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黃耆萃取物治療第三型小腦萎縮症之隨機雙盲臨床試驗

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Astragalus Membranaceus Extract for Patients with Spinocerebellar Ataxia type III – A Randomized Double-Blind Clinical Trial

Graduate Student: Chung-Min Chiu

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中國醫藥大學博士班研究生
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謝辭

終於完成了這項研究與博士班學業，這一路以來真的如同剛念研究所時抽到的籤詩一般:「...雖然多阻滯，花發再重榮」- 艱辛、又精彩。與小腦萎縮症認識的這段美麗緣份，起因於在彰基中醫部做住院醫師時，羅綸謙主任鼓勵年輕人走向中西合作，去神經罕病特診的劉副院長門診學習，因此開啓了一連串的故事。過程中最要感謝兩位指導老師: 彰化基督教醫院的劉青山副院長全程指導與協助，反覆修正研究與文章內容，隨時精進最新文獻與思考方向，不斷勉勵我要抱持著對病人最有實質幫助的臨床試驗，就是方便可近的中藥研究；張恒鴻所長積極地指導研究與整合資源，一步一步地監督研究過程與資料分析，帶領我走過完整的博士班訓練。

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消息而有機會加入招募。

最後,感謝我的家人與父母的鼓勵,以及門診小腦病人的積極參與和支持,讓我心理得到滿滿的支持與努力不懈的動力。原本以為念了博士會很懂研究,現在才覺得自己還是很不足。但經歷過這些,似乎又更有一些勇氣,能去建構可行的臨床研究與未來方向,也明白了這學位代表的真正涵義。

走進小腦萎縮症研究的領域是我人生中一場美麗的意外,很符合我理想型的性格,希望能以中醫好好地輔助小腦萎縮症患者,讓他們得到良好的治療,更期待在有生之年,看到這個疾病被宣告可以治癒。

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中文摘要

題目：黃耆萃取物治療第三型小腦萎縮症之隨機雙盲臨床試驗
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目的：

第三型小腦萎縮症（Spinocerebellar ataxia type 3, SCA3）是最常見的遺傳性小腦共濟失調。胰島素/類胰島素生長因子 1（insulin/insulin-like growth factor 1, IGF1）系統（IIS）途徑已被提出為潛在的疾病修飾治療目標。傳統中藥黃耆（*Astragalus membranaceus*）可能可以調節 IIS 途徑並減少神經退化，而血漿神經細絲輕鏈（neurofilament light chain, NfL）則可作為疾病進展的生物標記，本團隊擬在臨床上進行實證研究。

方法：

本研究為一項隨機、雙盲、安慰劑對照、交叉設計臨床試驗，共納入32位SCA3患者。受試者先接受黃耆萃取物或安慰劑治療三個月，停藥一個月後，再交叉至另一組治療三個月，其後再停藥一個月；治療前後及試驗結束均予訪視評估，測量血漿 NfL 與 IIS 相關指標。

結果：

共有 23 位受試者完成試驗。分組後兩組在基線資料並無顯著差異，且停藥一個月並未發現延續效應。意向性分析顯示，黃耆治療後組內的血漿 NfL 有輕度下降（27.6±10.7 vs. 25.6±9.8 pg/mL，P=0.040），且 insulin 水平顯著上升（12.5±15.2 vs. 21.1±15.5 μIU/mL，P=0.004），然而與安慰劑組比較，NfL 之下降未達顯著水準（P=0.127），且治療後 NfL 的下降與 insulin 上升無顯著相關。IGF1 與 IGF 結合蛋白未見顯著變化。探索性亞組分析顯示：在疾病後期患者中，NfL 下降幅度較大

（32.6±10.8 vs. 30.4±9.3 pg/mL，*P*=0.033），且 insulin 顯著上升（8.0±5.6 vs. 19.8±15.3 μIU/mL，*P*=0.003）。

結論：

在 32 個 SCA3 病人中，黃耆的治療與 NfL 輕度下降及 insulin 增加有相關，然而 NfL 的組間比較未達顯著水準，且 NfL 與 insulin 的變化未呈現相關，仍需進一步研究以釐清其作用機制與長期療效。

關鍵字：黃耆、第三型小腦萎縮症、神經細絲輕鏈、胰島素/類胰島素生長因子 1 系統、神經退化、臨床試驗

Abstract

Title: *Astragalus membranaceus* extract for patients with spinocerebellar ataxia type 3 - A randomized double-blind clinical trial

Student: Chung-Min Chiu

Advisor: Hen-Hong Chang

Co-Advisor: Chin-San Liu

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Background:

Spinocerebellar ataxia type 3 (SCA3) is the most common form of hereditary cerebellar ataxia. The insulin/insulin-like growth factor 1 (IGF1) system (IIS) pathway has been proposed as a potential target for disease-modifying therapy. *Astragalus membranaceus*(AM), a traditional herbal extract, may modulate the IIS pathway and reduce neurodegeneration, as indicated by plasma neurofilament light chain (NfL), a biomarker of disease progression. Our research team plans to conduct an evidence-based clinical study.

Methods:

A randomized, double-blind, placebo-controlled crossover trial was conducted in 32 patients with SCA3. Participants received either AM extract or placebo for three months, followed by a one-month washout, then crossed over to the alternate treatment. Participants were assessed before and after treatment and at the end of the study, with plasma NfL and IIS-related markers measured at each visit.

Results:

A total of 23 participants completed the trial. There were no significant differences in baseline characteristics between the two groups after

randomization. Furthermore, no carryover effect was observed following the one-month washout period. Intention-to-treat analysis revealed a modest within-treatment reduction in NfL levels (27.6±10.7 vs. 25.6±9.8 pg/mL, *P*=0.040) and a significant increase in insulin levels (12.5±15.2 vs. 21.1±15.5 μIU/mL, *P*=0.004) after AM treatment, but no significance in NfL between AM and placebo groups (*P*=0.127). There was no correlation between NfL reduction and insulin elevation. No significant changes were observed in IGF1 or IGF-binding proteins. Exploratory subgroup analysis of later-stage patients showed greater reductions in NfL (32.6±10.8 vs. 30.4±9.3 pg/mL, *P*=0.033) and insulin elevation (8.0±5.6 vs. 19.8±15.3 μIU/mL，*P*=0.003).

Conclusions:

In 32 participants of SCA3, AM was associated with a borderline reduction in plasma NfL levels and significantly increased insulin levels. However, there was no significance in NfL between AM and placebo groups, and no correlation was found in NfL reduction and insulin elevation. Further studies are needed to elucidate the underlying mechanisms and long-term effects.

Keywords: *Astragalus membranaceus*; Spinocerebellar ataxia type 3; Neurofilament light chain; Insulin/insulin-like growth factor 1 system; Neurodegeneration; Clinical trial

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第一章 前言

脊髓小腦萎縮症（Spinocerebellar ataxias，SCAs）是一群以顯性遺傳為主的神經退化性疾病。其中第 3 型（Spinocerebellar Ataxia type 3，SCA3），亦稱 Machado-Joseph Disease，為全球及台灣最常見之亞型，且在亞洲地區尤為盛行[1, 2]。SCA3是成人時期發病的神經系統罕見疾病，常在中年前後發病，未提早避孕的情況下，容易導致整個家族多人同時罹病。此疾病起因於第 14 號染色體長臂（14q32.1）基因突變，導致 CAG 重複序列異常擴增。CAG 轉譯出來為麩醯胺酸 glutamine，正常人的 CAG 重複序列為 12～44 個，這種短鏈的多麩醯胺酸（polyglutamine，polyQ）為無害的，而 SCA3 的病人重複序列達 62 個 CAG 以上[3]，進而在神經系統中形成過長的、具毒性的 polyQ 蛋白聚集，無法被細胞代謝回收，最終造成小腦及其週邊部位的神經細胞流失[4]。這些 polyQ 聚集體（aggregates）會破壞蛋白質體內平衡，損害細胞清除機制，並引發細胞凋亡，導致漸進性運動功能缺損與神經退化。

臨床上，SCA3 患者主要表現為步態不穩、眩暈、構音障礙、全身疲倦、複視、吞嚥困難，並可能出現錐體徵或錐體外徵[1]。由於直接針對疾病的基因治療在實際操作上存在技術挑戰與倫理困境[5]，因此目前 SCA3 的治療方向傾向於疾病修飾治療（disease-modifying therapies，DMT），目標為延緩疾病進展[6]。除藥物治療外，非藥物介入亦逐漸受到重視，例如高強度復健與協調性訓練，以及新興技術如經顱磁刺激（Transcranial Magnetic Stimulation，TMS）與經顱直流電刺激（Transcranial Direct Current Stimulation，tDCS），皆被視為潛在治療策略之一[7, 8]。在藥物臨床試驗方面，近期已有進入第二期或第三期臨床試驗的治療候選者，包括海藻糖（trehalose）、Troriluzole、臍帶來源或脂肪來源的人類間質幹細胞[9]。以上這些研究均為未來治療提供了新的展望，然而新興藥物治療所費不貲，因此探索常見且安全的傳統中藥來輔助延緩疾病病程，應屬治療神經退化疾病的一個選項[10]。

利用提升胰島素/類胰島素生長激素 1 系統（insulin/insulin-like growth factor 1 system，IIS）來活化腦部，是神經退化疾病的治療策略之一。活化 IIS 能啓動 PI3K/Akt 與 MAPK 路徑[11, 12]，抑制細胞凋亡，清除毒蛋白，或減少氧化損傷等，這些也都與 SCA3 的病理機轉相同[1]。事實上，目前已有一些臨床研究利用鼻噴 insulin 來治療阿茲海默症[13]，或者使用 IGF1 皮下注射來治療 SCA3[14]，並獲得有潛力的療效。然而直接使用 insulin 或 IGF1 確實仍有相關風險，比如 insulin 造成病人血糖不穩定，或者 IGF1 有致癌的可能。因此，選擇一個長期使用安全且便宜的中藥，比如黃耆，是更具臨床應用潛力的策略。

在華人的醫療文化裡，黃耆（*Astragalus membranaceus*，AM）一直是家喻戶曉、在進補時時常應用到此中藥。耆，有耆老之意，即有很安全、促進長壽、抗老化的意涵。在神農本草經裡，也把黃耆列為上品，即無毒、安全、長期服用對人體有益健康之意[15]。中醫認為它能補氣、抗疲勞、抗衰老，也有助於血糖穩定。現代藥理學則認為它能抗毒、調節免疫、改善神經退化等等[16]，尤其可能活化 IGF1 或生長激素（growth hormone，GH）[17, 18]，或者調節 insulin 來穩定血糖[19]。曾有研究報告以黃耆為主的中藥組方，來協助改善 SCA 病人症狀的個案經驗[20]。另外，也有 SCA3 細胞研究顯示，黃耆皂苷 IV（Astragloside IV，AST）能減少毒蛋白的聚集與異常 ataxin-3 蛋白質的表現量，改善粒腺體功能與增加抗氧化的能力[21]。因此，在本研究中我們選用黃耆當作治療 SCA3 的疾病修飾療法。

由於 SCA3 的病程退化緩慢，以臨床最常用的神經學檢查（共濟失調評估量表 scale for the assessment and rating of ataxia，SARA）來看，每年平均退步大約 1.60 分（總分 40 分）[22]，分數愈高代表神經退化愈嚴重，而罹病至臨終大約為 20~25 年[1]，因此要評估一項藥物的臨床效果，可能須要長期觀察。如果使用 SARA 來嚴謹評估一年期的臨床治

療，估計須要高達202位受試者，監測到50%的SARA程度改善，統計上才能有檢力（power），而這在個案數稀少的罕見疾病是非常困難的[23]。因此，利用血漿生物指標（plasma biomarker）可能是用來短期評估藥物療效的一個好方法。近年來，血液中的神經細絲輕鏈（neurofilament light chain，NfL）已被公認適合用來評估SCA3的病程變化與疾病嚴重度，也適合用以評估臨床治療的反應程度[24]。

基於上述原因，本研究設計一個隨機、雙盲、安慰劑對照交叉（crossover）試驗，進行三個月的治療，經過 washout 一個月後，再交換至另一組，旨在探討黃耆在 SCA3 中的潛在治療效益，並以 IIS 相關指標與 NfL 作為評估指標。

第二章 文獻探討

第一節 SCA3 的分子病理與臨床特徵

SCA3 的核心病理特徵在於 *ATXN3* 基因第 10 個外顯子（exon 10）中的 CAG 三核苷酸序列異常擴增。此遺傳缺陷導致其表現產物——ataxin-3 蛋白的羧基端（C-terminus）產生異常過長的 polyglutamine（polyQ）胜肽鏈，進而引發後續一系列複雜的細胞毒性級聯反應[25]。

在生理狀態下，ataxin-3 蛋白具備去泛素化酶（deubiquitinating enzyme）之功能，負責調節細胞內蛋白質的降解速率與蛋白質體內平衡（proteostasis）。然而，帶有異常 polyQ 擴增的突變型 ataxin-3 會發生蛋白質錯誤折疊（misfolding），這不僅使其原本正常的去泛素化（deubiquitination）生理功能喪失，更進一步干擾了重要蛋白質的降解路徑，造成細胞內蛋白質代謝與調控系統的失衡[26, 27]。

異常摺疊的 ataxin-3 蛋白傾向於在神經細胞內形成具毒性的聚集體（aggregates）。這些 aggregates 會破壞細胞內的蛋白質體平衡，並損害細胞自身的清除機制（如自噬作用或蛋白酶體系統）。有研究指出，儘管 SCA3 與 SCA1、2、6 型同屬 polyQ 疾病，雖然病變基因各異，但皆具有相似的病理特徵，即異常重複的 CAG 片段所產生的毒性蛋白累積，最終引發神經細胞凋亡[28]。

這種由蛋白聚集引發的細胞毒性具有高度組織特異性，主要造成腦幹、小腦、中腦、脊髓、紋狀體與丘腦等關鍵部位的神經細胞流失。臨床上，這類病理變化表現為漸進式的運動功能缺損，包括平衡失調、走路不穩、協調性差、吞嚥困難、構音困難及複視等症狀[1]。值得注意的是，除了運動症狀外，明顯的「疲勞感」（fatigue）亦是 SCA3 以及其他 polyQ 疾病患者共同且顯著的臨床特徵[29]。

第二節 SCA3 的臨床研究現況與挑戰

2-1. 基因療法的前景與限制

隨著分子生物學技術的突破，針對 SCA3 的「基因沉默」（Gene Silencing）技術被視為最具潛力的 DMT 手段。其核心策略是透過反義寡核苷酸（Antisense Oligonucleotides，ASOs）[30]或小干擾 RNA（siRNA/RNAi）[31]精準結合突變的 *ATXN3* mRNA，從源頭減少毒性 polyQ 蛋白的產生。然而，儘管在動物模型中展現了顯著療效，進入臨床試驗後仍面臨多重瓶頸。

目前臨床試驗多採行「鞘內注射」（Intrathecal injection），屬侵入性醫療行為，且需定期重複施作，增加了臨床操作的難度與患者的心理負擔。此外藥物進入腦脊髓液後，如何有效瀰散至較深層的受損組織（如腦幹與基底核）仍是一大挑戰[32]。另外，外源性核酸進入中樞神經系統可能引發非特異性的免疫反應或神經炎症，而長期抑制 *ATXN3* 表現是否會干擾蛋白質體內平衡（Proteostasis）的基礎代謝，目前尚缺乏長達數十年的追蹤數據，這使得基因療法在早期介入（Pre-symptomatic stage）時面臨高度的倫理與安全審查[33]。

基於上述基因療法的技術瓶頸與高昂成本，尋找具備多靶點保護功能、高安全性且能長期口服的藥物（如黃耆及其活性成分）顯得尤為重要。天然化合物不僅能透過調節 IIS 提供神經保護，且能作為基因療法的輔助治療，或在基因療法尚未普及前，提供患者一個更具可近性（Accessibility）的選擇。

2-2. 非藥物治療的實證

在現行的臨床實務中，物理治療、職能治療及語言治療仍是 SCA 患者維持功能的核心支柱[34]。研究指出，針對 SCA 患者進行短期且高強度的協調性訓練（Coordinative training），能顯著改善其共濟失調症

狀[35]。透過密集的運動介入，患者能有效延緩疾病的自然進程，甚至可能在運動表現上「贏回」至少一年的退化時間。Ilg 等人與 Miyai 等人的研究分別證實，經過 8 週與 24 週的訓練計畫後，患者的運動表現呈現持續性的改善[36, 37]。目前最長期的追蹤研究顯示，這類訓練的效果可穩定維持長達一年[38]；若在幾例 SCA6 與 SCA31 的病人觀察研究上，運動的保護效果可長達至少六年[39]。這種介入並非改變基因缺陷，而是透過強化腦部殘餘神經元的突觸可塑性（Synaptic plasticity）與大腦皮質的功能重組（Functional reorganization），來補償小腦功能的流失。

除了傳統復健外，非侵入性腦刺激技術如 TMS 與 tDCS，已被視為具備潛力的輔助治療策略[8]。TMS 主要是透過電磁感應產生感應電流，調節小腦與大腦皮質之間的聯絡通路（Cerebello-thalamo-cortical pathway）。研究顯示重複性 TMS（rTMS）能改善 SCA3 患者的平衡能力，甚至臨床上亦可考慮配合復健治療來縮短療程[40]。tDCS 則是利用微弱電流改變神經元的靜息膜電位（Resting membrane potential），藉此活化小腦神經元，增加 Purkinje 細胞的興奮性，進而改善運動協調功能，也有研究顯示搭配五天的短期步態訓練即可改善部分平衡項目[41]。

儘管上述非藥物療法能暫時緩解症狀並改善生活品質，但其效果往往受限於患者的配合度、訓練強度以及疾病本身的退化速率。更重要的是，這些介入難以直接逆轉細胞內毒性蛋白的累積。因此，如何將「非藥物療法」與「具備疾病修飾潛力的藥物」相結合，透過協同作用（Synergistic effect）從物理刺激與生化調控雙管齊下，可能是未來延緩 SCA3 病程的最佳策略。

2-3. DMT 藥物研究現況

目前已在進行臨床試驗中的小分子藥物有海藻糖與 Troriluzole，另

亦有發展中的間質幹細胞治療等，以下對此三者進行現況探討:

海藻糖是一種天然的雙醣類，已被證實具備獨特的化學伴侶（Chemical chaperone）功能，其主要機轉在於活化轉錄因子 EB，進而啟動細胞內的自噬-溶酶體路徑（Autophagy-lysosome pathway）。在 SCA3 細胞模型中，海藻糖能促進錯誤折疊的 ataxin-3 蛋白降解，減少毒性聚集體的累積並保護粒線體功能[42]。雖然在馬來西亞的一期臨床試驗中顯示出良好的安全性與治療潛力[43]，後續第二期臨床試驗的期中分析，口服海藻糖並未在 SCA3 實驗組當中獲得顯著的療效[44]，靜脈注射海藻糖的相關研究目前也中止了[45]。

Riluzole 原本應用在漸凍人的治療上，它能減少神經元的麩胺酸（glutamate）分泌並且增加麩胺酸的回收，因而減少過度興奮的毒性（excitotoxicity）和神經元損傷，藉此保護小腦蒲金氏細胞（Purkinje cell）[46]。過去十多年來，關於 Riluzole 有一些臨床治療 SCA 的研究，但規模小、追蹤時間短暫，而且並非每個研究都顯示有效。這幾年研究方向改成藥物動力學上更穩定、且口服利用率更高的 Troriluzole（Riluzole 的前驅藥物 prodrug），目前也進行臨床試驗中[47]，雖然該研究並未完全結束，但已有一些相關結果透露出其治療的部分成效[48-50]。

間質幹細胞（mesenchymal stem cell，MSC）是取之於骨髓、脂肪、或臍帶血的多功能性幹細胞，其調節免疫、減輕發炎、或再生的功能，在近年來受到廣泛應用。SCA3 的轉殖基因鼠植入 MSC 能提升小腦的 IGF1 等各種生長因子與熱休克蛋白 70（heat shock protein 70）的路經，進而減少 ataxin-3 毒蛋白[51]。由於植入 MSC 到腦部的效果短暫，使用多次的靜脈針劑注射效果更能維持運動平衡功能，以及腦部病理徵象的緩解[52]。目前第一期的安全性臨床試驗已經通過[53]，後續大規模的研究仍在進行當中[54]。

然而，這三種療法分別面臨高昂成本（新藥開發）、給藥途徑複雜（如鞘內注射）、生物利用度受限、或效果不顯著等問題。相比之下，黃耆的活性成分（如 AST）展現了類似海藻糖的自噬調節能力，同時又能像 MSCs 一樣透過調節 IIS 系統提供神經營養支持。最重要的是，黃耆具備極高的口服安全性與成本效益。這使得本研究探討黃耆作為一種「多標靶、低負擔」的 DMT 策略，在罕見疾病的長期管理上展現了獨特的臨床應用價值。

第三節 IIS 與神經退化

3-1. IIS 在中樞神經的功能

IIS 在大腦中支持神經元存活與功能方面具有關鍵作用，對認知功能與神經保護尤為重要。Insulin 與 IGF1 可啟動多種訊息傳遞途徑，促進神經元的生長與修復，增強突觸可塑性並促進記憶形成。其抗凋亡與抗發炎效應能減輕神經元損害，對抗神經退化，同時維持神經元能量供應與最佳腦部功能[55, 56]。而外周的 insulin 與 IGF1 能透過可飽和性運輸系統穿越血腦屏障，並經腦血管內皮細胞運輸進入中樞[57, 58]。

雖然目前尚不清楚 SCA3 患者的中樞 insulin 水平，但有研究指出 SCA3 患者的週邊血液 insulin 濃度明顯低於健康對照組[59]。在阿茲海默症中，已有不經血腦障壁（blood-brain barrier）的鼻噴胰島素、升糖素樣胜肽-1（GLP-1）類似物及 PPARγ 致效劑等藥物被應用於促進腦內 insulin 訊號傳導[60]。在 GH - IGF1 軸中，SCA3 84Q 轉殖基因小鼠接受 GH 治療後，其蒲金氏細胞得以保存，並減少 ataxin-3 聚集；而長達 9 個月的 IGF1 治療則改善了小鼠的運動功能並減緩小腦退化，推測其作用機轉可能與調控粒線體功能、氧化壓力與自噬功能相關[61, 62]。2014 年在西班牙的一項臨床研究，使用 IGF1 皮下注射長達一至二年，確實能延緩 SCA3 病人大約 1 年的病程[14]。然而，長期刺激生長軸可能增加癌症風險，因此更理想的方式是尋找常用且安全的中藥材，能

夠刺激胰島素或 IGF1，以作為 SCA3 潛在的疾病修飾治療候選者。

3-2. IGF 結合蛋白

由於 IGF1 在體內代謝率很高，因此須要一群能運輸 IGF1 的結合蛋白（binding proteins，BPs）來延長停留在血液的時間，並協助其分布到適當位置。人體常見有六種 IGFBPs，分別為 IGFBP1~6，其中的 IGFBP3 含量最高，也能結合最多的 IGF1。IGFBP1 受到 insulin 的負向調控，即飢餓時 insulin 下降，肝臟會分泌較多 IGFBP1 來運輸 IGF1，調節生理功能。這些 IGFBPs 和 insulin、IGF1 一起被歸於 IIS，常被監測以評估體內的 IIS 狀態[63]。

研究顯示，當機體處於禁食、代謝壓力或全身性發炎狀態時，IGFBP1 濃度會顯著升高[64]。在慢性疾病的病人身上，高濃度的 IGFBP1 通常與較低的身體質量指數（BMI）、肝功能異常及較差的預後相關。對於 SCA3 而言，儘管已知 NfL 是反映神經元損傷與共濟失調嚴重度的重要生物指標，但目前仍缺乏能精確捕捉患者全身性代謝失調的指標。過去研究指出， SCA3 與其他小腦萎縮症患者的 IIS 路徑可能存在廣泛受損，除了外周 insulin 降低外，IGFBP1 的異常波動亦暗示了代謝訊號傳導障礙與疾病狀態之間存在關聯[59, 65]。然而，過去關於 GH-IGF1 軸的介入研究多受限於樣本量不足或缺乏與神經退化指標（如 NfL）的直接關聯探討[14, 61, 62]。因此，深入分析 IGFBP1 與臨床 SARA 評分、NfL 損害程度及 BMI 之間的交互作用，亦將有助於闡明 SCA3 患者在神經退化過程中，全身性能量代謝失衡所扮演的角色，並評估其作為監測藥物療效之潛在生物指標的價值。

3-3. SCA3 的 IIS 異常

在 2011 年 Saute 等的研究當中，SCA3 病人的 IGF1 和健康人一致，然而 IGF1/IGFBP3（可用來代表間接測量的 free IGF1，即顯示 IGF1 的生物利用率，若此比值高，代表可自由被利用或分解的 free

