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Article

Growth Hormone (GH) and Rehabilitation Promoted Distal Innervation in a Child Affected from a Syndrome of Caudal Regression

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Abstract: Caudal regression syndrome (CRS) is a congenital abnormality characterized by an incomplete development of the spinal cord (SC) and other abnormalities. We studied a 9-months old CRS child presenting: interruption of SC at L2-L3 level, sacral agenesis, lack of innervation of the inferior limbs (flaccid paraplegia) and neurogenic bladder and bowel. Given the effects of growth hormone (GH) on the proliferation, differentiation and migration of neural stem cells (NSCs), we treated him with GH and rehabilitation, trying to induce the recovery of main sequelae. GMFM-88 test score was 12.31%. After a blood analysis, GH treatment (0.3 mg/day, 5 days/week, 3 months and then 15 days without GH) and rehabilitation commenced. This protocol was followed during 5 years, being the last GH dose 1 mg/day. Blood analysis and physical exams were performed every 3 months initially and every 6 months later. Six months after commencing the treatment GMFM-88 score increased to 39.48%. Responses to sensitive stimuli appeared in most of the territories explored; 18 months later sensitive innervation was complete and the patient moved any muscle over the knees and controlled his sphincters. Three years later he walked with the help of canes, there was plantar flexion and GMFM-88 score was 78.48%. In summary, GH plus rehabilitation may be useful for innervating distal territories, below the level of the incomplete spinal cord in CRS. Most likely, GH acts on ependymal SC NSCs, as the hormone does in the neurogenic niches in the brain.

Keywords: GH; syndrome of caudal regression; sacral agenesis; physiotherapy; neurogenic bladder; flaccid paraplegia

1. Introduction

Caudal regression syndrome (CRS) is a very rare congenital abnormality, mainly characterized by an incomplete development of the spinal cord (SC). However, a number of many different abnormalities may also appear in association with this syndrome. Among them, urological abnormalities such as renal agenesis and neurogenic bladder, tethered-cord, sacral agenesis, lipomyelomeningosel, anorectal atresia, orthopaedic deformations and even cardiac malformations [1]. While it is likely that most of these and other abnormalities observed in the syndrome occur as a consequence of the incomplete SC development during the fetal period, some of them may be the consequence of a genetic polymalformative syndrome. This would explain the wide spectrum of clinical presentations of CRS.

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The denomination of syndrome of caudal regression was first used by Duhamel in 1961 [2], describing it as an embryonal defect in the formation of the caudal region. In fact, this developmental abnormality has been related to neurulation alterations during the first 28 days of fetal life or malformations occurring during the fetal differentiation phase [3]. However, the later may be a consequence of the former, since the expression of genes involved in fetal development occurs in a progressive and sequential manner. This would agree with the first description of Duhamel [2], showing that the syndrome can present a wide spectrum of malformations, being the siren monstrosity and malformations of the anal region the two extremes of it (maximal and minimal, respectively).

The exact cause of CRS is unknown yet. It occurs in 2 live births per 100.000 newborns although its incidence increases to 1 in 350 when uncontrolled gestational diabetes exists [4, 5]. This means about 150-fold higher incidence compared to general population. However, no explanation has been given to this finding.

Apart from mother gestational diabetes, a number of potential factors that might play a role in the development of CRS has been suggested. Among them: alcohol, retinoic acid, deficient supply of oxygen to the fetus or putative amino acids imbalances, but no evidences exist about the possible involvement of any of them in the pathogenesis of the syndrome. Therefore, genetic reasons seem to be the most possible causes.

During fetal development GH and Insulin-like growth factors (IGF-I and II) play a key and different role, as we recently proposed [6]. Fetal GH seems not to be responsible for fetal growth; most likely the hormone is involved on the developmental program of virtually all tissues and organs [6]. In turn, Insulin and IGFs would be the factors responsible of growth but also, mainly IGF-II, on the developmental program of the fetus [6].

Apart of its effects on brain repair after an injury [7-12], we first reported that GH administration was able to regenerate transected sciatic nerve in rats [13], a finding also reported by other authors [14, 15], and still unpublished data from our group indicate that GH and rehabilitation improve the sensitive and motor functions of patients with SC injuries, below the level of the spine damage, at least in ASIA B and C patients [16].

