MECHANISMS IN ENDOCRINOLOGY

The multiple facets of GHRH/GH/IGF-I axis: lessons from lifetime, untreated, isolated GH deficiency due to a GHRH receptor gene mutation

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Abstract

Twenty years ago, we described kindred of 105 individuals with isolated GH deficiency (IGHD) in Itabaianinha County, in northeast Brazil, carrying a homozygous mutation in the GH-releasing hormone receptor gene. These subjects exhibit markedly reduced GH responsiveness to stimulatory tests, and anterior pituitary hypoplasia. Serum concentrations of IGF-I, IGF binding protein type 3 and the acid-labile subunit are markedly reduced, with a lesser reduction of IGF-II. The most striking physical findings of these IGHD individuals are the proportionate short stature, doll facies, high-pitched voice and visceral obesity with reduced fat-free mass. There is neither microphallus, nor neonatal hypoglycemia. Puberty is delayed, menopause anticipated, but fertility is preserved in both genders. The reduction in bone sizes is not even, with mean standard deviation scores for height of –7.2, total maxillary length of –6.5, total facial height of –4.3 and cephalic perimeter of –2.7. In addition, the non-osseous growth is not uniform, preserving some organs, like pancreas, liver, kidney, brain and eyes, and compromising others such as thyroid, heart, uterus and spleen. These subjects present higher prevalence of dizziness, mild high-tones sensorineural hearing loss, reduction of vascular retinal branching points, increase of optic disk, genu valgum and increased systolic blood pressure. Biochemically, they have high low density lipoprotein cholesterol and C-reactive protein levels, but maintain increased insulin sensitivity, and do not show premature atherosclerosis. Finally, they have normal immune function, and normal longevity. This review details the findings and summarizes 20 years of clinical research carried out in this unique population.

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Invited Author's profile

Manuel Hermínio de Aguiar Oliveira MD, PhD is a full-time professor in the Department of Medicine at Federal University of Sergipe, Brazil, and the founder of the Endocrinology Unit of the University Hospital. He is a general clinical endocrinologist, and his research is mainly in genetic endocrinology. His principal focus of interest is isolated growth hormone deficiency, due to a mutation in the GH-releasing hormone receptor gene, found in 'the Itabaianinha dwarfs'.

Introduction

In 1657, the British physician William Harvey wrote: ‘... nor is there any better way to advance the proper practice of medicine than to give our minds to the discovery of the usual laws of nature, by careful investigation of cases of rarer forms of disease’. We report an example of this wise advice, as congenital isolated growth hormone (GH) deficiency (IGHD) is quite rare, but can teach us a lot about the functions of GH (1). Over the past 2 decades, we have had the unique opportunity of studying an extended kindred with high prevalence of subjects affected by congenital inherited IGHD due to an inactivating mutation (c.57+1G>A) in the GH-releasing hormone (GHRH) receptor (GHRHR) gene (*GHRHR*. OMIM n. 612781). Most of the affected patients reside in the rural county of Itabaianinha, in the northeastern Brazilian state of Sergipe (2). Sergipe is the smallest state in Brazil. Due to its smallness, it is linked to GH disorders since 1890, when de Souza leite, born there, presented together with Pierre Marie, the first descriptions of GH excess syndrome (3). Since 1994 we have been studying the other side of this spectrum, a cohort of 105 (some deceased) subjects with mostly untreated IGHD, exhibiting a pattern of recessive heritage in a pedigree traced back 200 years, including 1570 individuals in eight generations (2). The high frequency of consanguineous unions and the lack of mobility of this population were the principal causes for the spread of this mutation.

Although the presence of a hypothalamic GH-releasing factor had been hypothesized, it was not until the eighties that two groups separately discovered GHRH from pancreatic tumors causing GH excess (4, 5). The GHRH gene and its receptor were later cloned and sequenced (6, 7, 8). With the demonstration of a second GH stimulatory peptide, ghrelin, the natural ligand of the growth hormone secretagogue receptor, first in the stomach, and later in the hypothalamus (9, 10), the stimulatory control of pituitary GH gained in complexity, with several studies attempting to establish the hierarchic role of each peptide. Furthermore, the balance between the growth promoting and not growth-related actions of GH was not clear. While the longitudinal bone growth involves the direct action of GH (11), the exact roles of the insulin like peptide type I (IGF-I), or type II (IGF-II) are still not completely understood. GHRH also exert direct actions, such as promoting somatotroph cells development (12). Extra pituitary GH (as in immune cells in the brain and the eye), and tissue growth factor production and action, confer more complexity to the GH-IGFs system. Brain,

immune and vision functions are likely important for environmental adaptation, an impressively multifactorial system involving both genetic and environmental factors (13).

