Cognitive performance during senescence in untreated congenital isolated GH deficiency


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Abstract

Individuals with untreated isolated GH deficiency (IGHD) due to a mutation in the GHRH receptor gene from Itabaianinha Brazil have increased insulin sensitivity, normal life expectancy, and an extended healthspan, i.e., the period of life free from disabilities. We hypothesize that their prolonged healthspan is accompanied by a delayed cognitive decline in senescence. To test this hypothesis, we have administered the Literacy Independent Cognitive Assessment (LICA) to 15 IGHD individuals aged over 50 years and 15 controls matched by age, sex, years of education, and percentage of illiteracy. All individuals were negative for HIV and syphilis serology, and there were no differences in serum levels of folate, vitamin B12 and TSH between the 2 groups, while free T4 was higher in the IGHD group. IGHD subjects had a higher total LICA score than controls, 215 (22.7) vs. 204.2 (18.1), without reaching statistical significance. Scores of memory, visuoconstruction, language and calculation were similar between the two groups, with better attention [9.5 (1.4) vs. 8.3 (1.1), p= 0.01], and executive function [38.3 (4.8) vs. 35.1 (2.5), p= 0.03] scores in IGHD. MANCOVA revealed that group (but no age) had a significant effect on the LICA variables (partial eta squared of 0.455, power of 0.812, p= 0.02). This effect is verified on attention (partial eta squared 0.216, power of 0.749, p= 0.01) and executive function (partial eta squared 0.154, power of 0.570, p= 0.03. In conclusion, IGHD in senescence is associated with similar total cognitive performance, but better attention and executive function than controls.
Introduction

GH has been reported to have a role in improving cognitive function, both by inducing cognitive behaviors related to learning and memory and by affecting excitatory circuits involved in synaptic plasticity (1). GH also has a protective effect on the central nervous system, with beneficial effects of GH therapy reported in patients with spinal cord injury. Accordingly in animal models GH appears to stimulate neurogenesis (1, 2, 3). However, brain function, like immune and visual function, seems to depend more on extra-pituitary circuits, namely insulin (4, 5), IGF2 (6), brain GH (7) and brain IGF1 and IGF2 (8, 9) than on the classical somatotrophic axis (pituitary GH and circulating IGF1) (10, 11).

GH administration seems ameliorate poor cognitive performance in GH deficient (GHD) subjects (12), and GH releasing hormone (GHRH) modulates neuronal exosome biomarkers in mild cognitive impairments (13). While a GHRH agonist appeared to improve cognitive function in humans with mild cognitive impairment (14), a ghrelin agonist (MK-677) had no clinical effect on Alzheimer Disease progression (15).

Genetically modified mice with changes in GH signaling have been used to assess the interaction of the somatotrophic system with aging, including the brain. Most of these studies show that impairing GH signaling results in delayed aging, including of the brain, with increased lifespan and healthspan, i.e., the period of life free from important morbidities (16). Accordingly, GH deficient GHRH knockout mice show cognitive decline markedly later than control animals (17). In a similar “nature’s experiment” we have described in the Brazilian city of Itabaianinha a large cohort of individuals with severe isolated GH deficiency (IGHD) caused by a homozygous (c.57+1G→A) mutation in the GHRH receptor (GHRHR) gene (GHRHR OMIM n.618157) (18). Most affected adults have not received GH replacement and exhibit normal lifespan (19), with an
extended healthspan (10), reflected by greater insulin sensitivity (20) and high levels of
GLP1 (21), with a favorable profile of miRNAs (22). Our hypothesis was that their
extended healthspan is accompanied by a delayed cognitive decline. The instruments
often used to assess cognitive function are appropriate for people who know how to write
and read, but their application to illiterate and low-education individuals is questionable.
The influence of educational level on the assessment of cognitive impairment was
confirmed in two Brazilian studies (23, 24). To overcome this problem, we used the
Literacy Independent Cognitive Assessment (LICA) (25, 26), comparing subjects with
IGHD with local controls, both little or no formal education
Subjects and Methods