On this basis, we decided to follow a similar treatment in a 9-months old child with CRS. His SC development had been interrupted at L2-L3 level, there was sacral agenesis and right renal agenesis, neurogenic bladder and bowel and lack of innervation of inferior limbs. After five years of treatment, most of nerve affectations have been corrected, including sphincters control, and the child is able to walk with the help of canes. This is, as far as we know, the first case in which CRS may be partially corrected (all but renal and sacral agenesis), despite that it is accepted that, since the primary pathology is irreversible, treatment of this syndrome is only supportive [4].

2. Results

Despite that the patient commenced with physiotherapy for rehabilitation early in his life, no significant results had been achieved when he was admitted at our Center.

2.1. Physiotherapy

At admission, the score in the GMFM-88 was 12.31 because the patient was unable to reach any punctuation in dimensions C, D and E. The Asshworth scale indicated that no spasticity existed (0/0 and 0/0, both hemibodies).

Four months after commencing with GH administration and rehabilitation, sensitivity to painful stimulation was detected at L4, L5 and S1 level, indicating that, despite that the SC had been interrupted at L2-L3, sensitive innervation was beginning to be developed below the level of the lesion.

Six months after commencing the treatment, the total score in GMFM-88 increased to 39.48. This clear increase was mainly due to improvements in dimensions A and B, remaining unchanged the other dimensions of the test (0 points in every item of them) with the only exception of item 38 in the dimension C, in which the patient reached a maximum punctuation of 3, since he was able to crawl

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forward 1.80 meters. At this time, the physical evaluation indicated that the patient significantly improved in sensitivity and motor functions. There was responses to sensitive stimuli in quadriceps, ischio-tibialis, tibiales and peroneal muscles, gastrocnemius and feet. In addition, the patient reacted to pressor stimuli in the first phalanx of both feet, but not in the other phalanges. Interestingly, an important improvement was observed in the amplitude of the movement of his knees, beginning to be able to realize a small active flexion of them.

One year later, evoked osteo-tendon reflexes were present in patellar and Achilles tendons (mild and weak responsiveness, respectively).

A new control performed two years after commencing with GH and rehabilitation, showed that sensitive innervation was complete (Movie 2), while at the motor level the patient was able to voluntarily move quadriceps, ischio-tibialis, adductors and abductors, but still not any muscle below the knee. He began to walk with the help of a walker (Movie 3) and he was able to ride in tricycle (Movie 4). Gastrocnemius muscles still were atrophics, but Achilles reflex was clearly evoked. His legs and feet were in external rotation, most likely because of the existing hips luxation.

Very important, the child fully had acquired a complete voluntary control of sphincters.

With regard to his left kidney problem, studies performed in two different hospitals indicated that no vesicoureteral reflux already existed.

One year later the patient was walking with the help of canes (Figure 2).

Figure 2.- The patient is walking with the help of canes.

Three years after commencing the treatment the patient was able to began to walk with the help of canes, but the lack of sacrum produced a flexion of the hips while walking. Notice that the muscles of the legs still lacked a clear development, while there was an increase in the mass of quadriceps and

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ischio-tibialis. Clubfoot persists. White arrow shows where the vertebral column ends, as indicated by a bulging bone in the back.

GH dose was increased to 0.8 mg/day, and melatonin (50 mg/day, orally, before going to bed) was prescribed for counteracting a possible increased production of oxygen free radicals due to the physical effort that walking, without the support of the sacrum and hips luxation, means.

The last control, carried out 5 years after commencing with the combined treatment with GH and rehabilitation, showed that the muscles of the legs began to be developed, mainly on the right side, and also that the three middle fingers of the right foot were able to make flexion and extension movements. Left leg was about 2 cm shorter than the right. Both feet clearly changed their look from the beginning of the treatment, although the left foot looked more hypotrophic than the right. However, the patient was able to make, with both feet, plantar flexion against resistance and a weak dorsiflexion (Movie 5).

The height of the child is now in normal percentiles (p15) and GH dose is 1 mg/day (5 days/week). Melatonin continues being given at a daily dose of 50 mg.

In summary, after 5 years of treatment significant sensorial and motor improvements had been reached, as the last GMFM-88 test performed revealed: 78.48 (maximum = 100). This means that despite that the SC had been interrupted at the L2-L3 level (Figure 4), a significant innervation occurred below the level of the lesion.

No cardiac or respiratory problems existed along the treatment period.

Voluntary control of sphincters persists, but the parents informed that a grade II vesicoureteral reflux had been again detected in the left kidney.

2.2. Imaging exams.

A magnetical resonance imaging (MRI) of the vertebral column, carried out 7-days after birth, showed that the vertebral development had been interrupted at the L2-L3 level, SC was tethered at L3 level and that there was sacral agenesis and right renal agenesis (Figure 1).