GHRHR is one of the few genes for which a naturally occurring mutation was discovered in mice before humans. The *little* mouse, a widely used model of IGHD was discovered to have missense mutation in this gene in 1993 (14). Three years later, the first human mutation was discovered in two IGHD cousins from a consanguineous Indian family (15). The importance of the finding of *GHRHR* mutations in understanding GH secretion regulation and GH effect was stressed by one of the discoverers of GHRH, Michael Thorner, who wrote: ‘this mutation in the *GHRHR* gene has been described in large Brazilian kindred with severe GH deficiency and extreme short stature. These experiments of nature demonstrate that a defect in the GHRH impairs normal growth and emphasizes the vital importance of GHRH’ (16).

Our purpose is to summarize and discuss, in an overview, the large list of identified features of IGHD due to a single inactivating *GHRHR* gene mutation from this unique case series followed over 25 years. These features have been published in 42 different publications (2, 19, 22, 25, 27, 29, 30, 31, 32, 33, 36, 37, 38, 40, 41, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 56, 57, 61, 62, 63, 64, 66, 67, 68, 74, 75, 76, 77, 78, 79, 80). We have used this experiment of nature to discover ‘usual laws of nature’ as suggested by William Harvey, and to understand some aspects of more common conditions such as short stature, insulin resistance, diabetes, atherosclerosis, osteoporosis, cancer and ultimately longevity.

Understanding the model

GHRH is released from neurosecretory nerve terminals of arcuate neurons, and through the hypothalamo-hypophyseal portal system reaches the somatotroph cells of the adenohypophysis and binds to its receptor, causing both GH secretion and cell proliferation. In humans, the *GHRHR* gene is located on chromosome 7p14.3. It has a complex structure spanning about 15kb, containing 13. The GHRHR protein is mostly expressed in the anterior pituitary gland, consisting of 423 amino acids with an extracellular N-terminal and an intracellular C-terminal portion linked by seven α -helices transmembrane domains. The binding of GHRH causes conformational changes, resulting in the activation of the G_{α} subunit, stimulation of the membrane-bound adenylyl cyclase,

increase of intracellular cyclic adenosine monophosphate (cAMP) and activation of protein kinase A (PKA) pathways. This translocates to the nucleus and phosphorylates the transcription factor cAMP-response element-binding-protein (CREB) causing the transcription of GH through binding to response elements in the target genes promoter region (17).

The Itabaianinha *GHRHR* mutation is a transversion (c.57+1G>A) in the consensus GT of the 5' splice donor site of intron 1, required for correct splicing of intron 1 from RNA transcripts. This mutation likely leads to retention of intron 1 and insertion of a premature stop codon 213 bases from exon-intron junction, similarly to what was later described for a different mutation in the same splice site (18). A founder effect has been demonstrated in this cohort and in other Brazilian patients (residing in different areas of Brazil) with the same mutation (19). It is chronologically the second discovery of about twenty inactivating *GHRHR* mutations described to date (14), but the one involving the largest number of individuals. The second largest kindred was reported in eighteen IGHD subjects residing in the Pakistani province of Sindh (20, 21), affected by the historically first *GHRHR* mutation (E72X) (15). Although well described initially (20, 21), the Sindh cohort was not accessible to further studies in the following years. Therefore, the Itabaianinha IGHD individuals constitute the only opportunity to assess the consequences of lifetime and untreated IGHD due to *GHRHR* mutations.

