. J Clin Endocrinol Metab. 2005;90:1420-7.
22. Cianfarani S, Tondinelli T, Spadoni GL, Scirè G, Boemi S, Boscherini B. Height velocity and IGF I assessment in the diagnosis of childhood onset GH insufficiency: Do we still need a second GH stimulation test? J Clin Endocrinol Metab. 2002;57:161-7.
23. Cianfarani S, Liguori A, Boemi S, Maghnie M, Iughetti L, WasniewskaM,*et al*. Inaccuracy of insulin-like growth factor (IGF) binding protein (IGFBP)-3 assessment in the diagnosis of growth hormone (GH) deficiency from childhood to young adulthood: association to low GH dependency of IGF-II and presence of circulating IGFBP-3 18-kilodalton fragment. J Clin Endocrinol Metab. 2005;90: 6028-34.
24. Pampanini V, Pedicelli S, Gubinelli J, ScirèG, Cappa M, BoscheriniB,*et al*. Brain magnetic resonance imaging as first-line investigation for growth hormone deficiency diagnosis in early childhood. Horm Res Paediatr. 2015;84:323-30.
25. Alatzoglou KS, Dattani MT. Genetic causes and treatment of isolated growth hormone deficiency—an update. Nat Rev Endocrinol. 2010;6:562-76.
26. Mullis PE. Genetic control of growth. Eur J Endocrinol. 2005;152:11-31.
27. Online Mendelian Inheritance in Man, OMIM (TM). McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD). Availablefrom:*http://www.ncbi.nlm.nih.gov/omim/.* Accessed January 01, 2017.
28. Desai MP, Mithbawkar SM, Upadhye PS, Rao SC, Bhatia V, Vijaykumar M. Molecular genetic studies in isolated growth hormone deficiency (IGHD). Indian J Pediatr. 2013;80:623-30.
29. Birla S, Khadgawat R, Jyotsna VP, Jain V, Garg MK, Bhalla A, *et al*. Identification of novel *PROP1* and *POU1F1*mutations in patients with combined pituitary hormone deficiency. Horm Metab Res. 2016;48:822-7.
30. Birla S, Khadgawat R, Jyotsna VP, Jain V, Garg MK, Bhalla AS, *et al*. Identification of novel *GHRHR* and *GH1* mutations in patients with isolated growth hormone deficiency. Growth Horm IGF Res. 2016;29:50-6.