IGF1 多，雖然容易被利用，但也很快就用完了；若此比值低，代表可被自由利用或分解的 free IGF1 少，所以在血液裡會維持較久的恆定，也代表目前身體並非處於須生長因子的狀況）明顯比較高，代表身體處於須要生長因子的狀態。另外，也觀察到 SCA3 患者 insulin 比健康組的還低，但血糖（blood sugar）數值完全正常，即 SCA3 病人的 insulin 敏感性（sensitivity）是高的，而 insulin 阻抗（resistance）是低的[59]。在轉殖基因 SCA3 果繩研究中，發現調升 insulin receptor 敏感度的果繩，明顯會改善視神經細胞，使得原本會遭受毒蛋白影響的視網膜保存較為完整[66]。因此利用 insulin 或 IGF1 為標的來作為此疾病的修飾治療，具有明顯的潛力[67]。

在 SCA3 的 IGFBPs 中，只有 IGFBP1 明顯比正常人高，而且 IGFBP1 能與發病年齡（age at onset）負相關、或與基因重複序列 CAG repeat number 有正相關，代表 IGFBP1 能反應一些 SCA3 疾病狀態或面貌[59]。另外 IGF2，也是一種生長因子，雖然不是人體最主要的，常與神經退化疾病有相關性，比如巴金森症或失智症，然而在 SCA3 中也缺乏研究資料，因此也值得一併探索[68]。

第四節 黃耆作為 IIS 誘導劑的潛力

4-1. 黃耆的基原與中醫論述

常見的黃耆稱為北耆、又叫白皮耆，為豆科多年生草本植物蒙古黃耆 (*Astragalus membranaceus* (Fisch.) Bunge var. *mongholicus* (Bunge.) P.K.Hsiao) 或膜莢黃耆 (*Astragalus membranaceus* (Fisch.) Bge.) 的根。主產於內蒙古、山西、甘肅、黑龍江等地。春、秋二季採挖，除去鬚根及根頭，曬乾。生用或蜜炙用。與黃耆相近的為晉耆、又稱紅皮耆，表皮為紅棕色，甜味較重，在華人地區較常入藥饈或補湯中，氣味香甜[69]。

Chen 等人曾指出，多種中藥可經由作用於泛素-蛋白酶體

（Ubiquitin-Proteasome）系統來調控 SCA3[70]，然而，目前仍缺乏這些中藥嚴謹且隨機化的研究設計。在亞洲，黃耆被廣泛應用於補氣、調節血糖恆定、抗疲勞與延緩衰老，其主要成分包括多醣體、皂苷及黃酮類，中醫認為它性甘、微溫，歸脾、肺經，能補氣升陽、益衛固表、利水消腫、托瘡生肌[71]，明代醫家李東垣則認為黃耆:「補五臟諸虛不足，瀉陰火。無汗則發之，有汗則止之。」臨床上 SCA3 的症狀即容易有疲勞、虛弱，神經退化的表現如同提早進入衰老階段—肌無力、肌少症、複視、顫抖、構音困難等[1]，中醫認為都可能是因為五臟虛損。尤其臨床觀察許多 SCA3 病人容易疲勞、容易自汗[72, 73]，這些都是氣虛的指徵，而黃耆特別從後天調補脾肺，能補氣止汗、抗疲勞，因此在中醫理論上可能有輔助治療 SCA3 的潛力。

4-2. 黃耆主成份的藥理作用

黃耆的成份主要有多醣體、皂苷類、與類黃酮等。黃耆多醣體（Astragalus polysaccharides，APS）的藥理作用包括免疫調節、抗腫瘤、抗細胞凋亡以及抑制異常 polyQ 聚集[74]。APS 可透過活化 AKT 與調控 IGF1 路徑來增進胰島素敏感性，亦能促進胰臟 β 細胞的增生與 insulin 分泌[75]。目前 APS 已有在台灣上市的懷特血寶注射液，適應症為改善癌症化療後疲憊[76]。黃耆皂苷類當中，最具代表性的為 AST，具有顯著的抗氧化、抗發炎與神經保護作用[77]。AST 在糖尿病（diabetes mellitus，DM）模型中能降低血糖並提升血漿 insulin 濃度[78]，其機制涉及調控腸道菌相、AMPK/SIRT1 以及 PI3K/AKT 訊息傳遞路徑[79]。黃耆類黃酮則是具有抗氧化、抗發炎與抗衰老作用，對心血管疾病、神經退化性疾病及慢性腎病具有潛在的治療與保健功效[80]。

4-3. 有關黃耆的臨床實驗

近年來黃耆的科學中藥應用在中風上有不少研究，比如 2012 年陳春忠等發表黃耆用於出血性中風的隨機雙盲對照研究，顯示黃耆能有

助於患者昏迷指數（Glasgow outcome scale）的恢復與功能獨立性評估（functional independence measure）的提升[81]。劉崇祥等人則探討了黃耆對於改善中風後疲勞的隨機雙盲對照研究，證明黃耆對於中風後的疲勞、認知、社會功能、與個人生活品質都能顯著改善[82]。在血管瘤破裂引起的蛛網膜下腔出血的中風病人，服用黃耆同樣也提升急性期的恢復速度（包括昏迷指數與巴氏量表），減少發炎數值[83]。總之，黃耆應用在急性腦中風能減少發炎因子，清除自由基與抗氧化，促進血管新生與神經再生[84]。

在韓國的轉骨方 HT042 的臨床研究中，黃耆為當中的主要成份，結果顯示服用 HT042 的身材矮小兒童能因此提升血液中的 IGF1 和 IGFBP3[85]。若在運動前服用黃耆，在運動誘發肌肉損傷之後可以明顯觀察到血中 IGF1 上升[86]。在我們先前的一則個案報導中，以黃耆為主要成分的複方中藥被證實可改善一位 SCA3 患者的生活品質[20]。在 3-acetylpyridine 誘發的 SCA3 小鼠模型，注射黃耆能提升血漿中的 IGF1，與小腦組織的 IGF1 mRNA 表現[87]。基於上述考量，本研究選擇黃耆作為 SCA3 患者的 DMT。

第五節 神經細絲輕鏈 NfL 與其他可能作為 SCA3 的生物指標探討

5-1. 血漿中的 NfL 作為 SCA3 的生物指標

腦脊髓液中的 NfL 可反映神經軸突損傷，而血漿中的 NfL 濃度與腦脊髓液的 NfL 濃度高度相關。血漿 NfL 具有穩定性高、敏感度佳、可靠性強及具預測性等特點，已廣泛應用於多種神經退行性疾病的病程追蹤，如多發性硬化症、阿茲海默症、肌萎縮性側索硬化症、脊髓損傷及毛細血管擴張性共濟失調等。高敏感度檢測技術顯示，血漿 NfL 可在數週至數月內捕捉與疾病相關的變化，因此適合作為短期臨床試驗的動態生物標記[88]。其特性亦能降低受試者負擔，使之成為合理且可行的臨床指標。近年來，NfL 已逐漸被用於 SCA3 的評估[89]，除了與 SARA 呈正相關性，也能用來區分未發病與已發病的 SCA3 族

群。在轉殖基因小鼠中，血漿 NfL 的濃度變化甚至早於 ataxin-3 毒蛋白的表現[90]，因此作為監測疾病進程變化或治療前後變化，NfL 都是目前最合適的血液指標[91]。

5-2. 其他可能的 SCA3 生物指標

在臨床工作中，醫師常持續尋求既具可及性（accessibility）又具效率的神經功能評估工具。目前，SARA 量表是神經科醫師最常用來評估小腦臨床疾病進程的神經學檢查[92]，其他的各種指標都以 SARA 作為標準。SARA 是一種半定量的評估，從 0-40 分，評估八個面向: 步態、站立、坐姿、口語、手指追蹤、指鼻測試、快速手部交替、跟脛滑動測試[93]。

在影像學與生物標記方面，形態測量核磁共振（Morphometric MRI）能提供關鍵的結構變化資訊，例如小腦、基底核、腦幹及間腦的體積縮減，這對於證實並加深對疾病的理解至關重要[94]。功能性核磁共振（functional MRI）則顯示了 SCA3 患者特有的「小腦/皮質解離模式」，有助於評估大腦—小腦運動網絡內的潛在功能連結性[95]。MRI 相關研究較耗費醫療經費與資源，因此較適合用在開發新的治療技術，或少數個案的藥物療效與神經機轉探討。其他生物標記部分，研究發現 SCA3 患者的尿液 polyQ ataxin-3 蛋白水平升高[96, 97]，而血漿或周邊血液單核細胞中的 polyQ ataxin-3 含量亦與疾病嚴重程度相關[97, 98]，然而這兩種方法學目前仍不普及，沒有廣泛地被科學界應用。

因此，除了 SARA 量表外，上述影像檢查與生物標記普遍存在價格昂貴或操作不便的問題。開發一種簡便且經濟（easy and affordable）的評估工具是臨床上的迫切需求。

5-3. 握力作為 SCA3 的生物指標潛力

手部握力（Handgrip strength，HGS）為使用手持式動力計即可測

量，已成為評估老年人健康狀況的實用工具。研究顯示 HGS 能預測社區高齡族群在認知、功能狀態及死亡率上的長期衰退[99]；英國生物樣本庫（UK Biobank）數據亦指出，失智症發病率與死亡率與低握力具有獨立相關性[100]。在帕金森氏症中，握力能預測因纹狀體多巴胺損傷及運動單元退化所導致的功能喪失[101]。對於脊髓損傷患者，握力臨界值是男性功能獨立性與輪椅操控技能的良好預測指標[102]。

在小規模試驗中，HGS 可能反映退化性小腦萎縮症患者的功能能力或運動表現[103]。在動物模型中，HGS 已被視為預測神經退化性疾病進程的生理標記[104-106]。特別是在 SCA3 轉殖基因猴模型中，症狀組的年齡相關握力下降明顯優於野生型或無症狀組[107]。儘管如此，目前關於 HGS 與 SCA3 患者臨床狀態之間確切關係的研究仍屬空白，具有進一步探討的價值。

第三章 材料與方法

第一節 受試者招募

本研究於彰化基督教醫院（Changhua Christian Hospital，CCH）中醫門診招募 SCA3 患者，所有參與者皆簽署知情同意書。納入條件包括：（1）經神經專科醫師以基因學檢查確診之 SCA3 患者；（2）年齡介於 20 至 80 歲。排除條件為：（1）正在服用中藥或草藥者；（2）對中藥或草藥過敏；（3）懷孕或哺乳中；（4）同時罹患其他重大疾病，如癌症、中風、心衰竭或腎衰竭等；（5）糖尿病（DM）。預計收集的基本人口學資料包括性別、年齡、身高、體重、身體質量指數（body mass index，BMI）、發病年齡、病程長度、用藥紀錄，以及 CAG 三核苷酸重複數等。在 SCA3 病人招募完成後，本研究同時招募一群符合上述招募條件，但是沒有罹患 SCA3 的健康受試者為對照組，也收集上述基本資料，簽署知情同意書。

第二節 研究設計

本研究為隨機分派、雙盲、安慰劑控制交叉試驗，自 2021 年 5 月 31 日至 2022 年 9 月 26 日於 CCH 進行。本研究獲 CCH 人體試驗委員會核准（編號 200703，附錄 1），並已登錄於 ClinicalTrials.gov（NCT05038306）。對照組招募亦獲 CCH 人體試驗委員會核准（編號 200730，附錄 2），惟對照組僅進行抽血檢測，並未進行藥物服用流程，用以與 SCA3 組的基本資料進行比較研究。

SCA3 受試者依門診招募順序隨機分配至 A 組或 B 組，且對試驗藥物成分不知情（受試者知情同意書內容僅告知實驗組會服用中藥 WT，但不知道 WT 是什麼成份）。A 組將先服用黃耆（AM）治療 12 週，再經過 4 週 washout 後，改為服用安慰劑 12 週；B 組則採相反順序（圖 1）；其後兩組均停藥 4 週，再作最後一次檢測。研究期間允許持續既有西藥治療，但不得額外使用其他中藥或草藥。研究助理透過電話定

期追蹤藥物遵從性及副作用，並要求受試者須填寫未服藥記錄表（若未服藥則填寫之，如附錄 3）。此外，研究亦分析中途退出者的特徵，以評估退出是否影響試驗結果解釋。

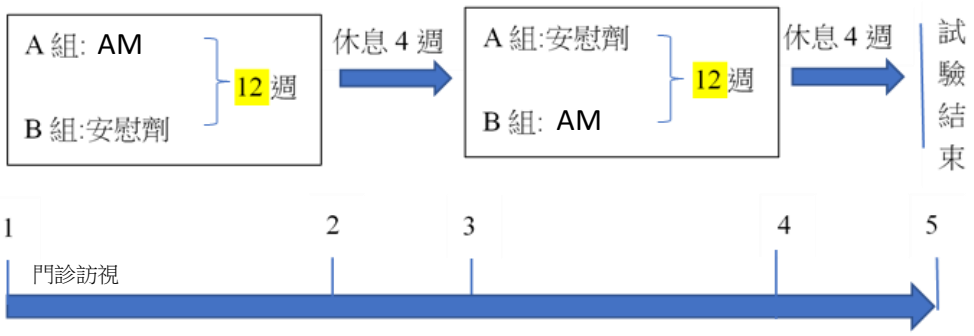

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圖 1. 隨機雙盲交叉設計試驗流程圖

第三節 隨機化與雙盲

本研究委託順天堂藥廠股份有限公司（Sun Ten Pharmaceutical Co.，Ltd.）制定隨機分派表: 採用塊狀隨機分派（block randomization），區塊大小（block size）為 4，以維持小樣本研究中組別的平衡。順天藥廠將分派表交由中藥局依序發藥。分派過程全程隱藏，研究人員、受試者及藥局發藥的藥師均對分派情況不知情，隨機化編碼由藥廠保存，直至試驗結束才解盲。

第四節 樣本數估算

在此交叉設計研究中，樣本數之推算設定顯著水準為 0.05（雙尾檢定），檢定力（power）為 0.8，並考慮相同受試者治療前後的標準差變異。若假設治療前後的差異達 50%，則估計需 33 位受試者以拒絕虛無假設。若考慮 25%的退出率，則需招募 44 位參與者。根據 Wilke

等人對血清 NfL 的研究，若效果量（effect size）為 20%，每組約 15 位受試者即可達到顯著差異[90]。

第五節 黃耆與安慰劑的製備

黃耆和安慰劑由順天堂藥廠提供。黃耆由生藥黃耆藥材經標準化濃縮製程製成藥粉。安慰劑由澱粉（starch）與麥芽糊精 (maltodextrin)（澱粉:麥芽糊精比例 7:3）組成，並加入少量焦糖色素（caramel）以模擬黃耆藥粉之外觀、味道及顏色。根據動物實驗，成人安全劑量低於 20 gm/天不會造成腎毒性[108]。根據黃耆用在出血性腦中風恢復及中風後疲勞兩項研究的使用劑量，9 gm/天並未顯示明顯副作用[81, 82]。因此，結合上述研究與臨床經驗，本研究藥物劑量設定為 3 gm/包（sachet），每日兩次，於早餐及晚餐前各服用一包，共 12 週。藥物遵從性藉由藥包回收數量及未服藥記錄表監測，要求受試者於每次回診時攜回未服用的藥包，交還中藥局。

原料黃耆來源為中國山西省，為豆科植物 *Astragalus membranaceus* var. *mongholicus*（Bunge）P.K. Hsiao 之乾燥根部。該藥材由財團法人必安研究所（Non-Profit Organization Brion Research Institute of Taiwan）依據《臺灣中藥典》（2021 年版）與《中華人民共和國藥典》（2020 年版）進行基原鑑定，並透過顯微與基因體學檢驗確認其真偽，同時保存標本（voucher specimen）編號 BVRSR115A140。原料藥材均依臺灣政府標準進行嚴格品質管制，包括微生物、重金屬與農藥殘留等檢測。每 1 公克活性中藥萃取顆粒（批號：20020432）係由 3.34 公克（約台制 1 錢）黃耆生藥製成，其中包含 0.66 公克萃取物與 0.34 公克生藥研磨粉，並採流體化床造粒法（fluid-bed granulation）製備。台灣中藥典（第四版）黃耆每日建議劑量為 9-30 克，本研究每日試驗用藥總劑量 6 克（約為 6 錢生藥黃耆）為安全範圍。其後，該萃取物經品質控制與分析，使用高效液相層析儀（high-performance liquid chromatography）（Pump: Waters 600；Autosampler: Waters 717plus，

Milford，Massachusetts，USA）及蒸發光散射偵測器（evaporative light scattering detector）（Sedex75，Olivet，France）進行分析。以黃耆活性成分黃耆皂苷 IV（Astragaloside IV）作為對照標準品（圖 2）。中華藥典規定黃耆皂苷 IV 不得低於 0.04%即為合格品，本次研究藥材的黃耆皂苷 IV 鑑定為 0.15%。

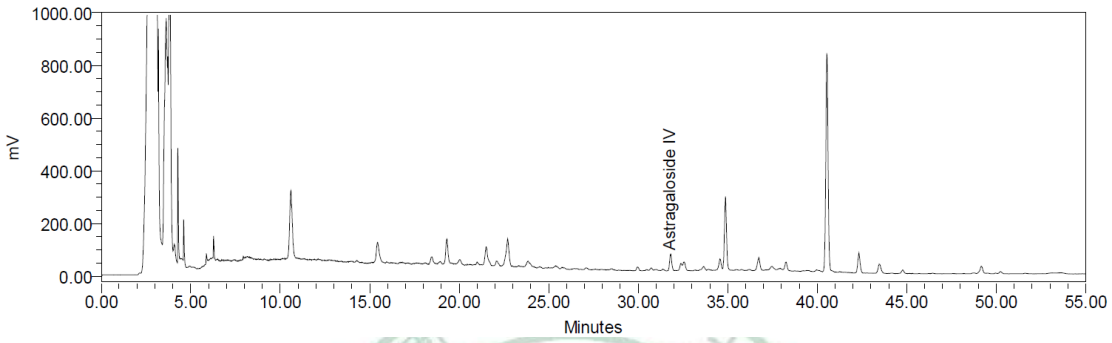


圖 2. 以黃耆皂苷 IV 為指標成分之黃耆高效液相層析／蒸發光散射偵測器指紋圖譜分析

第六節 安全性監測

黃耆在《神農本草經》中被列為上品，表示其無毒、可長期使用。動物實驗亦顯示黃耆具肝腎保護作用[109, 110]。在臨床研究中，針對中風後疲勞患者進行一個月黃耆治療，未見對血液學、肝功能或腎功能的不良影響[81]。然而，本研究仍於每次門診時抽血監測血清肌酸酐（creatinine，Cr）、估算腎絲球過濾率（estimated glomerular filtration rate，eGFR）、血尿素氮（blood urea nitrogen，BUN）、Glutamic Oxaloacetic Transaminase (GOT)、Glutamic Pyruvic Transaminase (GPT)及全血計數/分類計數（complete blood count / differential blood count，CBC/DC），以確保 SCA3 受試者的安全性。

第七節 研究結果測量

抽血時間在午餐後時間。病人服用中式餐點（大約 600-700kcal，

有均衡的營養組成: 碳水化合物、蛋白質、油脂），餐點內容由受試者自己選擇，不由本團隊提供。抽血由同一位研究護理師負責，大約在餐後二小時[111]，下午 2 點左右進行。初始的神經學檢查（SARA）評估則由同一位神經科醫師執行。

本試驗的預設主要結果為血漿 NfL 濃度的變化。由於 SCA3 患者已知存在胰島素系統、IGF1 及其結合蛋白（IGFBPs）的異常[59]，因此 IIS 也被視為潛在治療標的[67]。自由型 IGF1（free-form IGF1）可由 IGF1/IGFBP3 比值反映，因為 IGFBP3 為 IGF1 主要的結合蛋白[112]。動物實驗發現，IGFBP1 在轉殖基因小鼠腦部增加時，會抑制髓鞘軸突（myelin axons）與寡樹突膠細胞（oligodendrocyte）數量，可能參與 SCA3 的病理機轉[113]。另外，由於 IGF1 與 IGFBP1 的血液濃度可能會被服用 Q10 干擾[114]，而 Q10 是 SCA3 病人常在服用的藥物之一，因此分析服用 Q10 是否影響基線資料是必要的。氧化 DNA 產物 8-羥基-2-去氧鳥苷（8-OHdG）則為阿茲海默症及 SCA3 小鼠中常見的氧化壓力標記[62, 115]。因此，本研究欲探討黃耆是否能改善 SCA3 患者的 NfL、IIS 或氧化壓力 8-OHdG。

7-1. 血液生化檢查

CBC/DC 由 Beckman Coulter DxH 900 血液分析儀（Beckman Coulter，Miami，Florida，USA）檢測。血漿 GOT、GPT、BUN 與 Cr 由 AU5800 臨床化學分析儀（AU5811；Beckman Coulter，Brea，CA，USA）測定，由 CCH 檢驗醫學部執行。

7-2. 血漿中 8-OHdG 與 NfL 的測定

血漿 8-OHdG 濃度使用高敏感度 8-OHdG 檢測 ELISA 試劑盒（JaICA-#KOG-HS10E）。NfL 濃度則採用 Simoa® NF-light™ Advantage Kit（#103186），並以 Simoa HD-X 超高敏蛋白分子檢測儀（Quanterix，MA，USA）定量分析[116]。

7-3. 血漿中 IIS 相關檢測

血液收集於 10 mL BD Vacutainer® EDTA 管中，於 4˚C、3000 rpm 離心 15 分鐘後取血漿，-80˚C 冷凍保存直至分析。檢測項目包括葡萄糖（glucose）、胰島素（insulin）、IGF1、IGF2、IGFBP1 及 IGFBP3，分別使用以下檢測套組：Glucose Colorimetric Assay Kit (Cayman Chemical-10009582)、Insulin ELISA Kit (Mercodia 10-1113-01)、Human IGF1/IGF1 Immunoassay ELISA Kit (R&D Systems-DG100B)、Human IGF2 Quantikine ELISA Kit (R&D Systems-DG200)、Human IGFBP-1 ELISA Kit (RayBiotech-ELH-IGFBP1-1)、Human IGFBP3 ELISA Kit (Abcam-ab100541)。Free-form IGF1 由 IGF1/IGFBP3 的比值來推估，這是一個間接的預測值，無法取代直接測量 free IGF1。另外，血漿的 glucose-to-insulin ratio（G/I ratio）的計算為 glucose (mg/dl) 除以 insulin (μIU/ml)，用來作為 insulin 敏感度的估計值，特別是用在餐後的抽血狀態時，這是相對於空腹的 insulin 敏感度常用 Homeostatic Model Assessment 2[117] 或者 Quantitative Insulin Sensitivity Check Index[118]而言。

7-4. 握力測量

由固定一名醫師使用 Jamar Plus+ 手握力計（Sammons Preston Rolyan, Bolingbrook，IL，USA）來協助病人測量握力。受試者需坐於有直背的椅子上，肘關節屈曲 90 度，並以握把第 2 段位置握持測力計，該位置為握把至測力計固定端距離 4.8 公分[119]。受試者以慣用手持續用最大力量握緊 3 秒。手握力共測量三次，每次間隔休息 15 秒[120]。三次測量中的取其中一次最大值作為最終握力結果。

7-5. 動物模型與握力測量

C57BL/6 野生型（wild type，WT）小鼠購自 National Laboratory Animal Center（臺北，臺灣），而 SCA3 84Q 轉殖基因小鼠（C57BL/6 背景）先前已有文獻報導[62]。所有動物實驗均經 CCH 動物照護與使用

委員會核准（核准編號：CCH-AE-108-021）。

SCA3 84Q 小鼠之基因型係以取自鼠尾的 DNA 樣本進行聚合酶鏈反應（PCR）確認（primer sequences for forward: 5´-TGG CCT TTC ACA TGG ATG TGAA-3´，reverse: 5´-CCA GTG ACT ACT TTG ATT CG-3´）。出現 430 bp 片段者判定為 SCA3 84Q 陽性。小鼠餵以一般標準飼料（未給予黃耆），並飼養於溫控環境下之 12 小時明暗循環條件中。每週記錄小鼠體重以監測其健康狀況。野生與 SCA3 小鼠的握力測試僅於 18 個月齡時進行。

小鼠握力採用 Digital Force Gauge（Model DPS-5R: range of 0–5 kgf，Imada，Japan）測量小鼠四肢握力。測試時將小鼠置於金屬網格上，輕柔且平行地向後拉其尾巴，當小鼠鬆手時儀器自動記錄峰值力量。最大力量（克）作為四肢握力；相對握力則以小鼠體重進行校正。每隻小鼠以 1 分鐘間隔進行三次握力測試[121]。

第八節 統計方法

本研究使用 SPSS v.22.0（(IBM Corp.，Armonk，NY)）進行數據分析，依據資料分布採用常態或非常態統計方法。由於存在缺失值（missing cases），且樣本數偏少（small sample size），我們採用意向性治療分析（intention-to-treat，ITT）以維持隨機化，也反應真實世界情況，並使用末次觀察值攜入法（Last Observation Carried Forward，LOCF）補足缺失值，並比較完成者與未完成者的基線特徵（baseline characteristics），以評估偏差（bias）。

交叉設計中的延遲效應（carryover effects）以混合模型分析（mixed model analysis），檢視隨機化交叉設計的品質。考慮年齡、基線 NfL 水平、給藥順序（安慰劑/藥物哪個先）、治療類型（安慰劑/藥物）、介入次序（前三個月/後三個月）、治療-次序交互作用