Exploring the GH-IGFs system

In the Itabaianinha IGHD subjects serum concentrations of IGF-I, IGF-II and IGF binding protein type 3 (IGFBP3), and the acid-labile subunit (ALS) are all markedly reduced, with complete separation from normal individuals at all ages (Fig. 1) (22). To compare the relative proportions of IGF-I, IGF-II and IGFBP-3, we calculated their molar ratios (Fig. 2). In IGHD children and adults, there was a profound reduction in the ratio of IGF-I to IGFBP-3, a measure of IGF-I bioavailability, indicating that IGF-I is more severely affected than IGFBP-3 by the absence of GH. Conversely, the IGF-II/IGFBP3 ratio was significantly increased. Therefore, the combined IGF-I plus IGF-II/IGFBP-3 ratio is similar in IGHD children and age matched controls, whereas in adults this ratio is almost double in IGHD than in controls. These findings suggest a differential effect of IGHD on the level of these proteins. This may be due to IGF-I being more GH dependent. Data

Figure 1

Serum concentrations of IGF-I (A), IGF-II (B), IGFBP-3 (C) and ALS (D) throughout life in GHD (closed circles) and healthy control (open circles) subjects recruited from the same area and stratified by age. IGF-I, IGF-II, IGFBP-3 and ALS were centrally assayed. Serum IGF-I was measured by immunoradiometric assay (IRMA) after acid-alcohol extraction (Diagnostic Systems Laboratories, Inc., Webster, TX, USA) with a sensitivity of 0.8 ng/mL. Serum IGF-II was measured using an in-house IRMA, with a sensitivity of 30 ng/mL. Serum IGFBP-3 was measured using an IRMA (Diagnostic Systems Laboratories), with a sensitivity of 0.5 mg/L. Total serum ALS was measured using an enzyme-linked immunosorbent assay (Diagnostic Systems Laboratories) with a sensitivity of 0.07 mg/L.

from GH resistance (Laron syndrome) (LS) are conflicting depending on different studies and IGFBP-3 assays (23, 24). IGFBP-3 concentrations also reflect the amount of IGF-II, which is not as severely reduced as IGF-I. Despite the reduction in absolute concentration, there

Figure 2

Comparison of molar ratios of IGF-I and IGF-II to IGFBP-3 in GHD and control children (A) and in GHD and control adults (B). Boxes represent the interquartile range.

does appear to be an increase in IGF-II relative to IGFBP-3 in IGHD that continues into adult life. The reason for this is unclear, but it may reflect an upregulation of IGF-II to compensate for the diminished IGF-I concentration (22) guaranteeing enough IGF bioavailability to some vital tissues like the brain and the eye, as will be discussed later.

We also established the diagnostic power of separation between IGHD and controls among several measures and calculations of the GH-IGFs axis, by dividing the minimum value in the control group by the maximum value in the IGHD group. IGF-I calculated in the ternary complex (IGF-I/IGFBP-3/ALS) and total IGF-I provided the greatest separation between IGHD and controls in childhood. Similarly, in older adults the best separation was achieved with IGF-I in the ternary complex, with free ALS being optimal in younger adults. IGFBP-3 exhibited a low separation power, and consequently should not be

used in the diagnosis of GHD in the common setting of short stature (22).

GHRH unresponsiveness in these subjects was demonstrated by the lack of GH response to GHRH stimulation, even when patients were pre-treated with injections of GHRH for six days (2). GHD was further confirmed by abnormal GH responsiveness (with peak values less than 1 ng/mL) to clonidine (CL) and insulin tolerance test (ITT) (2, 25). There was a small but significant GH increase during ITT, but not during CL test, showing that some response to hypoglycemia can occur despite complete lack of GHRHR function (25). Similarly, we found a small but significant response to a GH secretagogue (GHRP-2) that acts on the ghrelin receptor, suggesting a GHRH-independent effect of this substance on the somatotroph cells, despite their hypoplasia (26). The low but detectable serum GH response to ITT and GHRP-2 classify this IGHD model at type 1B (autosomal recessive with low but measurable serum GH) (27). This model is different from the IGHD type 1A (also autosomal recessive) caused by deletion or nonsense mutations in the GH-encoding gene (*GH1*), resulting in no detectable GH secretion. This finding may have physiological implications, contributing to differences among these severe IGHD models despite similar degrees of IGF-I deficiency. Probably the difference of magnitude of these genetic IGHD models comes from the fact that the patients in our IGHD cohort are still able to secrete small but detectable amounts of GH (25, 26) causing some residual effect. It is also possible that the timing of these two genes expression during fetal and postnatal development may have additional consequences.

