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LHX4 Mutation: New Perspective

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1. Abstract

Some transcription factors orchestrate the ontogeny of the pituitary gland, maintain the differentiated state and mediate the coordinated expression of specific cell type and their alteration can be involved in isolated and combined deficits. Among them, LHX4 gene mutations can manifest as a combined pituitary hormone deficiency and are associated with an ectopic posterior pituitary and/or a sella turcica defect. We describe a patient with growth failure and recurrent sleep apneas. We excluded the most frequent causes of growth failure. Obstructive sleep apnea syndrome (OSAS) is reported. A genetic analysis revealed a mutation in the LHX4 gene, as part of an in-depth genetic study in idiopathic short stature. The brain MRI showed no abnormalities. At the age of 6.8 years, we started a trial therapy with rhGH 33 µg//kg/day, but due to onset of behavioral disorder and the lack of improvement the treatment was suspended after 6 months. Our patient has a mutation that is already described as putative, but he doesn't have any pituitary deficit nor any alteration at the brain MRI. His short stature can't be explained by a GHD, moreover the trial treatment with rhGH didn't work. The central OSAS could be related to the LHX4 mutation since studies on mice demonstrated a ventral motor neuron defect that impair respiratory movements. This could be a possible manifestation in humans which let us broaden the clinical manifestation associated with such mutation. Further studies are needed to better elucidate the relationship between short stature and LHX4 mutations.

2. Introduction

Congenital hypopituitarism (CH) is defined as the deficiency of one or more hormones produced by the anterior pituitary (AP) or released from the posterior pituitary (PP). Its estimated incidence is between 1 in 4,000 and 1 in 10,000 live births [1]. The pituitary gland is the central regulator of growth, metabolism, reproduction and homeostasis. It is located within the sella turcica and consists of three lobes of dual embryologic origin. The

adenohypophysis (anterior and intermediate lobes) originates from Rathke's pouch, an invagination of the oral ectoderm, whereas the neurohypophysis (posterior lobe) develops from the neural ectoderm of the ventral diencephalon. The PP releases antidiuretic hormone (ADH or AVP) and oxytocin, stored in vesicles, as part of axonal projections from hypothalamic cells, known as the hypothalamic-hypophyseal tract. The intermediate lobe contains melanotrophs which produce proopiomelanocortin (POMC), a major precursor to endorphins, and alpha-melanocyte stimulating hormone (αMSH), although in humans is typically an embryological remnant and may not be present. The AP consists of five different cell lineages producing six hormones: somatotrophs (growth