（treatment-order interaction）後，NfL 差異並無統計顯著性（*P*=0.174），因此本研究結果採合併序列（pooled sequence）分析。

在 IGFBP1 的樣本檢測中，多數對照組樣本的吸光值（Optical Density）落於標準曲線的下限或低於該下限。由於缺乏實驗室特定的偵測極限，本研究採用該分析法的靈敏度（5 pg/ml）作為替代下限（Surrogate lower limit）。針對這些低於閾值的數值，參考處理左偏態截斷（Left-censored）生物標記數據的既有方法，將其插補（Imputed）為靈敏度除以√2（約 3.5 pg/ml）[122]。為了評估 IGFBP1 及其他 IIS 在區分 SCA3 早期與晚期階段的能力，本研究進行了 Receiver Operating Characteristic Curve（ROC curve）分析，並利用曲線下面積（area under the curve，AUC）來比較各指標的診斷效能。此外，基線 insulin 水平依據三分位數（Tertiles）分為三組，使每組病例數大致相等（T1: <3.65 μIU/ml；T2: 3.65 – 9.35 μIU/ml；T3: >9.35 μIU/ml）。針對 insulin 三分位組別間的差異，先採用 Kruskal-Wallis 檢定進行分析，隨後使用 Dunn's 事後檢定（Post hoc test）進行兩兩比較。

其他統計檢定包括：連續變項的組間比較採獨立樣本 t 檢定或 Mann-Whitney U 檢定，類別變項則使用卡方檢定（Chi-square test）或費雪檢定（Fisher's exact test）；組內比較則採配對 t 檢定或 Wilcoxon 檢定。另建構多元迴歸模型（multiple regression model），以調整會讓 NfL 下降的潛在混雜因子（potential confounders），提供更穩健的療效評估。最後進行相關性分析（correlation analysis），以探索血漿 NfL 變化與 IIS 相關生物標記變化的關聯，符合常態分布者使用 Pearson correlation，未符合者使用 Spearman rank test，若須校正性別年齡者則使用 Partial correlation。在黃耆研究的亞組分析，由於第一組人數過少（n= 2），因此使用 effect size 來描述統計量，，統計顯著性設定為 *P*<0.05。

第四章 結果

第一節 實驗組與對照組基本資料與 IIS 比較

本研究共納入 32 位 SCA3 實驗受試者，與招募自彰化基督教醫院門診的 36 位性別年齡相近的非 SCA3 對照組。兩組在年齡上無顯著差異〔median，49.0 (IQR，34.3–54.8) vs. 47.5 (34.8–55.0) 歲；*P*=0.754〕，性別分佈亦無顯著不同（male， 62.5% vs. 61.1%；*P*=0.906）。然而，SCA3 組的 BMI 顯著低於健康對照組〔21.8 (19.5–25.4) vs. 23.6 (21.8–25.6) kg/m^2；*P*=0.040〕。此外，SCA3 組的 NfL 水平較對照組顯著升高〔27.8 (20.5–32.5) vs. 6.3 (4.2–7.6) pg/ml；*P*<0.001；表 1〕。

表 1. 健康對照組與 SCA3 受試者的基線資料與 IIS

	Controls n=36	SCA3 n=32	*P*
Demographics			
Sex (male，%)	22, 61.1%	20, 62.5%	0.906
Age (yr)	47.5 (34.8-55.0)	49.0 (34.3-54.8)	0.754
BMI (kg/m^2)	23.6 (21.8-25.6)	21.8 (19.5-25.4)	**0.040***
Age at onset (yr)	N/A	35.0 (29.8-45.0)	
Duration (yr)	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***
Insulin/IGF1 system			
IGF1 (ng/ml)	55.2 (34.0-68.1)	92.6 (67.9-108.6)	**<0.001***
IGF2 (ng/ml)	312.8 (271.7-361.4)	336.2 (316.2-395.0)	**0.002***
IGFBP1 (pg/ml)	3.5 (3.5-41.5)	252.9 (72.8-740.4)	**<0.001***
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***
Glucose (mg/dl)	95.9 (73.6-116.5)	98.9 (81.2-112.7)	0.708
Insulin (μIU/ml)	19.0 (8.8-28.1)	6.1 (2.6-11.0)	**<0.001***
G/I ratio	6.0 (3.3-9.3)	17.1 (8.6-35.0)	**<0.001***

BMI，身體質量指數。SARA，共濟失調評估量表。NfL，神經細絲輕鏈。IGF1，類胰島素生長因子 1。IGFBPs，類胰島素生長因子結合蛋白。G/I ratio，葡萄糖與胰島素比值。統計顯著性設定為*P<0.05。所有數據均以中位數（四分位距）表示，性別以例數（百分比）表示。

IIS 部分，SCA3 患者體內 IGFBP1、IGF2 及 free-form IGF 水平升高，且 insulin 水平不足。研究顯示，與對照組相比，SCA3 患者血漿中的 IGFBP1 顯著增加 [252.9 (72.8-740.4) vs. 3.5 (3.5-41.5) pg/ml；*P*<0.001]。SCA3 組的總 IGF1 水平同樣較高 [92.6 (67.9-108.6) vs. 55.2 (34.0-68.1) ng/ml；*P*<0.001]，IGF2 水平亦然 [336.2 (316.2-395.0) vs. 312.8 (271.7-361.4) ng/ml；*P*=0.002]。在 IGFBP3 水平方面則未觀察到顯著差異(*P*=0.873)；然而，代表 free-form IGF1 的 IGF1/IGFBP3 比值在 SCA3 患者中顯著較高 [1.92 (1.35-2.49) vs. 1.27 (0.96-1.75)；*P*=0.002]，這暗示了 IGF1 的生物利用度（bioavailability）有所增強（表 1）。

為了評估 SCA3 受試者補充輔酶 Q10 是否會造成干擾，本研究針對接受 Q10 治療（n=17）與未接受治療（n=15）的患者，比較了所有人口學變項及 IIS 相關變項。結果顯示兩組之間並無顯著差異。(表 2)

表 2. 輔酶 Q10 使用與否之 SCA3 受試者特徵及 IIS 相關變項比較

	SCA3 w/ Q10 n=17	SCA3 w/o Q10 n=15	*P*
Demographics			
Sex (male, %)	10, 58.5	10, 66.7	0.647
Age (yr)	49.0 (41.5-55.0)	47.0 (33.0-55.0)	0.521
BMI (kg/m^2)	22.7 (21.0-25.7)	21.2 (18.4-25.1)	0.141
Age at onset (yr)	43.0 (29.5-49.0)	34.0 (29.0-38.0)	0.308
Duration (yr)	8.0 (5.5-11.5)	10.0 (3.0-18.0)	0.909
CAG repeat number	71.0 (68.5-74.5)	72.0 (70.0-74.0)	0.985
SARA	14.0 (9.8-21.0)	15.5 (5.0-21.5)	0.910
NfL (pg/ml)	28.3 (24.0-34.7)	20.8 (18.0-32.8)	0.355
Insulin/IGF1 system			
IGF1 (ng/ml)	85.6 (64.7-111.1)	9836 (80.0-110.0)	0.396
IGF2 (ng/ml)	365.7 (316.2-403.3)	366.7 (305.4-390.4)	0.664
IGFBP1 (pg/ml)	315.3 (19.9-805.1)	208.9 (84.3-451.0)	0.970
IGFBP3 (ng/ml)	41.2 (35.8-69.2)	43.6 (38.1-52.1)	0.925
IGF1/IGFBP3 ratio	1.7 (1.3-2.4)	2.1 (1.4-3.2)	0.355
Glucose (mg/dl)	101.3 (85.1-130.2)	90.6 (81.2-101.6)	0.146
Insulin (μIU/ml)	6.2 (3.0-11.8)	6.0 (2.1-10.5)	0.720
G/I raito	14.6 (9.7-36.6)	19.1 (8.4-32.1)	0.895

BMI，身體質量指數。SARA，共濟失調評估量表。NfL，神經細絲輕鏈。IGF1，類胰島素生長因子 1。IGFBPs，類胰島素生長因子結合蛋白。G/I ratio，葡萄糖與胰島素比值。統計顯著性設定為*P<0.05。所有數據均以中位數（四分位距）表示，性別以例數（百分比）表示。

鑑於觀察到 IGFBP1 水平升高，本研究進一步評估了上游的 insulin 相關參數，以探索潛在的調節機制。結果顯示，SCA3 組與對照組之間的血糖水平相近 [98.9 (81.2-112.7) vs. 95.9 (73.6-116.5) mg/dl；P=0.708]；然而，SCA3 患者的 Insulin 水平顯著下降 [6.1 (2.6-11.0) vs. 19.0 (8.8-28.1) μIU/ml；P<0.001]。因此，作為評估胰島素缺乏（insulin deficiency）或敏感性（sensitivity）之替代指標的血糖/胰島素比值（G/I ratio），在 SCA3 患者中顯著增加 [17.1 (8.6-35.0) vs. 6.0 (3.3-9.3)；P<0.001]，這顯示其胰島素信號傳導受損（表 1）。

第二節 IGFBP1 區分 SCA3 病人共濟失調程度，與臨床嚴重度相關

本研究將 SCA3 患者分為前期（SARA≤11）與後期（SARA>11）兩組。結果顯示，與前期患者相比，後期共濟失調患者的血漿 IGFBP1 水平顯著升高 [415.5 (207.9-913.2) vs. 72.5 (13.1-108.1) pg/ml；*P*=0.003]，且 NfL 水平亦較高 [29.5 (25.1-38.1) vs. 18.8 (17.0-28.3) pg/ml；*P*=0.002]。這些發現顯示，IGFBP1 與 NfL 均能有效區分 SCA3 的前期與後期（表 3）。

表 3. SCA3 患者依 SARA 分級基線資料之亞組分析

	Stage I (SARA≤11) N=11	Stage II (SARA>11) N=21	*P*
Demographics			
Sex (male, %)	7, 63.6%	13, 61.9%	>0.999
Age (yr)	49.0 (29.0-51.0)	49.0 (41.5-59.5)	0.150
BMI (kg/m^2)	25.4 (22.1-26.9)	21.2 (18.8-22.9)	**0.005***
Age at onset (yr)	33.0 (28.0-45.0)	35.0 (32.5-46.5)	0.945
Duration (yr)	4.0 (0-6.0)	11.0 (9.0-15.0)	**<0.001***
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***
NfL (pg/ml)	18.8 (17.0-28.3)	29.5 (25.1-38.1)	**0.002***
Insulin/IGF1 system			
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)	72.5 (13.1-108.1)	415.5 (207.9-913.2)	**0.003***
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 (μIU/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

BMI，身體質量指數。SARA，共濟失調評估量表。NfL，神經細絲輕鏈。IGF1，類胰島素生長因子 1。IGFBPs，類胰島素生長因子結合蛋白。G/I ratio，葡萄糖與胰島素比值。統計顯著性設定為*P<0.05。所有數據均以中位數（四分位距）表示，性別以例數（百分比）表示。

正如預期，後期患者的發病病程較長 [11.0 (9.0-15.0) vs. 4.0 (0-6.0) 年；P<0.001]，且 BMI 較低 [21.2 (18.8-22.9) vs. 25.4 (22.1-26.9) kg/m^2；P=0.005]，這與疾病負擔（disease burden）較重的情況相符。然而，其他 IIS 相關參數（包括 glucose、insulin、G/I ratio、IGF1、IGF2、IGFBP3 及 IGF1/IGFBP3 ratio）在不同病程階段之間並無顯著差異（均為 P>0.05；表 2）。

此外，ROC 曲線分析證實，在區分早期與晚期病程方面，IGFBP1 展現出最強的辨別能力（AUC=0.829；95% CI: 0.646-1.000；*P*=0.003）。若以 139.1 pg/ml 作為最佳切點值（Cutoff value），其敏感度達 91%，特異度為 82%，表現優於其他 IIS 參數（其他參數之 AUC 範圍介於 0.385-0.541 之間）（圖 3）。

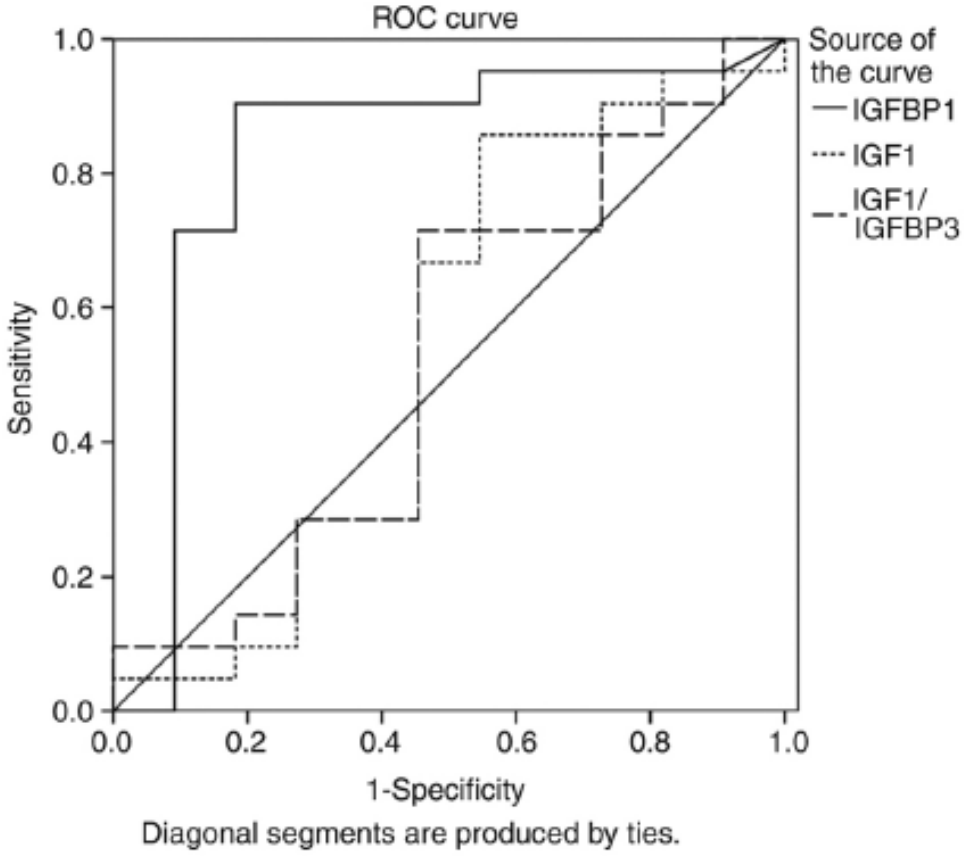


圖 3. 利用 IGFBP1 及其他 IIS 相關標記之 ROC 曲線分析，以區分早期與晚期 SCA3

在 SCA3 的資料中，Spearman 相關分析顯示，IGFBP1 水平與 SARA 評分（r=0.401；*P*=0.023）及 NfL 水平（r=0.538；*P*=0.001）呈正相關，並與 BMI 呈顯著負相關（r=-0.667；*P*<0.001）。然而，IGFBP1 與 CAG 重複次數之間並未觀察到顯著關聯（r=0.141；*P*=0.440）（圖 4）。儘管數據中存在數個較高的 IGFBP1 數值，但所有測量值均被視為具有生理上的合理性，因此分析中未剔除任何數據點（關於 IGFBP1 作為 SCA3 代謝與神經退化的生物指標，詳見附錄 4）。

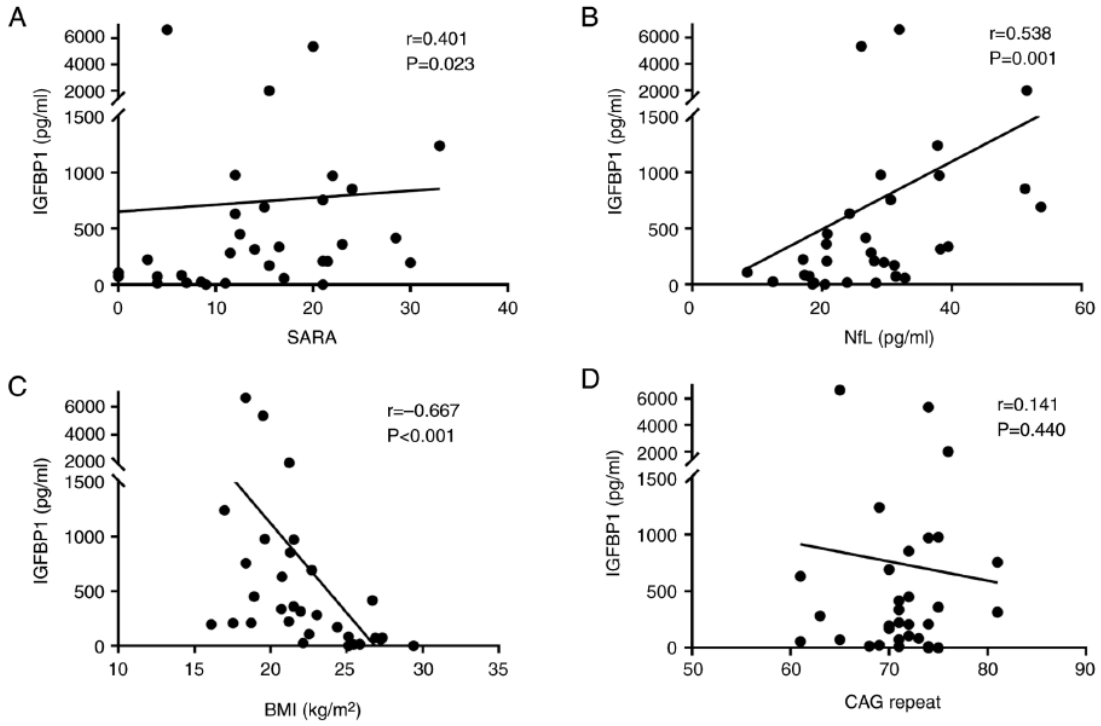


圖 4. 血漿 IGFBP1 與臨床指標的相關性分析

SCA3 受試者的基線資料以 Spearman's correlation 比較 IGFBP1 與 (A)SARA，(B)NfL，(C)BMI，和(D)CAG 的相關性

第三節 SCA3 與對照組的握力比較

與對照組相比，SCA3 組表現出較弱的握力 HGS [24.3 (19.2-38.2) vs. 40.7 (27.1-49.3) kg；*P*<0.001；圖 5A]，以及較低的 HGS/BMI 比值 [1.19 (0.95-1.55) vs. 1.38 (1.11-1.79) kg/(kg/m^2)；*P*<0.001]。此外，如圖 5B 所示，無論是女性患者 [20.10 kg (18.60-25.10) vs. 26.80 kg (25.45-30.4)，*P*= 0.007] 還是男性患者 [35.10 kg (23.30-38.33) vs. 47.20 kg (42.35-55.25)；*P*<0.001]，SCA3 受試者的握力均較正常受試者顯著下降。

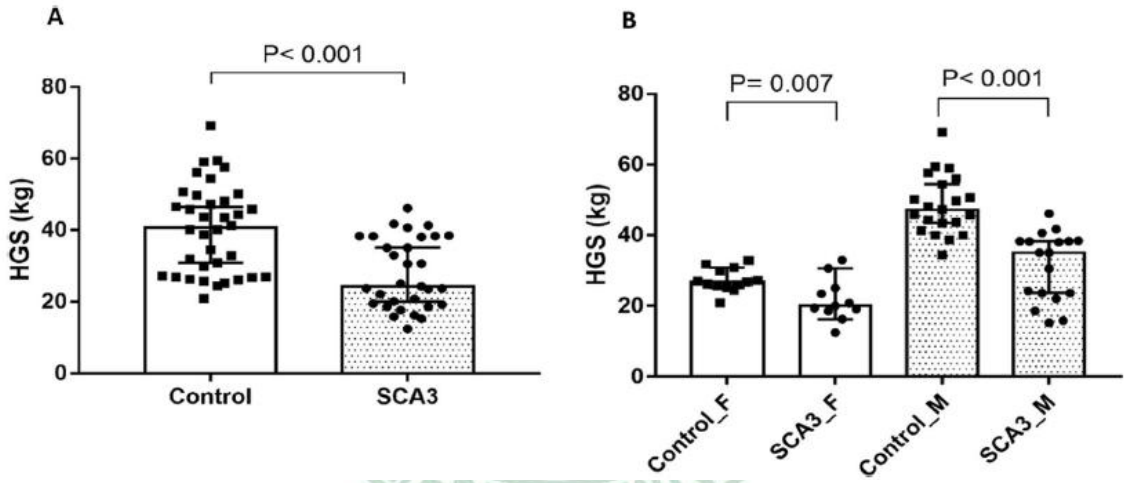


圖 5. SCA3 和健康對照組的握力 HGS 比較

數值以中位數與 95%信賴區間來呈現，比較(A)兩組，與(B)兩組兩性之比較；*P*: Mann-Whitney U test

在 18 月齡時，SCA3 84Q 轉殖基因小鼠的握力較野生型（WT）小鼠顯著下降（*P*=0.003，如圖 6A 所示）。在校正體重因素後，SCA3 小鼠的握力與野生型小鼠相比，依然維持在較低水平（*P*=0.007，圖 6B）。

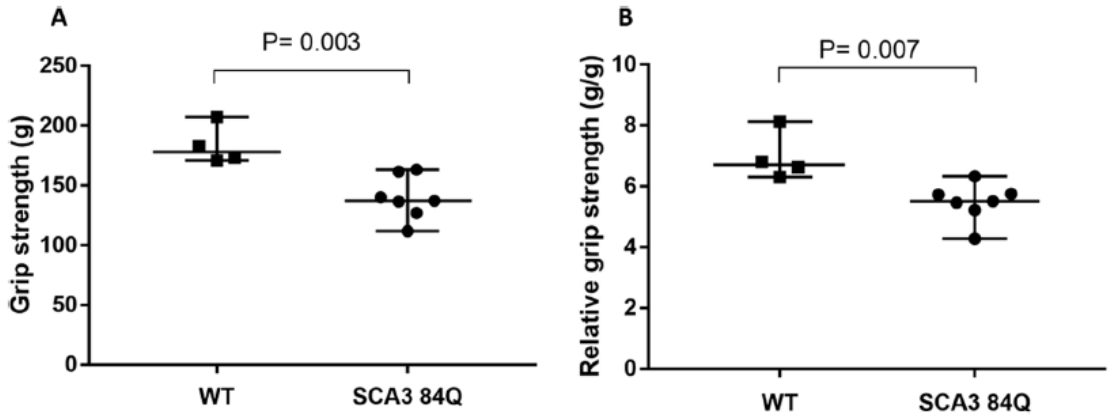


圖 6. 野生 WT 小鼠與 SCA3 84Q 小鼠的(A)四肢握力，與(B)體重相對握力之比較

資料以中位數與 95%信賴區間呈現；野生 WT，n=4；SCA3 84Q，n=7；*P*: Mann-Whitney U test

第四節 SCA3 的握力與臨床指標相關

在 SCA3 患者中，較強的握力可預測較低的 SARA 評分（r= -0.548，*P*=0.001，圖 7A）與較低的 NfL 水平（r= -0.359，*P*=0.048，如圖 7B）。此外，如表 4 所示，較大的握力與 SARA 各項子分項的低分均顯著相關，包括：步態（r= -0.525，*P*=0.002）、站姿（r= -0.487，*P*=0.005）、坐姿（r= -0.636，*P*<0.001）、言語障礙（r= -0.522，*P*=0.003）、手指追逐（r= -0.510，*P*=0.003）、指鼻測試（r= -0.355，*P*=0.050）、手部快速輪替動作（r= -0.505，*P*=0.004）以及跟脛滑動試驗（r= -0.403，*P*=0.025）。