Other hormonal findings

Affected IGHD subjects have higher basal cortisol levels than controls, likely reflecting increased activity of the enzyme 11 beta-hydroxysteroid dehydrogenase, which converts cortisone to cortisol (28). The high circulating cortisol levels together with the small amount of detectable circulating GH may contribute to the lack of neonatal symptomatic hypoglycemia episodes in *GHRHR* mutations (22), which typically occurs in severe IGHD due to *GH1* gene defects causing complete absence of GH protein. Notably, a child with type 1A IGHD without hypoglycemia and with elevated cortisol levels has recently been reported (29). Basal levels of serum TSH are mildly increased in the IGHD subjects (26), often within the upper normal range. This may be due to a reduced

effect of IGF-I on hypothalamic somatostatin, which reduces TSH secretion. Therefore, a modest elevation of TSH in a child with severe short stature may suggest IGHD rather than subclinical hypothyroidism. The IGHD subjects have also a reduced serum total T3 and increased serum free T4, suggesting a reduction in the function of the deiodinase system (30). Serum prolactin levels are similar in IGHD and control subjects (31). There is a reduction in prolactin level limited to climacteric period, possibly due to anticipated climacteric and reduced estrogen exposure (32). In IGHD males, testosterone and SHBG levels are higher than in controls, without difference in free testosterone (31). Gonadotropins are normal in post pubertal subjects.

Pituitary magnetic resonance imaging (MRI)

MRIs from adults and children of Itabaianinha kindred show obvious anterior pituitary hypoplasia (APH) (33), as it had been previously shown in 4 adult individuals from the Sindh kindred (17). APH is not due to reduction of cranial volume (33). Because somatotroph cells account for 50–60% of pituitary cell mass, their blunted development in absence of GHRH stimulus is the probable cause of APH (similarly to that described in the *little mouse*) (34). However, pathology data from humans are so far not available. Whether APH is present at birth or develops during early childhood is not known.

Overall homozygous phenotype

The most striking findings of these IGHD individuals are the proportionate short stature, the doll facies, the high-pitched voice and the truncal obesity. These four aspects will be described separately. In this section, we will summarize the other aspects. The skin appears markedly wrinkled and thin, and the pigmentation of the hair is delayed in children and teenagers (most with thin blonde hair). Curiously, hair graying is practically absent, even in old age. Microphallus is not observed, suggesting that this finding is a sign of gonadotropin deficiency, or of even more marked IGF-I deficit as seen in LS patients. However, it seems that IGF-I has only a modulatory role on the postnatal sexual maturation, as LS patients reach full sexual development despite delayed puberty (35) or lack of direct effect as in severe congenital IGF-I deficiency due to IGF-I gene deletion (36). Puberty usually occurs with delay in the IGHD untreated subjects, without fertility or breast-feeding abnormalities. IGHD

women tend to have fewer children than normal statured women from the same area, possibly due to the delayed age of first intercourse, and to the necessity to perform Cesarean section because of cephalic/pelvic disproportion. Menopausal age is somehow anticipated (32). Surprisingly, IGHD individuals show normal quality of life as assessed by a validated GHD specific international questionnaire (37). They generally do not complain of tiredness, even at advanced age (38). Compared with controls from the same area, these untreated IGHD subjects report dizziness more frequently, and have mild high-tones sensorineural hearing loss (39). This sensorineural hearing loss is less severe than the profound sensorineural deafness found in a patient with deletion of the IGF-I gene (36).

They also present a mild retinal phenotype, with a moderate reduction in vascular retinal branching points associated to an increase in optic disc size, but with similar thickness of the macula (40), as also observed in LS (24). Therefore, an increase in optic disc size in a child with severe short stature may suggest congenital IGHD (31). The vascular retinal hypoplasia may be protective in the setting of diabetic retinopathy. This is important, as these individuals can develop diabetes, with prevalence in adults (assessed by OGGT) of 15% (41).

Recently, there has been great interest in the report that adult individuals with GH resistance from large LS Ecuadorian kindred do not have diabetes or cancer (42). One possible explanation is that the self-reporting approach may have underestimated the real prevalence of diabetes. Indeed, diabetes and its complications have been described elsewhere in patients with LS (43). The prevalence of cancer also seems to be low in our cohort, but three skin cancers, one lethal, and a giant medulloblastoma (in a patient who had been treated with GH for 4 years as a child) were diagnosed during the past 20 years. These data suggest that low GH secretion associated to very low IGF-I levels does not guarantee absolute protection against neoplasia development.