hormone, GH), gonadotrophs (follicle stimulating hormone, FSH,

and luteinising hormone, LH), corticotrophs (adrenocorticotropic hormone, ACTH), thyrotrophs (thyroid stimulating hormone, TSH), and lactotrophs (prolactin, PRL). CH refers to a deficiency of one or more pituitary hormones resulting from events during fetal development. This may be the result of genetic mutation, antenatal insult, or as is commonly the case, be idiopathic. The overall incidence of genetic mutations in these patients is 16%, indicating that probably many genes remain to be identified [2]. CH may present as isolated or combined pituitary hormone deficiencies (CPHD) and may be part of a syndrome involving extra- pituitary abnormalities. Pituitary deficiencies may be detected during the neonatal period; however, in some individuals' pituitary deficiencies may not manifest until later in childhood. Furthermore, the severity can be variable [3]. Embryological pituitary development involves a complex interplay of transcription factors, extrinsic and intrinsic to the oral ectoderm and neuroectoderm which develop to form the mature pituitary. Abnormalities in genes expressed early in development such as SOX2, HESX1 and GLI2 more frequently effect other nearby structures as the eye, olfactory bulbs, midline structures, and forebrain, whereas those expressed later such as PROP1 and POU1F1 typically have localized effects [4]. Transcription factors may be induced, upregulated or downregulated by multiple other transcription factors and the degree and site of expression can vary across the embryological development [5]. There is considerable phenotypic variability within known genetic causes of hypopituitarism, with some forms having incomplete penetrance, and presentation ranging from asymptomatic, to severe neonatal onset forms. Among the different transcription factors, the structurally related LHX3 and LHX4 proteins are members of the LIM-homeodomain (HD) family of transcription factors LIM-HD proteins. They feature two amino-terminal LIM domains, which are required for multiple roles, including protein- protein interactions, and a central DNA-binding HD [6]. Studies of LHX3 and LHX4 mutations have shown that both genes drive the formation of the pituitary gland. Although LHX4 is required for the proliferation of lineage precursors, LHX3 is necessary to establish the fate of pituitary precursor cells [7]. While LHX3 and LHX4 share marked similarities in protein structure, the genes have different expression patterns, and their overlapping but distinct roles in development have been revealed by single and combined gene targeting in mice [6]. Studies on humans and rodents have observed expression of the LHX4 gene in the developing hindbrain, cerebral cortex, pituitary gland and spinal cord. Haploinsufficiency for LHX4 is sufficient to affect pituitary and brain development in humans, whereas heterozygous mice are unaffected [8]. In the pituitary, LHX3/4 proteins have been implicated in the regulation of genes including prolactin, TSHβ, FSHβ, and the PIT1 transcription factor [9, 10]. In LHX4 -/- mice all anterior pituitary cell types differentiate in the subsequent course of cell lineage specification, but with markedly diminished cell numbers resulting in a hypoplastic AP [11]. Mice with a homozygous LHX4 gene disruption die shortly after birth from lung defects, whereas heterozygous animals are apparently unaffected [12]. Death within 24 hours after birth in LHX4 -/- mice is attributed to ventral motor neuron defects that impair respiratory movements, and they also have lung hypoplasia [13, 8]. The LHX4 gene is found in humans at chromosome 1q25.2 with autosomal dominant expression causing 0.9-1.4% of hypopituitarism cases [14], [15] with variable penetrance and asymptomatic effected parents being a common occurrence [16]. LHX4 mutation typically results in CPHD [17], but milder presentations, including transient isolated GHD, have been reported [18]. In humans, the range of pituitary malformations in most cases comprises hypoplasia of the anterior lobe; however, an enlarged [19] or normal [20] anterior pituitary gland has also been observed. Cystic lesions within the pituitary have been reported in two families [20, 21]. Other brain abnormalities can also be present such as an ectopic posterior gland and a hypoplastic sella turcica as well as corpus callosum hypoplasia, cerebellar malformation or Chiari syndrome [1, 22]. Cardiac defects have been reported in 2 cases and respiratory distress in 4 cases [23]. In humans, only one family of recessive lethal LHX4 has been reported with severe combined hypopituitarism and fatal respiratory distress thought to be secondary to their hypopituitarism [24]. In this article we would like to present the case of a patient with short stature and an identified mutation of LHX4 gene.

3. Case Presentation

We describe a patient who came to our service of Pediatric Endocrinology for growth failure. From the family history point of view, no relevant pathologies are reported; the parents are not related and their final height is 158 cm for the mother and 168 cm for the father (target height 169.5 cm). The patient is born at term, with normal delivery and a regular pregnancy, without problems reported with prenatal ultrasounds. Birth weight was 3040 g (-1.1 SDS), length 48 cm (-1.5 SDS), head circumference 33 cm (-1.5 SDS). To calculate the anthropometric data, we use the Italian Neonatal Study (Bertino charts) [25]. At the clinical evaluation conducted at birth, no dysmorphisms nor malformations were present. His growth appears to be regular until about 6 months of age, when a slowdown in statural and ponderal growth begins to be observed. The first pediatric endocrinology visit was at the age of 2 years and 10 months. The height was measured at 84.7 cm (-2.4 SDS) and weight at 11 kg (-2.6 SDS) (Figure 1). X-rays revealed a delayed bone age of about 10 months, with metaphyseal striations probably due to calcium and vitamin D deficiency; Since he was not on supplementation, we therefore gave him vitamin D 2.000 UI dayly and calcium 800 mg/day with a stabilization in vitamin D laboratory values over the years (Vitamin D: 82.3 nmol/L after 6 months). Nighttime apneas were also reported during this visit. Due to this, a polysomnography was scheduled and an obstructive