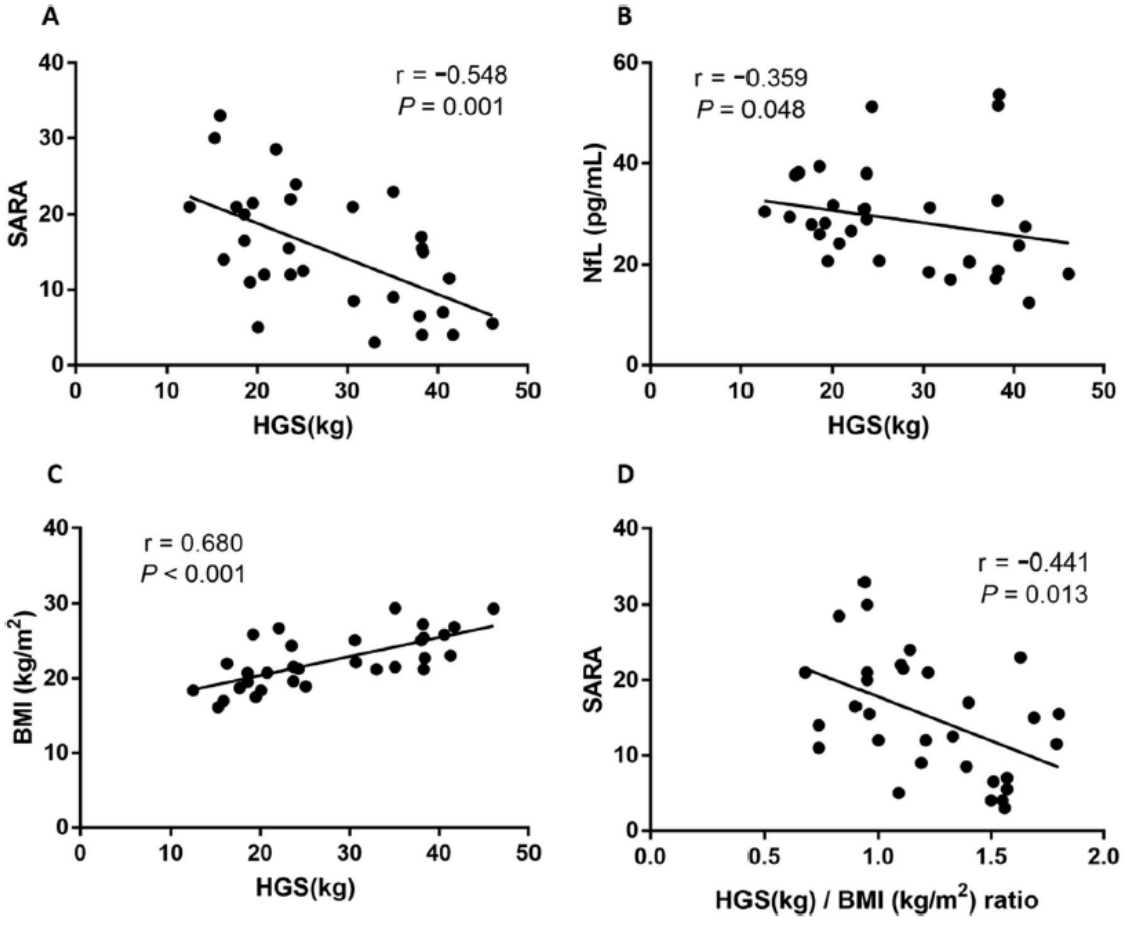


圖 7. 握力 HGS 與(A)SARA，(B)NfL，(C)BMI，(D)HGS/BMI 的相關性分析

r: 相關係數，*P*: Spearman rank test

表 4. SCA3 握力與 SARA 八個子項目的相關性

Variable	Correlation coefficient	*P*-value
HGS		
Gait	−0.525	**0.002***
Stance	−0.487	**0.005***
Sitting	−0.636	**<0.001***
Speech disturbance	−0.522	**0.003***
Finger chase	−0.510	**0.003***
Finger-nose test	−0.355	**0.050***
Fast alternating hand movements	−0.505	**0.004***
Heel-shin slide	−0.403	**0.025***

HGS，握力。SARA，共濟失調評估量表。採用 Spearman rank test 進行相關性分析。統計顯著性設定為*P<0.05

在 SCA3 組中，握力能有效反映 BMI 的狀況（r= 0.680，P<0.001，圖 7C）。BMI 已被證實為 SCA3 疾病進展的預測因子[123, 124]，而本研究顯示其與 SARA 呈顯著負相關（r= -0.420，P=0.019）。鑑於多數研究指出 BMI 與握力具有相關性[125, 126]，本研究計算了校正 BMI 後的新變量—HGS/BMI ratio。結果顯示，該比值與 SARA 評分仍呈負相關（r= -0.441，P=0.013，圖 7D）。即便將性別與年齡作為控制變項進行考量，握力與 SARA、NfL 及 BMI 之間仍維持一致的關聯。此外，在校正年齡後，較低的握力亦可預測 SCA3 患者具有較長的 CAG 重複次數（r= -0.396，P=0.030）（關於 SCA3 握力預測疾病進程的研究，詳見附錄 5）。

第五節 黃耆研究分派與流程

32 位 SCA3 受試者隨機分派為 A 組與 B 組，各 16 人（圖 8）。在 A 組中，於黃耆治療的前三個月內無人退出。在 washout 期間，有兩位受試者要求退出：其中一位因在試驗開始前六個月停用抗癲癇藥物而發生癲癇，另一位因反覆鼻炎且希望減少用藥而中止。在之後三個月的安慰劑治療中，有一人因交通問題而退出。在 B 組中，於前三個月的安慰劑治療期間有四人退出：一人因交通問題，一人因心臟事件，另兩人因四肢麻木而退出。在 washout 期間，又有兩人退出：一人因交通問題，一人因焦慮症發作。在隨後的三個黃耆治療中，無人退出。依從性良好，藥物紀錄顯示，定期追蹤的參與者在研究期間剩餘未服用的藥包數均少於 10 包。

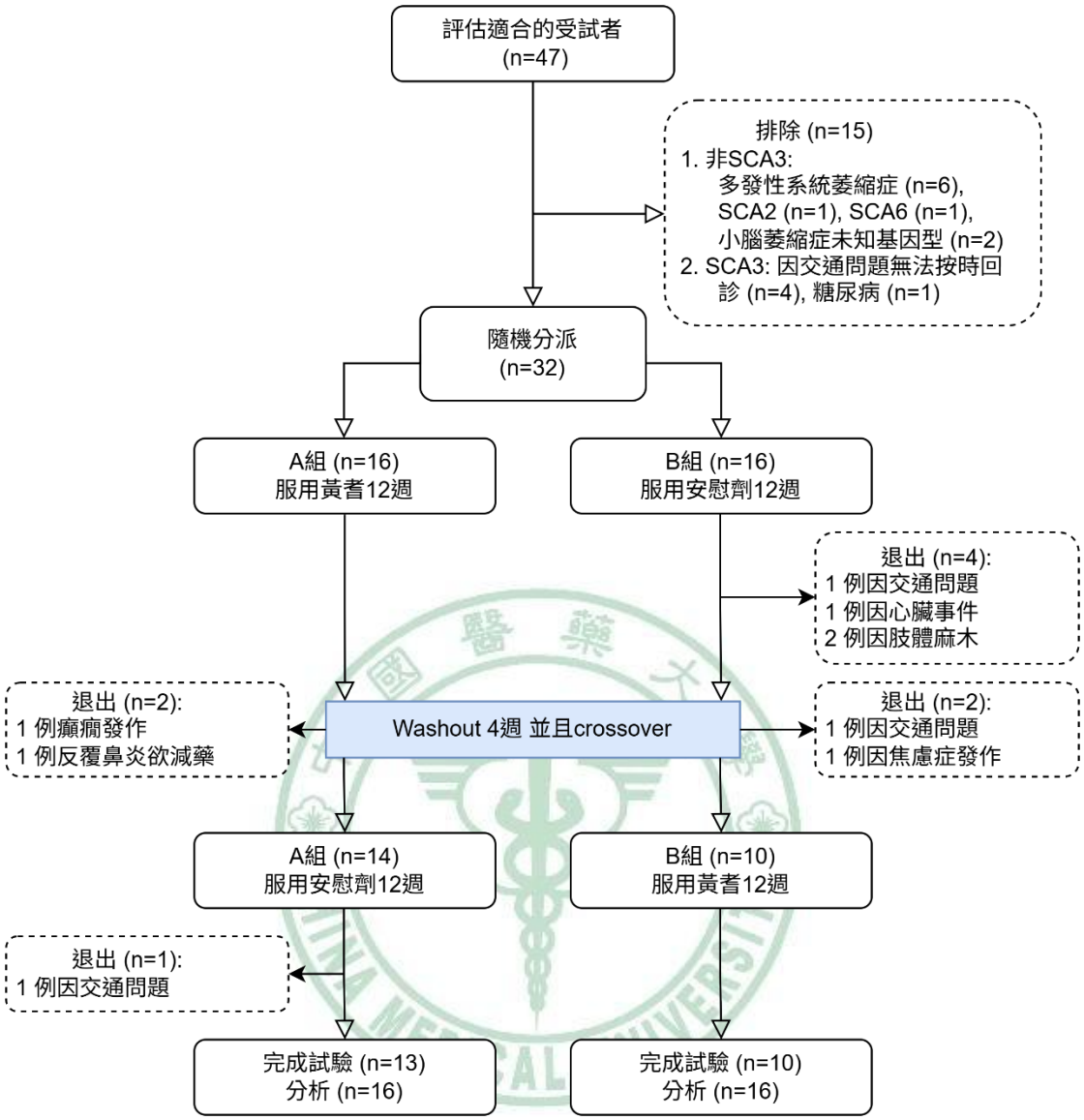


圖 8. 研究收案流程圖

最終，共有 23 位參與者完成研究，整體退出率為 28%。流失偏倚評估（attrition bias assessment）顯示，完成者與未完成者之間無顯著差異。研究設計採用 ITT 分析與序列合併（pooling sequence），共納入全部 32 位患者，其中 26 人接受黃耆，25 人接受安慰劑。A 組與 B 組的基線資料列於表 5，兩組在基本特徵、共濟失調臨床表現及 IIS 方面皆無顯著差異。

表 5. 黃耆研究受試者的分組基線資料與 IIS

	Group A (mean ± SD)	Group B (mean ± SD)	*P*
Basic features			
Case number	16	16	
Sex (male, %)	12 (75)	8 (50)	0.144
Age (yr)	47.5 ± 13.2	45.9 ± 14.1	0.739
BMI (m^2/kg)	22.7 ± 3.8	21.9 ± 3.1	0.520
SCA features			
Age at onset (yr)	36.9 ± 9.8	37.5 ± 14.5	0.887
Duration (yr)	10.6 ± 7.5	8.4 ± 5.6	0.344
CAG count	72.2 ± 3.6	70.3 ± 5.5	0.264
SARA	14.9 ± 10.8	14.2 ± 6.0	0.818
NfL (pg/mL)	28.9 ± 12.6	27.6 ± 9.1	0.748
8-OHdG (ng/mL)	3.37 ± 0.63	3.95 ± 2.00	0.955
Insulin/IGF1 System			
Glucose (mg/dL)	97.7 ± 20.0	101.6 ± 27.9	0.792
Insulin (μIU/mL)	5.7 ± 3.2	9.2 ± 7.5	0.187
IGF1 (ng/mL)	97.1 ± 33.1	85.0 ± 23.0	0.239
IGFBP1 (pg/mL)	441.4 ± 554.1	1043.0 ± 1974.2	0.806
IGFBP3 (ng/mL)	55.5 ± 27.0	45.0 ± 21.7	0.258
IGF1/IGFBP3 ratio	1.96 ± 0.86	2.18 ± 0.98	0.366

BMI，身體質量指數。SCA，脊髓小腦萎縮症。SARA，共濟失調評估量表。NfL，神經細絲輕鏈。8-OHdG，8-羥基-2-去氧鳥苷。 IGF1，類胰島素生長因子 1。IGFBPs，類胰島素生長因子結合蛋白。G/I ratio，葡萄糖與胰島素比值。統計顯著性設定為*P<0.05。所有數據均以中位數（四分位距）表示，性別以例數（百分比）表示。

第六節 服藥安全性監測

在 A 組與 B 組的黃耆治療三個月期間，無參與者於服用黃耆期間退出。在黃耆與安慰劑治療後，大部分血漿生化監測參數均未出現顯著變化（表 6）。雖部分數值有變化，但仍在正常範圍內：黃耆組中 Cr 顯著下降（P=0.028）、eGFR 顯著上升（P=0.016）；安慰劑組中白血球（WBC）顯著下降（P=0.004）。肝腎功能方面，兩組皆未見明顯異常。

表 6. 服用黃耆或安慰劑 12 週前後的安全性監測

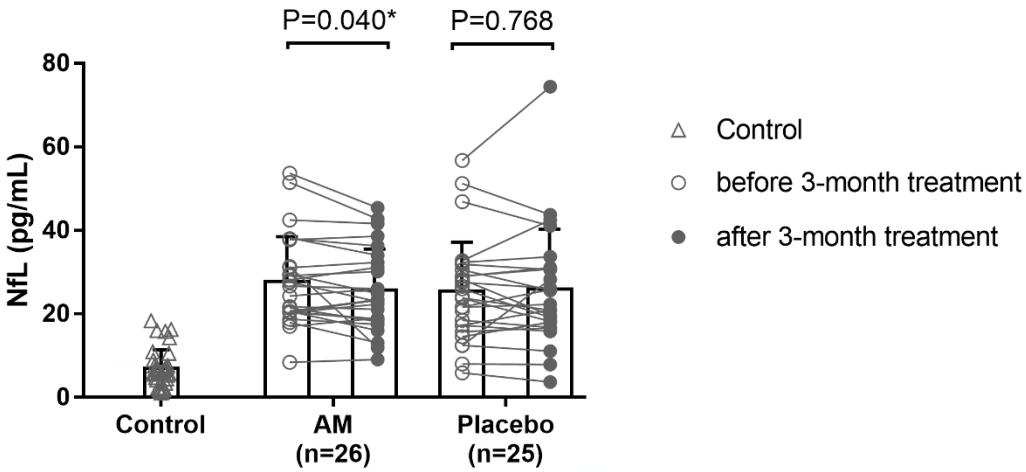
	AM (n=26)		Placebo (n=25)	
	mean ± SD	***P***	**mean ± SD**	***P***
WBC (10^3/μl)				
Before	7.10 ± 2.17	0.090	7.30 ± 1.69	**0.004***
After	6.36 ± 1.77		6.29 ± 1.16	
RBC (10^6/μl)				
Before	4.78 ± 0.49	0.197	4.72 ± 0.38	0.441
After	4.71 ± 0.42		4.74 ± 0.44	
Hemoglobin (g/dl)				
Before	14.32 ± 1.45	0.478	13.98 ± 1.67	0.090
After	14.22 ± 1.24		14.14 ± 1.85	
Platelet (10^3/ul)				
Before	274.1 ± 60.8	0.722	295.8 ± 121.5	0.602
After	276.4 ± 70.6		292.2 ± 98.6	
GOT (U/l)				
Before	22.6 ± 4.7	0.323	21.7 ± 5.6	0.366
After	21.8 ± 5.2		22.5 ± 4.5	
GPT (U/l)				
Before	23.6 ± 10.8	0.337	24.6 ± 17.2	0.726
After	22.3 ± 13.6		22.9 ± 10.2	
BUN (mg/dl)				
Before	14.4 ± 4.7	0.759	14.2 ± 4.1	0.174
After	14.3 ± 3.1		13.3 ± 3.3	
Creatinine (mg/dl)				
Before	0.74 ± 0.19	**0.028***	0.72 ± 0.17	0.445
After	0.69 ± 0.19		0.73 ± 0.18	
eGFR (ml/min/1.73m^2)				
Before	110.2 ± 24.7	**0.016***	114.3 ± 25.4	0.479
After	120.6 ± 29.5		112.2 ± 24.9	

WBC，白血球。RBC，紅血球。GOT，天門冬胺酸轉胺酶。GPT，麩胺酸丙酮酸轉胺酶。BUN，血中尿素氮。eGFR，估算腎絲球過濾率。統計顯著性設定為*P<0.05。

第七節 黃耆治療後的 NfL、8-OHdG 與 IIS

本試驗的主要指標—血漿 NfL—在黃耆治療後呈現下降，由 27.6±10.7 降至 25.6±9.8 pg/mL（*P*=0.040；圖 9A，n=26），大約減少 7%，而安慰劑組則無顯著改變（25.4±12.5 vs. 25.8±14.3 pg/mL，*P*=0.768）。在多元迴歸模式（multiple regression model）中調整潛在混雜因子（BMI 和 CAG）後，黃耆引起的 NfL 降低幅度仍高於安慰劑（standard error: 黃耆-2.372，BMI -0.0049，CAG -0.129），但組間差異未達統計顯著（*P*=0.127）。為避免缺失資料偏差，採 LOCF 分析後亦證實黃耆可帶來輕度但顯著的 NfL 降低（*P*=0.041），而安慰劑組無顯著變化（*P*=0.767）。

A



B

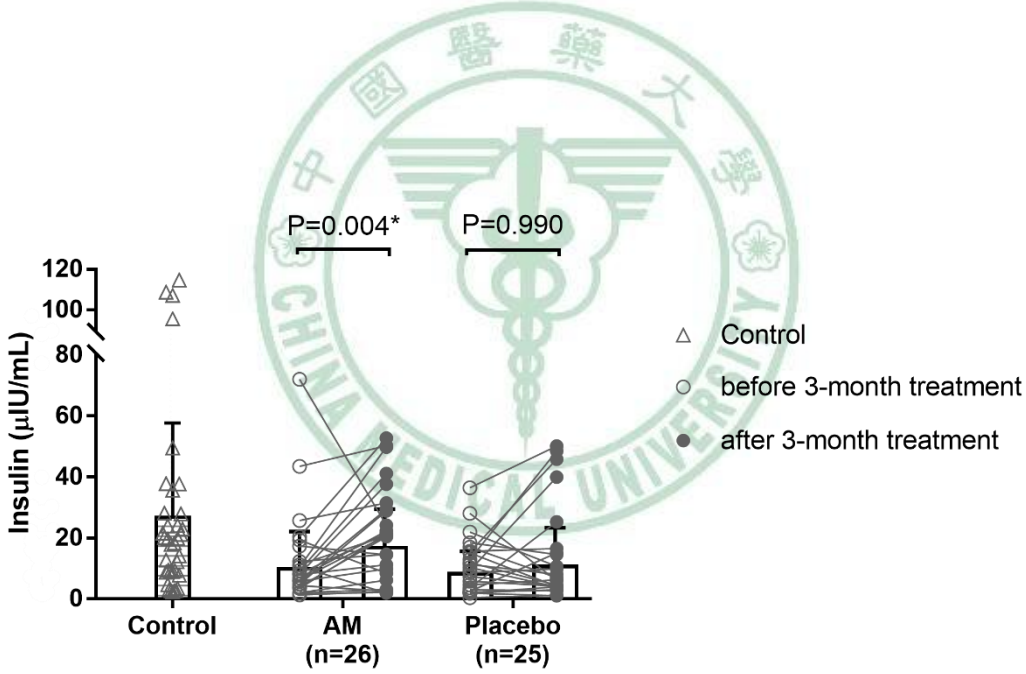


圖 9. NfL 與 insulin 在接受三個月黃耆 AM (A) 或安慰劑 (B) 介入前後的合併分析

在 insulin 與 glucose 代謝方面，兩組 glucose 水準均無改變，但黃耆治療後 insulin 濃度幾近倍增（12.5±15.2 → 21.1±15.5 μIU/mL，*P*=0.004；圖 9B），而安慰劑組則無顯著變化（10.7±9.0 vs. 13.6±15.6 μIU/mL，*P*=0.990）。NfL 降低幅度與 insulin 上升之間無顯著相關，但呈現正向趨勢（r=0.165，*P*=0.420）。其他生物標誌物，包括 8-OHdG、IGF1、IGFBP1、IGFBP3 及 IGF1/IGFBP3 ratio，在黃耆與安慰劑治療後皆無顯著變化（表 7）。

表 7. 黃耆或安慰劑治療 12 週前後的血漿生物指標與 IIS 變化

		AM (n=26)		Placebo (n=25)	
		mean ± SD	***P***	**mean ± SD**	***P***
NfL (pg/mL)					
	Before	27.6 ± 10.7	**0.040***	25.4 ± 12.5	0.768
	After	25.6 ± 9.8		25.8 ± 14.3	
8-OHdG (ng/mL)					
	Before	3.89 ± 1.88	0.770	3.87 ± 1.66	0.864
	After	3.78 ± 1.46		4.01 ± 1.86	
Glucose (mg/dL)					
	Before	103.5 ± 23.5	0.101	104.1 ± 23.0	0.696
	After	118.5 ± 39.0		103.6 ± 22.3	
Insulin (μIU/mL)					
	Before	12.5 ± 15.2	**0.004***	10.7 ± 9.0	0.990
	After	21.1 ± 15.5		13.6 ± 15.6	
IGF1 (ng/mL)					
	Before	95.2 ± 32.6	0.647	91.7 ± 29.6	0.412
	After	97.4 ± 30.3		95.3 ± 30.0	
IGFBP1 (pg/mL)					
	Before	406.5 ± 592.9	0.732	798.3 ± 1608.9	0.459
	After	350.9 ± 540.7		411.3 ± 520.7	
IGFBP3 (ng/mL)					
	Before	55.4 ± 26.6	0.849	51.1 ± 23.5	0.989
	After	54.8 ± 25.7		51.7 ± 24.3	
IGF1/IGFBP3 ratio					
	Before	1.93 ± 0.84	0.517	2.09 ± 1.03	0.326
	After	1.99 ± 0.81		2.18 ± 1.16	

IIS，胰島素/類胰島素生長因子 1 系統。NfL，神經細絲輕鏈。8-OHdG，8-羥基-2-去氧鳥苷。 IGF1，類胰島素生長因子 1。IGFBPs，類胰島素生長因子結合蛋白。統計顯著性設定為*P<0.05。

第八節 黃耆治療後 NfL、8-OHdG 與 IIS 的亞組分析

由於受試者病程嚴重度不同，黃耆的效果可能存在差異。依據 SARA 進展分級[24, 127]，將受試者分為：前臨床期（SARA<3，n= 2）、第一期（SARA≤ 11，n= 9）、第二期（SARA> 11，n= 15）（表 8）。前臨床期樣本數過少，因此僅呈現效應量（effect size）而非 *P* 值：NfL 降低 2.1 pg/mL（16%），胰島素上升 21.7 μU/mL（344%）。其他參數則呈現波動變化，未見一致趨勢。

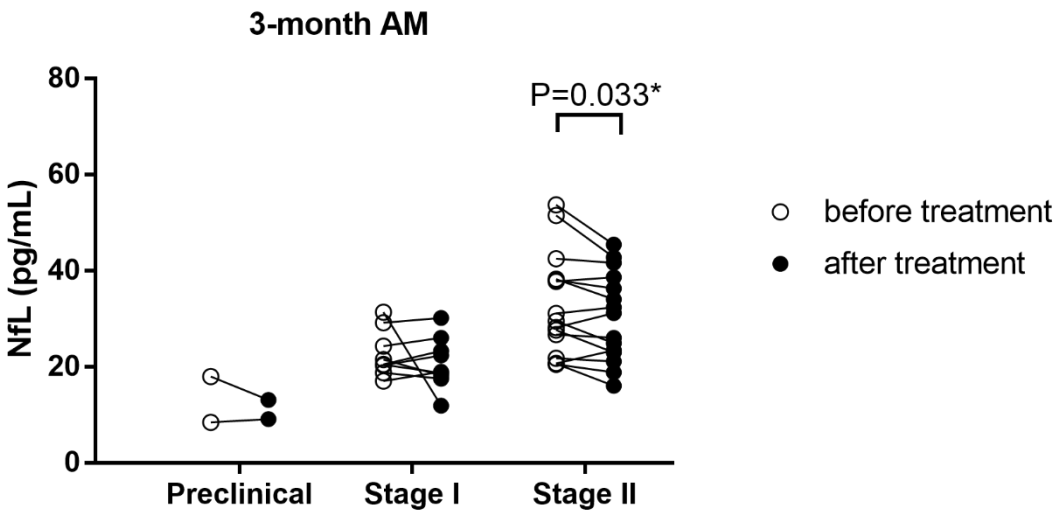
表 8. 黃耆組受試者治療 12 週前後血漿生物指標與 IIS 的亞組分析

	Preclinical (n=2)	Stage I (n=9)		Stage II (n=15)	
	mean ± SD	mean ± SD	*P*	mean ± SD	*P*
NfL (pg/mL)					
Before	13.2 ± 6.7	22.6 ± 4.8	0.458	32.6 ± 10.8	**0.033***
After	11.1 ± 2.9	20.8 ± 5.3		30.4 ± 9.3	
8-OHdG (ng/mL)					
Before	3.84 ± 0.87	3.97 ± 1.77	**0.028***	3.85 ± 2.12	0.334
After	3.88 ± 0.25	3.49 ± 1.45		3.93 ± 1.60	
Glucose (mg/dL)					
Before	94.6 ± 1.7	94.9 ± 16.2	0.117	111.4 ± 26.2	0.344
After	92.4 ± 1.1	107.2 ± 28.4		102.9 ± 19.8	
Insulin (μIU/mL)					
Before	6.3 ± 6.0	21.5 ± 22.9	0.594	8.0 ± 5.6	0.003*
After	28.0 ± 30.8	21.9 ± 14.5		19.8 ± 15.3	
IGF1 (ng/mL)					
Before	101.7 ± 21.7	91.6 ± 37.6	0.483	96.4 ± 31.7	0.571
After	115.0 ± 1.5	99.4 ± 33.8		93.8 ± 30.5	
IGFBP1 (pg/mL)					
Before	90.8 ± 24.5	353.2 ± 735.9	0.260	480.6 ± 544.3	0.865
After	113.8 ± 161.0	237.1 ± 509.1		450.8 ± 587.5	
IGFBP3 (ng/mL)					
Before	67.8 ± 40.4	51.1 ± 26.0	0.678	56.3 ± 27.0	0.820
After	74.4 ± 29.0	50.0 ± 23.7		55.2 ± 26.9	
IGF1/IGFBP3 ratio					
Before	1.99 ± 1.64	2.01 ± 0.94	0.199	1.87 ± 0.75	0.955
After	1.67 ± 0.63	2.29 ± 1.07		1.86 ± 0.63	

