

# IGFBP1 as a metabolic-neurodegenerative biomarker in spinocerebellar ataxia type 3

CHUNGMIN CHIU<sup>1,2</sup>, WENLING CHENG<sup>3</sup>, TATSUNG LIN<sup>3</sup>, HUIJU CHANG<sup>4</sup>, YUJUN CHANG<sup>5</sup>, SHIH LI SU<sup>6,7</sup>, CHIAJU LEE<sup>8</sup>, HENHONG CHANG<sup>2,9,10</sup> and CHINSAN LIU<sup>2,3,8</sup>

<sup>1</sup>Department of Chinese Medicine, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>2</sup>Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung 40447, Taiwan, R.O.C.; <sup>3</sup>Vascular and Genomic Center, Institute of ATP, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>4</sup>Center of Regenerative Medicine and Tissue Repair, Institute of ATP, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>5</sup>Big Data Center, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>6</sup>Vascular Medicine and Diabetes Research Center, Institute of ATP, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>7</sup>Division of Endocrinology and Metabolism, Department of Internal Medicine, Diabetes Education Center, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>8</sup>Department of Neurology, Changhua Christian Hospital, Changhua 50006, Taiwan, R.O.C.; <sup>9</sup>Department of Chinese Medicine, China Medical University Hospital, Taichung 40447, Taiwan, R.O.C.; <sup>10</sup>Chinese Medicine Research Center, China Medical University, Taichung 40447, Taiwan, R.O.C.

Received September 9, 2025; Accepted January 9, 2026

DOI: 10.3892/etm.2026.13100

**Abstract.** Spinocerebellar ataxia type 3 (SCA3) is a progressive neurodegenerative disorder for which reliable metabolic biomarkers are lacking. Insulin-like growth factor binding protein 1 (IGFBP1), a stress-responsive protein regulated by insulin signaling, serves as an indicator of neurodegenerative burden. The present study aimed to measure the plasma levels of insulin, glucose, IGF1, IGF2, IGFBP1, IGFBP3 and neurofilament light chain (NfL) in patients with genetically confirmed SCA3 and age-matched controls. In addition, the association between the above molecules and clinical severity were assessed using the scale for the Assessment and Rating of Ataxia score, body mass index (BMI) and NfL levels, whereas metabolic-neurodegenerative interactions were assessed by stratifying patients by insulin tertiles. A total of 32 individuals with SCA3 and 36 age- and sex-matched controls were enrolled in the current study. The results demonstrated that patients with SCA3 exhibited markedly elevated IGFBP1, IGF2 and free IGF1 levels, as well as reduced insulin and higher glucose-to-insulin ratios, thus indicating disrupted insulin signaling. IGFBP1

was positively associated with SARA score and NfL levels and negatively associated with BMI. Notably, patients in the lowest insulin tertile (<3.65  $\mu$ IU/ml) showed significantly higher IGFBP1 and NfL levels compared with the remaining groups, thus suggesting that the insulin/IGFBP1/NfL axis was associated with ataxia severity. Collectively, IGFBP1 could be a promising peripheral biomarker reflecting both metabolic and neurodegenerative processes in SCA3 and could facilitate monitoring of disease stages.

## Introduction

The insulin/insulin-like growth factor 1 (IGF1) signaling (IIS) pathway plays a notable role in regulating systemic metabolism, growth and cellular survival; it involves insulin, IGF1 and IGF binding proteins (IGFBPs), which collectively modulate glucose homeostasis and energy utilization (1,2). In the central nervous system (CNS), insulin and IGF1 are involved in neuronal maintenance, synaptic plasticity and myelination via regulation of metabolic and trophic signaling pathways. Dysregulation of the IIS pathway is involved in the onset of several neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease and Huntington's disease (3,4).

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, is a progressive autosomal dominant neurodegenerative disorder which is characterized by cerebellar ataxia, pyramidal signs, peripheral neuropathy and autonomic dysfunction (5). The disease is caused by a CAG repeat expansion in the ataxin-3 (*ATXN3*) gene, eventually leading to neurodegeneration. Neurofilament light chain (NfL) has emerged as a reliable blood biomarker reflecting neuronal injury and ataxia severity (6,7); however, additional biomarkers capturing metabolic dysfunction or systemic catabolism remain understudied.

*Correspondence to:* Professor Chinsan Liu, Department of Neurology, Changhua Christian Hospital, 7th Floor, 235 Syuguang Road, Changhua 50006, Taiwan, R.O.C.  
E-mail: liu48111@gmail.com

Professor Henhong Chang, Department of Chinese Medicine, China Medical University Hospital, 2 Yude Road, North, Taichung 40447, Taiwan, R.O.C.  
E-mail: temchh55@gmail.com

**Key words:** Machado-Joseph disease, IGFBP1, neurofilament light chain, biomarker, metabolic stress, ataxia stage, insulin

Early studies in SCA3 and other cerebellar ataxias indicate potential alterations in the IIS pathway, including reduced circulating insulin and IGFBP1 levels, suggesting an association between impaired metabolic signaling and disease status (8,9). IGFBP1, one of the six high-affinity IGFBPs, plays a central role in modulating IGF1 bioavailability and insulin sensitivity. Produced primarily in the liver, IGFBP1 is markedly downregulated by insulin and increased during fasting, metabolic stress and catabolic states. A study demonstrated that in contrast to insulin, IGFBP1 exhibited slower kinetics, making it a more stable indicator of sustained metabolic adaptation (10). Elevated IGFBP1 levels have also been associated with reduced body mass index (BMI), hepatic dysfunction and adverse outcomes across chronic diseases, such as diabetes, chronic liver disease and systemic inflammatory states (11,12).

Furthermore, animal study using growth hormone demonstrated transient motor improvement and neuroprotective effects in SCA3 model (13), while IGF1 supplementation shows similar effects in both animal models and patients (14,15). However, these earlier investigations were limited by small sample sizes, variable treatment durations and the lack of direct association with objective biomarkers of neurodegeneration, leaving the relevance of the IIS pathway, particularly IGFBP1, uncertain in the context of SCA3 pathophysiology. To address this gap, the present study investigated whether plasma IGFBP1 levels are associated with clinical manifestations and neurodegenerative burden in SCA3. Particularly, the associations among IGFBP1, the Scale for the Assessment and Rating of Ataxia (SARA) (16), NfL, BMI (17) and insulin were assessed. Accordingly, a case-control study was performed comparing individuals with SCA3 with age- and sex-matched healthy controls to evaluate IGFBP1 as a potential biomarker of ataxia severity.

