The GH–IGF system in amyotrophic lateral sclerosis: correlations between pituitary GH secretion capacity, insulin-like growth factors and clinical features

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Background and purpose: The growth hormone (GH) and insulin-like growth factor (IGF) system may be involved in neurodegenerative processes, and some abnormalities have been reported in amyotrophic lateral sclerosis (ALS). Our aim was to investigate the GH–IGF axis in patients with ALS and evaluate correlations between this endocrine system and clinical features.

Methods: Serum levels of GH, IGF-I, IGF-II, insulin, IGF-binding protein 1 (IGF-BP1), and IGF-binding protein 3 (IGF-BP3) were measured in 25 patients with ALS and 25 age-, gender-, and BMI-matched healthy controls. A GHRH plus arginine test was performed in patients and controls. Clinical status of patients was evaluated with the ALS Functional Rating Scale – Revised (ALSFRS-R) and upper motor neuron (UMN) score.

Results: GHRH plus arginine test showed GH deficiency (GHD) in 13 (52%) patients with ALS; severe GHD was found in 6 (24%) and partial GHD in 7 (28%) patients. IGF-I levels were significantly higher in patients with ALS than in healthy controls (182.9 ± 90.8 vs. 139.4 ± 58.1 ng/ml; $P = 0.015$). IGF-I levels were higher in patients with ALS with UMN score >10 than those with UMN score <10 (217.8 ± 100.8 vs. 155.5 ± 74.6 ng/ml, $P = 0.05$). IGF-II levels were significantly lower in patients with ALS than in healthy controls (720.9 ± 215 vs. 1001.9 ± 475.4 ng/ml; $P = 0.03$).

Conclusions: The results demonstrate an impairment of the GH–IGFs system in ALS. The degenerative process in ALS might lead to a compensatory increase in IGF-I in an attempt to provide additional support to motor neurons or degenerating muscle fibers. The decrease in IGF-II levels may also be of pathological significance.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal, usually sporadic, neurodegenerative disease affecting upper and lower motor neurons. There are many hypotheses about the pathogenesis of sporadic ALS, including autoimmune reactions to calcium channels on motor neurons, oxidative damage, accumulation of intracellular aggregates, mitochondrial dysfunction, defects in axonal transport, glial cell pathology, glutamate excitotoxicity, and loss of neurotrophic support to motor neurons [1,2].

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The insulin-like growth factors (IGF-I and IGF-II) are neurotrophic factors expressed in the central nervous system promoting survival and differentiation of neuronal cells including motor neurons [3–5]. There is evidence that IGF-I rescues motor neurons *in vitro* and in animal models [6,7], but therapeutic trials of human recombinant IGF-I in patients with ALS gave inconsistent results [8–10]. A clinical trial with recombinant growth hormone (GH) also failed to show any effect on disease progression in patients with ALS [11]. However, a high prevalence of GH deficiency (GHD) has been recently reported in patients with ALS [12].

There is evidence that the peripheral and the brain IGF systems are functionally linked. In fact, IGF-I and IGF-II can cross the blood brain barrier and uptake of circulating IGFs into the cerebrospinal fluid has been

demonstrated [13,14]. Partial and controversial data on IGF system have been reported in patients with ALS, but correlations with clinical features of the disease have been poorly investigated [15–18].

The present study aims at providing a comprehensive profile of the GH–IGF system in patients with ALS and at evaluating any correlation between this endocrine system and ALS clinical features.

Patients and methods

Subjects

Twenty-five patients (16 men, nine women, mean age 54.3 ± 9.2, range 31–71) with definite or probable ALS, according to El Escorial revised diagnostic criteria [19], and 25 control subjects (16 men, nine women, mean age 55.2 ± 9.4, range 32–73) were enrolled in the study. Patients were individually matched to a healthy control based on gender, age (± 3 years), and body mass index (BMI) (± 1.5 kg/m^2). BMI was 24.4 ± 2.1 kg/m^2 in patients and 24.7 ± 2.4 kg/m^2 in controls. None of the patients and controls had diabetes mellitus or had been under treatment with sex hormones, and all patients were in a good nutritional state. Table 1 summarizes demographic and clinical features of patients with ALS.