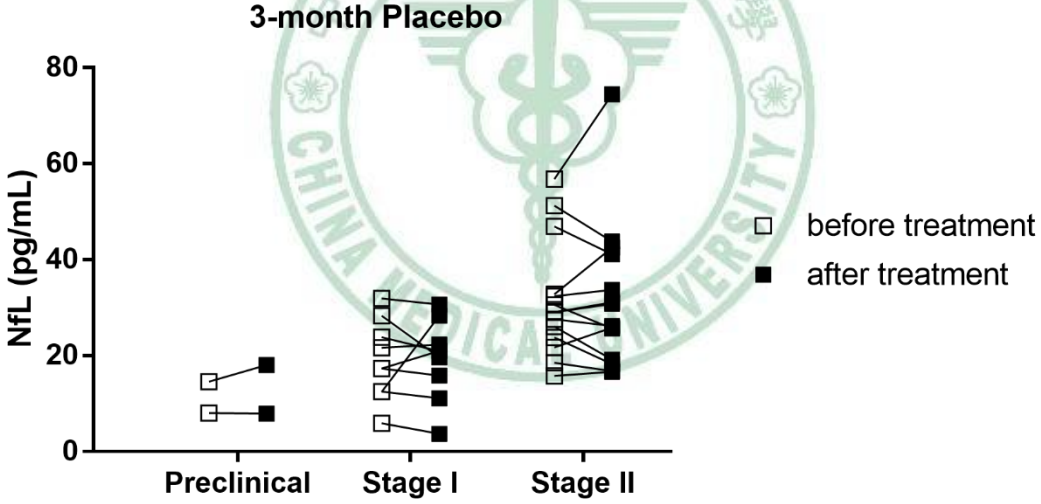
IIS，胰島素/類胰島素生長因子 1 系統。NfL，神經細絲輕鏈。8-OHdG，8-羥基-2-去氧鳥苷。IGF1，類胰島素生長因子 1。IGFBPs，類胰島素生長因子結合蛋白。由於臨床前期的樣本數極少（n=2），因此沒有進行檢定，僅以描述性數值呈現。統計顯著性設定為*P<0.05。

在 NfL 方面，第一期患者於黃耆治療後無變化；而第二期患者則顯著下降（32.6±10.8 vs. 30.4±9.3，*P*=0.033，大約減少 7%；圖 10A）。安慰劑組在不同期別均無變化（圖 10B）。glucose 水準在各期均維持穩定。insulin 方面，第二期患者在黃耆治療後顯著上升（8.0±5.6 → 19.8±15.3 μIU/mL，*P*=0.003；圖 10C），而安慰劑組無變化（圖 10D）。然而，NfL 與 insulin 變化間並無顯著相關，可能與 insulin 系統與中樞神經之間的複雜交互作用有關。氧化壓力方面，第二期患者的 8-OHdG 無差異，但第一期患者有輕度下降（3.97±1.77 → 3.49±1.45，*P*=0.028；表 8）。IGF1、IGFBPs 及 IGF1/IGFBP3 比值則均無變化。

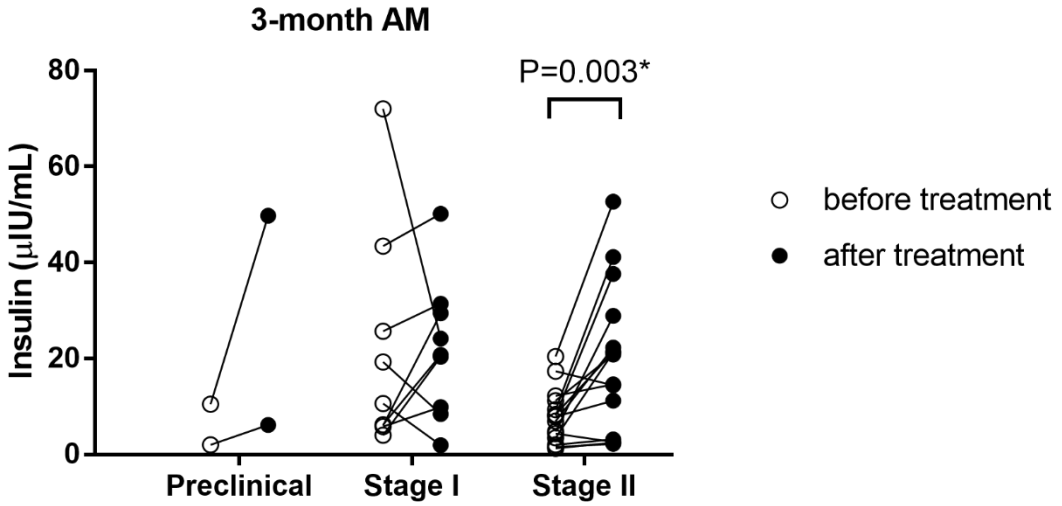
A


3-month AM
P=0.033*
NfL (pg/mL)
80
60
40
20
0
Preclinical
Stage I
Stage II
before treatment
after treatment

B


3-month Placebo
NfL (pg/mL)
80
60
40
20
0
Preclinical
Stage I
Stage II
before treatment
after treatment

C



D

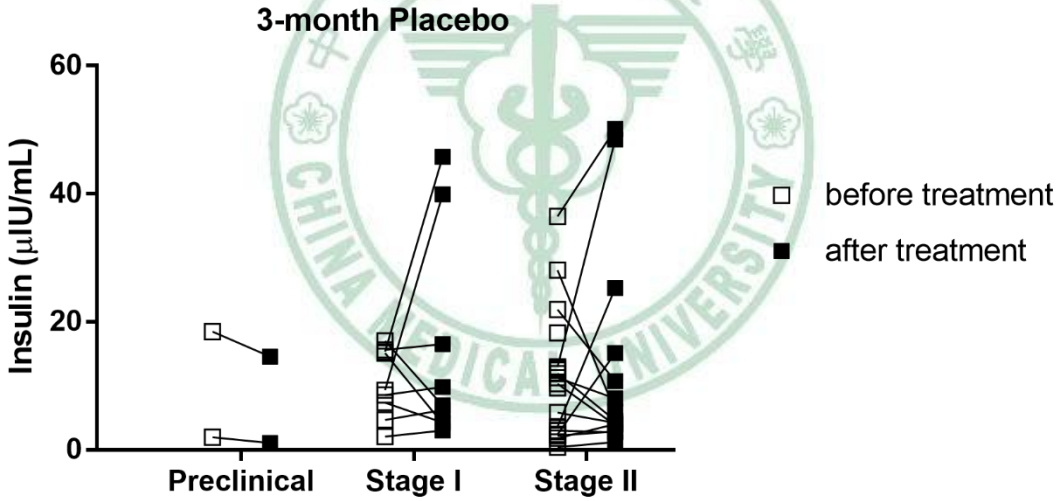


圖 10. NfL 與 insulin 在接受三個月黃耆 AM (A 與 C) 或安慰劑 (B 與 D) 介入前後的亞組分析

臨床前期（SARA< 3）有 2 名受試者，第一期（SARA≤ 11）有 9 名，第二期（SARA> 11）有 15 名

第九節 患者自述之正面效果與疑似副作用

除血漿中的生物指標外，本研究亦記錄了受試者自述的藥物反應與疑似副作用（表 9）。在正面效果方面，三位患者在服用黃耆後表示平衡感改善，兩位表示疲勞減輕；而在安慰劑組中，無人報告平衡改善，但有五人表示疲勞減輕。疑似副作用方面，黃耆組有四人報告口乾，三人報告失眠；安慰劑組則有兩人報告口乾，一人報告失眠。然而，黃耆與安慰劑兩組間比較皆無顯著差異（$P > 0.05$）（關於黃耆治療 SCA3 的臨床研究，詳見附錄 6））。

表 9. 服用黃耆或安慰劑的 SCA3 病人自述的正向反應與疑似副作用

	AM (n=26) n (%)	**Placebo (n=25) n (%)**	***P***
Patient-Reported Positive Effects			
Balance improvement	3 (11.5)	0 (0)	0.235
Fatigue improvement	2 (7.7)	5 (20.0)	0.248
Headache relief	2 (7.7)	2 (8.0)	1
Sleep improvement	1 (3.8)	3 (6.0)	0.350
Vision improvement	0 (0)	1 (4.0)	0.490
Smooth bowel	0 (0)	1 (4.0)	0.490
Appetite enhancement	0 (0)	1 (4.0)	0.490
Suspected Adverse Effects			
Dry mouth	4 (15.4)	2 (8.0)	0.668
Insomnia	3 (11.5)	1 (4.0)	0.610
Abdominal dullness	1 (3.8)	0 (0)	1
Constipation	1 (3.8)	0 (0)	1
Muscle rigidity	1 (3.8)	1 (4.0)	1
Numbness	0 (0)	2 (4.0)	0.235

AM，黃耆。*P* 值為比較 AM 與安慰劑組的差異。統計顯著性設定為*$P<0.05$。

第五章 討論

目前，對於 SCA3 患者仍缺乏確立的治療基準，凸顯了急需有效的症狀治療或疾病修飾療法（DMT）。利用血漿 NfL 濃度監測治療前後的變化，提供了一種兼具時間與成本效率的評估方式。在本研究的組內比較，黃耆組在治療後觀察到邊緣（borderline）顯著的 NfL 降低，並伴隨明顯的胰島素上升，然而兩者的變化沒有相關性，且組間比較 NfL 的變化並未達顯著。對一個長達 20 年自然病程的罕見疾病，此交叉設計試驗得到下降約 7%的 NfL 生物學訊號（biological signaling），仍是一個有潛力的假說生成（hypothosis-generating）指標，值得未來更長期試驗。

亞組分析顯示，第二期（後期）患者對黃耆的反應較為顯著，但可能受限於分層後的樣本數不足，因此只適合作為探索性發現。本研究首次在 SCA3 臨床治療中使用 NfL 作為評估指標，也是首次採用雙盲隨機交叉設計，並應用中藥於 SCA3 的試驗。當黃耆使血漿 insulin 回升至接近正常值時[59]，亦觀察到 SCA3 軸突損傷的減輕（大約 7%），而此數值變化量呈現的生物訊號，並未代表確切的臨床意義。我們假設，黃耆可能透過調節 insulin 訊號或其他途徑，而非 IGF1 途徑，來減少神經退化。然而 NfL 與 insulin 並沒有直接相關性，因此這些初步結果僅能作為一個，值得在更大規模的臨床試驗中驗證探索性的早期發現。

第一節 insulin 系統

中樞與周邊 insulin 在調控代謝及神經過程中具有關鍵作用。小鼠研究顯示，周邊 insulin 上升可調節下視丘基因轉錄[128]。相反地，DM 小鼠的 insulin 下降與腦內膽固醇合成基因表現下降相關，影響髓鞘形成與神經膜功能（neuronal membrane function）[129]。與其他神經退行性疾病不同，SCA3 患者多數表現為血糖正常但相對低的胰島素血症（hypoinsulinemia）[59, 130]。周邊 insulin 下降可能導致腦內 insulin 不足[131]，並可能因 polyQ 存在而惡化[132]。較低的 BMI 與升高的 insulin 敏感性，可能反映了對 insulin 不足的代償[59, 133]。黃耆介入後，SCA3 患者的周邊 insulin 上升，接近一般人族群。雖然 insulin 上升與 NfL 降低間呈正向趨勢，但未達統計顯著，可能反映中樞—周邊間交互作用的複雜性。

聚焦 IIS 的合理性來自於黃耆與 IIS 相關的多重證據。以往研究顯示，APS 在第一型 DM 低胰島素（hypoinsulinemia）模型中可保護胰島 β 細胞超微結構並減少胰島炎症[16]。APS 亦能降低 insulin 阻抗、改善 glucose 恆定，甚至可能藉由修正代謝功能來降低阿茲海默症的風險[134]。AST 則可能影響中樞 insulin 調控，在大鼠精神分裂模型中可促進脈絡叢（choroid plexus）血清素釋放，進而增強腦內 insulin 訊號[135, 136]。另有探索性研究顯示，在橄欖-小腦退化（olivo-cerebellar degeneration）的大鼠模型中，黃耆增加血清與小腦的 IGF1 表現並支持下橄欖（inferior olive）神經元存活[87]。在臨床上，黃耆被廣泛應用於華人社區之第 2 型 DM，動物與臨床研究均顯示其能改善高血糖、降低 insulin 阻抗並提升 insulin 敏感性[137]。綜合來看，黃耆在 SCA3 的神經保護作用，可能部分透過周邊及中樞的 IIS 調節。

第二節 NfL 降低與其他可能機轉

本研究發現黃耆治療後血漿 NfL 輕度下降，尤其是在第二期患者。由於 NfL 反映軸突損傷，此結果提示黃耆可能具神經保護作用。雖然我們原始假設聚焦於 IIS 的調控，但 IGF1 與 IGFBPs 並未顯著變化，因此 NfL 的降低可能涉及其他或互補性途徑。以往研究顯示，APS 可透過啟動 Klotho-Nrf2 抗氧化途徑，提高 HO-1、NQO1 與 TrxR1 表現，減少活性氧物質（reactive oxygen species，ROS）導致的神經傷害[138]。AST 則展現強效抗發炎與抗氧化作用，在阿茲海默模型中可抑制小膠質細胞（microglial）活化、NADPH 氧化酶表現，並降低 ROS 與促發炎細胞激素（TNF-α、IL-1β、IL-6），減輕神經炎症與損傷[139]。此外，黃耆萃取物可逆轉澱粉樣蛋白（amyloid）誘發的記憶缺失，並促進軸突再生與突觸重建[140]。

本研究顯示第一期患者的血漿 8-OHdG（氧化壓力指標）顯著下降，提示黃耆可能於早期 SCA3 發揮抗氧化作用，不過第一期的病人數目不足（n= 9），僅能作為探索性發現。一些動物或細胞研究亦顯示黃耆及其成分能減少氧化性 DNA 損傷。例如，AST 可降低腎臟損傷並下調 8-OHdG[141]，APS 可在高糖環境下保護心肌細胞，減少粒線體損傷與凋亡，並降低 8-OHdG[142, 143]。這些證據支持黃耆可能緩解 SCA3 的氧化壓力相關神經退化，但其詳細機制仍待進一步研究。

第三節 IGF1、IGF2 與相關 IGFBPs

在 DM 患者中，高胰島素血症（insulinemia）或 insulin 補充可促進肝臟 IGF1 生成[143]。然而，在本研究中，黃耆雖提升了 insulin，但並未使 SCA3 患者的總 IGF1 或 free-form IGF1 上升，也未改善主觀疲勞。同樣地，作為 SCA3 潛在生物指標的 IGFBP1 並未因黃耆而顯著變化[59]。既往研究曾指出，黃耆或含黃耆配方可促進 IGF1 或 IGFBP3 訊號，增進兒童的肌肉修復與生長[85, 144]；APS 亦可在 HepG2 細胞中改善 insulin 阻抗，提升 IGF1/IGF1R 表現，並活化 STAT5 與 AKT[75]。在我們的受試者中，free-form IGF1 起初偏高，可能反映對 insulin 不足的代償[59, 130]。黃耆治療後 IGF1 未再上升，或許是因患者基線 IGF1 已在正常範圍，顯示黃耆在此神經退化病中不太可能直接提升 IGF1。整體而言，黃耆調控 insulin 與 IGF1 的精確機制，仍待後續研究驗證。

本研究首次發現 SCA3 患者的周邊 IGF2 水平比健康受試者高。雖然 IGF2 並非主要研究標的，且其濃度與小腦共濟失調嚴重度（ataxia severity）無直接相關，但此現象可能反映了機體針對 insulin 信號受損或代謝壓力的一種補償性反應[145]。參考其他神經退化疾病模型，IGF2 被認為具有神經保護潛力[68, 146]。因此，SCA3 患者體內 IGF2 增加，可能是一種試圖抵消代謝失衡或蛋白質穩態失調的適應性機制，值得未來進一步探討。

第四節 IGFBP1 作為 SCA3 代謝-神經退化指標的潛力

本研究證實 SCA3 患者血漿 IGFBP1 顯著升高，且與 SARA（臨床嚴重度）、NfL（神經退化指標）及 BMI 相關。這顯示 IGFBP1 可反映疾病活動度而非僅是遺傳載荷（genetic load），提供優於 CAG 重複序列的另一種指標。相較於 insulin、IGF1 或 IGF2，IGFBP1 區分疾病前後期的效果更好，具備作為分期生物指標的潛力。

SCA3 患者呈現正常血糖（normoglycemic）的低胰島素血症（hypoinsulinemia）[59]。研究發現 insulin 最低者，其 IGFBP1 與 NfL 濃度最高（附錄 4），暗示週邊 insulin 不足可能加劇中樞神經損傷。患者的 G/I ratio 顯著升高，反映的是 insulin 分泌功能受損而非 insulin 阻抗，且此特徵在病程中保持穩定，不隨前後期改變。IGFBP1 透過高親和力結合 IGF1，會降低其生物活性[147]。在低 insulin、禁食或代謝壓力下，肝臟會增加 IGFBP1 合成[148]。由於 IGFBP1 可穿過血腦障壁[149]，外周 IGFBP1 水平升高可能導致中樞 IGF1 信號減弱[150]，進而損害 SCA3 患者的神經保護機制（如髓鞘修復）[151, 152]。此外，發炎因子（如 IL-6）與晚期消瘦（低 BMI）也會誘導 IGFBP1 上調[153-155]。因此，IGFBP1 不僅反映代謝壓力，更可能透過抑制 IGF1 信號加劇神經退化[113]。

第五節 握力作為 SCA3 評估的新工具

目前的 SCA3 病程評估主要依賴 SARA 量表、NfL 及 BMI。本研究首次證實，握力 HGS 或 HGS/BMI 比值亦能獨立預測 SCA3 的疾病嚴重度（含 SARA 所有子項目）。較強的握力通常對應較高的 BMI 與較低的血漿 NfL 水平。然而在黃耆服藥三個月的研究中，握力並未顯示有顯著變化（未列出數據），可能短期黃耆治療並未足以改變功能性指標。

在臨床上，握力是一項極易取得且非侵入性的指標，即便考量了遺傳因素（CAG 重複序列數），HGS 仍能有效估算患者的疾病狀態。雖然握力受性別、年齡、慣用手及營養狀況影響[156]，且 SCA3 患者的握力顯著低於健康對照組，但本研究在校正上述因素並排除關節炎、中風、癌症等共病干擾後[119]，握力與疾病嚴重度仍呈現顯著負相關。這顯示握力是監測 SCA3 臨床表現的實用工具。

動物實驗顯示，135Q 與 84Q 型 SCA3 小鼠在疾病晚期皆出現明顯握力下降[157]。即便校正體重因素，其握力仍顯著弱於正常小鼠。研究發現，SCA3 小鼠的多處肌肉（如股四頭肌、腓腸肌）重量比值下降，且肌纖維橫截面積縮小[158]。這與 Akt/FoxO 萎縮信號通路及肌球蛋白重鏈（myosin heavy chain，MyHC）表現減少有關[159, 160]，證實了 SCA3 具有原發性肌肉病變或肌少症的特徵[158]。

肌肉無力是 SCA3 患者的常見特徵。臨床上，患者的肌肉力量與體重均低於健康對照組[155, 161]。這可能與多重因素相關：能量代謝- 運動過度（Hyperkinesia）導致能量消耗增加；營養攝取- 吞嚥困難（dysphagia）導致營養不足[155]。美國國立衛生研究院肌肉減少症計畫基金會研究建議，BMI 與握力可作為評估老人臨床進展的重要指標[162]。綜上所述，握力 HGS 或 HGS/BMI 比值反映了 SCA3 神經系統的嚴重度，更是篩查患者是否併發肌少症的非侵入性實用工具。

第六節 安全性與副作用

常規生化檢查顯示黃耆在血球計數及腎、肝功能上均屬安全。服用安慰劑組出現白血球輕度下降，但仍在正常範圍，可能屬隨機變異。黃耆組則出現輕度 Cr 下降，可能反映腎臟保護效應，值得進一步探討[82]。黃耆的耐受性良好，僅少數報告口乾或失眠，且發生率與安慰劑組相當，因果性不明。疲勞改善在兩組皆有少數個案發生，顯示安慰劑效應的可能性。

既往臨床試驗中，黃耆或 APS 注射僅有輕微副作用，如濕疹、發燒、頭痛與便秘，發生率不高於安慰劑，且均為短暫可逆[163, 164]。毒理研究亦支持黃耆具廣泛安全範圍：大鼠每日口服 150 mg/kg，連續 91 天無毒性；單次口服高達 5,000 mg/kg 無死亡；大鼠與犬的亞慢性研究顯示，即便劑量達人類等效劑量的 70 倍與 35 倍，仍無明顯毒性[108, 165]。本研究採用單一固定劑量，主要考量安全性與可行性；未來需在更大規模臨床中探討劑量反應關係。整體而言，黃耆耐受性良好，安全性與安慰劑相當。

第七節 本研究與既往 SCA3 臨床研究比較

我們的結果置於既往 SCA3 藥物試驗的脈絡下進行比較：口服海藻糖的期中分析顯示 SARA 分數無改善，部分原因在於招募不足與檢驗力不足，突顯短期臨床試驗難以觀察療效[44]。開放標籤（open-label）IGF1 的皮下注射研究中，報告 12 個月內小腦性共濟失調程度穩定，但因缺乏安慰劑對照，無法確立因果[14]。迄今所有藥物仍屬探索性，僅物理復健與神經調控（如 TMS 與 tDCS）等非藥物策略，被證實可暫時延緩疾病進展，且這些研究多未納入血漿生物指標[7]。相較之下，我們的隨機雙盲交叉試驗提供了短期生物指標改變的探索性證據，後續長期追蹤有助於判斷是否能轉化為持久的臨床效益。

第八節 研究限制

本研究預期招募 44 人，然而只招募到 32 人，因此可能導致本研究結果的統計檢定力受限，造成 NfL 在組間比較未達顯著。本研究退出率為 28%，主要與共病或交通問題有關；然而，在兩組黃耆治療的三個月期間，無人因服用黃耆而退出。完成者與未完成者的基線特徵相當，顯示偏倚風險（attrition bias）低。為降低缺失資料影響，我們使用 LOCF 分析，並驗證黃耆對 NfL 降低的效果。未來可考慮多重插補（multiple imputation）以加強結果穩健性。疾病分期的亞組分析樣本不足，特別是前臨床組（n=2）與第一期組（n=9），因此應視為探索性。多元迴歸分析顯示，黃耆對 NfL 的降低雖未顯著優於安慰劑，但其 *P* 值低於其他混雜因子（如 CAG 與 BMI），提示潛在訊號值得驗證。本研究介入期僅三個月，可能不足以觀察 SARA 分數的變化[23]。此外，黃耆降低 NfL 的詳細機制，包括 insulin 或其他途徑的交互作用，仍不明確。未來研究若能採用黃耆的純化成分（如 APS 或 AST），可能比粗提物（crude extract）更具針對性地增強 IIS。另外在握力方向，評估肌電圖、神經傳導檢查、甚至肌肉切片，是未來研究 SCA3 握力退化機轉的可能方案。在尋找生物指標部分，長期縱向（longitudinal）追蹤 SCA3 病人的 IGFBP1 或握力變化，也更能確認其作為可靠評估病情進展的指標。

第六章 結論

本項研究為隨機、雙盲、交叉的先導試驗，首次評估黃耆對 SCA3 患者，以血漿 NfL 作為療效指標，結果顯示黃耆治療有可靠的安全性，僅造成組內邊緣顯著的 NfL 降低與 insulin 上升，且兩者變化並無相關性，提示其為探索性發現，可能減緩 SCA3 患者的神經退化。鑒於效果量小且統計僅達顯著邊緣，仍需更大規模，長期研究以確認療效並釐清其機制。

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附錄

附錄 1. 彰化基督教醫院人體試驗委員會核准函 (IRB No.200703)

彰化基督教醫院
CHANGHUA CHRISTIAN HOSPITAL

同意臨床試驗證明書

Clinical Trials Approval Certificate(New Protocol)

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計畫中文名稱： 探討黃耆治療小腦萎縮症病人 IGF-1 之臨床研究- 前驅研究
計畫主持人： 劉青山 / 協同主持人：林廉証、邱重閎
試驗機構名稱： 彰化基督教醫療財團法人彰化基督教醫院
本會編號：200703
核准日(審查通過日)： 西元 2020 年 10 月 06 日
核准臨床試驗期間： 西元 2020 年 10 月 06 日 至 西元 2021 年 10 月 05 日止

計畫書： Version 2.0，2020-09-15
受試者同意書： Version 2.0，2020-09-15
問卷： 1. SARA 量表：Version 1.0，2020-06-04
2. 修訂版疲勞影響程度量表：Version 1.0，2020-06-04
3. Epworth 嗜睡量表：Version 1.0，2020-06-04
4. 中醫體質問卷：Version 1.0，2020-06-04
資料及安全性監測計畫書： Version 2.0，2020-09-15
其他： 個案資料收集表 Version 2.0，2020-09-15

未預期事件或藥品嚴重不良反應通報、後續定期追蹤之程序及應注意事項，請參閱背面。

彰化基督教醫院 人體試驗委員會 Institutional Review Board Changhua Christian Hospital

彰化基督教醫院

Protocol Title: Radix Astragali for Elevating IGF-1 of Patients with Spinocerebellar Ataxia - Pilot Study
Principal Investigator(s): Chin San LIU / Co Investigator：Lin, Lien-Cheng、Chiu, Chung-Min
Institution: CHANGHUA CHRISTIAN HOSPITAL
CCH IRB No. : 200703
Date of Approval: Oct 06, 2020
Duration of Approval: from Oct 06, 2020 to Oct 05, 2021

Protocol: Version 2.0, Sep 15, 2020
Informed Consent: Version 2.0, Sep 15, 2020
Questionnaire: 1. SARA 量表：Version 1.0, Jun 04, 2020
2. Modified Fatigue Impact Scale：Version 1.0, Jun 04, 2020
3. Epworth 嗜睡量表：Version 1.0, Jun 04, 2020
4. 中醫體質問卷：Version 1.0, Jun 04, 2020
Data and Safety Monitoring Plan (DSMP)： Version 2.0, Sep 15, 2020
Other: 個案資料收集表 Version 2.0, Sep 15, 2020

See the back of this page for the procedures for reporting unanticipated problems, or drug serious adverse reactions, or interim, and other important notes.