Subjects

In a cross-sectional study, individuals with IGHD residing in Itabaianinha were recruited by telephone by the senior author (M.H.A.O). Inclusion criteria for IGHD were homozygosity for the c.57+1G→A GHRHR mutation, while homozygosity for the wild-type GHRHR allele was required for the control group. Exclusion criteria were age less than 50 years, previous GH replacement therapy, and history of traumatic brain injury and liver and kidney diseases, hypothyroidism or thyrotoxicosis, positivity to HIV and syphilis serology, and reduced serum levels of folate or vitamin B12. Presently there are 54 homozygous IGHD subjects in the Itabaianinha cohort (27), of which 15 individuals are aged 50 or over. All the 15 agreed to participate, without any compensation. From our database of genotyped homozygous normal individuals (18), we included 15 individuals, in the same age group, in the same households or neighborhood, who did not meet our exclusion criteria. Therefore, the groups included 15 IGHD subjects (6 men, range 53-84 years old) and 15 controls (6 men, range 56-78 years old). The Federal University of Sergipe Institutional Review Board approved these studies, and all subjects gave informed consent.

Study protocol

The “Literacy Independent Cognitive Assessment” (LICA) was administered in the participants' homes, and blood samples were taken in the week following the LICA, in a group of 3 individuals per day. Head circumference was measured with an inelastic measuring tape over the most prominent point on the back of the skull (occipital) and over the eyebrows. These steps were carried out between May and November 2022.

Laboratory analyses
Blood samples were collected between 09:00 and 10:00 at the University Hospital. TSH, free T4, HIV, syphilis serology, and vitamin B12 were measured by standard techniques.

**LICA administration**

We translated the English version of the previously published and validated South Korean LICA model (25, 26) into Portuguese (28), and adapted it to the local culture to assess the cognitive function of people with little or no formal education, for the purposes of research. The translation protocol was adapted from Beacon's guidelines (29) and carried out in six stages by a multidisciplinary team of 17 members (English teachers, nutritionist, psychologists, geriatrician, endocrinologists, and statistician). LICA was used 59 times, 5 in the pilot study, 24 in the variability studies and 30 in the experimental step. Inter- and intra-observer variability was 99 and 96%, respectively. The Cronbach's alpha was 0.76, indicating good instrument reliability.

**Literacy screen**

Participants were separated into illiterate or literate groups by a brief reading and writing test. The participants were asked to read aloud the following two sentences: “Carlos was very thirsty after exercising. Carlos opened the refrigerator door”. They were then asked to write about what would happen next. The individual should write that "Carlos drank water". A participant who was able to read the sentences and write the appropriate response was considered literate. The same trained researcher (V.O.B.) administered the LICA to all subjects.

**Literacy Independent Cognitive Assessment**

LICA is a 300-point test, consisting of 13 subtests assessing memory, visuoconstruction, executive function, attention, language, and calculation. The story recall task involves immediate and delayed recall after approximately 20 minutes, and
recognition. The word recall task involves three learning trials of ten nouns, an immediate recall trial, a 20-minute delayed recall trial, and recognition. Visuoconstruction and memory are assessed using copy construction and recognition of 10 pictures of stick formations, which were modified from the Stick Pattern Reversal Test. Executive functions are assessed using the Digit Stroop test and the animal fluency task. Attention, concentration, and working memory are evaluated using forward and backward repetition tapping tasks of nine Corsi blocks. It involves mimicking a researcher as he taps on a sequence of up to nine spatially separated identical blocks. The sequence starts using two blocks but becomes more complex until the subject's performance fails. Language is assessed using a fifteen-item confrontation-naming task with animals, fruits, and vegetables and the Color and Object Recognition Test (CORT). CORT was modified from semantic knowledge tests on the visual form and color of objects. Scores below 186.0 and 154.5 define dementia in literacy and illiteracy, LICA was understood easily by literate and illiterate people.

**Statistical Analysis**

Anthropometric measures, blood pressure (BP), vitamin B12, folate, free T4, TSH, the LICA’s domains and the total LICA score were expressed as mean (standard deviation) and compared by Student’s t test. Sex and illiteracy were expressed as n, (percentage) and compared by the Fisher’s exact test. MANCOVA using all the LICA domains (memory, visuoconstruction, executive function, attention, language, and calculation), and the total score as dependent variables, with two factors (age and group) as independent variables, was performed to examine main and interaction effects of the factors on the defined multiple dependent variables This is accomplished by estimating their effect size by the partial eta-square, so defined: values for partial eta squared: 0.01, small effect size; 0.06, medium effect size; 0.14 or higher, large effect size.
The statistical software SPSS/PC 20.0 (SPSS, Inc., Chicago, IL) was used. Statistical significance was set at $p< 0.05$.