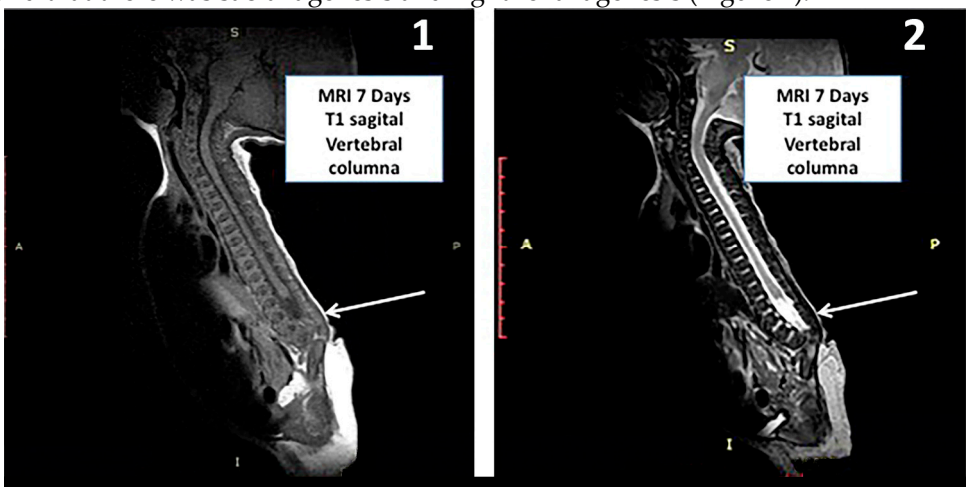

Figure 1.- MRI of the vertebral column MRI performed at 7-days of age.

1.- The white arrow shows that the vertebral column development had been interrupted at L2-L3 level and that the spinal cord was tethered. 2.- A small lipoma can be seen at the end of the conus medullaris (arrow), there was sacral agenesis too. A = Anterior; P = Posterior; S = Superior; I = Inferior.

At 21-days of age an abdominal ultrasound study showed luxation of both hips with an incomplete development of both acetabulae.

At 13-months of age, that is 4-months after commencing with GH treatment and rehabilitation, a MRI study indicated that no changes existed with regard to the first study carried out 7-days after birth. The study indicated agenesis of the sacrum and of lumbar vertebral bodies L4 and L5. L3 was reduced to a simple vestige; iliac bones were articulated in the midline and both femoral heads were luxated. The spinal canal terminated at L3 level, and a tissue with fat intensity, most likely a lipoma,

could be observed in the bottom of the spinal canal. The conus medullaris ended at the level of thoracic T12 vertebra, and both in the conus medullaris and in the total SC a prominent central ependymal canal could be observed. This ependymal canal was enlarged in the region of the conus medullaris and in the cervical SC (data not shown).

At age 3-years old a new MRI study of the SC indicated that there was not any modification in the findings observed in the previous study. That is, there was agenesis of the sacrum and of vertebral bodies L4 and L5 with hypoplastic L3, where the spinal canal ended. The conus medullaris ended at the level of D12 vertebra, and in its lower and posterior part presented a small lipoma, most likely corresponding to a fatty terminal filum. The SC was normal in its caliber and morphology showing a minimal enlargement of the central ependymal canal in the lower cervical and dorsal segments; however, as referred by the radiologist, this enlargement was lower than in the previous study (Figure 3).

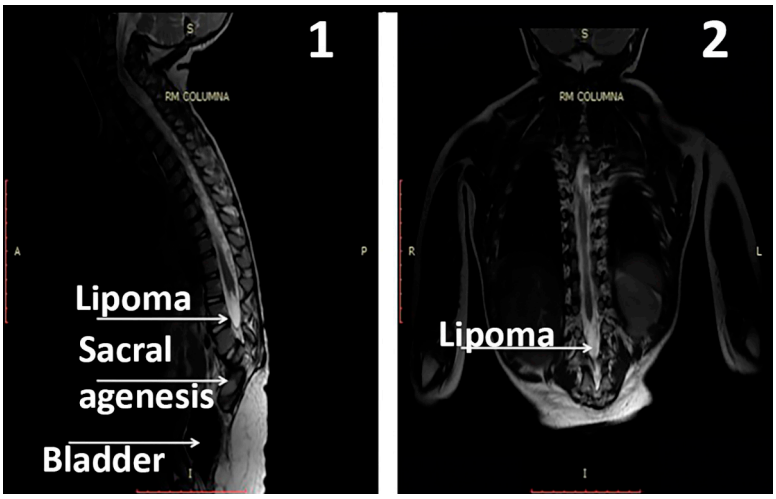

Figure 3.- MRI performed at age 3-years old.