## Materials and methods

**Participants.** A total of 32 patients with genetically confirmed SCA3 were recruited from the Changhua Christian Hospital (Changhua, Taiwan) between May 2021 and February 2022. The study protocol was approved by the Institutional Review Board of Changhua Christian Hospital (IRB nos. 200703 and 200730 for healthy control), and written informed consent was obtained from all participants. The inclusion criteria were as follows: i) Patients with a confirmed CAG repeat expansion in the *ATXN3* gene; and ii) age 20-80 years. The exclusion criteria included comorbidities that could affect glucose or insulin metabolism, such as a cancer, stroke, heart failure, renal failure or diabetes mellitus, and conditions known to affect insulin or IGF signaling, including marked hepatic, renal or thyroid dysfunction, based on medical record review. Medication histories were also screened, verifying no use of drugs known to affect IGF1 or IGFBP1 levels except coenzyme Q10 (18), which was analyzed separately. Ataxia severity was assessed using SARA (16), a semi-quantitative scale (range, 0-40) evaluating gait, stance, limb coordination and speech (19). Based on SARA scores, patients were classified into the following three disease stages (20,21): Preclinical (SARA <3), stage I (SARA ≤11) and stage II (SARA >11). The control group consisted of 36 age- and sex-matched healthy individuals who met the

same exclusion criteria to ensure comparable metabolic and clinical backgrounds. These participants were recruited from the outpatient clinics at Changhua Christian Hospital. Basic demographic and clinical data, including sex, age, BMI, age at onset, disease duration and CAG repeat number, were recorded for all participants.

**Plasma biochemistry of NfL and IIS components.** All blood samples were collected under standardized postprandial conditions. Participants consumed a typical Taiwanese-style lunch (600-700 kcal) with balanced macronutrient composition (carbohydrates, protein and fat). The meal was self-selected by the participants and the details of the intake were self-reported prior to the study measurements to confirm consistency with the required caloric and macronutrient range. The participants then followed a fixed sampling schedule, with blood drawn at ~2:00 p.m., corresponding to a consistent 2-h postprandial interval. To minimize acute metabolic variability, no additional food, caffeine, caloric beverages, strenuous physical activity or nutritional supplementation were allowed between lunch and blood collection (22). Samples were drawn in 10 ml BD Vacutainer® EDTA tubes (Becton, Dickinson and Company), centrifuged at 1,400 x g at 4°C for 15 min to obtain plasma and stored at -80°C until analysis. All biochemical analyses were performed using plasma to ensure methodological consistency and minimize clot-related pre-analytical variability. Plasma NfL levels were quantified using the Simoa® NF-light™ Advantage Kit (cat. no. 103186) and on the Simoa HD-X ultra-sensitive protein molecular detection instrument (both Quanterix) (23). Additionally, the plasma levels of IGF1, IGF2, IGFBP1, IGFBP3, glucose, and insulin were quantified using the Human IGF1/IGF1 Immunoassay kit (cat. no. DG100b) and the Human IGF2 Quantikine ELISA Kit (cat. no. DG200) (both R&D Systems China Co., Ltd.), the Human IGFBP-1 ELISA Kit (cat. no. ELH-IGFBP; RayBiotech Life), the Human IGFBP3 ELISA Kit (cat. no. AB100541; Abcam), the Glucose Colorimetric Assay Kit (cat. no. 10009582; Cayman Chemical Company) and the Insulin ELISA Kit (cat. no. 10-1113-01; Mercodia AB), respectively, according to the manufacturer's instructions. Free IGF1 levels were estimated using the IGF1/IGFBP3 ratio, a commonly used surrogate marker for bioavailable IGF1. This ratio provides an indirect estimate and does not replace direct measurement of free IGF1. Plasma glucose-to-insulin ratio (G/I ratio) was calculated as glucose (mg/dl) divided by insulin (μIU/ml) and used as a practical surrogate for insulin sensitivity in blood samples obtained 2 h postprandially, in contrast to fasting-based indices such as the Homeostatic Model Assessment 2 (24) or the Quantitative Insulin Sensitivity Check Index (25).

**Statistical analysis.** The normality of data distribution was assessed using the Shapiro-Wilk test, and most demographic and IIS-related variables showed non-normal distributions and were therefore summarized as medians and interquartile ranges (IQRs). Group differences were compared using the Mann-Whitney U test, and correlations were assessed using Spearman's rank correlation. Non-parametric methods were applied throughout the analyses due to skewed distributions and presence of potential outliers. Categorical variables, such as sex, were analyzed using the  $\chi^2$  test or Fisher's exact test,

Table I. Demographics and insulin/IGF1 system of healthy controls (n=36) and patients with SCA3 (n=32).

Parameter	Control	SCA3	P-value
<b>Demographic</b>			
Male sex	22 (61.1)	20 (62.5)	0.906
Age, years	47.5 (34.8-55.0)	49.0 (34.3-54.8)	0.754
BMI, kg/m <sup>2</sup>	23.6 (21.8-25.6)	21.8 (19.5-25.4)	0.040 <sup>a</sup>
Age at onset, years	N/A	35.0 (29.8-45.0)	
Duration, years	N/A	10.0 (4.0-12.0)	
CAG repeat number	N/A	71.5 (69.3-74.0)	
SARA	N/A	14.5 (7.4-21.0)	
NfL, pg/ml	6.3 (4.2-7.6)	27.8 (20.5-32.5)	<0.001 <sup>a</sup>
<b>Insulin/IGF1 system</b>			
IGF1, ng/ml	55.2 (34.0-68.1)	92.6 (67.9-108.6)	<0.001 <sup>a</sup>
IGF2, ng/ml	312.8 (271.7-361.4)	336.2 (316.2-395.0)	0.002 <sup>a</sup>
IGFBP1, pg/ml	3.5 (3.5-41.5)	252.9 (72.8-740.4)	<0.001 <sup>a</sup>
IGFBP3, ng/ml	43.3 (37.8-47.9)	41.8 (38.1-54.6)	0.873
IGF1/IGFBP3 ratio	1.27 (0.96-1.75)	1.92 (1.35-2.49)	0.002 <sup>a</sup>
Glucose, mg/dl	95.9 (73.6-116.5)	98.9 (81.2-112.7)	0.708
Insulin, $\mu$ IU/ml	19.0 (8.8-28.1)	6.1 (2.6-11.0)	<0.001 <sup>a</sup>
G/I ratio	6.0 (3.3-9.3)	17.1 (8.6-35.0)	<0.001 <sup>a</sup>