Metabolic and cardiovascular consequences

GH is an important anabolic, lipolytic and hyperglycemic hormone (effect due to increase in hepatic gluconeogenesis and glycogenolysis). IGF-I has anabolic, lipotropic and hypoglycemic effects. The exact role that each hormone exerts on metabolism is still not completely clear. We found that the metabolic consequences of severe IGHD are already present in early childhood and persist throughout the life. As expected, IGHD children, adolescents (44) and adults

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(45) from the Itabaianinha kindred have decreased fat-free mass (FFM) and increased percent fat mass (FM). The GH/IGF-I axis has important interactions with the alimentary system and with the balance between energy intake (EI) and energy requirement. Reduced EI has been described in adult-onset GHD. However, when corrected by body weight, we found that EI was higher in IGHD than in controls. In addition, these IGHD individuals consume in percentage more proteins, less carbohydrates and equal amount of fat in comparison to controls (46). This could ensure greater caloric intake, which would have adaptive advantages for small sized individuals, in an environment with limited access to food. The higher EI per body weight suggests a possible increase of orexigenic mechanisms. It is tempting to speculate that due to reduced feedback by IGF-I, there may be an increased secretion of the orexigenic hormone ghrelin, resulting in greater caloric intake. Due to logistic problems, plasma ghrelin measurements have not yet been done.

These IGHD subjects exhibit high serum total and LDL cholesterol levels throughout life (45, 47). There is also a mild increase in systolic blood pressure in adults, and arterial hypertension in the elderly, without evidence of cardiac hypertrophy. This suggests that the very low GH/IGF-I levels might counterbalance the effects of the increased blood pressure on left ventricular mass (45). Similarly, these individuals do not exhibit increase in carotid intima medial thickness (IMT), or evidence of coronary atherosclerosis when assessed by stress echocardiograms (48). In addition, they have similar coronary (49) and aortic abdominal calcium scores as controls (50). Not surprisingly, they do not show any evidence of increased mortality (51). These apparently surprising findings could be attributed to the dual role of IGF-I in atherosclerosis pathophysiology: while it promotes atherogenesis by increasing vascular smooth muscle cells proliferation, it also protects against it by increasing nitric oxide formation, vascular compliance and insulin sensitivity. It has been suggested that a very low level of IGF-I might have a protective role, whereas a milder decrease (as seen in adult-onset GH deficiency) might be noxious (48). In addition, the increase in serum adiponectin (protein influenced by GH level) may contribute to such a beneficial outcome (52). They also exhibit normal urinary albumin excretion. Finally, increased insulin sensitivity (IS) is present in these subjects. We assessed IS by homeostasis model assessment index of insulin resistance (IR), quantitative IS check index, oral glucose IS in 2 h (OGIS2) and 3 h (OGIS3) (44). β -cell function was assessed by homeostasis model assessment index- β , insulinogenic index and area under the curve of

insulin–glucose ratio. These IGHD subjects have increased IS and reduced β -cell function (53). In addition, the prevalence of nonalcoholic fatty liver disease (NAFLD) is increased in the IGHD adults. Nevertheless, they do not progress to advanced hepatitis forms. This finding contrasts with the association of severe forms of NAFLD in acquired hypopituitarism, and weakens the hypothesis of a direct role of GH/IGF-I deficiency in the pathogenesis of advanced NAFLD (54).

Truncal adiposity is one of the most important clinical findings of GHD and GH resistance, due to the reduced lipolysis by the GH-sensitive lipase. This finding may be a direct effect of the lack of GH, as it is not reversed by IGF-I treatment in GH insensitivity syndrome (55). It is noteworthy that the truncal obesity of the Itabaianinha IGHD subjects is indeed the expression of real visceral adiposity. The reduced IR despite this feature suggests that there is a threshold of GH secretion necessary for visceral adiposity to impair IS (28). Given the lack of increased IR, it is not surprising that these individuals exhibit normal longevity (51).

Immune function

GH is important for the development and function of the immune system, but there is controversy on whether GHD is associated with immune disorders. While no case of tuberculosis was observed in IGHD subjects during the 20 years of clinical and research activities in this kindred (56), they have an increased prevalence of periodontal diseases (57).

We have studied the frequency of infectious diseases and the immune function using a clinical questionnaire, physical examination, serology for trypanosomiasis, leishmaniasis, HIV, tetanus, hepatitis B and C, skin reaction to BCG, candidin, streptokinase and serum immunoglobulin G, M, E and A measurement (56). The untreated IGHD adults do not exhibit increased frequency of infections, or a significant alteration in humoral immune response. However, they have reduced total IgG levels, smaller papule diameter after streptokinase test and a non-significant tendency to reduced cellular response with higher frequency of all the three negative skin tests. These differences seem without clinical relevance in daily conditions, but may contribute to an unfavorable outcome in situations of more severe infections (56).