1 and 2 show sagital and antero-posterior images (respectively) of the spinal cord. In 1 sacral agenesis and hypoplastic L3 vertebra can be clearly seen (white arrow). In both images a small lipoma can be seen in the lower part of the conus medullaris. As the images show the SC was normal in its caliber and morphology, but a small hydrosyringomyelia can be seen. In 2 an asymetry between the right and left hip can be observed. A= Anterior; P = Posterior; S = Superior; I = Inferior; R = Right side: L = Left side.

At age 4-years old, a 3D reconstruction of a CT-SCAN allowed to clearly see the abnormalities occurred during the development of the vertebral column and SC. As Figure 4 shows, vertebral development had been interrupted at L2-L3 level. Sacrum did not exist; iliac bones were articulated in the midline, hips were rotated, the development of both acetabulae had been incomplete and both femoral heads were luxated.

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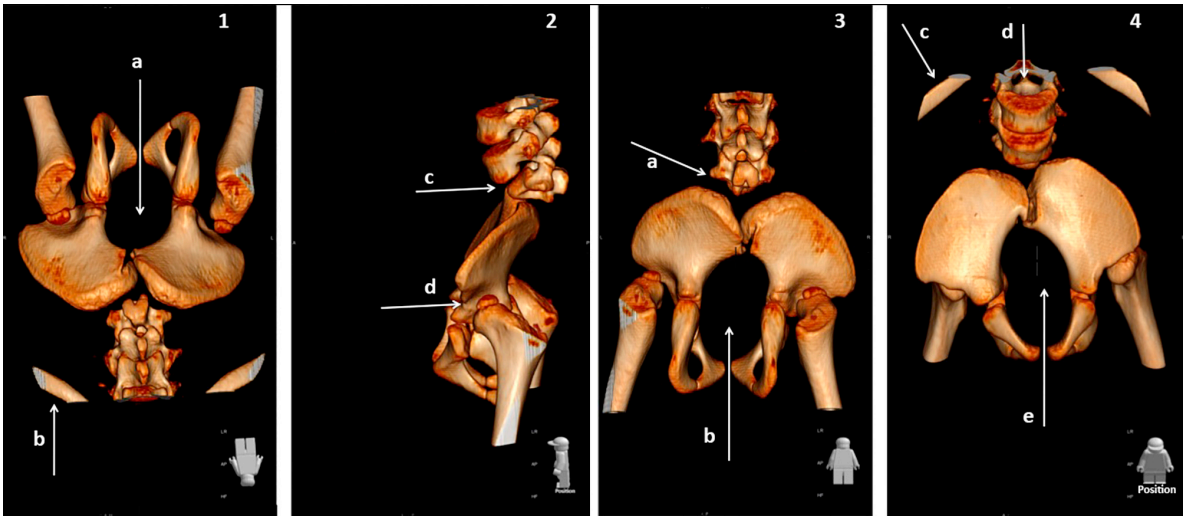

Figure 4.- 3D reconstruction of a CT SCAN showing the vertabral and hips abnormalities.

1.- Inverted position for better see the hypoplastic L3 vertebra. The arrow named a shows the sacral agenesis, while the arrow b indicates the 12th rip (for knowing where the lumbar column begins). 2.- In this sagital caption, arrow c shows the hypoplastic L3 vertebra and arrow d shows the lack of articular congruence between the head of the femur and the abnormal left hip acetabulum. 3.- Posterior caption where arrow a shows the abnormal L3 and arrow b shows sacral agenesis. Notice too the iliac bones articulated in the midline, the rotation of the hips and the incomplete development of both acetabulae, and femoral heads luxated. 4.- Oblique caption for seeing the spinal cord (arrow d). Arrow c shows the 12th rip and arrow e shows the sacral agenesis. Notice again the articulation of iliac bones and the rotation of the left hip.

Movie 6, shows the motor evolution of the child before he began to walk with canes.

2.3. Blood Analysis

Pre-treatment blood analyses were practically normal, excepting for a slightly elevated plasma creatinine value (0.51 mg/dL; normal range: 0.2-0.49 mg/dL) and low plasma IGF-I value (48 ng/mL; normal range for his age 50-354 ng/mL), while IGF-Binding protein 3 (IGFBP3) was normal (2.4 µg/mL; normal range: 0.7-3.9 µg/mL). Erythrocytes and Hb were in normal values, despite that serum iron was low (28 µg/dL; normal range: 40-100 µg/dL). Plasma proteins were in normal values. Thyroid Stimulating Hormone (TSH) was normal (3.02 µUI/mL), as it was free Thyroxine (fT4): 1.2 ng/dL; plasma cortisol at 8 am was also normal (18 µg/dL).