彰化基督教醫院
第一人體試驗委員會
主任委員： 蘇式立

Sincerely Yours
ShihLi Su, Ph.D.
Chairman
Institutional Review Board Committee A
Changhua Christian Hospital, Taiwan

彰化基督教醫院 人體試驗委員會 Institutional Review Board Changhua Christian Hospital

Lin Chang 2020 Oct 06
(signature, date)

本會組織與執行皆符合 ICH-GCP
The Institutional Review Board performs its functions according to written
Operating procedures and complies with ICH-GCP and with the applicable regulations.

附錄 2. 彰化基督教醫院人體試驗委員會核准函 (IRB No.200730)

彰化基督教醫院

變更案同意臨床試驗證明書
Clinical Trials Approval Certificate(Amendments)

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計畫中文名稱：探討罕見遺傳神經疾病其基因，生物指標，臨床及影像之相關分析
計畫主持人： 劉青山 / 協同主持人：陳彥廷、李佳儒、張明裕、張通銘、蔡世峯
試驗機構名稱： 彰化基督教醫療財團法人彰化基督教醫院
本會編號：200730
核准日(審查通過日)： 西元 2023 年 03 月 08 日
核准臨床試驗期間： 西元 2023 年 03 月 08 日 至 西元 2023 年 09 月 06 日止

計畫書： 第九版，2023-01-18
受試者同意書： 1. 第 12 版，2023-02-24
2. 兒童版 第 10 版，2023-02-24
其他： 1. 次世代基因定序 (NGS)研究發現之告知方式說明表 第一版，2023-02-24
2. 次世代基因定序 (NGS)研究結果之告知說明暨同意書 第一版，2023-02-24
新增協同主持人：蔡世峯

未預期事件或藥品嚴重不良反應通報、後續定期追蹤之程序及應注意事項，請參閱背面。

Protocol Title: Explore the correlation analysis of genes, biomarkers, clinical and imaging of rare inherited neurodegenerative diseases
Principal Investigator(s): Chin San LIU / Co Investigator：Yan-Ting Chen、Chia-Ju Lee、MingYuh Chang、TungMing Chang、Shih-Feng Tsai
Institution: CHANGHUA CHRISTIAN HOSPITAL
CCH IRB No. : 200730
Date of Approval: Mar 08, 2023
Duration of Approval: from Mar 08, 2023 to Sep 06, 2023

Protocol: Version 9, Jan 18, 2023
Informed Consent Form: 1. Version 12, Feb 24, 2023
2. 兒童版 Version 10, Feb 24, 2023
Other: 1. 次世代基因定序 (NGS)研究發現之告知方式說明表 Version 1, Feb 24, 2023
2. 次世代基因定序(NGS)研究結果之告知說明暨同意書 Version 1, Feb 24, 2023
Add Co-Investigator: Shih-Feng Tsai

See the back of this page for the procedures for reporting unanticipated problems, or drug serious adverse reactions, or interim, and other important notes.

彰化基督教醫院
第一人體試驗委員會
主任委員： 蘇矢立

Sincerely Yours
ShihLi Su, Ph.D.
Chairman
Institutional Review Board Committee A
Changhua Christian Hospital, Taiwan

Shih-Li Su 2023. Mar. 08
(signature, date)

本會組織與執行皆符合 ICH-GCP
The Institutional Review Board performs its functions according to written Operating procedures and complies with ICH-GCP and with the applicable regulations.

附錄 3. 未服藥記錄表

未服藥記錄表

1. 若您因忘記或任何因素沒有服藥，請在下表勾選並填寫。

2. 若不確定什麼狀況是否須停藥，請聯絡計劃主持人邱重閔醫師(0982506067)。

3. 請於下次回診時**把剩藥帶回，與此表單**一同繳回**中醫門診**。

未服藥日期	哪一包藥	未服藥原因
月　日	□早 □晚	□忘記 □其他:_____
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月　日	□早 □晚	□忘記 □其他:_____

附錄 4. 已接受的期刊文章 IGFBP1 as a metabolic-neurodegenerative biomarker in spinocerebellar ataxia type 3

SPANDIDOS PUBLICATIONS EXPERIMENTAL AND THERAPEUTIC MEDICINE 31: 105, 2026

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

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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 μIU/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.

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

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.

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 IGFI/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 χ^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
Demographic			
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^2	23.6 (21.8-25.6)	21.8 (19.5-25.4)	0.040[a]
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[a]
Insulin/IGF1 system			
IGF1, ng/ml	55.2 (34.0-68.1)	92.6 (67.9-108.6)	<0.001[a]
IGF2, ng/ml	312.8 (271.7-361.4)	336.2 (316.2-395.0)	0.002[a]
IGFBP1, pg/ml	3.5 (3.5-41.5)	252.9 (72.8-740.4)	<0.001[a]
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[a]
Glucose, mg/dl	95.9 (73.6-116.5)	98.9 (81.2-112.7)	0.708
Insulin, μIU/ml	19.0 (8.8-28.1)	6.1 (2.6-11.0)	<0.001[a]
G/I ratio	6.0 (3.3-9.3)	17.1 (8.6-35.0)	<0.001[a]

Data are presented as median (interquartile range), with the exception of sex which is n (%). Sex was analyzed using the χ^2 test, whereas all other continuous variables were analyzed using the Mann-Whitney U test; [a]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/$\sqrt{2}$ (≈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 μIU/ml; T2: 3.65-9.35 μIU/ml; T3: >9.35 μIU/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^2; 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≤11)	Stage II (SARA>11)	P-value
Demographics			
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^2	25.4 (22.1-26.9)	21.2 (18.8-22.9)	0.005[a]
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[a]
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[a]
NfL, pg/ml	18.8 (17.0-28.3)	29.5 (25.1-38.1)	0.002[a]
Insulin/IGF1 system			
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[a]
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, μIU/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; [a]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) μIU/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 ≤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^2; 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 yielded, outperforming other IIS parameters (AUC range for other parameters, 0.385-0.541) (Fig. 1; Table SII).

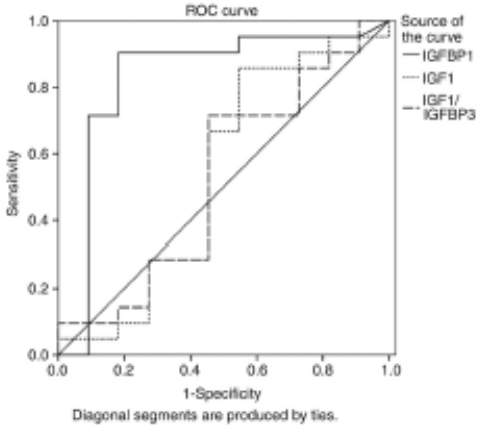


Figure 1. ROC curve analysis of IGFBP1 and other insulin/IGF1 signaling-associated markers for distinguishing early- and advanced-stage spinocerebellar ataxia type 3. ROC, receiver operating characteristic; IGF, insulin-like growth factor; IGFBP, IGF binding protein.

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

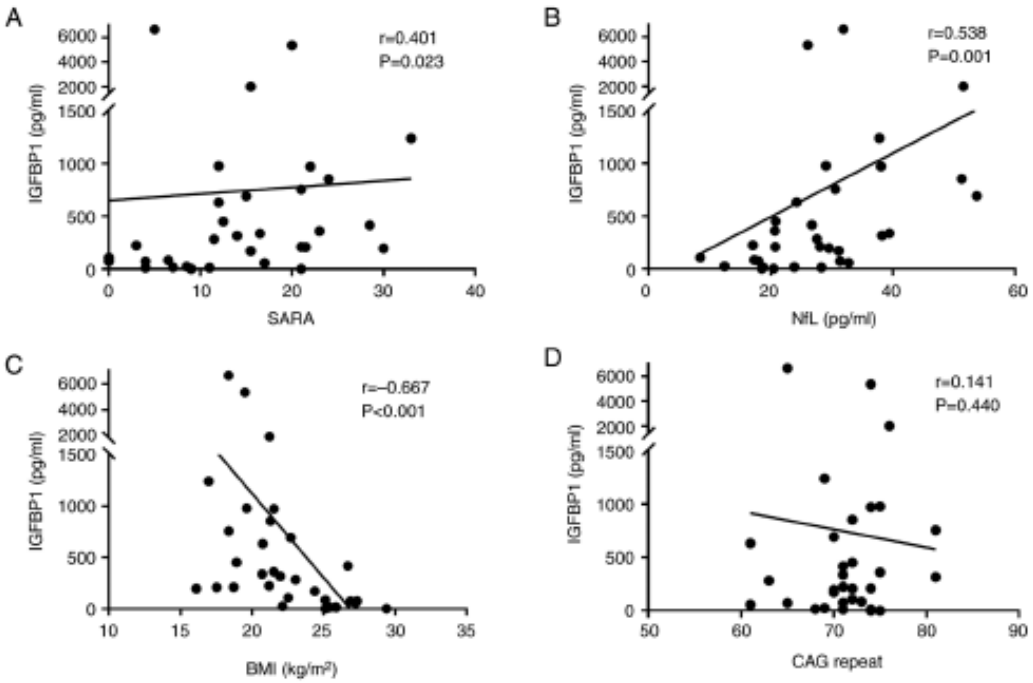


Figure 2. Correlation between plasma IGFBP1 and clinical parameters. Spearman's correlation analyses between IGFBP1 and (A) scale for the assessment and rating of ataxia, (B) neurofilament light chain, (C) body mass index and (D) CAG repeat length in patients with spinocerebellar ataxia type 3. IGFBP1,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

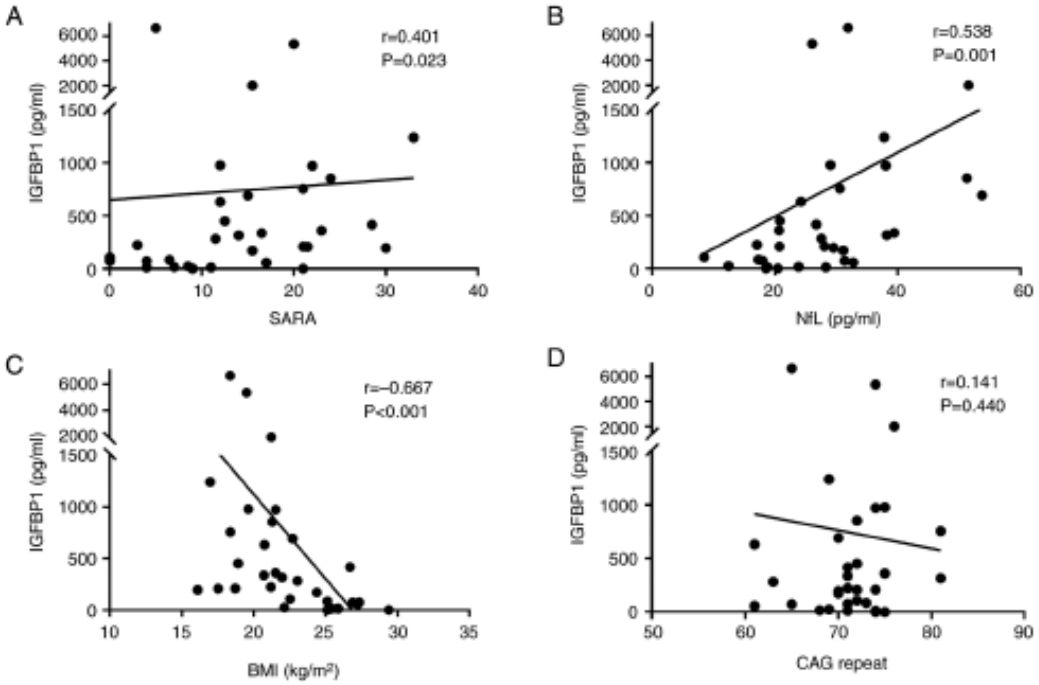
positively correlated with SARA scores (r=0.401; P=0.023) and NfL (r=0.538; P=0.001), and negatively correlated with BMI (r=-0.667; P<0.001). No significant association with CAG repeat length was observed (r=0.141; P=0.440) (Fig. 2). Although several higher IGFBP1 values were present, all measurements 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 β-cell dysfunction or altered insulin kinetics, the present results



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

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.

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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.

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附錄 5 已接受的期刊文章 A pilot study: handgrip as a predictor in the disease progression of SCA3

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A pilot study: handgrip as a predictor in the disease progression of SCA3

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Abstract

Background Spinocerebellar ataxia type 3 (SCA3) is an inherited, autosomal, and rare neurodegenerative disease. Serum/plasma biomarkers or functional magnetic resonance imaging used to assess progression, except for neurological examinations, is either inconvenient or expensive. Handgrip strength (HGS) may be considered as a biomarker to predict the progress of SCA3 and align with the alteration of plasma neurofilament light chain (NfL) and Scale for the Assessment and Rating of Ataxia (SARA).

Methods Patients with SCA3 and healthy subjects were recruited from Changhua Christian Hospital. SARA, body mass index (BMI), and NfL were obtained for both groups. HGS was measured using a Jamar Plus + hand dynamometer.

Results This study recruited 31 patients and 36 controls. HGS in the SCA3 group revealed a profound decrease ($P<0.001$) compared with normal subjects. HGS also had a negative correlation with SARA ($r=-0.548$, $P=0.001$), NfL ($r=-0.359$, $P=0.048$), and a positive correlation with BMI ($r=0.680$, $P<0.001$). Moreover, HGS/BMI ratio correlated with SARA ($r=-0.441$, $P=0.013$). Controlling for gender and age, HGS still correlated with the above clinical items. The initial hypothesis was also proved in SCA3 84Q transgenic mice, showing grip strength weakness compared to normal mice.

Conclusions HGS can be an alternative tool to assess the clinical severity of SCA3. Further research is needed to investigate the underlying mechanisms.

Keywords Spinocerebellar ataxia type 3, Handgrip strength, Neurofilament light chain, Scale for the assessment and rating of ataxia, Body mass index

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Background

Spinocerebellar ataxia (SCA) is an autosomal inherited disease and one of the rare neurodegenerative diseases. SCA type 3 (SCA3), also known as Machado-Joseph disease, caused by abnormal CAG trinucleotide expansion greater than 62 at the gene of *ataxin-3*, is the most frequent SCA in most countries [1]. Common symptoms include imbalance, incoordination, muscle rigidity or spasticity, dysarthria, diplopia, and depression [2]. Scientists have been searching for disease-modifying therapies in recent years, such as varenicline, valproic acid, or Trehalose, because SCA3 is challenging to treat [3]. SCA3 is a slowly progressive neurodegenerative disease; however, monitoring the change of clinical status by neurological scales in a short period would be challenging [4].

Physicians are on the lookout for assessment tools that are both accessible and efficient when it comes to neurological status assessment for ataxia. The Scale for the Assessment and Rating of Ataxia (SARA) is a clinical scale dedicated to detecting disease progression of cerebellar symptoms [5]. Morphometric magnetic resonance imaging (MRI) offers vital insights into structural changes, such as volumetric reduction in cerebellum, basal ganglia, brain stem, and diencephalon. These are instrumental in confirming and enhancing our understanding of this disease [6]. Functional MRI has revealed cerebellar/cortical dissociation pattern specific to SCA3 patients, as opposed to healthy controls. This finding shed light on the evaluation of potential functional connectivity within cerebral-cerebellar motor networks [7]. Neurofilament light chain (NfL) is an emerging blood biomarker to evaluate neuroaxonal damage such as Alzheimer's disease, multiple sclerosis, postoperative delirium, multiple system atrophy, and amyotrophic lateral sclerosis [8–10]. NfL also correlates with the disease severity of SCA3 and predicts its pre-ataxic and ataxic stages [11, 12]. Urinal polyglutamine ataxin-3 protein is elevated in patients with SCA3 [13]. while polyglutamine ataxin-3 in plasma or peripheral blood mononuclear cells correlates with disease severity [14, 15]. However, fMRI and the above biomarkers, except for SARA, are either expensive or inconvenient. Developing an easy and affordable assessment tool is highly anticipated and much needed for clinical use.

Handgrip strength (HGS), which is measured using a portable hand-held dynamometer, has been a practical tool to assess the well-being of the elderly. HGS could predict longitudinal declines in cognition, functional status, and mortality in older community populations [16]. The United Kingdom Biobank data reported that higher dementia incidence and mortality were independently associated with lower grip strength [17]. Grip strength could also assess muscle activity, which predicts functional loss in patients with Parkinson's disease, where striatal dopamine and impaired motor unit degeneration are recruited [18]. Proper grip strength cutoff points are good predictors of functional independence and wheelchair skills for males with spinal cord injuries [19]. HGS might relate functional capacity or motor performance to cerebellar disorders in a small pilot study, including three patients with degenerative cerebellar ataxia, without mentioning the genetic background [20]. For animal models related to neurodegeneration diseases, HGS can be identified as a physiologic biomarker to forecast the disease course [21–23]. In a transgenic marmoset model of SCA3 diseases, age-associated decrease of HGS were significantly more prominent in transgenic symptomatic marmosets compared to either wide-type or asymptomatic marmosets [24]. However, the relationship between HGS and the clinical status of patients with SCA3 remains unknown.

Herein, we aim to establish the validity of HGS inspection as a predictor of the disease progress in patients and mice with SCA3, and its application in ataxia clinic for the disease following.

Result

Demographic data

This study enrolled 31 participants with SCA3 and 36 healthy controls. No significant difference was found in age or gender between the two groups (age: $P=0.517$, gender: $P=0.988$), as shown in detail in Table 1. The BMI of participants in the SCA3 group was lower than that of the control group (21.5 kg/m^2 [19.5–25.5] vs. 23.6 kg/m^2 [21.8–25.6], $P=0.043$), consistent with the previous studies [25, 26]. The SCA3 group exhibited weaker HGS (24.3 kg [19.2–38.2] vs. 40.7 kg [27.1–49.3], $P<0.001$, Fig. 1A) and lower HGS/BMI ratio compared to the control group (1.19 kg/(kg/m^2) [0.95–1.55] vs. 1.38 kg/(kg/m^2) [1.11–1.79], $P<0.001$). A decrease in HGS was observed in either SCA3 female (20.10 kg [18.60–25.10] vs. 26.80 kg [25.45–30.4], $P=0.007$) or SCA3 male (35.10 kg [23.30–38.33] vs. 47.20 kg [42.35–55.25], $P<0.001$) patients compared with normal subjects in Fig. 1B. Additionally, a notable difference was observed in the significantly elevated plasma level of NfL in patients with SCA3 as compared to that in normal subjects (28.01 pg/mL [20.65–32.75] vs. 6.35 pg/mL [4.22–7.63], $P<0.001$, Table 1). An elevated NfL level was found to predict the severity of SARA within the SCA3 group ($r=0.436$, $P=0.014$; see Additional file 1), consistent with previous reports [11, 12].

Correlation between HGS and clinical items

The high strength of HGS can predict low scoring on SARA ($r=-0.548$, $P=0.001$, Fig. 2A) and NfL

Table 1 Demographic data for patients with SCA3 and control subjects

	Control	SCA3	*P* value
Case number	36	31	
Male (%)	22 (61.1%)	19 (61.3%)	0.988
Age (years)	47.0 (32.0–53.5)	49.0 (40.0–55.0)	0.517
BMI (kg/m^2)	23.6 (21.8–25.6)	21.5 (19.5–25.5)	0.043*
Handgrip strength (kg)	40.7(27.1–49.3)	24.3 (19.2–38.2)	< 0.001*
Handgrip strength (kg)/BMI (kg/m^2)	1.38 (1.11–1.79)	1.19 (0.95–1.55)	< 0.001*
Disease status			
The age of onset (years old)	N/A	36 (32–45)	
Duration (years)	N/A	10 (5–12)	
CAG repeat count	N/A	71.0 (69.0–74.0)	
SARA	N/A	15.0 (8.5–21.0)	
Plasma			
NfL (pg/mL)	6.35 (4.22–7.63)	28.01 (20.65–32.75)	< 0.001*

Values are median with interquartile range

The intergroup comparison for gender was analyzed using the chi-square test, while the Mann–Whitney U test was used for other variables

BMI Body mass index, *SARA* Scale for the assessment of rating of ataxia, *NfL* Neurofilament light chain, *N/A* Not applicable, *$P < 0.05$

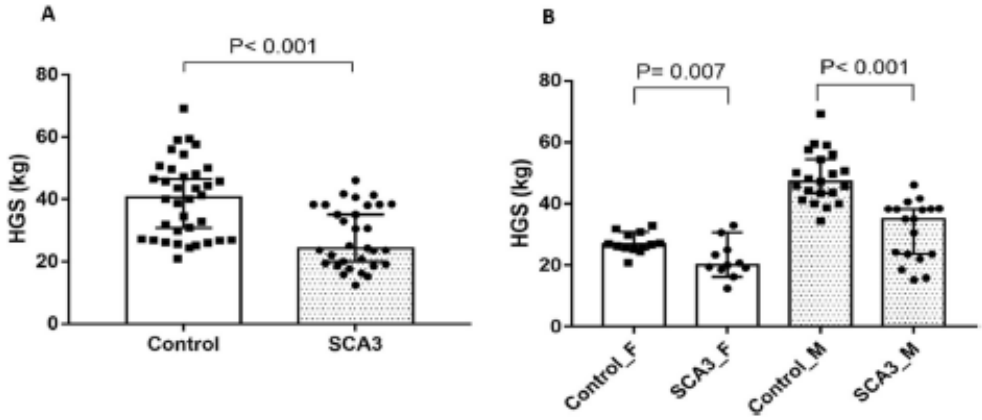

Fig. 1 Handgrip strength of patients with SCA3 and healthy controls. Values are given as median with 95% coefficient interval (CI) for comparison of both groups (**A**) and both genders (**B**). *P*: by Mann–Whitney U test

($r = -0.359$, $P = 0.048$, Fig. 2B) in SCA3 patients. Additionally, greater HGS was associated with lower scores in each subscale of SARA, including gait ($r = -0.525$, $P = 0.002$), stance ($r = -0.487$, $P = 0.005$), sitting ($r = -0.636$, $P < 0.001$), speech disturbance ($r = -0.522$, $P = 0.003$), finger chase ($r = -0.510$, $P = 0.003$), finger-nose test ($r = -0.355$, $P = 0.050$), fast alternating hand movements ($r = -0.505$, $P = 0.004$), and heel-shin slide ($r = -0.403$, $P = 0.025$), as shown in Table 2.

In the SCA3 patient group, HGS was effectively indicative of BMI ($r = 0.680$, $P < 0.001$, Fig. 2C). BMI has been demonstrated as a disease progression predictor in SCA3 [25, 26], and our study revealed a significant negative correlation with SARA ($r = -0.420$, $P = 0.019$; see Additional file 1). BMI has been correlated with HGS in most studies [27, 28]; thus, the HGS/BMI ratio was calculated as a new variable after adjusting HGS for BMI. The HGS/BMI ratio still exhibited a negative correlation with SARA scores ($r = -0.441$, $P = 0.013$, Fig. 2D). Even after considering gender and age as controlling factors, HGS continued to illustrate a consistent relationship with SARA, NfL, and BMI. Moreover, lower HGS can predict SCA3 patients with a higher CAG repeat number under adjusting for age ($r = -0.396$, $P = 0.030$; see Additional file 2).