Data are presented as median (interquartile range), with the exception of sex which is n (%). Sex was analyzed using the  $\chi^2$  test, whereas all other continuous variables were analyzed using the Mann-Whitney U test; <sup>a</sup>P<0.05. SCA3, spinocerebellar ataxia type 3; IGF1, insulin-like growth factor 1; BMI, body mass index; SARA, scale for the assessment and rating of ataxia; NfL, neurofilament light chain; IGFBP, insulin-like growth factor binding protein; N/A, not applicable.

as appropriate based on expected cell counts. For IGFBP1, the majority of control samples displayed optical density values at or below the lower limit of the standard curve. In the absence of a laboratory-specific detection limit, the sensitivity of the assay (5 pg/ml) was used as a surrogate lower limit, and values below this threshold were imputed as sensitivity/ $\sqrt{2}$  ( $\approx$ 3.5 pg/ml), following established approaches for handling left-censored biomarker data (26). Receiver operating characteristic (ROC) curve analyses were performed to evaluate the ability of IGFBP1 and other IIS markers to discriminate between early and advanced SCA3 stages, with area under the curve (AUC) values used to compare their diagnostic performance. Insulin levels were categorized into tertiles to produce three groups with an approximately equal number of cases in each group (T1, <3.65  $\mu$ IU/ml; T2: 3.65-9.35  $\mu$ IU/ml; T3: >9.35  $\mu$ IU/ml). Group differences between insulin tertiles were assessed using the Kruskal-Wallis test, followed by Dunn's post hoc test. P<0.05 was considered to indicate a statistically significant difference. All data analyses were performed using IBM Statistical Package for the Social Sciences for Windows, version 22.0 (IBM Corp.).

## Results

**Participant characteristics and baseline differences.** A total of 32 genetically confirmed patients with SCA3 and 36 age- and sex-matched healthy controls were enrolled in the present study. There were no significant differences in age [median, 49.0 (IQR, 34.3-54.8) vs. 47.5 (34.8-55.0) years; P=0.754]

or sex distribution (male, 62.5 vs. 61.1%; P=0.906) between groups. BMI was significantly lower in patients with SCA3 compared with the healthy controls [21.8 (19.5-25.4) vs. 23.6 (21.8-25.6) kg/m<sup>2</sup>; P=0.040]. In addition, plasma NfL levels were significantly elevated in the SCA3 group compared with the control group [27.8 (20.5-32.5) vs. 6.3 (4.2-7.6) pg/ml; P<0.001; Table I].

**Elevated IGFBP1, IGF2 and free IGF, and insufficient insulin levels in patients with SCA3.** Patients with SCA3 exhibited a significant increase in plasma IGFBP1 compared with controls [252.9 (72.8-740.4) vs. 3.5 (3.5-41.5) pg/ml; P<0.001]. Total IGF1 levels were also higher in the SCA3 group [92.6 (67.9-108.6) vs. 55.2 (34.0-68.1) ng/ml; P<0.001], as were IGF2 levels [336.2 (316.2-395.0) vs. 312.8 (271.7-361.4) ng/ml; P=0.002]. No significant difference was observed in IGFBP3 levels (P=0.873); however, the IGF1/IGFBP3 ratio, representing free IGF1, was significantly higher in patients with SCA3 [1.92 (1.35-2.49) vs. 1.27 (0.96-1.75); P=0.002], suggesting enhanced IGF1 bioavailability (Table I).

To assess potential confounding by coenzyme Q10 supplementation, all demographic and IIS-related variables were compared in patients treated (n=17) or not (n=15) with Q10. The results revealed no significant differences between the two groups (Table SI).

Given the observed elevation in IGFBP1, upstream insulin-related parameters were further assessed to explore potential regulatory mechanisms. Glucose levels were comparable between the SCA3 and control groups [98.9 (81.2-112.7)

Table II. Subgroup analysis of SARA stage I (n=11) vs. Stage II (n=21) in patients with spinocerebellar ataxia type 3.

Parameter	Stage I (SARA $\leq$ 11)	Stage II (SARA $>$ 11)	P-value
<b>Demographics</b>			
Male sex	7 (63.6)	13 (61.9)	>0.999
Age, years	49.0 (29.0-51.0)	49.0 (41.5-59.5)	0.150
BMI, kg/m <sup>2</sup>	25.4 (22.1-26.9)	21.2 (18.8-22.9)	0.005 <sup>a</sup>
Age at onset, years	33.0 (28.0-45.0)	35.0 (32.5-46.5)	0.945
Duration, years	4.0 (0-6.0)	11.0 (9.0-15.0)	<0.001 <sup>a</sup>
CAG repeat number	71.0 (68.0-73.0)	72.0 (70.0-75.0)	0.402
SARA	5.0 (3.0-8.5)	20.0 (14.5-22.5)	<0.001 <sup>a</sup>
NfL, pg/ml	18.8 (17.0-28.3)	29.5 (25.1-38.1)	0.002 <sup>a</sup>
<b>Insulin/IGF1 system</b>			
IGF1, ng/ml	73.0 (59.4-124.8)	94.2 (76.4-107.1)	0.577
IGF2, ng/ml	365.7 (320.1-406.9)	366.7 (310.6-395.0)	0.996
IGFBP1, pg/ml	48.3 (13.0-137.0)	415.5 (207.9-913.2)	0.002 <sup>a</sup>
IGFBP3, ng/ml	40.2 (36.4-72.5)	41.9 (35.8-53.8)	0.907
IGF1/IGFBP3 ratio	1.54 (1.23-3.17)	1.96 (1.36-2.48)	0.815
Glucose, mg/dl	99.1 (81.2-104.4)	98.8 (81.2-124.1)	0.584
Insulin, $\mu$ IU/ml	6.0 (4.0-9.3)	6.9 (2.2-12.2)	0.969
G/I ratio	18.2 (10.8-21.7)	16.1 (8.5-38.4)	0.696