**Results**

Table 1 shows the comparison of anthropometric, biochemical, blood pressure data and LICA’s domains between the two groups. As expected, height, weight and head circumference were lower, while FT4 was higher in the IGHD group, ($p= 0.02$), as previously published (30). IGHD subjects had a higher total LICA score than controls, without reaching statistical significance. However, they showed better attention ($p= 0.01$) and executive function ($p= 0.03$).

MANCOVA revealed that group (but no age) had a significant effect on the LICA variables (partial eta squared of 0.455, power of 0.812, $p= 0.02$). This effect is verified on attention (partial eta squared 0.216, power of 0.749, $p= 0.01$) and executive function (partial eta squared 0.154, power of 0.570, $p= 0.03$). Both partial eta-square values were greater than 0.14, showing a large effect size that translates into superior cognitive performance in the IGHD group.

Test application time was in controls 36.2 (9.1) vs. 47.8 (4.6) min. literate vs. illiterate, $p= 0.02$. In IGH, it was 41.8 (7.6) vs. 49.3 (7.2) min, literate vs. illiterate, $p= 0.089$.

**Discussion**

Population aging has become a worldwide phenomenon, with projection that the number of people aged ≥65 years will be approximately 1 billion by 2030 (31). The consequences of GHD on brain aging remains controversial. Patients with acquired GHD may have several confounding factors: different etiologies of GHD, pituitary surgery, irradiation, use of anticonvulsants, deficits of other pituitary hormones with inadequacy of the respective replacement therapies (27). IGHD is rare, and often treated with GH
replacement therapy during childhood. Therefore, the Itabaianinha cohort, with severe congenital and mostly untreated IGHD, constitutes a valuable opportunity to study the effect of GHD on brain aging. In this work we have found that individuals with lifetime untreated IGHD older than 50 years exhibited a trend toward a higher total LICA score than the control group, without reaching statistical significance. However, they showed better attention and executive function than controls. These data contradict the widespread, though never proven, concept that reduced function of the somatotropic axis (i.e., GHRH, pituitary GH, and circulating IGF1) seen during aging causes the cognitive impairment often found in senescence. Accordingly, there has been no description of cases of dementia in the almost thirty years of follow-up of this cohort, nor a mention of dementia as a cause of death in the death certificates of IGHD deceased subjects born since 1892 (19).

The results of this study expand on our previous observations that in these subjects the deficiency of GH, except for severe short stature, possibly results in more benefit than harms (10, 11, 22). The harms involve unpleasant high-pitched voice, mild hearing loss, mild vestibular (10, 11, 25) and hip problems (32), increased total and LDL cholesterol, and higher systolic blood pressure (10, 11, 22). The benefits include reduced prevalence of vertebral fractures (32) with appropriate muscle function (33), increased insulin sensitivity (20), increased GLP-1 response to mixed meal (21), delayed atherosclerosis (34), protection against common cancers (such as breast, lung, intestine, and prostate), and prolonged healthspan (10, 11, 22). Remarkably, we also found a significant upregulation of age-related miRNAs, miR-100-5p, miR-195-5p, miR-181b-5p and miR-30e-5p, which are known for targeting pathways associated with longevity such as mTOR, AKT, NFκB, and IRS1s (22). As a result, despite the cardiovascular
disease risk factors, their longevity is similar to that of their unaffected siblings (19). The
excellent quality of life of these IGHD individuals is also notable (35).