Patients with ALS with spinal (n = 21) and bulbar (n = 4) onset were included. All patients but two were receiving riluzole. Disease duration was 3.4 ± 3.7 years. Clinical status of each patient was evaluated with the ALS Functional Rating Scale – Revised (ALSFRS-R) and upper motor neuron (UMN) score. ALSFRS-R item scores are summed to produce a reported score of between 0 = worst and 48 = best, mostly reflecting lower motor neuron involvement. UMN item scores are summed to produce a score of between 0 = best and 16 = worst, reflecting UMN involvement. The mean (SD) ALSFRS-R score was 28.9 (8.5) with a range of 16–47; the mean (SD) UMN score was 8.1 (4.9), with a range of 0–16.

The study was approved by the Ethic Committee at the Second University of Naples. An informed consent was obtained by all subjects.

Study protocol

Venous blood samples were drawn in the morning after an overnight fast to evaluate baseline GH, IGF-I, IGF-binding protein 1 (IGF-BP1), IGF-binding protein 3 (IGF-BP3) and insulin levels. GH releasing hormone (GHRH) plus arginine test was then performed as follows: arginine (arginine hydrochloride, 30% solution) was infused iv over 30 min at the dose of 30 g, and 100 μg in bolus of GHRH (Geref; Serono Diagnostics, Norwell, MA, USA) was administered iv whilst starting arginine infusion. Blood samples were drawn every 30 min for 2 h, immediately centrifuged, and plasma samples were frozen (−20°C) until GH measurement. To reduce analytical variance, samples were batch analyzed using the same assay lot. According to the GH response to GHRH plus arginine test, a diagnosis of severe GHD was performed when GH peak was below 11.5, 8.0 and 4.2 ng/ml for lean, overweight and obese patients respectively, whereas a diagnosis of partial GHD was performed in case of GH peak between the cut-off of severe GHD and the cut-off of normal response, namely 16.5 ng/ml [20].

Table 1 Demographic and clinical features of patients with amyotrophic lateral sclerosis (ALS)

#	Age	Gender	BMI	Disease duration (years)	Cinical onset	UMN score	ALSFRS-R score
1	53	M	21.2	3	Spinal	12	27
2	62	M	27.05	4	Spinal	13	25
3	55	M	22.49	6	Spinal	8	19
4	61	M	23.73	3	Spinal	3	27
5	55	M	24.09	3	Bulbar	11	32
6	60	F	25	2	Spinal	13	22
7	57	M	27.85	19	Spinal	2	31
8	65	M	25.71	4	Spinal	2	25
9	53	F	26.72	2	Bulbar	7	37
10	47	F	24.97	2	Spinal	3	27
11	31	M	21.87	4	Spinal	6	16
12	42	M	22.49	1	Spinal	16	16
13	40	F	23.87	2	Spinal	15	30
14	44	M	23.51	1	Spinal	11	47
15	57	M	25.21	1	Spinal	5	23
16	47	M	22.85	1	Spinal	11	43
17	52	F	29.38	9	Spinal	2	31
18	57	M	23.38	4	Spinal	6	27
19	71	F	25.8	2	Spinal	5	37
20	49	F	22.89	1	Spinal	13	25
21	69	M	24.54	2	Bulbar	3	33
22	56	F	25.51	3	Spinal	0	36
23	62	M	25.28	1	Spinal	9	45
24	63	M	23.45	2	Bulbar	13	22
25	50	F	20.28	2	Spinal	15	19

ALSFRS-R, ALS Functional Rating Scale – Revised; UMN, upper motor neuron.