sleep apnea syndrome (OSAS) with central predominant component of mild degree was identified. It also showed a mild degree desaturation index, but no snoring. Given the mild degree of the problem, there was no indication to start a treatment (Continuous Positive Airway Pressure, CPAP). Even in the following years, the disorder remained stable. A reevaluation at the age of 4 years and 5 months showed a height of 93.6 cm (-2.7 SDS) and weight of 13.6 kg (-2.4 SDS). Weight and height still fell below the normal limits for gender and age; therefore, we programmed some investigations to exclude the most common causes of growth failure. In this occurrence, we performed an X-rays of the wrist to evaluate the bone age, that appeared to be delayed by about a year, according to the Atlas of Skeletal Development of the Hand and Wrist by Greulich e Pyle [26]. The celiac disease was excluded by the negativity of the immunoglobulin A tissue transglutaminase antibodies; thyroid function was found to be in the normal range (TSH 1.61 mUI/L; FT4 12.8pmol/L); there were no chromosomal abnormalities (Karyotype: 46 XY), and there wasn't SHOX gene alteration. We didn't find a GH deficiency (GHD), specifically, the Arginine load curve revealed a basal GH of 0.7 µg/L, with a maximum value after stimulation of 11 µg/L. Finally, no other deficiencies in pituitary hormonal function were found: ACTH 2.64 pmol/L, Cortisol 5.54 g/dL, Somatomedin C (IGF-1) 9.41 nmol/L. At this point we performed a genetic analysis as we usually do in our clinic to investigate idiopathic short stature, it is a panel of more than 100 genes more frequently involved in short stature [27]. The analysis revealed a heterozygous mutation in the variant c.250C>T p. (Arg84Cys) in the LHX4 gene with autosomal dominant transmission; subsequently the same mutation was also detected in the father. On this occasion, the patient's height was 104.1 cm (-3 SDS), weight 16.2 kg (-2.7 SDS). Since growth was still at the lower limits of normality, both in terms of height and weight, we decided to perform a second curve to definitely exclude GH deficiency. So, at the age of 6 years and 6 months, we accomplished a Glucagon load curve that revealed a basal GH of 0.32 µg/L, with a maximum value after stimulation of 11.4 ug/L. As a clinical complement, we performed a brain MRI that showed no abnormalities in the hypothalamic-pituitary region nor other encephalic defects. Subsequently, the control assessments confirmed the absence of pituitary hormonal function deficiencies. Given the mutation and the growth failure persistence, despite the fact that there were no criteria for starting GH therapy, at the age of 6 years and 9 months, we started a trial therapy with rhGH 33 µg/kg/day, asking to the regional GH hormone Committee the authorization to start the treatment, relying on several studies in the literature that describes the benefits of treatment in patients with idiopathic short stature [28-30]. After six months of treatment, the parents reported difficulties in continuing therapy due to poor compliance; in particular, behavioral alterations (increased aggressiveness) and mood swings were observed. Analyzing the growth curve, we found out that the statural and weight growth did not achieve a meaningful improvement: the height went from -3,00 SDS to -2,85 SDS six months later. Due to these difficulties, and seeing that the treatment was not effective, we decided to suspend rhGH therapy; at the re-evaluation of the patient, six months after we stopped the treatment, we found once again a height stable at -3,00 SDS.