All data are presented as median (interquartile range), with the exception of sex which is n (%). Sex was analyzed using the Fisher's exact test as appropriate, whereas all other continuous variables were analyzed using the Mann-Whitney U test; <sup>a</sup>P<0.05. SARA, scale for the assessment and rating of ataxia; BMI, body mass index; NfL, neurofilament light chain; IGF1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; G/I ratio, glucose/insulin ratio.

vs. 95.9 (73.6-116.5) mg/dl; P=0.708], whereas insulin levels were significantly decreased in patients with SCA3 [6.1 (2.6-11.0) vs. 19.0 (8.8-28.1)  $\mu$ IU/ml; P<0.001]. Consequently, the G/I ratio, a surrogate marker of insulin deficiency or sensitivity, was significantly increased in patients with SCA3 [17.1 (8.6-35.0) vs. 6.0 (3.3-9.3); P<0.001], indicating impaired insulin signaling (Table I).

*IGFBP1 distinguishes ataxia severity in SCA3.* Patients with SCA3 were divided into early-stage (SARA  $\leq$ 11) and advanced-stage (SARA >11) groups. Compared with early-stage patients, those with advanced ataxia exhibited significantly higher plasma IGFBP1 levels [415.5 (207.9-913.2) vs. 72.5 (13.1-108.1) pg/ml; P=0.003] as well as higher NfL levels [29.5 (25.1-38.1) vs. 18.8 (17.0-28.3) pg/ml; P=0.002]. These findings indicate that both IGFBP1 and NfL effectively distinguish between early and advanced stages of ataxia in SCA3. As expected, patients in the advanced stage also had longer disease duration [11.0 (9.0-15.0) vs. 4.0 (0-6.0) years; P<0.001] and lower BMI [21.2 (18.8-22.9) vs. 25.4 (22.1-26.9) kg/m<sup>2</sup>; P=0.005], which was consistent with greater disease burden. Other IIS-related parameters, including glucose, insulin, the G/I ratio, IGF1, IGF2, IGFBP3 and the IGF1/IGFBP3 ratio, did not differ significantly between stages (all P>0.05) (Table II). Furthermore, ROC curve analysis demonstrated that IGFBP1 had the strongest discriminatory ability for differentiating early from advanced disease stages (AUC=0.829; 95% CI, 0.646-1.000; P=0.003). With an optimal cutoff value of 139.1 pg/ml, a sensitivity of 91% and specificity of 82% was

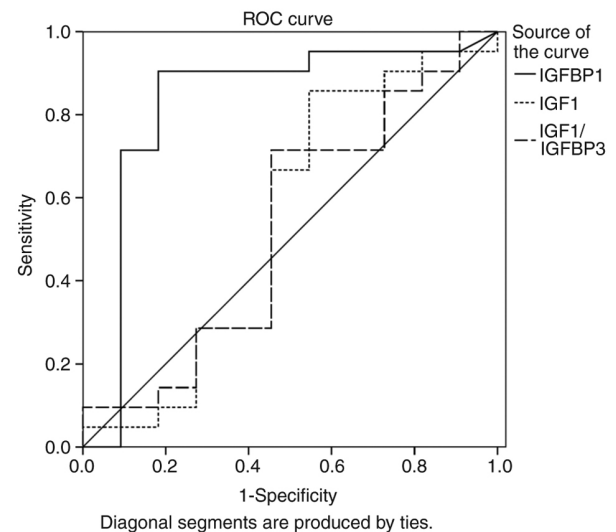


Figure 1. ROC curve analysis of IGFBP1 and other insulin/IGF1 signaling-associated markers for distinguishing early- and advanced-stage spinocerebellar ataxia type 3. ROC, receiver operating characteristic; IGF, insulin-like growth factor; IGFBP, IGF binding protein.

yielded, outperforming other IIS parameters (AUC range for other parameters, 0.385-0.541) (Fig. 1; Table SII).

*IGFBP1 is associated with clinical severity and neurodegenerative indicators.* In the SCA3 cohort, Spearman's correlation analysis revealed that IGFBP1 levels were