Hormone assays

Serum GH, IGF-II and insulin were measured by immunoradiometric assay (IRMA) using commercially available kits (Diagnostic Systems Laboratories, Webster, TX, USA). For GH, the sensitivity of the assay was 0.01 ng/ml, whereas the intra-assay coefficients of variation (CVs) were 3.1%, 3.9%, and 5.4% and the inter-assay CVs were 5.9%, 11.5%, and 8.8% for low, medium, and high points of the standard curve, respectively. For IGF-II, the sensitivity was 0.13 ng/ml, whereas the intra-assay CVs were 4.3%, 7.2%, 4.3%,

and the inter-assay CVs were 9.5%, 6.3%, and 10.4%. For insulin the sensitivity was 0.3 μUI/ml, whereas the intra-assay CVs were 2.6%, 2.1%, 2.2% and the inter-assay CVs were 4.7%, 2.9% and 4.8%.

Serum IGF-I, IGF-BP1, and IGF-BP3 were measured by enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Diagnostic Systems Laboratories). For IGF-I, the sensitivity of the assay was 0.01 ng/ml, whereas the intra-assay CVs were 8.6%, 4.5%, and 6.3% and the inter-assay CVs were 6.8%, 6.0%, and 3.3%. For IGF-BP1, the sensitivity of the assay was 0.25 ng/ml, whereas the intra-assay CVs were 4.6%, 2.5%, and 1.7% and the inter-assay CVs were 7.6%, 6.8%, and 6.2%. For IGF-BP3, the sensitivity of the assay was 0.04 ng/ml, whereas the intra-assay CVs were 9.6%, 9.5%, and 7.3% and the inter-assay CVs were 11.4%, 10.4%, and 8.2%.

Statistical analysis

For statistical purposes, patients were divided into two groups based on the ALSFRS-R score <25 and >25 and the UMN score <10 and >10. The statistical significance of the differences between patients with ALS and controls was analyzed using the Mann–Whitney test. Significance was set at 5%. Correlation analysis was performed calculating Pearson's or Spearman's coefficient, as appropriate. Results were expressed as mean $\pm$ SD.

Results

Baseline serum GH levels were similar in patients with ALS and controls ($P = 0.46$; Table 2). The peak GH responses to GHRH plus arginine test tended to be lower in patients with ALS than in healthy controls ($P = 0.08$; Table 2). According to the GH response to GHRH plus arginine test, 13 (52%) patients with ALS met the criteria for GHD: six (24%) patients had severe GHD and seven (28%) partial GHD. The prevalence of GHD was similar in female and male patients (55% vs. 50%) as well as between patients with bulbar and spinal ALS (50% vs. 52.4%). No significant difference in GHD prevalence was found between patients with ALSFRS-R score <25 or >25 (50% vs. 53%) or between patients with UMN score <10 or >10 (57% vs. 45%). No significant correlation was found between peak GH levels and age, disease duration, BMI, ALSFRS-R, and UMN scores in patients with ALS.

Insulin-like growth factor (IGF-I) levels were significantly higher in patients with ALS than in healthy controls ($P = 0.015$; Table 2). In patients with ALS, IGF-I levels were not significantly different between those with normal GH secretion and those with GHD, and they did not correlate with age, disease duration, and BMI. IGF-I levels were similar in patients with spinal and bulbar onset (186.8 $\pm$ 94.6 vs. 162.2 $\pm$ 74.3 ng/ml, $P = 0.59$). IGF-I levels were higher in patients with ALS with UMN score >10 than those with UMN score <10 (217.8 $\pm$ 100.8 vs. 155.5 $\pm$ 74.6 ng/ml, $P = 0.05$), but were similar in patients with an ALSFRS-R score <25 and >25 (178.1 $\pm$ 57.6 vs. 186.1 $\pm$ 109.4 ng/ml, $P = 0.84$). A significant direct correlation was found between IGF-I and baseline GH levels ($r = 0.400$, $P = 0.048$) as well as between IGF-I and IGF-BP3 ($r = 0.582$, $P = 0.002$), whereas a significant inverse correlation was found between IGF-I and IGF-BP1 levels ($r = -0.432$, $P = 0.031$).