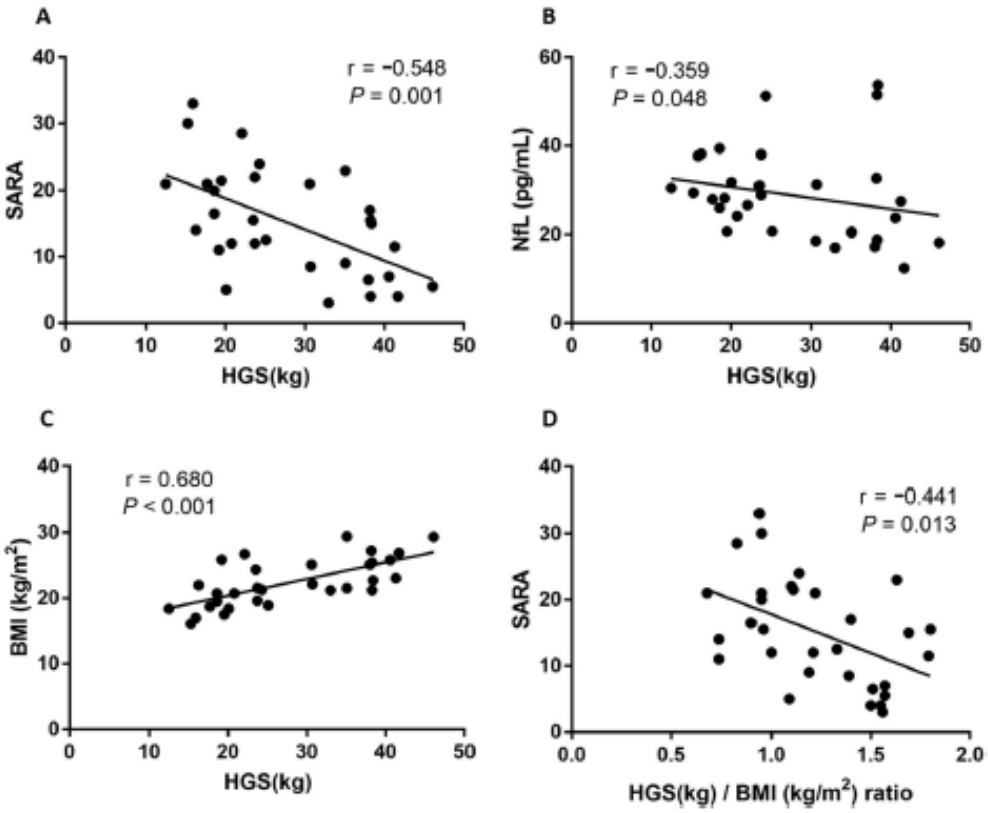

Fig. 2 Correlation between HGS and SARA, NfL, and BMI (as shown in **A**–**C**). Correlation between HGS/BMI ratio and SARA (**D**). r: correlation coefficient, *P*: by Spearman rank test

Table 2 Correlation between HGS and eight subscales of SARA

Variable	Correlation coefficient	*P* value
HGS		
Gait	−0.525	0.002
Stance	−0.487	0.005
Sitting	−0.636	< 0.001
Speech disturbance	−0.522	0.003
Finger chase	−0.510	0.003
Finger-nose test	−0.355	0.050
Fast alternating hand movements	−0.505	0.004
Heel-shin slide	−0.403	0.025

Spearman rank test was used for correlation

HGS Handgrip strength, *SARA* Scale for the assessment and rating of ataxia

Grip strength in SCA3 mice

The grip strength of SCA3 84Q transgenic mice was significantly decreased at the age of 18 months than that of WT mice ($P=0.003$, Fig. 3A). After correcting for body weight, the grip strength of SCA3 mice remained lower when compared to that of the WT mice($P=0.007$, Fig. 3B).

Discussion

The current assessment of disease progression in SCA3 involves neurological examinations, such as SARA, for evaluating ataxia. NfL [12] and BMI [26] have established themselves as reliable predictors of SCA3, and our research uncovered a robust relationship between HGS and SARA, even after adjusting for gender and age. The increased strength of HGS can predict higher BMI and lower plasma levels of NfL. Additionally, a higher HGS/BMI ratio indicated lower scores in the SARA evaluation. Moreover, our study demonstrated that patients with SCA3 exhibited significantly lower HGS compared to healthy controls. This study represents the first instance where HGS or the HGS/BMI ratio has independently predicted the disease severity of SCA3, including all eight subscores of SARA. HGS can serve as an easily accessible

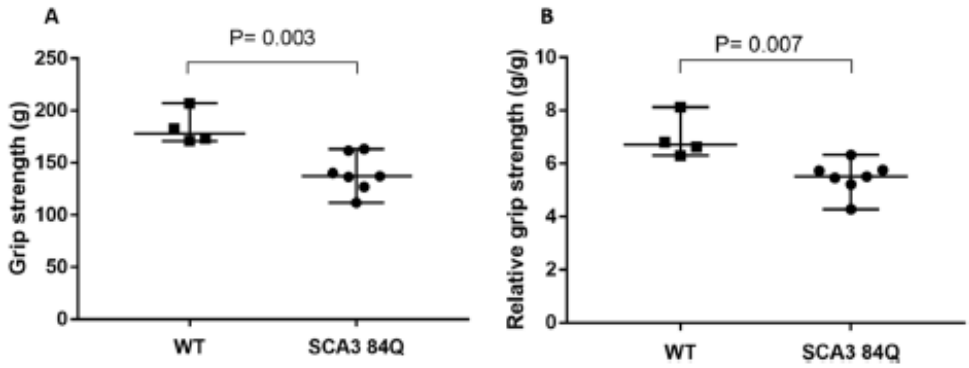

Fig. 3 Four-limb grip strength (**A**) and relative grip strength (**B**) in micewith WTand transgenic SCA384Q mice. Data expressed as median with 95% CI (WT, n = 4; SCA3 84Q, n = 7). *P*: by Mann–Whitney U test

tool to estimate the disease status of SCA3 in a clinical setting, even when considering the gene loading of SCA3-CAG repeat expansion.

HGS is a widely used measure of muscular strength, but various factors can influence its accuracy. HGS in patients with SCA3 may relate to myopathy, neuropathy or other comorbidity. Our study revealed a significant inverse correlation between HGS and disease severity, even after controlling for common factors. Various conditions, including arthritis, tendinitis, and major vascular or neurological disorders, can affect grip strength. Other factors, such as sex, age, handedness, and nutritional status, also impact HGS [29]. Participants with severe comorbidities, such as stroke, cancer, heart failure, or kidney failure, were excluded, and the absence of arthritis or tendinitis was screened to ensure the accuracy of our study. Handedness was determined according to the standard protocol for using a hand dynamometer [30], while sex and age were considered during statistical analysis.

Accordingly, recent literature revealed a report investigating grip strength in transgenic SCA3 135Q mice without mentioning the pathogenesis that contributes to the reduction of grip strength [31]. In the present study, we found a similar result in SCA3 84Q mice with weak grip strength compared with WT mice during the late disease stage. To account for the potential confounding effect of decreased body weight in SCA3 84Q mice at 18 months, we adjusted for body weight and still detected weaker grip strength in SCA3 mice compared to WT mice. Our previous studies have also demonstrated a decrease in the ratios of muscle mass and body weight in the quadriceps, gastrocnemius, tibialis, extensor digitorum longus, and soleus muscles compared to WT mice. The cross-sectional area of muscle fibers was found to be reduced in SCA3 84Q mice [32]. Atrophic signaling involving Akt/Forkhead box-O and myosin heavy chain (MyHC) expressions were implicated in these findings, indicating the existence of sarcopenia or muscle disease [33, 34]. Specifically, our study revealed a significant decrease in phosphorylated AKT and the muscle cell differentiation marker, MyHC, in SCA3 84Q mice compared to WT mice which indicated the possible muscular pathogenesis involved in the disease of SCA3 [32].

Muscle weakness is a common feature in patients with SCA3, and recent studies indicate that myopathic origin may contribute to distal muscle weakness in these individuals [35]. Further, evidence indicates a potential association between SCA3 and sarcopenia, as patients with SCA3 display lower muscle strength and lean mass than healthy controls [36]. Therefore, the muscle atrophy pathway may be related to the lower HGS observed in patients with SCA3 who have sarcopenia. Our study revealed that sarcopenia-related BMI and HGS could provide valuable insights into the clinical progression of patients with SCA3. A declining BMI signifies deterioration in the condition of patients with SCA3 and may indicate the possible occurrence of comorbidity with sarcopenia. Another report suggests that hyperkinesia may lead to increased energy expenditure, while dysphagia results in decreased nutrition intake, both potentially contributing to a body composition resembling sarcopenia [36]. The Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project also recommends grip strength measurements, such as HGS and HGS/BMI ratio, as non-Dual-energy X-ray absorptiometry approaches to identify sarcopenic patients among the elderly [37]. Our study revealed that HGS not only helps assess the clinical severity of

SCA3 but is also significantly related to BMI. HGS can be regarded as a valuable tool for evaluating the progression of SCA3 patients with sarcopenia.

Therefore, further investigation into the possible mechanism of decreased HGS, including peripheral neuropathy, such as electromyography, nerve conduction studies, or even muscle biopsy, should be considered as the next step. In the future, we will conduct long-term longitudinal observations to examine the relationship between HGS and disease progression and expand our sample size to include more SCA3 patients and normal subjects.

Conclusions

This is the first report on HGS in patients with SCA3. HGS exhibited an inverse correlation with the neurological status, namely SARA and Nfl., and demonstrated a strong correlation with BMI, which is another predictor of disease progression. The increased HGS/BMI ratio consistently showed a relationship with SARA, providing valuable insights into disease severity. Combined with the grip strength information obtained from SCA3 84Q mice in the present study, HGS can serve as a practical and accessible tool to evaluate the clinical status of SCA3.

Methods

Patient recruitment

Subjects diagnosed with SCA3 by genetic mutational screening were recruited at Changhua Christian Hospital (CCH), Taiwan. The inclusion criteria for patients were a SARA score of ≥ 3 points [38]. Informed consent was collected from May 2021 to January 2022 (CCH-IRB approval No:200703). Participants were aged from 20 to 80 years. Exclusion criteria were pregnancy, comorbidity with stroke, cancer, heart failure, or renal failure. Patients with < 3 Points on the SARA scale were excluded. Demographic data such as age, age of disease onset, disease duration, CAG trinucleotide repeat number, and body mass index (BMI) were recorded. Age- and sex-matched individuals with negative genetic screening for mutant *ataxin-3* were recruited as healthy controls from the normal population of staff in CCH (CCH-IRB approval No:200730).

HGS

A single physician measured the HGS with a Jamar Plus+hand dynamometer (Sammons Preston Rolyan, Bolingbrook, IL, USA). Participants were asked to sit on a straight back chair, keep their elbow flexed at 90 degrees, and hold the dynamometer with handle position 2, which is the distance of 4.8 cm from the handle to the fixed part of the dynamometer [30]. Participants had to apply maximal power for 3 s by the dominant hand. Grip strength was measured three times, with rest for 15 s between each effort [39]. The handgrip strength was the maximal value of the three measurements.

SARA

A single experienced neurologist measured SARA for the SCA3 group. SARA is a universal scale to assess the severity of ataxia. SARA has eight subscores, including gait, stance, sitting, speech disturbance, finger chase, nose-finger test, fast alternating hand movements, and heel-shin slide [40]. The total scores ranged from 0 to 40, with higher points indicating worse cerebellar conditions.

Plasma NfL

Blood was collected in BD EDTA tubes and then centrifuged at 2500 × g for 10 min at 4 °C to obtain plasma for each participant. Plasma samples were diluted at a ratio of 1:4. The single-molecule (Simoa) array technology by an ultra-high sensitivity protein molecular detection instrument (Simoa HD-X, Quanterix, MA, USA) and the Simoa NfL Advantage kit (Quanterix, MA, USA) measured the plasma NfL level [41]. All NfL values were within the linear ranges of the assays. The average intraassay coefficient of variation was 4.94%.

Animal model and grip strength

C57BL/6 wild-type (WT) mice were obtained from the National Laboratory Animal Center (Taipei, Taiwan), and SCA3 84Q transgenic mice (C57BL/6 background) have been previously described [42]. The Institutional Animal Care and Use Committee of Changhua Christian Hospital approved all animal experiments (approval number: CCH-AE-108-021). The genotype of SCA3 84Q mice was confirmed through the polymerase chain reaction of a DNA sample obtained from the mouse tail (primer sequences for forward: 5′-TGGCCTTTCACA TGGATGTGAA-3′, reverse: 5′-CCAGTGACTACT TTGATTCG-3′). The 430-bp molecule refers to the positive of SCA3 84Q. Mice received standard diets and were housed under a 12-h light/dark cycle in a temperature-controlled room. The body weights of the mice were recorded weekly to monitor their health status. Grip strength tests were performed only on 18-month-old mice. A Digital Force Gauge (Model DPS-5R: range of 0–5 kgf, Imada, Japan) was used to measure the mice's four-limb grip strength. The mouse was placed on a metal grid, and its tail was gently pulled back in parallel, and the apparatus automatically recorded the peak force when the mouse released its grip. The maximal force (grams) was represented as four-limb grip strength, while relative grip strength was normalized to the weight of the mouse. Each mouse underwent a grip strength test three times at 1-min intervals [43].

Statistics

Continuous variables were presented as the medians and interquartile ranges (25th–75th percentile), whereas the categorical variables were presented as numbers and percentages because most of the continuous variables did not follow a normal distribution. The Mann–Whitney U test was used to assess the difference in continuous variables between the healthy and diseased populations, while the chi-square test was employed for categorical variables. Additionally, the Spearman rank test was used to determine the strength of the relationship between the two variables. The partial correlation was used to adjust for age and gender. All data were analyzed using IBM Statistical Package for the Social Sciences for Windows, Version 22.0 (IBM Corp., Armonk, NY). A P value of < 0.05 was considered statistically significant.

Abbreviations

SCA	Spinocerebellar ataxia
SCA3	SCA type 3
SARA	Scale for the assessment and rating of ataxia
MRI	Magnetic resonance imaging
NfL	Neurofilament light chain
HGS	Handgrip strength
CCH	Changhua Christian Hospital
BMI	Body mass index
WT	Wild-type
MyHC	Myosin heavy chain

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13023-023-02948-3.

Additional file 1: Fig. S1. C orrelation between NfL and SARA in the SCA3 group. Plasma NfL positively correlated with SARA. **Fig. S2.** Correlation between BMI and SARA in the SCA3 group. BMI negatively correlated with SARA.

Additional file2 : Table 1. Correlation between HGS and A ge, Gender, SARA, NfL, BMI and CAG repeat count.

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Author contributions

CMC, CSL and HHC conceptualised and contributed to the study design. WLC, TTL and HJC execution for patient recruitment and performed the experiments. CMC, WLC and YSL contributed to the data analysis, interpretation and wrote the manuscript. CMC and YJC contributed to the data analysis of statistics. CMC and CJL verified the underlying data. All authors contributed to the article and editing of the final version of the manuscript. All authors read and approved by the final manuscript.

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Availability of data and materials

We understand the importance of providing a data availability statement as part of our research manuscript. While we acknowledge the significance of transparency and reproducibility, due to privacy and confidentiality concerns, we are unable to publicly disclose the data used in this study. We assure you that the data supporting our findings will be made available to interested researchers upon reasonable request, subject to ethical and legal considerations. For inquiries regarding access to the data, please contact Chin-San Liu (liu48111@gmail.com) and Chung-Min Chiu (shongdiah@gmail.com). We are committed to promoting scientific integrity and collaboration while maintaining the confidentiality of sensitive information.

Declarations

Ethics approval and consent to participate

All procedures of this study were approved by the Independent Ethics Committee of the Changhua Christian Hospital (CCH-IRB approval No:200703 and No:200730). In addition, written informed consent was obtained from all participants prior to study inclusion.

Consent for publication

All the patients included signed the consent for publication.

Competing interests

The authors declare that they have no competing interests.

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Astragalus membranaceus therapy in patients with spinocerebellar ataxia type 3

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ABSTRACT

Background and aim: Spinocerebellar ataxia type 3 (SCA3) is the most common form of hereditary cerebellar ataxia. The insulin/insulin-like growth factor 1 (IGF1) system (IIS) has been proposed as a potential target for disease-modifying therapy. *Astragulus membranaceus* (AM) may modulate the IIS pathway and reduce neurodegeneration, as indicated by plasma neurofilament light chain (Nfl.), a biomarker of disease progression.
Experimental procedure: A randomized, triple-blind, placebo-controlled crossover trial was conducted in 32 patients with SCA3. Participants received AM or placebo for three months, followed by a one-month washout, then crossed over to the alternate treatment. Plasma Nfl. and IIS-related markers were measured. Twenty-three participants completed the trial.
Results and conclusion: Intention-to-treat analysis revealed a modest within-treatment reduction in Nfl. levels (27.6 ± 10.7 vs. 25.6 ± 9.8 pg/mL, $P = 0.040$) and increased insulin (12.5 ± 15.2 vs. 21.1 ± 15.5 μIU/mL, $P = 0.004$) after AM. However, the adjusted regression model showed no significant between-treatment difference in Nfl. ($P = 0.127$), and changes in Nfl. were not correlated with insulin. No significant changes were observed in IGF1 or IGF-binding proteins. Exploratory subgroup analyses suggested larger biomarker changes in later-stage patients. No carryover effects or demographic differences were noted. Overall, AM administration was associated with modest within-treatment biomarker changes in Nfl. and insulin, although the adjusted between-treatment difference in Nfl. was not statistically significant. These findings should be interpreted cautiously as preliminary and hypothesis-generating observations rather than confirmatory evidence of therapeutic efficacy.

Abbreviations

AD	Alzheimer's disease	GH	growth hormone
AM	*Astragalus membranaceus*	GOT	glutamic oxaloacetic transaminase

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APS	astragalus polysaccharides	GPT	glutamic pyruvic transaminase
AST	astragaloside IV	IGF1	insulin-like growth factor 1
BMI	body mass index	IGFBP	IGF binding protein
BUN	blood urea nitrogen	ITT	intention-to-treat

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CA	cerebellar ataxia	IIS	Insulin/insulin-like growth factor 1 system
CBC/DC	complete blood count with differential count	Nfl	neurofilament light chain
CCH	Changhua Christian Hospital	PolyQ	polyglutamine
Cr	creatinine	SARA	scale for assessment and rating of ataxia
DCS	direct current stimulation	SCA	spinocerebellar ataxia
DM	diabetes mellitus	SCA3	spinocerebellar ataxia type 3
DMT	disease-modifying therapy	TMS	transcranial magnetic stimulation
eGFR	estimated glomerular filtration rate	WBC	white blood cell
LOCF	last observation carried forward	8-OHdG	8-hydroxy-2-deoxyguanosine

1. Introduction

Spinocerebellar ataxias (SCAs) are a group of dominantly inherited neurodegenerative diseases. Among them, Spinocerebellar Ataxia Type 3 (SCA3), also known as Machado-Joseph Disease, is the most common subtype globally and particularly prevalent in Asia.[1] The genetic mutation on chromosome 14q32.1 causing an abnormal expansion of CAG repeats and leading to toxic polyglutamine (polyQ) protein aggregation in the nervous system, ultimately causing neuronal cell loss in the brainstem, cerebellum, midbrain, spinal cord, striatum, and thalamus.[2] These aggregates disrupt protein homeostasis, impairing cellular clearance mechanisms and triggering apoptosis, leading to progressive motor deficits and neurodegeneration.

Clinically, SCA3 manifest as progressive gait imbalance, dizziness, dysarthria, general fatigue, diplopia, dysphagia, pyramidal or extrapyramidal signs.[1] Given the challenges and ethical dilemmas associated with disease-specific gene therapy,[3] current management of SCA3 leans towards disease-modifying therapies (DMT) aimed at slowing disease progression.[4] Non-pharmacological interventions such as intensive rehabilitation or coordination training, as well as emerging techniques like transcranial magnetic stimulation (TMS) and direct current stimulation (DCS), are among the novel intervention strategies.[5,6] Recent advancements in clinical pharmaceutical trials in phases II or III with agents such as trehalose, Troriluzole, human mesenchymal stem cells derived from the umbilical cord or adipose tissues.[7] These studies have all provided prospects for the treatment of the disease.

1.1. The therapeutic target niche for SCA3 within the insulin/insulin-like growth factor 1 system

In the brain, the insulin/insulin-like growth factor 1 (IGF1) system (IIS) supports neuronal survival and function, playing a crucial role in cognitive processes and neuroprotection. Insulin and IGF1 activate signaling pathways that promote neuronal growth and repair, enhancing synaptic plasticity and facilitating memory formation. Their anti-apoptotic and anti-inflammatory effects mitigate neuronal damage and protect against neurodegeneration, while ensuring energy supply to neurons and maintaining optimal brain function.[8,9] Peripheral insulin and IGF1 penetrate the blood-brain barrier via saturable transport systems and are further facilitated in crossing brain vascular endothelial cells.[10,11] Although central insulin levels in SCA3 patients are unknown, a study in 2011, indicated that peripheral insulin levels are lower in SCA3 patients compared to healthy individuals.[12] Nasal insulin spray, glucagon-like peptide-1 analogue, or PPARγ agonist, has been attempted in Alzheimer's disease (AD) to promote insulin signaling in the brain.[13] In the growth hormone (GH) -IGF1 axis, SCA3 84Q transgenic mice treated with GH showed preserved Purkinje cells and reduced ataxin-3 aggregation, while 9-month IGF1 treatment improved locomotor function and minimized cerebellar degeneration, potentially by modulating mitochondrial function, oxidative stress, and autophagy.[14,15] However, whether modulation of the IIS pathway translates into meaningful therapeutic effects in human SCA3 remains unclear. Moreover, long-term stimulation of the somatotropic axis may increase the risk of cancer. Therefore, more moderate approaches to influencing IIS activity may be of interest, and extracts from commonly used herbal medicines could be considered as potential candidates for DMT in SCA3.

1.2. Astragalus membranaceus as a possible IIS inducer

Chen et al. have identified several Chinese herbal medicines that target the ubiquitin proteasome pathway for SCA3.[16] However, rigorous and randomized research designs are scarce. In Asia, *Astragalus membranaceus* (AM, 黃耆Huang Qi) is widely used for tonifying qi, glycemic homeostasis, anti-fatigue, and anti-aging. Its main components include polysaccharides, saponins, and flavonoids. The pharmacological effects of Astragalus polysaccharides (APS) include immune modulation, anti-tumor activity, anti-apoptotic effects, and prevention of abnormal polyQ aggregation.[17] APS enhances insulin sensitivity through AKT activation and IGF1 pathway modulation, and it also promotes the proliferation and insulin secretion of pancreatic β cells.[18] Astragaloside IV (AST), one of its most important saponins, exhibits even remarkably antioxidant, anti-inflammatory, and neuroprotective.[19] AST protects against diabetes mellitus (DM) by triggering a decline in blood glucose levels and a rise in plasma insulin levels,[20] via regulating gut microbiota, AMPK/SIRT1 and PI3K/AKT signaling pathways.[21] In our previous study, the use of a herbal formula mainly composed of AM has been shown to assist in improving the quality of life of a SCA patient.[22] As mentioned above, AM has been reported to influence components of the IIS; nonetheless, whether this manifest as measurable neuroprotective effects in humans remains unclear.

1.3. Neurofilament light chain as outcome biomarker in SCA3

Cerebrospinal neurofilament light chain (Nfl) reflects neural axonal damage, with plasma levels correlating with those in the cerebrospinal fluid. Plasma Nfl demonstrates stability, sensitivity, reliability, and predictive value in tracking the course of neurodegenerative diseases such as multiple sclerosis, AD, amyotrophic lateral sclerosis, spinal cord injury, and ataxia-telangiectasia. High-sensitivity assays further indicate that plasma Nfl can capture disease-related changes within weeks to a few months,[23] making it a suitable biomarker for short-term monitoring. These features support its application as a dynamic outcome measure in clinical trials, particularly when aiming to minimize participant burden, making this approach both justifiable and feasible.[23] Nfl for assessing SCA3 has emerged as a novel approach in recent years.[24] In transgenic mice, alterations in plasma Nfl concentration precede even those of mutant ataxin-3.[25] The present study will not assess traditional neurological examinations, such as scale for assessment and rating of ataxia (SARA), because changes in SARA for SCA3 patients within a short timeframe are not anticipated. The mean annual progression in SARA was 1.56 points out of a total score of 40 (SE 0.08), and up to 202 patients would be needed to detect a 50% reduction in progression of the SARA in a one-year trial.[26]

Hence, we will conduct a three-month clinical trial using a randomized triple-blind placebo-controlled crossover design in patients of SCA3 with AM or placebo administration. We aim to observe whether AM could contribute alterations in plasma IIS indices and corresponding Nfl levels.

2. Material and method

2.1. Participants

Patients with SCA3 were recruited from the outpatient clinic of Changhua Christian Hospital (CCH) in Taiwan and provided informed

consent. Inclusion criteria comprised: (1) genetic diagnosis of SCA3 by a neurologist, (2) aged between 20 and 80 years. Exclusion criteria included: (1) current use of other herbal or traditional Chinese medicine, (2) allergy to herbal or traditional Chinese medicine, (3) pregnancy or lactation, (4) concurrent major illnesses such as cancer, stroke, heart failure, or renal failure, (5) DM. Basic demographic data including gender, age, body mass index (BMI), age at onset, duration of illness, and CAG trinucleotide repeat number were collected from participants.

2.2. Study design

This was a randomized, triple-blind, placebo-controlled crossover study, conducted at CCH from May 31, 2021, to September 26, 2022. It was approved by the CCH Institutional Review Board (No. 200703), and it has also been registered on ClinicalTrials.gov (NCT05033306).