These IGHD individuals, despite visceral obesity (36), do not exhibit insulin
resistance (on the contrary they have increased insulin sensitivity), as shown by lower
homeostasis model assessment index of insulin resistance (HOMA-IR) (p= 0.04), and
trend to elevation of quantitative insulin sensitivity check index and oral glucose insulin
sensitivity in 2 hours (p= 0.06 and 0.09, respectively) (20). Insulin resistance is a
crossroads of metabolic and cognitive disorders in humans (37). The hypothalamus,
frontal and striatal regions are particularly sensitive to insulin. Memory, hippocampal,
and visual brain regions are also modulated by insulin. Healthy insulin signaling
modulates brain networks involved in homeostatic control, human eating behavior reward
processing, and cognitive function. Thus, insulin might benefit memory function (37),
and support neuronal survival by triggering the release of glial cell neurotrophic factors
(38). In addition, insulin input controls neurotransmitter systems (norepinephrine and
acetylcholine) with relevance to memory function (36). Conversely, brain insulin
resistance can be considered a potential link between obesity, type 2 diabetes, and
dementia (39) or major depressive disorder (40), and peripheral insulin-resistance
assessed by HOMA-IR is negatively associated with cognitive function (41). Compared
with normal subjects, insulin-resistant subjects have reduced glucose metabolism in
middle temporal gyrus, middle frontal gyrus, right precentral gyrus, right inferior frontal
gyrus, right cuneiform lobe, and bilateral cerebellar regions as assessed by PET imaging
(42). These findings substantiate the insulin resistance-executive function hypothesis,
demonstrated by the differential impact of insulin-resistance on processing speed and
specific aspects of executive function (42). Based on all the above literature, we
hypothesize that the increased insulin sensitivity of these IGHD individuals (20) may contribute to their better attention and executive function.

Another possible explanation of our findings is the increased GLP-1 secretion (21). Modulation of GLP-1 activity may influence amyloid β peptide aggregation in Alzheimer's disease, and dopamine levels in Parkinson's disease. GLP-1 receptor agonists might also exert a beneficial effect on the cognitive impairment induced by diabetes or obesity, improving learning and memory by modulating synaptic plasticity (43).

While IGHD subjects have a mildly increased FT4, likely due to reduced function of the deiodinase system (30), their TSH is not different from controls. Therefore, we do not think that the FT4 difference is involved in the difference in brain function findings.

There are some reports of cognitive impairments in adult-onset GHD (AO-GHD), with somewhat positive (44), though inconsistent, effects of GH replacement therapy, particularly on attention (45) and self-reported memory (46), but no effects in patients with normal cognitive performance at baseline (47, 48). In this setting, GH therapy seems improve intellectual tasks, but it is not able to reduce the subjects’ distorted perception of body image (49). In addition, one placebo-controlled study found improved memory and attentional performance in 48 patients with CO-GHD (17 with IGHD) (50). However, AO-GHD is a different condition from childhood onset GHD. Indeed, IGHD subjects from Itabaianinha have normal quality of life (QoL), and treatment with GH depot for 6 months did not result in further total QoL improvement, except in satisfaction with physical endurance (35).

Looking at cognitive function in another model of genetic cause of GH/IGF-1 alteration (GH resistance), there appears to be some discrepancy between the Ecuadorian and Israeli Laron Syndrome cohorts in terms of cognitive function, reported as normal or improved in the former (51), and somehow compromised in the latter (52), (despite the
same genetic mutation), possibly indicating an effect of environment. There are differences between the Laron and Itabaianinha syndromes, likely due to some residual action of GH in the latter, not found in the former. Finally, we cannot exclude a specific effect of lack of GHRH receptor function in other areas of the brain, as, while the expression of the full-length receptor is mostly localized in the pituitary gland, splice variants are expressed in several areas of the brain (53).

This study has several strengths, such as the application of LICA by the same experienced examiner, and the match between the IGHD and control groups. A possible limitation of this study could be an adverse influence of the COVID pandemic on our results, as cognitive impairment may follow the resolution of acute COVID-19 infection (54). Such effect is unlikely, as cognitive function, assessed after the end of the pandemic in Brazil, was excellent in both groups. Furthermore, we have previously shown no difference in the prevalence of anti-SARS-CoV-2 IgM and IgG antibody positivity between IGHD subjects and matched normal stature individuals in October 2020, during the acceleration of the pandemic (55). The relatively low number of subjects is a limitation of the present study. We studied all the IGHD subjects above 50 years of age and one control for each IGHD subject, matched for gender, age, literacy, neighborhood, and socio-economic conditions. While recognizing that increasing the proportion of controls for each case would have increased the power of the study, we think that the careful matching of the groups supports the conclusions of similar cognitive function between cases and controls. It is noteworthy that no individual presented a score compatible with dementia in either of the two groups. This work expands the description of a rare genetic syndrome in a cohort that will not last forever due to the improvement of their geographic isolation, the reduction in consanguineous marriages, and the availability of GH therapy in childhood, all resulting in the reduction in the number of GH-naive individuals.
throughout their lives. Considering the above, we believe that the approach of collecting maximum information from this cohort is highly desirable rather than applying a sample size approach (56).