Table 2 Serum hormonal levels in patients with amyotrophic lateral sclerosis (ALS) and healthy controls

	Patients ($n=25$)	Controls ($n=25$)	*P*-value
Baseline GH (ng/ml)	1.5 (1.4)	1.2 (1.5)	0.46
Peak GH (ng/ml)	16.9 (8.0)	19.8 (2.0)	0.08
IGF-I (ng/ml)	182.9 (90.8)	139.4 (58.1)	**0.015**
IGF-II (ng/ml)	720.9 (215)	1001.9 (475.4)	**0.03**
Insulin (μUI/ml)	22.8 (15.7)	16.7 (4.1)	0.24
IGF-BP1 (ng/ml)	18.1 (12.8)	22.8 (11.8)	0.109
IGF-BP3 (ng/ml)	4417 (1104)	4656 (1984)	0.94

GH, growth hormone; IGF, insulin-like growth factor. Significant *P* values are bold.

Insulin-like growth factor (IGF-II) levels were significantly lower in patients with ALS than in healthy controls ($P = 0.03$; Table 2). In patients with ALS, IGF-II levels were not significantly different between those with normal GH secretion and those with GHD, and they did not correlate with age, disease duration and BMI. IGF-II levels were significantly lower in patients with bulbar than spinal ALS (482.4 $\pm$ 61 vs. 766.4 $\pm$ 203 ng/ml; $P = 0.019$). IGF-II levels were significantly and inversely correlated with ALSFRS-R score ($r = -0.451$, $P = 0.024$). Conversely, no significant correlation was found between IGF-II levels and UMN score. A significant inverse correlation was also found between IGF-II and baseline GH levels ($r = -0.475$, $P = 0.016$).

The GH response to GHRH plus arginine test, IGF-I and IGF-II levels were similar in male and female patients.

No significant difference was found in serum insulin ($P = 0.24$; Table 2), IGF-BP1 ($P = 0.109$; Table 2), and IGF-BP3 ($P = 0.94$; Table 2) levels between patients with ALS and healthy controls.

Discussion

The results of the current study demonstrate that patients with ALS might present some impairment of

the GH–IGFs system. A deficit of GH secretion was found in half of the patients with ALS, with a similar proportion of severe and partial GHD without gender differences. These results are in partial disagreement with a previous study reporting a 73% prevalence of GHD amongst patients with ALS, with a higher frequency in men than women [12]. This discrepancy may be as a result of a different age of patients with ALS, as our patients were younger than those included in the previous study. Moreover, although in a previous study the interference of riluzole on GH response has been excluded in a small sample of patients with ALS evaluated before and after starting riluzole [21], a possible influence of riluzole treatment on GH response cannot be completely ruled out in our patients. Consistent with Morselli *et al.,* the results of the current study demonstrate the absence of any correlation between GH peak levels and age, BMI, or disease characteristics. It is difficult to understand whether GHD found in some patients may contribute to the pathogenesis of ALS or is a consequence of it. ALS is increasingly recognized as a multisystem disorder because of non-motor manifestations that can accompany it, especially behavioral and cognitive dysfunctions. We cannot exclude that hypothalamic neurones involved in the complex regulation of GH secretion may also be altered in some patients with ALS, but this hypothesis deserves further studies. It is noteworthy that clinical trials using recombinant GH in patients with ALS showed no effect on disease progression [11]; in this latter study, however, the GH secretory status was not assessed suggesting that a clinical trial with recombinant GH in selected patients with ALS with GHD is advisable to draw definitive conclusion about the meaning of GHD occurrence in a proportion of patients with ALS.