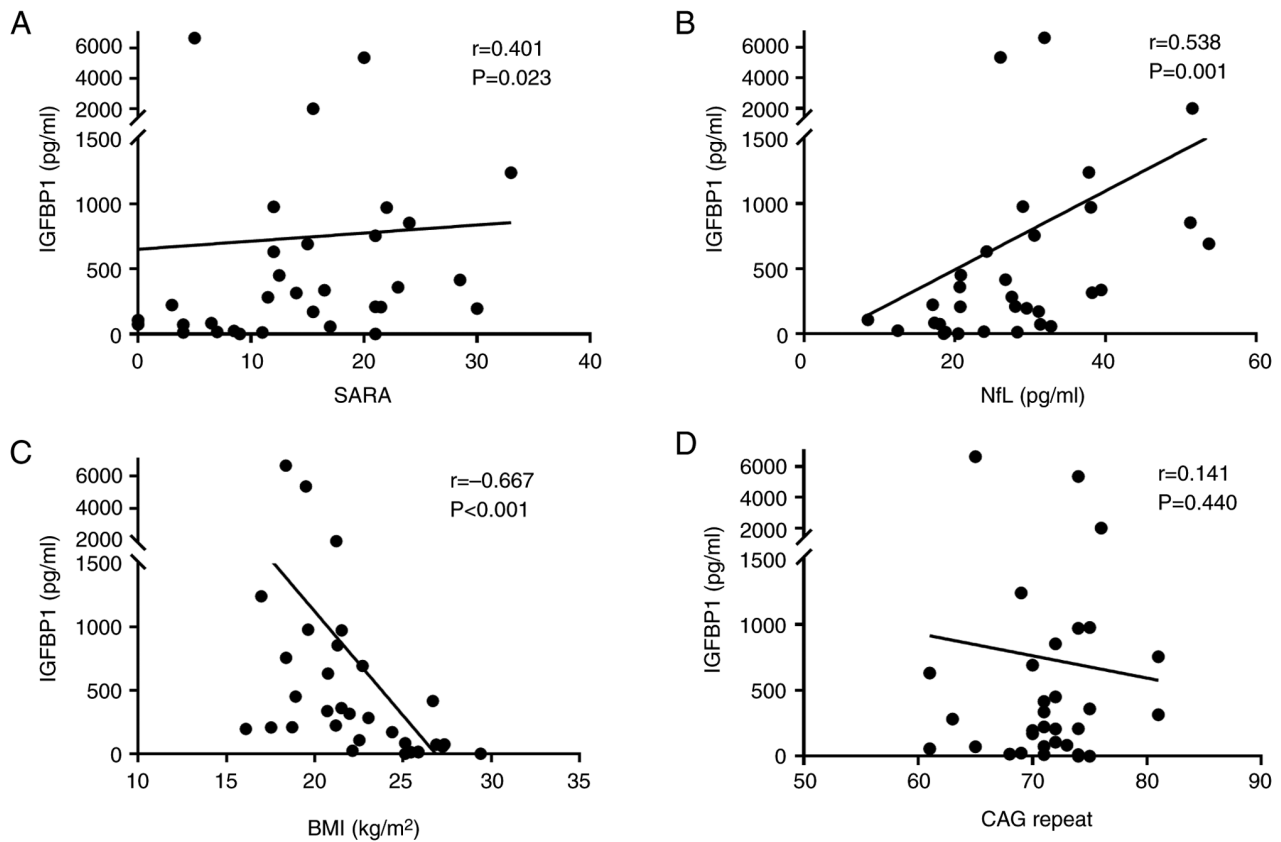


Figure 2. Correlation between plasma IGFBP1 and clinical parameters. Spearman's correlation analyses between IGFBP1 and (A) scale for the assessment and rating of ataxia, (B) neurofilament light chain, (C) body mass index and (D) CAG repeat length in patients with spinocerebellar ataxia type 3. IGFBP1, insulin-like growth factor binding protein 1; SARA, scale for the assessment and rating of ataxia; NfL, neurofilament light chain; BMI, body mass index.

positively correlated with SARA scores ( $r=0.401$ ;  $P=0.023$ ) and NfL ( $r=0.538$ ;  $P=0.001$ ), and negatively correlated with BMI ( $r=-0.667$ ;  $P<0.001$ ). No significant association with CAG repeat length was observed ( $r=0.141$ ;  $P=0.440$ ) (Fig. 2). Although several higher IGFBP1 values were present, all measurements were deemed physiologically plausible and therefore no data points were excluded from the analysis.

*Lower insulin is associated with elevated IGFBP1 and NfL.* To further investigate the interplay between insulin signaling and neurodegenerative markers, patients with SCA3 were stratified into tertiles based on baseline plasma insulin levels. Log-transformed IGFBP1 levels differed significantly among insulin tertiles (Kruskal-Wallis test,  $P<0.001$ ). Dunn's post hoc test revealed that patients in the lowest insulin tertile had significantly higher IGFBP1 levels than those in the middle ( $P<0.001$ ) and highest tertiles ( $P=0.009$ ), whereas no significant difference was observed between the middle and highest tertiles. Similarly, NfL levels also differed among insulin tertiles ( $P=0.046$ ), with higher levels in the lowest vs. middle tertile ( $P=0.042$ ), but no significant difference vs. the highest tertile ( $P=0.388$ ; Fig. 3A and B). These findings suggested that insulin insufficiency could be associated with increased IGFBP1 expression and greater neuroaxonal damage. Notably, insulin concentrations were lowest in the tertile with the highest IGFBP1 and NfL levels, further supporting an inverse association between insulin signaling and neurodegenerative indicators. However, the association between insulin tertiles

and IGFBP1 or NfL was not attributed to differences in ataxia severity or genetic factors, as SARA scores, age at onset and CAG repeat numbers were comparable across insulin tertiles (all  $P>0.05$ , Fig. S1).

### Discussion

Although alterations in the IIS pathway have been described in SCA3, the clinical significance of circulating IGFBP1 remains unclear. The present study showed that plasma IGFBP1 levels were significantly elevated in patients with SCA3 and was associated with clinical severity (SARA), neurodegeneration (NfL) and BMI. These associations suggested that IGFBP1 could reflect disease activity rather than genetic load, providing complementary information to CAG repeat length. While elevated IGFBP1 in SCA3 has been previously reported (8), the present study expanded these earlier findings by linking IGFBP1 to neurodegeneration biomarkers and directly comparing its performance with other IIS components. Notably, ROC analysis revealed that IGFBP1 outperformed other IIS components, including insulin, total/free IGF1 and IGF2, in distinguishing early from advanced disease stages, thus highlighting its potential as a clinically relevant and stage-sensitive biomarker.

Normoglycemic hypoinsulinemia was evident in the current SCA3 cohort, which was consistent with previous findings (8). Although this pattern could arise from pancreatic  $\beta$ -cell dysfunction or altered insulin kinetics, the present results