Given the pathology and the age of the patient we did not perform any provocative test for analyzing pituitary GH secretion, in spite of the low height and low plasma IGF-I values.

Blood analysis carried out after 3-months of GH treatment indicated that the slight abnormalities observed in pre-treatment study had disappeared. Plasma creatinine and IGF-I were now in normal values (0.40 mg/dL and 146 ng/mL, respectively).

Subsequent blood analysis carried out along the whole treatment always showed normal values in all the parameters analyzed. Plasma creatinine ranged between 0.3 and 0.4 mg/dL, and plasma IGF-I reached a maximal value of 254 ng/mL, while IGFBP3 oscillated around 3.5-4µg/mL.

GH administration did not produce any kind of adverse effects.

3. Discussion

In this study we describe the results obtained after treating with GH and rehabilitation a child born with CRS and sacral agenesis. To our knowledge, this is the first report about practically full innervation below the level of the SC affectation in this rare congenital syndrome, a fact that we have to attribute to the combined treatment with GH and rehabilitation that the child received since he

was 9-months old. Since the primary pathology has been reported as irreversible, treatments for it have been considered to be only supportive [4]. We know that this is only one case among the wide spectrum of abnormalities that can appear associated to the syndrome, and it is possible that other type of CRS could not have an evolution as favorable as our patient showed, but in any case GH treatment resulted in promoting sensitive and motor innervation, and bowel and bladder control.

As expected, the treatment used could not recover the lack of sacrum bone, nor renal agenesis or orthopedic anomalies, but, in our opinion, most of these could be solved surgically in the next years. Hence, the quality of life that the affected patient improved considerably with regard to the initial prognosis.

Despite of the successful results obtained as a result of the combined treatment with GH and rehabilitation, we can not know what was the exact role played by the hormone. However, it is clear that rehabilitation alone would not produce these results. In fact, before receiving GH the child had been treated exclusively with rehabilitation without improvements. Moreover, rehabilitation commenced early after birth, a period of time during which a high plasticity exists, at least at the nervous system level. This is consistent with a number of reports describing that the treatment of this syndrome is merely supportive, addressed to correct orthopedic and other abnormalities (cardiac, gastrointestinal, vertebral, respiratory, etc) if they exist, in order to improve the quality of life [1, 3-5, 17-19, 21).

At this point, in order to trying to understand how GH acted, we should recapitulate about the possible causes giving origin to the syndrome.

It has been proposed that CRS is associated with the presence of maternal diabetes and mutations in homeobox gene *HBLX9*, a gene that is also expressed in the pancreas [18, 20-23]. However, this was not the case of our patient. As described in the introduction, his mother had not diabetes and genetical studies of the child were normal. On the other hand, although the incidence of the syndrome increases to 1 in 350 when uncontrolled gestational diabetes exists [4, 5], it has been found that only 16 to 22% of the mothers of CRS patients have diabetes, therefore it seems to be clear that the syndrome is not specific to diabetes [24], at least in humans.

We and others demonstrated [13-15], in rats, that following sciatic nerve transection, GH administration leads to accelerated axonal regeneration, reduces muscle atrophy and promotes muscle reinnervation, and an increased number of Schwann cells that produce myelin. This and our unpublished studies in patients with SC injuries [16], indicates that GH is able to promote peripheral axonal growth and this might explain the effect of the hormone on the innervation observed in our CSR patient. However, a clear difference exists between repairing an injured nerve in a previously innervated zone and innervating a big area that never had received nervous stimulation because of the lack of innervation, as it happened in the CRS patient we treated.