Insulin-like growth factor (IGF-I) levels were increased in patients with ALS and appeared to be higher in patients with more severe UMN involvement. At the cellular level, IGF-I is described as a prosurvival factor, generally acting through the phosphatidylinositol 3-kinase/Akt pathway to activate antiapoptotic cascades [22]. The disruption of neurotrophic support provided by IGF has been proposed to contribute to the pathogenesis of ALS [23]. Our findings suggest that the degenerative process in ALS could lead to a compensatory increase in IGF-I in an attempt to provide additional support to motor neurons. It is known that under pathological conditions, such as inflammatory and excitotoxic processes, nerve cells may become resistant to IGF-I, and the loss of sensitivity to IGF-I leads to a compensatory increase of IGF-I synthesis through a feedback mechanism [24]. It is noteworthy that ALS is associated with a progressive decrease of skeletal muscle mass and strength and that IGF-I is an important factor for the maintenance of muscle structure and function [25]; on the basis of these evidences, the possibility that the increase in IGF-I represents an attempt to counteract the muscle impairment cannot be ruled out. Some previous studies found normal or reduced serum IGF-I levels [15–17] in patients with ALS. Methodological differences may account for these discrepancies, especially considering the small sample sizes of patient and/or control groups and the lack of age-, gender- and BMI-matched control populations in these previous studies. More recently, Hosback *et al.* [18] found increased IGF-I levels in surviving patients with ALS versus matched controls, further supporting a neuroprotective role for IGF-I in this disease. Interestingly, IGF-I levels were not correlated to peak GH levels in patients with ALS as also reported by Morselli *et al*. [12]. The divergence between IGF-I secretion and GH response to provocative stimuli is not surprising, as it has often been observed in other clinical conditions associated with GHD, such as traumatic brain injury and subarachnoid hemorrhage and even in the presence of multiple pituitary hormone deficiencies [26–28]. IGF-II levels were reduced in patients with ALS and appeared to be lower in patients with bulbar than spinal form of the disease. Unlike IGF-I, the role of IGF-II in the brain is less established. IGF-II is the most abundantly expressed IGF in the adult central nervous system [29], where it binds to IGF-II receptor that has been suggested to have a role in control of neuronal growth, differentiation and repair, in addition to transporting lysosomal enzymes [30]. Most studies investigating changes in the peripheral IGF system in patients with ALS have focused on IGF-I, excluding IGF-II secretion from their analysis. Only one study previously assessing circulating IGF-II levels in patients with ALS did not find any difference when compared to controls [18]. We cannot exclude that the decrease in IGF-II levels in ALS is just secondary to IGF-I increase, in line with the evidence of IGF-II decrease following the recombinant human IGF-I administration in patients with insulin-dependent diabetes mellitus [31,32]. Nevertheless, the decrease in IGF-II levels observed in our patients may be of pathological significance. It is noteworthy that, although IGF-II is also produced in the brain, the principal source of serum IGF-II is the liver. We may hypothesize that hepatocytes synthesize lower amounts of IGF-II from the beginning of the disease because of a dysregulation of IGF-II synthesis. In this latter case, a reduction in IGF-II concentration could lead to a decrease in survival signal to motor neurons in the early stages of disease. An upregulation of IGF-II receptors has been found in the spinal cord of ALS transgenic rats [33] and patients with ALS [34,35]. This could represent a compensatory

mechanism to the GHD and the IGF-I resistance for maintaining the trophic actions of IGF system in the nervous system. On the other hand, the upregulation of IGF-II receptors might also be a consequence of the primitive reduction of the IGF-II synthesis. As the IGF-II synthesis is mainly under genetic control, the possibility that a genetic predisposition to ALS is associated with a progressive decrease of IGF-II synthesis and secretion cannot be ruled out.

In conclusion, an impairment of GH–IGFs system, characterized by a GHD status in a proportion of patients together with increased IGF-I and decreased IGF-II levels, is associated with ALS. The results of the current study do not permit to infer if these endocrine abnormalities precede ALS development or if they occur as a consequence of the disease. Further studies are required to investigate the pathogenetic contribution of GH, IGF-I, and IGF-II abnormalities to the course of ALS as well as their potential implications for future therapeutic strategies.