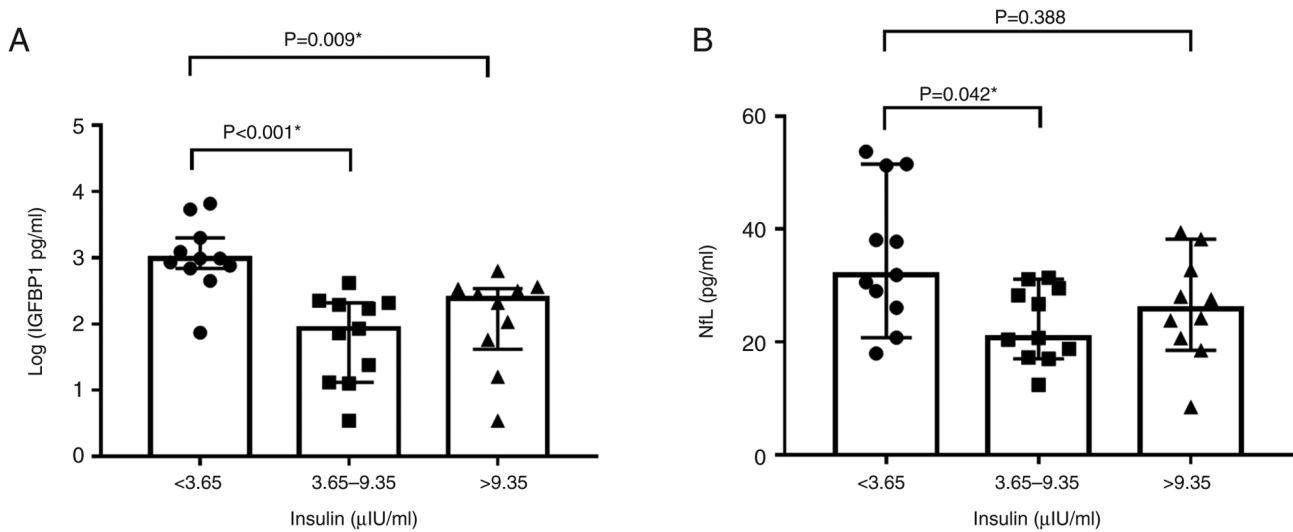


Figure 3. Associations between insulin levels, NfL and IGFBP1 in the spinocerebellar ataxia type 3 cohort. Participants were stratified into tertiles based on plasma insulin concentrations: First tertile (n=11; <3.65  $\mu$ IU/ml), second tertile (n=11; 3.65–9.35  $\mu$ IU/ml) and third tertile (n=10; >9.35  $\mu$ IU/ml). (A) Associations between insulin levels and log-transformed IGFBP1 is shown. (B) Association between insulin levels and plasma NfL are presented. Data are expressed as the median  $\pm$  interquartile range. Statistical significance was determined by Kruskal-Wallis test followed by Dunn's post hoc test. \* $P$ <0.05. NfL, neurofilament light chain; IGFBP1, insulin-like growth factor binding protein 1.

suggested that reduced insulin availability could be involved in the upregulation of hepatic IGFBP1 secretion. The present finding was supported by the observation that patients with the lowest insulin levels exhibited the highest IGFBP1 and NfL concentrations, thus indicating that peripheral insulin insufficiency could exacerbate central neurodegeneration. Notably, this coordinated elevation of IGFBP1 and NfL occurred even when clinical severity (SARA scores) and genetic factors (CAG repeat length) were comparable across groups, thus suggesting that the IGFBP1/NfL association could reflect a shared underlying biological process linking metabolic stress and neuroaxonal damage, rather than merely mirroring clinical ataxia severity. Furthermore, the significantly increased G/I ratio in patients with SCA3 compared with healthy controls indicated decreased insulin secretion rather than insulin resistance, despite the absence of overt hyperglycemia. Notably, this ratio remained comparable between early- vs. advanced-stage), suggesting that impaired insulin secretion is a stable metabolic characteristic in this patient cohort.

IGFBP1 serves a key role in regulating IGF1 availability by binding circulating IGF1 with high affinity, thereby reducing the fraction of free IGF1 capable of activating IGF1 receptors (27). Hepatic IGFBP1 synthesis can be increased under conditions of low insulin, fasting or metabolic stress, thereby further limiting IGF1 bioactivity (28). As IGFBP1 can cross the blood-brain barrier (29), elevated peripheral IGFBP1 levels can be translated into diminished IGF1 signaling within the CNS (30). Such inhibition could compromise IGF1-mediated neuroprotective pathways, which are particularly relevant in SCA3, where myelin and oligodendrocyte dysfunction can be involved in disease pathogenesis (31,32). In addition, a previous study demonstrated that IGFBP1 expression was affected by inflammatory mediators, such as IL-6, which were increased in the brain tissues of patients with SCA3 (33). The catabolic state associated with advanced SCA3, characterized by lower BMI and potential sarcopenia, can further drive IGFBP1 upregulation (12,34,35);

therefore, IGFBP1 could not only reflect metabolic stress but could also exacerbate neurodegeneration by inhibiting IGF1 signaling and myelination (10,36). Taken together, these data suggested that IGFBP1 could represent a point of convergence between peripheral metabolic insufficiency and central neurodegenerative processes in SCA3. Unlike CAG repeat number, which reflects genetic risk, IGFBP1 appears to reflect disease activity, thus providing a sensitive indicator for monitoring disease progression and therapeutic response. Although, this proposed mechanism, whereby circulating IGFBP1 could cross the blood-brain barrier and attenuate central IGF1 signaling, is intriguing, it should be regarded as a working model rather than a demonstrated pathway. Direct evidence is still needed to verify whether IGFBP1 could directly contribute to neurodegeneration or reflect consequences of disease-related metabolic dysregulation. Future studies incorporating measurements of IGFBP1 in cerebrospinal fluid or brain tissues from SCA3 models, as well as experiments exploring IGF-independent actions that could underly its association with ataxia severity, should be conducted.

The present study showed elevated circulating IGF2 levels in patients with SCA3, a finding not previously reported. Although IGF2 was not a primary focus of the present study, its elevation in SCA3 could reflect a compensatory response to impaired insulin signaling or enhanced catabolic stress (37). While IGF2 levels did not correlate with ataxia severity, prior studies in other neurodegenerative models suggest a potential neuroprotective role for IGF2 (38,39). These observations raise the possibility that increased IGF2 in SCA3 could represent an adaptive mechanism to counteract metabolic or proteostatic imbalance, warranting further investigation.

The present study has several limitations. Firstly, the sample size, while comparable to previous biomarker studies in rare neurodegenerative disorders (7,8,40), remains modest and could limit the statistical power to detect more subtle associations, including those between IGFBP1 levels and CAG repeat length. Consequently, the generalizability of

the findings should be interpreted with caution. Future and larger multi-center studies with more diverse SCA3 cohorts are needed to validate the results of this study and to further clarify disease-biomarker associations. Additionally, longitudinal data are also necessary to establish IGFBP1 as a reliable marker of disease progression over time. Mechanistic investigations are also warranted to assess whether IGFBP1 could exert a causal role in neurodegeneration or if it primarily reflects downstream disease-related metabolic dysregulation. Finally, therapeutic strategies aimed at modulating the IIS, through insulin sensitizers, IGF1 supplementation or IGFBP1 inhibition, warrant further investigation in future trials.