Our immunology data fit with the finding of normal longevity (51). In that study, five deaths were registered between 4 and 20 years, one due to infectious cause and the three others attributed to diarrheic diseases by verbal

reports. In the Ecuadorian LS cohort, 22 children died at an early age, apparently from infections and/or hypoglycemia (58). Among the four known deaths of the Israeli LS cohort, one was due to encephalitis (59). Therefore, both IGHD and GH resistant patients seem vulnerable to infectious diseases in childhood (51, 58, 59, 60).

Bone growth and structure

IGHD newborns have a size within normal range, and growth retardation becomes evident during the first year of age. Appropriately, the IGHD subjects describe themselves as 'shrunk', indicating a postnatal growth failure in which the height SDS decreases as age increases, reflecting the effects of cumulative GH deficiency until the achievement of adult height (37). Final adult height in untreated individuals is 128.7 ± 5.9 cm in males (range 117–137 cm), and 117.6 ± 5.7 cm in females (range 107–126 cm), showing an 18% variation in both genders. Adult height standard deviation score (SDS) ranges from -9.6 to -5.1 (37). The causes of this height variation are unknown. In children, we found a positive correlation between height SDS and serum ALS (16). Nevertheless, other factors, such as parental height, GH-independent mechanisms and environment may interact with the GH-IGFs axis, determining the final stature.

The dimensions of hands, feet, scapular and pelvic girth are also reduced, reflecting the effect of GH on bone growth (37). Conversely, the reduction of the cephalic perimeter (-2.7 SDS) is less accentuated than stature (26, 29) and facial height (-4.3 SDS) (61), causing a disproportion between the calvarium and the face (Fig. 3), resulting in a 'doll' or cherubim angel facies. Therefore, the consequence of IGHD on bone is not uniform.

The adult IGHD subjects present a typical cephalometric pattern with maxillary length being the most reduced cephalometric dimension (-6.5 SDS), with magnitude of reduction that is similar to the final height (Fig. 3) (61). These cephalometric features may help the diagnosis of IGHD, and – together with the underdevelopment of the larynx – lead to a typically high-pitched voice with high fundamental frequency in both genders (62, 63). Formal voice analysis showed higher values of most formant frequencies, suggesting smaller oral and pharyngeal cavities. The voice changes result in a reduction of the effect of gender on the structure of the formants, maintaining a pre-pubertal acoustic prediction (64). Therefore, GH plays a relevant role in the

Figure 3

Mean standard deviation scores for height, total maxillary length, total facial height and cephalic perimeter. The reduction in bone sizes is not even.

development of voice quality, mostly increasing vocal cords thickness (62, 63, 64).

Because the GH-IGF axis plays an important role in bone metabolism and IGF-I levels correlate with bone mineral density in population studies (65), one may expect increased prevalence of osteoporosis and fractures in these subjects. However, although smaller, the bones of these individuals seem to be structurally appropriated to their muscles. Reduced heel quantitative ultrasound and areal bone mineral density (BMD) scores reflect the small size of the bones, but calculated volumetric BMD are similar to controls (50, 66, 67), and the prevalence of vertebral fractures is actually reduced in older IGHD individuals. Accordingly, these individuals do not seem to be prone to fractures, including the ones playing in the local soccer team, which competes well with normal statured teams. Congenital IGHD causes hip joint problems and genu valgum, without apparent clinical consequences (67).

Non-osseous growth

Growth of non-osseous structures shows tissue-specific consequences of IGHD. Besides the already cited APH, thyroid volume (30) and left ventricular area mass (45) are reduced when corrected by body surface (BSA). This is probably due to the reduced trophic effect of IGF-I on the thyrocytes and cardiomyocytes. By using ultrasonography, we found that spleen and uterus show a relative reduction in volume, prostate and ovaries are proportionate to BSA, while pancreas, liver and kidney appear larger compared

to controls (68). This suggests in these three organs a possible compensatory overgrowth. The lower number of pregnancies may contribute to the reduced uterine size (25). Very recently, we studied the ocular axial length (AL) and its relationship with head circumference and stature. The average AL corresponds to 96%, head circumference to 93%, and stature to 78% of normal controls, suggesting a parallelism between eye and head circumference. The adequate ocular growth and head circumference (a measure of the development of the nervous central system) may contribute to the environmental adaptation and survival capacity of these individuals. Ocular and brain growth may involve different patterns of regulation from whole body growth, reflecting a physiologic hierarchy.