References

1. Rowland LP, Schneider NA. Amyotrophic lateral sclerosis. *N Engl J Med* 2001; **344:** 1688–1700.
2. Rothstein JD. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann Neurol* 2009; **65**(Suppl. 1)**:** S3–S9.
3. D'Costa AP, Prevette DM, Houenou LJ, *et al*. Mechanisms of insulin-like growth factor regulation of programmed cell death of developing avian motoneurons. *J Neurobiol* 1998; **36:** 379–394.
4. Pu SF, Zhuang HX, Marsh DJ, Ishii DN. Insulin-like growth factor-II increase and IGF is required for postnatal rat spinal motoneuron survival following sciatic nerve axotomy. *J Neurosci Res* 1999; **55:** 9–16.
5. Koopmans GC, Brans M, Gomez-Pinilla F, *et al*. Circulating insulin-like growth factor I and functional recovery from spinal cord injury under enriched housing conditions. *Eur J Neurosci* 2006; **23:** 1035–1046.
6. Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. Retrograde viral delivery of IGF-I prolongs survival in a mouse ALS model. *Science* 2003; **301:** 839–842.
7. Nagano U, Ilieva H, Shiote M, *et al*. Therapeutic benefit of intrathecal injection of insulin-like growth factor-I in a mouse model of Amyotrophic Lateral Sclerosis. *J Neurol Sci* 2005; **235:** 61–68.
8. Borasio GD, Robberecht W, Leigh PN, *et al*. A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis. *Neurology* 1998; **51:** 235–238.
9. Lai EC, Felice KJ, Festoff BW, *et al*. Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. *Neurology* 1997; **49:** 1621–1630.
10. Sorenson EJ, Windbank AJ, Mandrekar JN, *et al*. Subcutaneous IGF-I is not beneficial in 2-year ALS trial. *Neurology* 2008; **71:** 1770–1775.
11. Smith RA, Melmed S, Sherman B, Frane J, Munsat TL, Festoff BW. Recombinant growth hormone treatment of amyotrophic lateral sclerosis. *Muscle Nerve* 1993; **16:** 624–633.
12. Morselli LL, Bongioanni M, Genovesi R, *et al*. Growth hormone secretion is impaired in amyotrophic lateral sclerosis. *Clin Endocrinol* 2006; **65:** 385–388.
13. Reinhardt RR, Bondy CA. Insulin-like growth-factors cross the blood-brain barrier. *Endocrinology* 1994; **135:** 1753–1761.
14. Pulford BE, Ishii DN. Uptake of circulating insulin-like growth factors (IGFs) into cerebrospinal fluid appears to be independent of the IGF receptors as well as IGF-binding proteins. *Endocrinology* 2001; **142:** 213–220.
15. Bilic E, Bilic E, Rudan I, *et al*. Comparison of the growth hormone, IGF-I and insulin in cerebrospinal fluid and serum between patients with motor neuron disease and healthy controls. *Eur J Neurol* 2006; **13:** 1340–1345.
16. Braunstein GD, Reviczky AL. Serum insulin-like growth factor-I levels in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry* 1987; **50:** 792–794.
17. Torres-Aleman I, Barrios V, Berciano J. The peripheral insulin-like growth factor system in amyotrophic lateral sclerosis and in multiple sclerosis. *Neurology* 1998; **50:** 772–776.
18. Hosback S, Hardiman O, Nolan CM, *et al*. Circulating insulin-like growth factors and related binding proteins are selectively altered in amyotrophic lateral sclerosis and multiple sclerosis. *Growth Horm IGF Res* 2007; **17:** 472–479.
19. Brooks BRMR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 2000; **1:** 293–298.
20. Corneli G, Di Somma C, Baldelli R, *et al*. The cut-off limits of the GH response to GH-releasing hormone-arginine test related to body mass index. *Eur J Endocrinol* 2005; **153:** 257–264.
21. Morselli LL, Bongioanni P, Genovesi M, *et al*. Impairment of GH secretion in amyotrophic lateral sclerosis is not affected by riluzole treatment. *J Endocrinol Invest* 2007; **30:** 767–770.
22. Peruzzi F, Prisco M, Dews M, *et al*. Multiple signaling pathways of the insulin-like growth factor I receptor in protection from apoptosis. *Mol Cell Biol* 1999; **19:** 7203–7215.
23. Wilczak N, de Vos AI, De Keyser J. Free insulin-like growth factor (IGF)-I and IGF binding proteins 2, 5 and 6 in spinal motor neurons in amyotrophic lateral sclerosis. *Lancet* 2003; **361:** 1007–1011.
24. Trejo JL, Carro E, Garcia-Galloway E, Torres-Aleman I. Role of insulin-like growth factor I signaling in neurodegenerative diseases. *J Mol Med* 2004; **82:** 156–162.
25. Clemmons DR. Role of IGF-I in skeletal muscle mass maintenance. *Trends Endocrinol Metab* 2009; **20:** 349–356.
26. Gasperi M, Aimaretti G, Scarcello G, *et al*. Low dose hexarelin and growth hormone (GH)-releasing hormone as diagnostic tool for the diagnosis of GH deficiency in adults:comparison with insulin-induced hypoglycemia test. *J Clin Endocrinol Metab* 1999; **84:** 2633–2637.
27. Aimaretti G, Ambrosio MR, Di Somma C, *et al*. Traumatic brain injury and subarachnoid haemorrhage are conditions at high risk for hypopituitarism:screening study at 3 months after the brain injury. *Clin Endocrinol* 2004; **61:** 320–326.