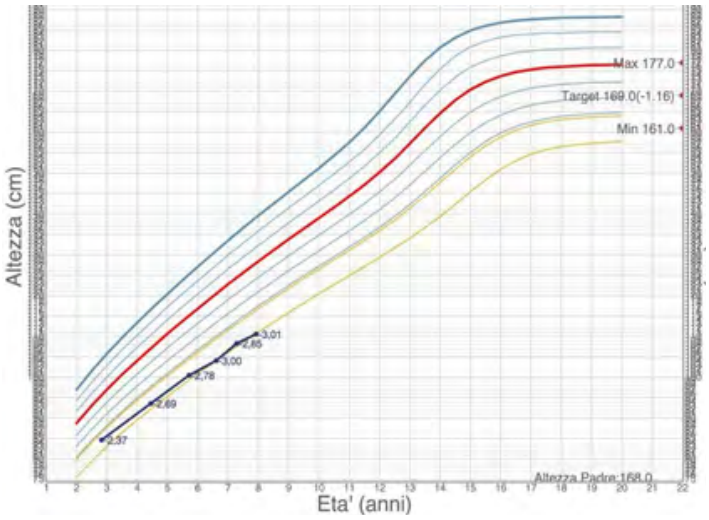
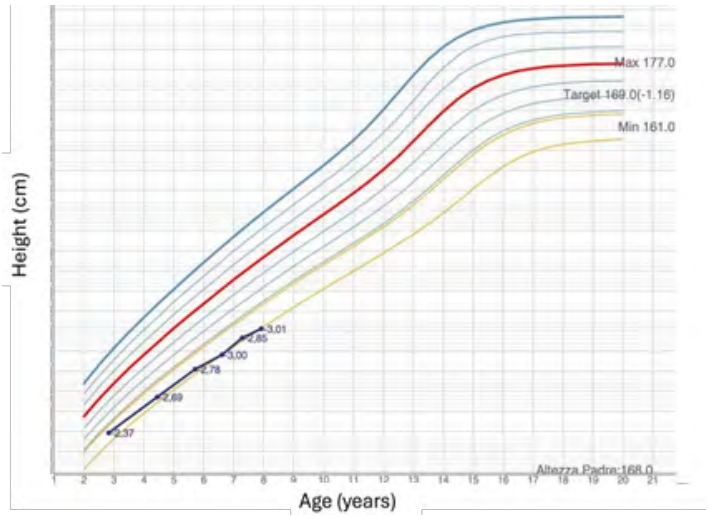


Fig. 1: growth chart



4. Discussion

The LHX4 mutation c.250C>T p. (Arg84Cys) of our patient is already described by Pfaeffle et al, [20]. As putative for CPHD (GHD and central hypothyroidism) with small AP and an ectopic PP. The altered protein, called R84C, has an intact Homeodomain (HD), that regulates gene expression and cell differentiation during early embryonic development. R84C bounds the DNA with similar efficiency to the wild-type protein, but apparently has a different stability because the quantity of the protein is lower compared to the wild type. The heterozygous condition of the LHX4 mutations described by Pfaeffle could be associated with several mechanisms, including dominant negative action of aberrant proteins or by a reduction in the activity of LHX4 to a level below critical

thresholds for developmental steps. Our patient doesn't present the typical clinical features: he doesn't have any pituitary deficit yet nor any alteration at the brain MRI. Usually, patients with LHX4 mutations present a short stature related to their growth hormone deficiency. Our patient doesn't have this deficit, however his height is -3 DS from the mean and the gene panel we performed ruled out other mutations on the most frequently involved genes. Furthermore, the trial treatment with rhGH didn't work because in 6 months he only gained + 0,15 DS. His father has the very same mutation, but his final height is -1.15 DS and, to our knowledge, he doesn't have any pituitary deficits. It is known that the penetrance and the severity of LHX4 mutations are variable [8] and that in some individuals' pituitary deficiencies may not manifest until later in childhood, so we cannot exclude that our patient will develop a pituitary deficit later; in particular, we'll have to explore the gonadotrophin axe at the puberty. To our knowledge, the only explanation for the short stature in those patients is a GHD: growth retardation manifests postnatally with a severe form of short stature and at birth weight and length are in the normal to low- normal range [31]. Patients with heterozygous LHX4 gene mutation represent a phenotypic spectrum with variable penetrance even within the same family. Concerning the respiratory problem of our patient, also the central OSAS could be related to this mutation. Studies on mice demonstrated that homozygous LHX4 mice have a ventral motor neuron defect that impair respiratory movements, associated whit lung hypoplasia; these mice die at birth from lung and ventral motor neuron developmental failure. This means that LHX4 gene mutation is involved also in respiratory symptoms, therefore the central OSAS could be a possible manifestation in humans, which let us widen the clinical manifestation associated with this mutation [32]. So, further studies are needed to elucidate the relationship between short stature and the LHX4 mutation besides the GHD and to assess the pulmonary involvement in those patients. We recommend evaluating the pulmonary function and the possible sleep apnea in patients with LHX4 mutation.

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