Overall, the results of the current study suggested that IGFBP1 could be a promising biomarker for ataxia severity and neurodegeneration in SCA3; its strong associations with NfL, SARA and BMI supported its potential clinical utility in disease monitoring and stratification. IGFBP1 could also represent a mechanistic link between metabolic dysfunction and CNS pathology in SCA3.

### Acknowledgements

The authors would like to thank Dr Tsung-Han Lee (Department of Chinese Medicine, Changhua Christian Hospital, Changhua, Taiwan) for providing assistance in submitting the required documents to the Institutional Review Board. The authors would also like to thank Professor. Jui-Chih Chang (Center of Regenerative Medicine and Tissue Repair, Institute of ATP, Changhua Christian Hospital, Changhua, Taiwan) for providing suggestions that aided in refining the presentation of the results and enhancing the clarity and depth of the discussion, which contributed meaningfully to the overall quality of the manuscript.

### Funding

The present study was supported by research grants from Changhua Christian Hospital, Taiwan (grant nos. 109-CCH-IRP-055 and 110-CCHMST-123). Additional funding was provided by the National Science and Technology Council, Taiwan (grant nos. NSTC 112-2320-B-039-046 and NSTC 112-2314-B-371-007-MY3), the Ministry of Science and Technology, Taiwan (grant no. MOST 109-2314-B-371-008-MY3), China Medical University Hospital (grant no. DMR-112-176) and the Chinese Medicine Research Center, China Medical University through the Featured Areas Research Center Program under the Higher Education Sprout Project, Ministry of Education, Taiwan (grant no. CMRC-CMA-0).

### Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

### Authors' contributions

CC, HeC, YC, SS and CLi conceived and designed the study. CC, WC and TL interpreted the results and wrote the manuscript. CC, WC, HuC, JC and CLe contributed to sample collection and data analysis. YC and SS analyzed data. CC,

HeC and CLi confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

### Ethics approval and consent to participate

All procedures in this case-control study were approved by the Independent Ethics Committee of the Changhua Christian Hospital (approval nos. 200703 and 200730) and written informed consent was obtained from all participants.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### References

- Sadagurski M and White MF: Integrating metabolism and longevity through insulin and IGF1 signaling. *Endocrinol Metab Clin North Am* 42: 127-148, 2013.
- Werner H: The IGF1 signaling pathway: From basic concepts to therapeutic opportunities. *Int J Mol Sci* 24: 14882, 2023.
- Majumder P, Roy K, Bagh S and Mukhopadhyay D: Receptor tyrosine kinases (RTKs) consociate in regulatory clusters in Alzheimer's disease and type 2 diabetes. *Mol Cell Biochem* 459: 171-182, 2019.
- Muddapu VR, Dharshini SAP, Chakravarthy VS and Gromiha MM: Neurodegenerative diseases-is metabolic deficiency the root cause? *Front Neurosci* 14: 213, 2020.
- Paulson H and Shakkottai V: Spinocerebellar ataxia type 3. In: *GeneReviews*® [Internet]. Adam MP, Bick S, Mirzaa GM, Pagon RA, Wallace SE and Amemiya A (eds.), Seattle (WA): University of Washington, Seattle, 1993.
- Chen Y, Jin Y, Hu Z, Qiu M, Li D, Cai Q, Tao C, Lou D, Qi L, Chen S, *et al*: Association between serum neurofilament light chain and neurochemistry deficits in patients with spinocerebellar ataxia type 3. *Cerebellum* 23: 92-100, 2024.
- Wilke C, Haas E, Reetz K, Faber J, Garcia-Moreno H, Santana MM, van de Warrenburg B, Hengel H, Lima M, Filla A, *et al*: Neurofilaments in spinocerebellar ataxia type 3: Blood biomarkers at the preataxic and ataxic stage in humans and mice. *EMBO Mol Med* 12: e11803, 2020.
- Saute JAM, da Silva ACF, Muller AP, Hansel G, de Mello AS, Maeda F, Vedolin L, Saraiva-Pereira ML, Souza DO, Arpa J, *et al*: Serum insulin-like system alterations in patients with spinocerebellar ataxia type 3. *Mov Disord* 26: 731-735, 2011.
- Torres-Aleman I, Barrios V, Lledo A and Berciano J: The insulin-like growth factor I system in cerebellar degeneration. *Ann Neurol* 39: 335-342, 1996.
- Crosby S, Tsigos C, Anderton C, Gordon C, Young R and White A: Elevated plasma insulin-like growth factor binding protein-1 levels in type 1 (insulin-dependent) diabetic patients with peripheral neuropathy. *Diabetologia* 35: 868-872, 1992.
- Busiguina S, Fernandez AM, Barrios V, Clark R, Tolbert DL, Berciano J and Torres-Aleman I: Neurodegeneration is associated to changes in serum insulin-like growth factors. *Neurobiol Dis* 7: 657-665, 2000.
- Lang CH and Frost RA: Role of growth hormone, insulin-like growth factor-I, and insulin-like growth factor binding proteins in the catabolic response to injury and infection. *Curr Opin Clin Nutr Metab Care* 5: 271-279, 2002.
- Wu S, Liu K, Cheng W, Su S, Lin Y, Lin T, Cheng Y, Chang J, Wu Y and Liu C: Growth hormone rescue cerebellar degeneration in SCA3 transgenic mice. *Biochem Biophys Res Commun* 529: 467-473, 2020.
- Arpa J, Sanz-Gallego I, Medina-Báez J, Portela LV, Jardim LB, Torres-Aleman I and Saute JA: Subcutaneous insulin-like growth factor-I treatment in spinocerebellar ataxias: An open label clinical trial. *Mov Disord* 26: 358-359, 2011.