28. Hilding A, Hall K, Wivall-Helleryd IL, Saaf M, Melin AL, Thoren M. Serum levels of insulin-like growth factor I in 152 patients with growth hormone deficiency, aged 19-82 years, in relation to those in healthy subjects. *J Clin Endocrinol Metab* 1999; **84:** 2013–2019.
29. Russo VC, Gluckman PD, Feldman EL, Werther GA. The insulin-like growth factor system and its pleiotropic functions in brain. *Endocr Rev* 2005; **26:** 916–943.
30. Hawkes C, Kar S. The insulin-like growth factor-II/mannose-6-phosphate receptor: structure, distribution and function in the central nervous system. *Brain Res Brain Res Rev* 2004; **44:** 117–140.
31. Cheetham TD, Taylor A, Holly JM, Clayton K, Cwyfan-Hughes S, Dunger DB. The effects of recombinant human insulin-like growth factor-I (IGF-I)administration on the levels of IGF-I, IGF-II and IGF-binding proteins in adolescents with insulin-dependent diabetes mellitus. *J Endocrinol* 1994; **142:** 367–374.
32. Cheetham TD, Holly JM, Baxter RC, *et al*. The effects of recombinant human IGF-I administration on concentrations of acid labile subunit, IGF binding protein-3, IGF-I, IGF-II and proteolysis of IGF binding protein-3 in adolescents with insulin-dependent diabetes mellitus. *J Endocrinol* 1998; **157:** 81–87.
33. Dagvajantsan B, Aoki M, Warita H, Suzuki N, Itoyama Y. Up-regulation of insulin-like growth-factor-II receptor in reactive astrocytes in the spinal cord of amyotrophic lateral sclerosis transgenic rats. *Tohoku J Exp Med* 2008; **214:** 303–310.
34. Doré S, Krieger C, Kar S, Quirion R. Distribution and levels of insulin-like growth factor (IGF-I and IGF-II) and insulin receptor binding sites in the spinal cords of amyotrophic lateral sclerosis (ALS) patients. *Brain Res Mol Brain Res* 1996; **41:** 128–133.
35. Kihira T, Suzuki A, Kubo T, Miwa H, Kondo T. Expression of insulin-like growth factor-II and leukemia inhibitory factor antibody immunostaining on the ionized calcium-binding adaptor molecule 1-positive microglias in the spinal cord of amyotrophic lateral sclerosis patients. *Neuropathology* 2007; **27:** 257–268.