The formation of vertebral column during embryogenesis is a critical process known as somitogenesis [25]. It follows a periodic organization along the anterior-posterior axis. This peculiar pattern is established when segments called somites bud off at an established place from the anterior tip of the presomitic mesoderm (PSM) of the embryo [25]. There is a rhythmic production of somites, triggered by three major signaling pathways: Notch, Wnt/ß-catenin, and fibroblast growth factor (FGF), whose activity is evident in PSM. These signaling pathways integrate into a molecular network that generates a traveling wave of gene expression along the embryonic axis, known as the "segmentation clock" [25]. Within the network a number of specific signaling circuits set the pace of the oscillations, synchronize gene expression cycles in neighboring cells, and therefore contribute to the periodicity and bilateral symmetry of somite formation [25]. Somites are the precursors of the vertebrae and structures related to them, such as muscles, nerves, blood vessels, tendons, ligaments and dorsal dermis [26]. The frontier, or limits, of somite formation and hence axial segmentation, implies a mesenchymal to epithelial transformation of the PSM and this coincides with intersection of oscillatory gene activity with the determination front. This front seems to be determined mainly by a gradient of FGF8 (androgen-induced growth factor) and WNT signaling in the caudal PSM that diminishes rostrally. However, this interesting model has been challenged recently, since it has been proposed that somites may have the capacity for self-organization independent of any clock and

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wavefront mechanism [27]. These authors demonstrated that non-somite mesoderm treated with Noggin generates many somites that form simultaneously, without cyclic expression of Notch-pathway genes, and they have normal size, shape and fate, as well as axial identity. However, these somites are not subdivided into rostral and caudal halves, which is necessary for neural segmentation [27]. In all, these authors propose that somites are self-organizing structures whose size and shape is controlled by local cell-cell interactions [27].

Recently, a novel role in somite segmentation and in the pathogenesis of vertebral anomalies has been described for an auto-catalitically activatable member of the proprotein convertase family of serine proteases, MBTPS1/SK1/S1P (membrane bound transcription factor protease, subtilisin kexin isozyme-1 or site 1 protease). In mice models in which the *Mbtps1* gene has lost its function or it has been deleted during embryogenesis, appear phenotypic changes localized to the lumbar/sacral vertebral region which mimic those observed in CRS. According to their data *Mbtps1* gene plays critical roles in regulating somatogenesis [26].

Although it was not the objective of this work, once we know how much critical is the development of the vertebral column, it is easy to understand that any single alteration during this period may lead to the appearance of many different abnormalities. Moreover, since this stage of the embryonic development occurs sequentially, depending on when the alteration has affected somitogenesis, the severity of the resulting abnormalities may be different. In addition, since somitogenesis occurs during a restricted period of time during embryogenesis, we can also understand why GH administration did not induce any positive change in the abnormal vertebral column of the patient we treated, in spite of there are close relationships between GH, FGF and Notch [6].

The role that GH and IGF-I play on neural development and neural injuries has been postulated years ago [28]. The GH receptor (GHR) is expressed in regions of the brain in which neurogenesis occurs during embryonic brain development [29, 30]. GH itself is also found in cells of the ventricular zone during embryonic neurogenesis [30], and is produced endogenously within the postnatal hippocampus [31-34]. Studies of the effects of GH on embryonic rat cerebral cortical [35], and hippocampal neuronal cultures of aged mice found that it induces the proliferation and differentiation of these neural stem cells (NSCs) [35-37].

Exogenously applied GH and PRL promote the proliferation and migration of NSCs derived from fetal human forebrains [38]. This agrees with previous preclinical data from our group and others, demonstrating that exogenous GH administration promotes the proliferation of hippocampal neural precursors after brain injury induced by kainate administration [37], and in a number of zones in the intact adult rat brain [39].

These and many other studies (for a detailed review about the GH effects on the brain, see Ref. [6]), indicate that GH may induce a positive effect together with specific neurorehabilitation after a brain injury in human patients. With such a combined treatment, we and others obtained significant improvements both in children with cerebral palsy [40, 41] and in patients that suffered traumatic brain injury (TBI) [7, 8, 11, 42], or in a patient suffering from a neurogenic dysphagia after oncological brain surgery [43].

Less known are the effects of GH in the SC, but it seems to be logical that the hormone may act there as it does in the brain. The SC ependyma holds a neurogenic potential [44], and a number of Nestin (a marker of neural progenitor differentiation towards neurons) immunoreactive cells has been detected at all three SC levels in humans died after an accident or nontraumatic causes [45], suggesting that in the SC exists a population of neural progenitor cells with the potential for proliferation, differentiation and migration, as it occurs in the brain. These SC NSCs have been proposed to play a protective role after a SC injury, restricting the loss of tissue induced by the injury [46], and even they might represent a potential source for repairing SC injuries [45, 47]. However, a recent study in which the gene expression profile of the SC ependymal region was analyzed in control subjects, patients with traumatic SC injury and patients with non-traumatic SC injuries, showed that the ependymal region is enriched only in 14 genes related to neurogenic niches [48]. Moreover, these authors demonstrated that the central canal is mainly absent in the adult human SC (beyond the age

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18 years) and is replaced by a structure morphologically and molecularly different from that described for rodents and other primates. Their data suggest that the ependymal region is more likely to be reminiscent of a low-grade ependymoma [48]. According to these data it remains to be established whether the ependymal NSCs play a protective and reparative role or they hold a latent danger of transformation. In any case, this does not apply to children, as the own authors affirm in their study [48].