Longevity

A highly debated issue is the relationship between the GH/IGF axis and longevity. Studies performed in several animal species suggest that blunting of the GH-IGF-I axis increases life span (69). This concept was challenged by a Swedish paper showing high cardiovascular mortality in non-GH replaced patients with hypopituitarism when compared with expected mortality (70). However, a more recent national Swedish hypopituitarism study

with 1286 deaths during the 1995–2009 period showed that the moderate excess of mortality (120 deaths vs 84.3 expected, SMR 1.42) was due to adrenal crisis and the late appearance of de novo malignant brain tumors in patients who previously received radiotherapy (71). Therefore, GHD per se may not cause increased mortality. Accordingly, the Itabaianinha kindred exhibits normal longevity (Fig. 4) (51). We observed no differences in the rate of cardiovascular death, despite the previously described cardiovascular risk factors, as well as in the rate of cancer deaths (51). These data are similar to those recently reported in the Ecuadorian LS patients (42). On the other hand, this finding differs from the reduced longevity described in kindred with IGHD type 1A, due to a large homozygous deletion in the GH-1 gene (72). As our subjects have measurable (albeit very low) serum GH levels, the different degrees of GH deficiency may have different impacts on longevity. Very recently, the Endocrine Society hypopituitarism treatment guideline endorsed the concept that untreated congenital GHD does not lead to shortened life expectancy (73).

GH treatment

For several reasons (high prevalence in the community, lack of obvious consequences other than short stature),

Figure 4

Kaplan–Meier survival curves. Solid lines, IGHD subjects; dotted lines, unaffected siblings. A and B, Both sexes; C and D, males; E and F, females. A, C and E, Individuals of all ages; B, D and F, individuals who reached age 20. Values under each graph are the numbers at risk by group.

the IGHD individuals from Itabaianinha and their parents do not have special interest in GH treatment. They consider themselves 'shrunk long-lived people', not patients. They are well accepted by the community, living and marrying with normal statured people. In this scenario, we offer, but do not oblige parents to provide GH treatment during childhood, and few decide to have their children continue treatment until achievement of optimal final adult stature. When treated, children and adolescent respond well to GH replacement therapy (GHRT), with no evidence of slowing response that may suggest the development of anti-GH antibodies. All the subjects have the expected initial lipolytic response to GHRT, with reduced FM percentage, increased FFM and normalization of cholesterol levels (74). We hope that all affected children will be engaged in GHRT thus reducing possible consequences of untreated IGHD.

We had the opportunity of administering a six-month treatment with bi-weekly depot GH to a subset of 22 adult IGHD individuals. Despite the only modest increase in the levels of IGF-I, GHRT improved lipid profile and body composition, and increased all the parameters related to LV mass, but also increased significantly carotid IMT, and induced the development of carotid atherosclerotic plaques (75). Fortunately, we saw arrest of the atherosclerosis progression after five years from the interruption of treatment (76). These observations should be considered before starting GHRT in adults with previously untreated congenital GHD.

This depot GH trial induced a biochemical pattern of bone anabolism persisting for at least six months after the end of treatment (66), and increase of serum T3 levels and thyroid volume (77), while (unsurprisingly), did not improve the excellent previous quality of life of these individuals (36).

Heterozygous (HTZ) phenotype

Different from the homozygous affected young adults, who exhibit severe short stature, with decreased FFM (kg) and increased percent of FM (45), young adult subjects HTZ for the Itabaianinha *GHRHR* mutation show no significant difference in height, but have lower FFM, no difference in the percent of FM and a trend of reduction in FM, when compared with homozygous normal controls from the same kindred (78). We hypothesized that a partial reduction in GH secretion might cause these changes in body composition, without affecting height. In addition, thyroid volume (30) and serum levels of the C-terminal cross-linking telopeptide of type I collagen (79) are also

lower in HTZ subjects, corroborating the presence of a mild phenotype. In contrast, older HTZ individuals are in average 4.2cm shorter than controls, showing age-associated height reduction, possibly suggesting frailty, a public health problem. These data indicate different effects of heterozygosity through lifespan (80). Table 1 summarizes the principal findings in this IGHD group.