In conclusion, congenital untreated lifelong IGHD due to a GHRHR mutation is associated with total cognitive performance similar to controls, and better attention and executive function.

**Declaration of interest:** R. Salvatori serves on Novordisk advisory board.

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Table 1. Demographic, anthropometric, biochemical, blood pressure (BP) and Literacy Independent Cognitive Assessment (LICA) data of 15 IGHD subjects and 15 controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IGHD</th>
<th>Controls</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66.0 (8)</td>
<td>66.4 (6.5)</td>
<td>-5.8 to 5.0</td>
<td>0.88</td>
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<td>Sex, male (n%)</td>
<td>6 (40)</td>
<td>6 (40)</td>
<td>-0.3 to -0.3</td>
<td>1</td>
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<tr>
<td>Weight (kg)</td>
<td>46.5 (15.1)</td>
<td>66.6 (10.3)</td>
<td>-29.7 to -10.4</td>
<td>&lt;0.0001</td>
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<td>Height (m)</td>
<td>1.2 (0.1)</td>
<td>1.6 (0.1)</td>
<td>-0.4 to -0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.2 (8.4)</td>
<td>27.4 (2.9)</td>
<td>-0.9 to 8.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>50.7 (2.8)</td>
<td>54.7 (2.2)</td>
<td>-5.9 to -2.1</td>
<td>&lt;0.0001</td>
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<tr>
<td>Systolic BP (mm Hg)</td>
<td>131 (21)</td>
<td>140 (13)</td>
<td>-23.2 to 5.3</td>
<td>0.21</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
<td>82 (15)</td>
<td>82 (6)</td>
<td>-9.4 to 9.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Vitamin B12 (pg/mL)</td>
<td>500 (175)</td>
<td>526 (271)</td>
<td>-197.0 to 144.2</td>
<td>0.75</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>14.7 (3.6)</td>
<td>12.7 (3.3)</td>
<td>-0.6 to 4.5</td>
<td>0.14</td>
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<tr>
<td>TSH (mUI/mL)</td>
<td>3.2 (2.7)</td>
<td>1.9 (1.1)</td>
<td>-0.3 to 2.8</td>
<td>0.11</td>
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<tr>
<td>Free T4 (ng/dL)</td>
<td>1.0 (0.2)</td>
<td>0.9 (0.1)</td>
<td>0.03 to 0.2</td>
<td>0.02</td>
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<tr>
<td>Illiteracy (n%)</td>
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<td>5(33.3)</td>
<td>-0.3 to 0.3</td>
<td>1</td>
</tr>
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<td>Education years</td>
<td>6.4 (5.5)</td>
<td>4.7 (4.1)</td>
<td>-1.9 to 5.3</td>
<td>0.33</td>
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<tr>
<td>LICA ratings:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory</td>
<td>89.2 (19.3)</td>
<td>83.6 (11.9)</td>
<td>-6.5 to 17.6</td>
<td>0.35</td>
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<td>Visuoconstruction</td>
<td>28.0 (2.8)</td>
<td>26.2 (3.5)</td>
<td>-0.6 to 4.2</td>
<td>0.13</td>
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<tr>
<td>Language</td>
<td>39.4 (3.8)</td>
<td>39.4 (3.9)</td>
<td>-2.9 to 2.9</td>
<td>1</td>
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<tr>
<td>Executive function</td>
<td>38.3 (4.8)</td>
<td>35.1 (2.5)</td>
<td>0.3 to 6.1</td>
<td>0.03</td>
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<td>Attention</td>
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<td>0.3 to 2.1</td>
<td>0.01</td>
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<td>11.7 (0.5)</td>
<td>-0.5 to 0.2</td>
<td>0.37</td>
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<tr>
<td>Total Score</td>
<td>215.8 (22.7)</td>
<td>204.2 (18.1)</td>
<td>-3.7 to 28.0</td>
<td>0.13</td>
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