A study in transgenic mice expressing a growth hormone antagonist demonstrated that, after birth, the neural effects of the hormone are most evident in the SC than in the brain; even more, the SC continues to show GH dependence into adulthood [49]. There is GH and GHR immunoreactivity in the embryonic SC of chickens [50]. GH overexpression coordinately increased nucleolar, nuclear, and cell body size in lumbar spinal motoneurons in transgenic mice, and the weight of SCs in these animals also was significantly increased in relation to littermate controls [51]. In a more recent study, the abundance and activity of acetylcholinesterase, a marker for cholinergic neurons and their synaptic compartments, was shown to be markedly reduced in the SC of GH deficient rats, indicating that GH positively affects the neuronal and synaptic compartments of the developing rat SC [52]. Moreover, topical application of GH or nanowired delivery of the hormone to a rat model of injured SC promotes neuroprotection, decreasing the degree of edema formation and neuronal SC injuries [53]. In addition, GH is expressed in the peripheral nervous system [49].

Taken together, these data indicate that GH may act in the SC as it does in the brain, inducing the proliferation, differentiation, migration and survival of ependymal stem cells. This would explain the results we obtained in this case of CRS. That is, the administration of GH may have led to increased proliferation and differentiation of ependymal stem cells allowing the formation of the neural components responsible for the development of the new innervation (sensitive and motor) observed in this case of CRS. Once GH provided the needed nerve support, rehabilitation improved the functional significance of afferent and efferent nerve pathways.

Since the patient lacks the sacrum bone and his hips are luxated, we are now trying to develop an artificial sacrum, made in the laboratory with a decellularized matrix proceeding from a died donnor and autologous mesenchymal stem cells expanded in GMP conditions. Theoretically, the implant of this sacrum would allow it to grow as the patient grows. Before doing it we will study in rats whether this is possible. Another option would be to implant an artificial sacrum able to grow by means of external screws manipulated by their therapists after radiological controls. Apart of these, the patient will need a very complex surgery for reconstructing the hips, detethering the SC and he will need to be carefully controlled for a possible increase of hydrosyringomyelia.

4. Materials and Methods

The patient was a 9-months-old male born of a nonconsanguineal marriage by scheduled caesarean, that presented a caudal regression syndrome with sacral agenesis (detected in utero by ultrasonography), right renal agenesis and left hydronephrosis, neurogenic bladder and bowel, absence of innervation (sensitive and motor) of legs, scoliosis, passive knees flexion and clubfoot. Apgar score at birth was 3/4 (1 minute/5 minutes), pH in blood cord was 7.3; his weight at birth was 2.634 Kg (p10) and his size was 41 cm (< p10). General conditions were very bad at birth and the patient had to be reanimated (oro-tracheal intubation).

According to the Medical Files Provided or Reported by the Family of the Patient

The patient was the first and unique child of a non-diabetic woman; his weight was normal for the gestational age. The mother had not any kind of toxic habits (alcohol, tobacco, drugs); she did not took any kind of pharmaceutical drugs during pregnancy, she was not exposed to toxics, organic fat solvents or radiation, and no remarkable incidences existed along gestation.

An X-ray exam of the vertebral column performed at day 1 of age showed agenesis of the sacrum and L5 vertebra, and that the hypoplastic portion of L4 was articulated with iliac pseudoartrosis. These correspond with a sacral agenesis Type IV, according to the Renshaw classification of this delopmental abnormality [17].

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For correcting clubfoot, feet were plastered at age 4-days. The patient suffered multiple urine infections and a cystography carried out 2-days after birth detected passive vesicoureteral reflux to the whole excretory way of the left kidney. At age 7-days old a MRI confirmed the lack of development of the vertebral column stopped in L3 (Figure 1), as well as the existence of sacral agenesis and a hypertense signal of fat intensity most likely corresponding to a lipoma.

An electromyogram revealed a slight innervation of the psoas and quadriceps, with full denervation of any other muscle of the legs and feet (data not shown).

Molecular exams (Multiplex ligation-probe amplification; MRC-Holland) carried out in his hospital of reference did not detect any alteration in the subtelomeric regions analyzed; karyotype was that of a normal male (46,XY) and the analysis of multiple genes that might be involved in the appearance of the syndrome was also normal.