Comparing Itabaianinha and Sindh dwarfs

The second largest kindred with mutated *GHRHR* was reported in the late 1990s. This includes 18 IGHD subjects, in one generation (the oldest age being 29 at the time of report), residing in the Pakistani province of Sindh (20, 21). They all share the same nonsense *GHRHR* mutation (E72X), which is prevalent in the Indian subcontinent (15). In both cohorts, body proportions are normal; head circumference is reduced (2.7 and 4.1 standard deviations below the norm, in Itabaianinha and Sindh cohort respectively) and no microphallus or neonatal hypoglycemia was observed. In the Itabaianinha

Table 1 Main findings in subjects with isolated GH deficiency from Itabaianinha due to *GHRHR* gene mutation.

- Very low activity of the GH-IGF-I axis throughout the life
- Severe short stature developing postnatally
- Uneven reduction in bone sizes with mean standard deviation scores for height of -7.2, total maxillary length of -6.5, total facial height of -4.3 and cephalic perimeter of -2.7
- Typical doll facies and high-pitched voice
- Overgrowth of pancreas, liver, kidney
- Preserved brain and eyes growth
- Reduced pituitary, thyroid, heart, uterus and spleen size corrected for body size
- Increased total and LDL cholesterol, C-reactive protein throughout the life and blood pressure in adulthood
- No history of hypoglycemia and increased insulin sensitivity
- Reduced fat-free mass with increase in the rate of fat mass and visceral obesity development
- Increased cortisol/cortisone ratio
- Increased adiponectin, normal leptin and urinary albumin excretion
- Normal volumetric bone density and higher frequency of genu valgum
- No microphallus, delayed puberty and anticipated menopausal age
- Preserved fertility
- Normal quality of life
- Higher prevalence of dizziness and mild high-tones sensorineural hearing loss
- Reduction of vascular retinal branching points and increase of optic disk
- Lack of premature atherosclerosis
- Increased optic disk and cup sizes
- Normal daily immune function
- Normal longevity

kindred adult height is very similar to what was observed in the Sindh kindred (130.3 ± 10.6 cm in males and 113.5 ± 0.7 cm in females) (20, 21). Biochemical findings of serum GH and IGF-I were also similar in the two kindreds. Interestingly, in four patients with the E72X mutation nocturnal plasma GH levels were significantly higher than daytime, with maintained pulsatility. However, there was no correlation between GH production and height or serum IGF-I level in this small group (81). In Itabaianinha IGHD children we found a positive correlation between height SDS and one parameter of the activity of the GH-IGF-I axis, namely ALS (22). Both kindreds have a blunted but detectable GH response to GH secretagogues (26, 82).

Both models exhibit anterior pituitary hypoplasia (33, 83). Another remarkable similarity between the two models is the normal volumetric BMD, suggesting that GH/IGF-I deficiency has relatively little impact on bone mineralization during the bone accretion phase (84). In summary, the phenotypic similarity between the two cohorts is evident. Subtle differences can arise from influence of other genetic and environmental factors.

Conclusion

The finding of *GHRHR* mutations causing IGHD demonstrates the fundamental role of GHRH for GH secretion. This model (IGHD with otherwise normal pituitary function) can help teasing out which of the consequences of hypopituitarism are truly due to GHD, avoiding confounders such as associated pituitary deficits, replacement therapies, etiologies and previous treatment (pituitary surgery or radiotherapy). What emerges from studying the large Itabaianinha IGHD cohort is that having severe lifelong GHD may be preferable than having normal GH secretion during infancy-childhood-puberty followed by adult-onset GHD. While the endpoint on growth may be very similar, studying different genetic models of GHD or GH resistance may help shed light on the individual role of each factor (GHRH, GH, IGF-I) in this complex system.

Our findings may not be necessarily applicable to sporadic IGHD. It is possible that these subjects also share other genetic traits that protect against the consequences of GHD. It is also possible that this congenital IGHD model causes some compensatory mechanisms, not found in acquired GHD. The knowledge of these mechanisms would be crucial, not only in the management of this

condition, but also to understand common disorders such as short stature, IR, diabetes, atherosclerosis, osteoporosis, cancer and aging.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the Declaration of Helsinki 1964 and its later amendments or comparable ethical standards.

Declaration of interest

R S serves on the advisory board of Pfizer.

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