15. Lin YS, Cheng WL, Chang JC, Lin TT, Chao YC and Liu CS: IGF-1 as a potential therapy for spinocerebellar ataxia type 3. *Biomedicines* 10: 505, 2022.
16. Schmitz-Hübsch T, du Montcel ST, Baliko L, Berciano J, Boesch S, Depondt C, Giunti P, Globas C, Infante J, Kang JS, *et al*: Scale for the assessment and rating of ataxia: Development of a new clinical scale. *Neurology* 66: 1717-1720, 2006.
17. Yang JS, Chen PP, Lin MT, Qian MZ, Lin HX, Chen XP, Shang XJ, Wang DN, Chen YC, Jiang B, *et al*: Association between body mass index and disease severity in Chinese spinocerebellar ataxia type 3 patients. *Cerebellum* 17: 494-498, 2018.
18. Alehagen U, Johansson P, Aaseth J, Alexander J and Brismar K: Increase in insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 1 after supplementation with selenium and coenzyme Q10. A prospective randomized double-blind placebo-controlled trial among elderly Swedish citizens. *PLoS One* 12: e0178614, 2017.
19. Zhou J, Lei L, Liao X, Wang J, Jiang H, Tang B and Shen L: Related factors of ICARS and SARA scores on spinocerebellar ataxia type 3/Machado-Joseph disease. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 36: 498-503, 2011.
20. Li QF, Dong Y, Yang L, Xie JJ, Ma Y, Du YC, Cheng HL, Ni W and Wu ZY: Neurofilament light chain is a promising serum biomarker in spinocerebellar ataxia type 3. *Mol Neurodegener* 14: 39, 2019.
21. Maas RPPWM, van Gaalen J, Klockgether T and van de Warrenburg BPC: The preclinical stage of spinocerebellar ataxias. *Neurology* 85: 96-103, 2015.
22. Hancox RJ and Landhuis CE: Correlation between measures of insulin resistance in fasting and non-fasting blood. *Diabetol Metab Syndr* 3: 23, 2011.
23. Chiu C, Cheng W, Lin Y, Lin T, Chang H, Chang Y, Lee C, Chang H and Liu C: A pilot study: Handgrip as a predictor in the disease progression of SCA3. *Orphanet J Rare Dis* 18: 317, 2023.
24. Levy JC, Matthews DR and Hermans MP: Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 21: 2191-2192, 1998.
25. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G and Quon MJ: Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85: 2402-2410, 2000.
26. Molina-Castro M, Rowitz B and Pepino MY: Glucagon-like peptide-1, fibroblast growth factor 21, and other endocrine responses to alcohol ingestion in women before and after metabolic surgery. *Front Pharmacol* 16: 1575156, 2025.
27. Rajaram S, Baylink DJ and Mohan S: Insulin-like growth factor-binding proteins in serum and other biological fluids: Regulation and functions. *Endocr Rev* 18: 801-831, 1997.
28. Lee PD, Conover CA and Powell DR: Regulation and function of insulin-like growth factor-binding protein-1. *Proc Soc Exp Biol Med* 204: 4-29, 1993.
29. Bunn RC, King WD, Winkler MK and Fowlkes JL: Early developmental changes in IGF-I, IGF-II, IGF binding protein-1, and IGF binding protein-3 concentration in the cerebrospinal fluid of children. *Pediatr Res* 58: 89-93, 2005.
30. Lewitt MS and Boyd GW: The role of insulin-like growth factors and insulin-like growth factor-binding proteins in the nervous system. *Biochem Insights* 12: 1178626419842176, 2019.
31. Ramani B, Panwar B, Moore LR, Wang B, Huang R, Guan Y and Paulson HL: Comparison of spinocerebellar ataxia type 3 mouse models identifies early gain-of-function, cell-autonomous transcriptional changes in oligodendrocytes. *Hum Mol Genet* 26: 3362-3374, 2017.
32. Schuster KH, Zalon AJ, Zhang H, DiFranco DM, Stec NR, Haque Z, Blumenstein KG, Pierce AM, Guan Y, Paulson HL and McLoughlin HS: Impaired oligodendrocyte maturation is an early feature in SCA3 disease pathogenesis. *J Neurosci* 42: 1604-1617, 2022.
33. Evert BO, Vogt IR, Vieira-Saecker AM, Ozimek L, de Vos RA, Brunt ER, Klockgether T and Wüllner U: Gene expression profiling in ataxin-3 expressing cell lines reveals distinct effects of normal and mutant ataxin-3. *J Neuropathol Exp Neurol* 62: 1006-1018, 2003.
34. Nolte AA, Movin M, Lundin H and Salminen H: IGFBP-1 predicts all-cause mortality in elderly women independently of IGF-I. *Growth Horm IGF Res* 25: 281-285, 2015.
35. Leite CMBA, Schieferdecker MEM, Frehner C, Munhoz RP, Ashizawa T and Teive HAG: Body composition in Spinocerebellar ataxia type 3 and 10 patients: Comparative study with control group. *Nutr Neurosci* 23: 49-54, 2020.
36. Ye P, Carson J and D'Ercole AJ: In vivo actions of insulin-like growth factor-I (IGF-I) on brain myelination: Studies of IGF-I and IGF binding protein-1 (IGFBP-1) transgenic mice. *J Neurosci* 15: 7344-7356, 1995.
37. Biadgo B, Tamir W and Ambachew S: Insulin-like growth factor and its therapeutic potential for diabetes complications-mechanisms and metabolic links: A review. *Rev Diabet Stud* 16: 24-34, 2020.
38. Arcos J, Grunenwald F, Sepulveda D, Jerez C, Urbina V, Huerta T, Troncoso-Escudero P, Tirado D, Perez A, Diaz-Espinoza R, *et al*: IGF2 prevents dopaminergic neuronal loss and decreases intracellular alpha-synuclein accumulation in Parkinson's disease models. *Cell Death Discov* 9: 438, 2023.
39. García-Huerta P, Troncoso-Escudero P, Wu D, Thiruvalluvan A, Cisternas-Olmedo M, Henríquez DR, Plate L, Chana-Cuevas P, Saquel C, Thielen P, *et al*: Insulin-like growth factor 2 (IGF2) protects against Huntington's disease through the extracellular disposal of protein aggregates. *Acta Neuropathol* 140: 737-764, 2020.
40. Coarelli G, Darios F, Petit E, Dorgham K, Adanyeguh I, Petit E, Brice A, Mochel F and Durr A: Plasma neurofilament light chain predicts cerebellar atrophy and clinical progression in spinocerebellar ataxia. *Neurobiol Dis* 153: 105311, 2021.