Early from birth he received rehabilitation in his hospital of reference according to the Vojta method therapy.

At admission in our Center (age 9 months), the patient had a height slightly below p3 for his age, mainly due to the marked hypotrophism of his legs, while his weight was in p50. Physical examination revealed a high hypotony in his legs (his thighs were practically composed by fatty tissue, without muscular tissue) and fully paralytic and hypotrophic clubfoot. Their knees were in an irreductible passive flexion of 68º and 80º (right and left knees respectively), as measured with a goniometer. There were important retractions in the pelvic musculature, mainly in hip flexors. The pelvic diameter was reduced because of an articulation of the iliac bones among themselves. There was luxation of both hips. Plantar flexion and foot dorsiflexion did not exist.

The Gross Motor Function Test (GMFM-88) and Modified Ashworth Scale were performed before commencing the treatment, in some of the controls carried out during it, and after completion thereof. The GMFM-88 is a scale constructed for evaluation of change in gross motor function in children with cerebral palsy and consists of 88 items grouped into five dimensions: dimension A (lying and rolling, 17 items), dimension B (sitting, 20 items), dimension C (crawling and kneeling, 14 items), dimension D (standing, 13 items) and dimension E (walking, running, and jumping, 24 items). Scores for each dimension are expressed as a percentage of the maximum score for that dimension, adding the scores for all dimensions, and dividing by 5 to obtain the total score. The Modified Asshworth Scale indicates if there is spasticity and the degree of it. Values range between 0 (no spasticity) and 4 (affected parts remain rigid in flexion or extension when they are moved passively).

No responses existed to any kind of sensitive stimulation in buttocks and legs. There was an almost continuous flow of urine and liquid feces. The child was able to maintain sedestation, but unable to perform a normal crawl, since he only utilized his arms (Movie 1).

After the physical examination, the Battelle Developmental Inventory Screening test (BDIST) was performed. This test screens and evaluates early childhood developmental milestones. Results from this evaluation indicated that no cognitive stimulation was needed, because the scores reached in each of the areas explored were normal or higher than those expected for the age of the patient, particularly at the cognitive level. The only exception was the motor area, a logical result given the pathology of the patient.

Routine blood analysis (hematimetry and biochemistry) and the analysis of some important hormones (plasma TSH, fT4, morning cortisol, IGF-I and IGFBP3) were carried out before commencing the GH treatment and at 3-months intervals during the first year of treatment, and at 6-months intervals after discharge from our Center.

Studies and treatments were conducted according to the protocols of Medical Center Foltra in compliance with national legislation and the Code of Ethics of the World Medical Association (Declaration of Helsinki). After obtaining signed informed consent of their legal representatives, the patient was scheduled for GH treatment and rehabilitation consisting of daily physical therapy (2 hours/day, 5 days/week). Once the patient was growing and significant sensitive and motor improvements were observed, a session of pelvic floor therapy was added (1 h/week) to to the two daily sessions of physiotherapy

GH treatment started in parallel with physiotherapy. Initially, GH (Nutropin, Ipsen) was given at a daily dose of 0.3 mg/day (5 days/week) during 3-months, followed by 15 days resting. After it the dose was increased to 0.5 mg/day, following the same schedule.

Blood analyses were repeated every 3-months during the first year of treatment and every 6-months after it.

In a first stage the patient remained in treatment in Medical Center Foltra during a period of seven months. After it, and because of working problems of his parents, he was discharged from our Center and referred to another Center of Physiotherapy closer to his home; there the same treatment procedures were followed, as indicated by us. Every 3-months he came back to the Medical Center Foltra for a control of his evolution, and new instructions for rehabilitation were given whether it was appropriate. One year later, the patient came back to our Center during 6-months; after it and given his good evolution, he was discharged and physiotherapy was carried out at home by his parents. GH administration and physical and analytical controls continued until now. Currently the patient is 6-years old and the GH dose is 1 mg/day.

Because of the existence of hydronephrosis in his unique kidney, renal function was periodically controlled by a nephrologist from his hospital of reference.

5. Conclusions

GH administration, together with a specific rehabilitation may improve the quality of life of some cases of CRS, hitherto considered only susceptible of receiving supportive measures. It is expected that an early GH treatment would produce better results in terms of innervation of the distal segments previously lacking it.

Supplementary Materials: Supplementary materials can be found at www.mdpi.com/link.

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Conflicts of Interest: "The authors declare no conflict of interest."

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