SCA3 participants were randomized into Group A or Group B according to the recruitment order, without knowledge of the experimental drug composition. Each group received either Chinese medicine or placebo first. Group A received AM for twelve weeks initially, followed by a four-week washout period, and then placebo for twelve weeks. Group B followed the reverse sequence. Participants were allowed to continue their previous western medication therapy during the study but were restricted from receiving other Chinese herbal medicines. A research assistant regularly monitored medication adherence and side effects via phone calls during the trial period. In addition, we analyzed the characteristics of dropout participants to evaluate whether attrition might bias the interpretation of trial outcomes.

2.3. Randomisation and blinding

Sun Ten Pharmaceutical Co., Ltd. designed a randomization list, using block randomization with a block size of four, which is commonly applied in small-sample studies to maintain balanced allocation between groups during recruitment, and provided it to the CCH Chinese Medicine Pharmacy for sequential medication distribution. Allocation was thus concealed: investigators, study staff and participants were blinded to assignment, and the randomization code was held by the manufacturer until study unblinding.

2.4. Sample size

In this crossover study, participant numbers needed were assessed quantitatively to estimate: a significant difference of 0.05% was set, utilising a two-tailed test, with a power set at 0.8, and calculating the variance in the same patient's standard deviation before and after. If the sample size was set to advance by 50% before and after, the paired number of cases was estimated to be 33 individuals to reject the null hypothesis. Assuming a dropout rate of 25%, the sample size would then require 44 individuals. In a study by Wilke et al. on serum NfL levels, it was suggested that with an effect size of 20%, approximately 15 participants were needed in both the study and control groups.[25]

2.5. Preparation of AM and the placebo

AM and placebo were provided by Sun Ten Pharmaceutical Co., Ltd. AM was manufactured at a rate of 3.0 g from every 3.3 g of crude AM. The placebo consisted of starch and maltodextrin (in a 7:3 ratio), with a small amount of caramel coloring to match the appearance, taste, and color of AM powder. Based on a rat model study, the safe dosage of AM for adults is less than 20 g per day without significant nephrotoxicity.[27] According to the dosage of AM extract used for stroke recovery and post-stroke fatigue, a daily dosage of 9 g has no significant side effects.[28,29] Therefore, combining clinical experience and the above research results, the dosage of AM in this study was set at 3 g per sachet, twice a day. Participants were required to take one sachet before breakfast and dinner for 12 weeks. Adherence was monitored through both returned sachet counts and review of participants' medication diaries. Participants were instructed to bring back any unused sachets at each follow-up visit.

2.6. Safety monitoring

AM has been documented as a top-grade herb medicine in the Shen Nong's Herbal Classic (Shennong Bencao Jing), indicating its non-toxic, safe, and suitable for long-term consumption. In animal studies, AM has demonstrated hepatorenal protective effects.[30,31] In a clinical trial for post-stroke fatigue, one-month administration of AM showed no side effects on peripheral blood levels, liver function, or renal function.[28] Nevertheless, we continued to monitor creatinine (Cr), estimated glomerular filtration rate (eGFR), blood urea nitrogen (BUN), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and complete blood count with differential count (CBC/DC) during each outpatient visit to ensure the safety in patients with SCA3.

2.7. Outcome measurements

The participants were instructed to undergo blood sampling with assistance from a designated assistant. The initial outpatient neurological assessment, SARA, was conducted by a consistent neurologist.

Plasma NfL reduction was designated as the pre-specified primary outcome of this trial. Insulin system, IGF1, and associated binding proteins (IGFBPs), exhibit anomalies in SCA3,[12] making the IIS a potential therapeutic target.[32] Free-form IGF1, represented by the IGF1/IGFBP3 ratio, reflects IGF1 activity, with IGFBP3 predominantly binding to regulate IGF1 activity. An increase in IGFBP1 levels within the brains of transgenic mice has been found to suppress the quantity of myelin axons and oligodendrocytes,[33] contributing to the possible pathogenesis of SCA3.[34] 8-hydroxy-2-deoxyguanosine (8-OHdG), a product of DNA oxidation, serves as an oxidative stress marker in neurodegenerative diseases such as AD and in SCA3 mice.[15,35] Our assessments aim to determine whether AM could ameliorate NfL, IIS or oxidative stress in SCA3 patients.

2.7.1. Clinical blood chemistry

The analyzed laboratory parameters included blood levels of CBC/DC were analyzed by Beckman Coulter DxH 900 hematology analyzer Beckman Coulter DxH 900 (Beckman Coulter, Miami, Florida, USA). The plasma levels of GOT, GPT, BUN, and Cr were measured using an AU5800 Series Clinical Chemistry Analyzers (AU5811; Beckman Coulter, Brea, CA, USA) by Department of Laboratory Medicine, CCH.

2.7.2. Determination of 8-OHdG and NfL in plasma

The plasma level of 8-OHdG was assessed using the Highly Sensitive 8-OHdG Check ELISA kit (JaICA-#KOG-HS10E). Quantification of NfL levels in plasma was using the Simoa® NF-light™ Advantage Kit (#103186) and an ultra-high sensitivity protein molecular detection instrument (Simoa HD-X, Quanterix, MA, USA), as previously described in the literature.[36]

2.7.3. Measurement of IIS in plasma

Blood samples were drawn into 10 mL BD Vacutainer® EDTA Tubes and centrifuged at 3000 rpm at 4 °C for 15 min to isolate plasma, which was then stored at −80 °C until analysis. The concentrations of glucose, insulin, IGF1, IGFBP1, and IGFBP3 were determined using specific assay kits: Glucose Colorimetric Assay Kit (Cayman Chemical-10009582), Insulin ELISA kit (Mercodia 10-1113-01), Human IGF1/IGF1 Immunoassay ELISA kit (R&D Systems-DG100B), Human IGFBP-1 ELISA Kit (RayBiotech-ELH-IGFBP1-1), and Human IGFBP3 ELISA Kit (Abcam-ab100541) respectively.

2.8. Statistics

We utilized SPSS v.22.0 to analyze variable changes in the study, employing both parametric and non-parametric statistical methods, depending on the distribution of the data. Due to missing cases and a small sample size, we employed intention-to-treat analysis (ITT) to maintain randomization and reflect real-world conditions. Missing values were handled using last observation carried forward (LOCF) under the ITT principle, and baseline characteristics of completers and non-completers were compared to assess potential bias. Carryover effects were assessed using mixed model analysis to evaluate the quality of the randomized crossover design. After considering variables such as age, baseline NfL plasma levels, drug administration sequence (placebo/drug first), treatment type (placebo/drug), intervention order (first/second three months), and treatment-order interaction, the difference in NfL levels was not statistically significant ($P = 0.174$). Therefore, we analyzed the results using a pooled sequence. We used independent t-tests and Mann-Whitney U tests for between-group comparisons, and paired t-tests or Wilcoxon tests for within-group comparisons. In addition, multiple regression models were applied to adjust for potential confounders of NfL reduction, providing a more robust assessment of treatment effects. Correlation analyses were conducted to explore relationships between changes in plasma NfL levels and changes in IIS-related exploratory biomarkers showing treatment-associated effects. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Study flow and demographics

This study recruited 32 subjects with SCA3, who were randomly assigned, with 16 patients entering Group A and 16 patients entering Group B (Fig. 1). In Group A, there were no participants who dropped out during the first three months of AM. During the washout period, two participants asked to exit the study, one had experienced a seizure due to discontinuing seizure medication six months before the beginning of the trial, and the other discontinued because of recurrent rhinitis and a desire to reduce medication usage. In the subsequent three months with placebo, one was asked to exit due to a transportation issue. In Group B, there were four participants who dropped out during the first three months of placebo: one due to a transportation issue, one due to cardiac event, and two due to numbness of limbs. During the washout period, two participants asked to exit: one due to a transportation issue and one due to an anxiety disorder attack. In the subsequent three months with AM, no participants dropped out. Adherence was satisfactory, with medication records showing that regularly followed-up participants had fewer than 10 sachets unconsumed during the study period.

In total, 23 participants completed the study, corresponding to an overall dropout rate of 28%. Attrition bias assessment revealed no significant differences between completers and non-completers (Supplementary Table 1). Our study design employed ITT analysis and pooling sequence, encompassing all 32 patients, with 26 receiving AM and 25 receiving placebos. Basic demographic data for Group A and Group B are provided in Table 1. There were no significant differences between the two groups in terms of demographic characteristics, SCA

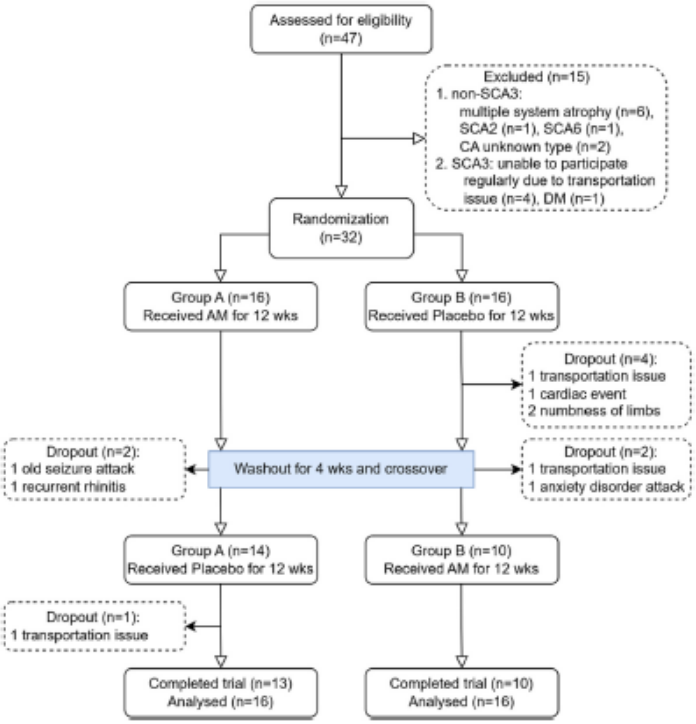

Fig. 1. Flowchart of study recruitment. SCA, spinocerebellar ataxia. CA, cerebellar ataxia. DM, diabetes mellitus. AM, *Astragalus membranaceus*.

Table 1
Demographic characteristics of participants.

	Group A (mean ± SD)	Group B (mean ± SD)	*P*
Basic features			
Case number	16	16	
Sex (male, %)	12 (75)	8 (50)	0.144
Age (yr)	47.5 ± 13.2	45.9 ± 14.1	0.739
BMI (m^2/kg)	22.7 ± 3.8	21.9 ± 3.1	0.520
SCA features			
Age at onset (yr)	36.9 ± 9.8	37.5 ± 14.5	0.887
Duration (yr)	10.6 ± 7.5	8.4 ± 5.6	0.344
CAG count	72.2 ± 3.6	70.3 ± 5.5	0.264
SARA	14.9 ± 10.8	14.2 ± 6.0	0.818
Nfl. (pg/mL)	28.9 ± 12.6	27.6 ± 9.1	0.748
8-OHdG (ng/mL)	3.37 ± 0.63	3.95 ± 2.00	0.955
Insulin/Insulin-like growth factor 1 system			
Glucose (mg/dL)	97.7 ± 20.0	101.6 ± 27.9	0.792
Insulin (μIU/mL)	5.7 ± 3.2	9.2 ± 7.5	0.187
IGF1 (ng/mL)	97.1 ± 33.1	85.0 ± 23.0	0.239
IGFBP1 (pg/mL)	441.4 ± 554.1	1043.0 ± 1974.2	0.806
IGFBP3 (ng/mL)	55.5 ± 27.0	45.0 ± 21.7	0.258
IGF1/IGFBP3 ratio	1.96 ± 0.86	2.18 ± 0.98	0.366

BMI, body mass index. SCA, spinocerebellar ataxia. SARA, scale for the assessment and rating of ataxia. Nfl., neurofilament light chain. 8-OHdG, 8-hydroxy-2′-deoxyguanosine. IGF1, insulin-like growth factor 1. IGFBPs, insulin-like growth factor binding proteins.

features, and IIS.

3.2. Safety monitoring

There were no participants who dropped out during the three months of AM in either Group A or Group B. Following interventions with AM and placebo, most monitored biochemistry parameters in plasma showed no significant changes (Supplementary Table 2). Although some parameters exhibited alterations, they remained within the normal range: Cr significantly decreased in the AM group ($P = 0.028$), eGFR significantly increased in the AM group ($P = 0.016$), and white blood cell (WBC) significantly decreased in the placebo group ($P = 0.004$). Regarding liver and kidney function, there were no obvious changes observed in either group.

3.3. Nfl, 8-OHdG, and IIS after intervention

Plasma Nfl, the primary outcome of this trial, decreased after AM administration from 27.6 ± 10.7 to 25.6 ± 9.8 pg/mL, representing an approximately 7% reduction ($P = 0.040$; Fig. 2A) in 26 patients with SCA3, whereas no significant change was observed in the placebo group (25.4 ± 12.5 vs. 25.8 ± 14.3 pg/mL, $P = 0.768$). However, in the multiple regression model adjusting for potential confounders, the between-treatment difference in Nfl reduction did not reach statistical significance (mean difference: −2.372, $P = 0.127$; Supplementary Table 3). To further address potential bias from missing data, LOCF analysis was applied and confirmed a modest but significant reduction in Nfl after AM ($P = 0.041$), with no significant change in the placebo group ($P = 0.767$).

Regarding insulin and glucose metabolism, glucose levels remained unchanged in both groups, but insulin nearly doubled after AM treatment (12.5 ± 15.2 to 21.1 ± 15.5 μIU/mL, $P = 0.004$; Fig. 2B), while no significant change was detected in the placebo group (10.7 ± 9.0 vs. 13.6 ± 15.6 μIU/mL, $P = 0.990$). Importantly, no significant correlation was observed between the magnitude of Nfl reduction and insulin elevation ($r = 0.165$, $P = 0.420$; Supplementary Fig. 1). Other biomarkers, including 8-OHdG, IGF1, IGFBP1, IGFBP3, and the IGF1/IGFBP3 ratio, did not exhibit significant changes following AM or placebo administration (Table 2).

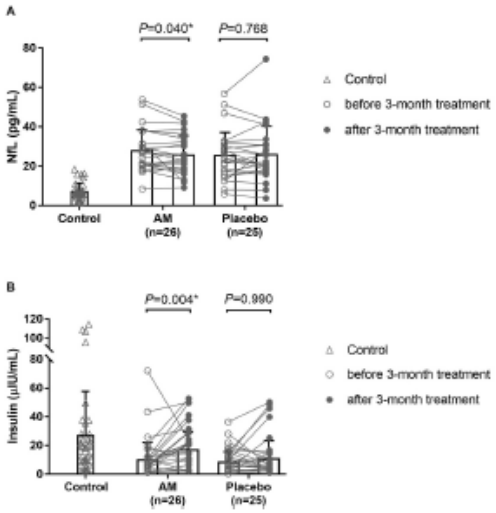

Fig. 2. Pooled analysis of Nfl and insulin before and after three months AM (A)/Placebo (B) intervention. The data for the control group were sourced from another study conducted by our team and comprised 36 sex- and age-matched healthy subjects. Nfl., neurofilament light chain. AM, *Astragalus membranaceus*.

Table 2
Plasma biomarkers and IIS before and after 12 weeks of treatment with AM or placebo.

	AM (n = 26)		Placebo (n = 25)	
	mean ± SD	*P*	mean ± SD	*P*
Nfl. (pg/mL)				
Before	27.6 ± 10.7	0.040*	25.4 ± 12.5	0.768
After	25.6 ± 9.8		25.8 ± 14.3	
8-OHdG (ng/mL)				
Before	3.89 ± 1.88	0.770	3.87 ± 1.66	0.864
After	3.78 ± 1.46		4.01 ± 1.86	
Glucose (mg/dL)				
Before	103.5 ± 23.5	0.101	104.1 ± 23.0	0.696
After	118.5 ± 39.0		103.6 ± 22.3	
Insulin (μIU/mL)				
Before	12.5 ± 15.2	0.004*	10.7 ± 9.0	0.990
After	21.1 ± 15.5		13.6 ± 15.6	
IGF1 (ng/mL)				
Before	95.2 ± 32.6	0.647	91.7 ± 29.6	0.412
After	97.4 ± 30.3		95.3 ± 30.0	
IGFBP1 (pg/mL)				
Before	406.5 ± 592.9	0.732	798.3 ± 1608.9	0.459
After	350.9 ± 540.7		411.3 ± 520.7	
IGFBP3 (ng/mL)				
Before	55.4 ± 26.6	0.849	51.1 ± 23.5	0.989
After	54.8 ± 25.7		51.7 ± 24.3	
IGF1/IGFBP3 ratio				
Before	1.93 ± 0.84	0.517	2.09 ± 1.03	0.326
After	1.99 ± 0.81		2.18 ± 1.16	

IIS, insulin/insulin-like growth factor 1 system. AM, *Astragalus membranaceus*. Nfl., neurofilament light chain. 8-OHdG, 8-hydroxy-2′-deoxyguanosine. IGF1, insulin-like growth factor 1. IGFBPs, insulin-like growth factor binding proteins. The *P* value was calculated for the comparison between before and after treatment. *P value less than 0.05.

3.4. Subgroup analysis of Nfl, 8-OHdG, and IIS after intervention

Due to variations in the severity of enrolled patients, AM may yield diverse effects. According to the classification derived from the SARA

progression scale,[37,38] individuals receiving AM with SARA scores <3 points were categorized into the preclinical group (n = 2), those with scores ≤11 were labeled as stage I (n = 9), and those with scores >11 were allocated to stage II (n = 15) (Table 3). For the preclinical subgroup, results are presented as effect sizes due to the very small sample size: plasma NfL decreased by 2.1 pg/mL (16%) after AM, while insulin increased by 21.7 μIU/mL (344%). Other parameters showed variable changes without consistent trends. Stage I results are presented with *P* values in the table; however, given the limited sample size, these findings should also be considered exploratory. In this group, only a modest reduction in 8-OHdG levels (3.97 ± 1.77 vs. 3.49 ± 1.45, $P = 0.028$) was observed after AM administration. In contrast, stage II patients exhibited a significant reduction in NfL (32.6 ± 10.8 vs. 30.4 ± 9.3, $P = 0.033$; Fig. 3A). No change in NfL was observed after the placebo across the two stages (Fig. 3B). Glucose levels remained consistent in each stage. Like NfL, insulin showed significant variation only in stage II patients (8.0 ± 5.6 vs. 19.8 ± 15.3, $P = 0.003$; Fig. 3C) after AM but not after the placebo (Fig. 3D). However, there was no direct significant correlation between the change in NfL and insulin after AM, possibly due to the complex interaction between the insulin system and the central nervous system. No variation was noticed in IGF1, IGFBP3, and IGF1/IGFBP3 ratio among the two stages.

3.5. Patient-reported positive effects and suspected adverse effects

In addition to plasma biomarkers, we recorded subjective responses and suspected side effects in patients after medication administration (Supplementary Table 4). Among patient-reported positive effects, three participants reported improved balance and two reported alleviations of fatigue after taking AM, while none reported improved balance and five reported alleviations of fatigue after taking placebo. Regarding suspected adverse effects, four reported dry mouth and three reported insomnias after taking AM, while two reported dry mouth and one reported insomnia after taking placebo. Nevertheless, no significant differences ($P > 0.05$) were found between the AM and placebo groups in comparison.

Table 3
Subgroup analysis of plasma biomarkers and IIS before and after 12 weeks of treatment in subjects with AM.

	Preclinical (n = 2)	Stage I (n = 9)		Stage II (n = 15)	
	mean ± SD	mean ± SD	*P*	mean ± SD	*P*
Nfl (pg/mL)					
Before	13.2 ± 6.7	22.6 ± 4.8	0.458	32.6 ± 10.8	0.033*
After	11.1 ± 2.9	20.8 ± 5.3		30.4 ± 9.3	
8-OHdG (ng/mL)					
Before	3.84 ± 0.87	3.97 ± 1.77	0.028*	3.85 ± 2.12	0.334
After	3.88 ± 0.25	3.49 ± 1.45		3.93 ± 1.60	
Glucose (mg/dL)					
Before	94.6 ± 1.7	94.9 ± 16.2	0.117	111.4 ± 26.2	0.344
After	92.4 ± 1.1	107.2 ± 28.4		102.9 ± 19.8	
Insulin (μIU/mL)					
Before	6.3 ± 6.0	21.5 ± 22.9	0.594	8.0 ± 5.6	0.003*
After	28.0 ± 30.8	21.9 ± 14.5		19.8 ± 15.3	
IGF1 (ng/mL)					
Before	101.7 ± 21.7	91.6 ± 37.6	0.483	96.4 ± 31.7	0.571
After	115.0 ± 1.5	99.4 ± 33.8		93.8 ± 30.5	
IGFBP1 (pg/mL)					
Before	90.8 ± 24.5	353.2 ± 735.9	0.260	480.6 ± 544.3	0.865
After	113.8 ± 161.0	237.1 ± 509.1		450.8 ± 587.5	
IGFBP3 (ng/mL)					
Before	67.8 ± 40.4	51.1 ± 26.0	0.678	56.3 ± 27.0	0.820
After	74.4 ± 29.0	50.0 ± 23.7		55.2 ± 26.9	
IGF1/IGFBP3 ratio					
Before	1.99 ± 1.64	2.01 ± 0.94	0.199	1.87 ± 0.75	0.955
After	1.67 ± 0.63	2.29 ± 1.07		1.86 ± 0.63	

IIS, insulin/insulin-like growth factor 1 system. AM, *Astragalus membranaceus*. Nfl., neurofilament light chain. 8-OHdG, 8-hydroxy-2′-deoxyguanosine. IGF1, insulin-like growth factor 1. IGFBPs, insulin-like growth factor binding proteins. The *P* value was calculated for the comparison between before and after treatment. Due to the very small sample size in preclinical group (n = 2), statistical testing was not performed; values are presented descriptively only. *P value less than 0.05.

4. Discussion

Currently, there is no established therapeutic modality for patients with SCA3, underscoring the urgent need for efficacious symptomatic management or DMT. Utilizing plasma NfL levels to monitor changes pre- and post-treatment presents an opportunity to streamline both time and cost efficiency. In this exploratory pilot crossover study, a borderline reduction in plasma NfL was observed within the AM group following administration, along with the notable increase in insulin levels; however, the adjusted between-treatment difference in NfL was not statistically significant. Although the approximately 7% reduction in plasma NfL may represent a potentially relevant biological signal, its clinical significance remains uncertain. Exploratory subgroup analyses numerically suggested larger biomarker changes in stage II patients, although these findings should be interpreted cautiously given the limited sample size after stratification. This study marks the first clinical intervention utilizing NfL assessment in SCA3 and the first triple-blind randomized crossover trial employing traditional Chinese medicine in cerebellar ataxias. Since the insulin rise was not significantly correlated with NfL change and IGF-related markers remained unchanged, the present data do not establish that AM reduced neurodegeneration through IIS signaling, and the observed NfL reduction should not be regarded as confirmatory evidence of efficacy. Rather, the biomarker pattern should be interpreted as a preliminary, hypothesis-generating signal that requires confirmation in larger, adequately powered trials. Accordingly, this study should be considered a pilot biomarker-guided exploratory trial rather than a definitive efficacy study.

Central and peripheral insulin play critical roles in regulating metabolic and neuronal processes. Elevated circulating insulin in mice has been shown to modulate hypothalamic gene transcription.[39] Lower circulating insulin levels in diabetic mice correlate with decreased gene expression and synthesis of brain cholesterol, which contributes to myelin formation and neuronal membrane function.[40] Unlike other neurodegenerative diseases, individuals with SCA3 often present with normal glycemia but relative hypoinsulinemia.[12] The decline in peripheral insulin levels might lead to a reduction in cerebral insulin,[41] which may be further aggravated by the presence of polyQ in the brain.[42] Lower BMI and elevated insulin sensitivity may represent compensatory adaptations to insufficient insulin.[12,43] After AM intervention, peripheral insulin levels in SCA3 patients increased, approaching those observed in the normal population. However, as the association between insulin elevation and NfL reduction was not statistically significant, no causal relationship can be inferred, and the present study does not establish whether the observed biomarker changes are mechanistically linked to IIS modulation. The rationale for focusing on IIS in SCA3 is supported by converging evidence linking AM to IIS. Prior studies demonstrate that APS preserves pancreatic β-cell ultrastructure in type 1 DM models with hypoinsulinemia, thereby mitigating islet inflammation.[44] APS has also been shown to reduce insulin resistance and improve glucose homeostasis, potentially lowering the risk of AD by addressing metabolic dysfunction at its root.[45] Moreover, AST may influence central insulin regulation; in postnatal rat models of schizophrenia, AST promoted serotonin release in the choroid plexus, which may enhance brain insulin signaling.[46,47] Additional exploratory preclinical data suggest that in a rat model of olivo-cerebellar degeneration, AM increased serum and cerebellar IGF1 expression and supported neuronal survival in the inferior olive.[48] In clinical practice, AM has been widely applied in Chinese communities for type 2 DM, where both animal and clinical studies indicate benefits in improving hyperglycemia, reducing insulin resistance, and enhancing

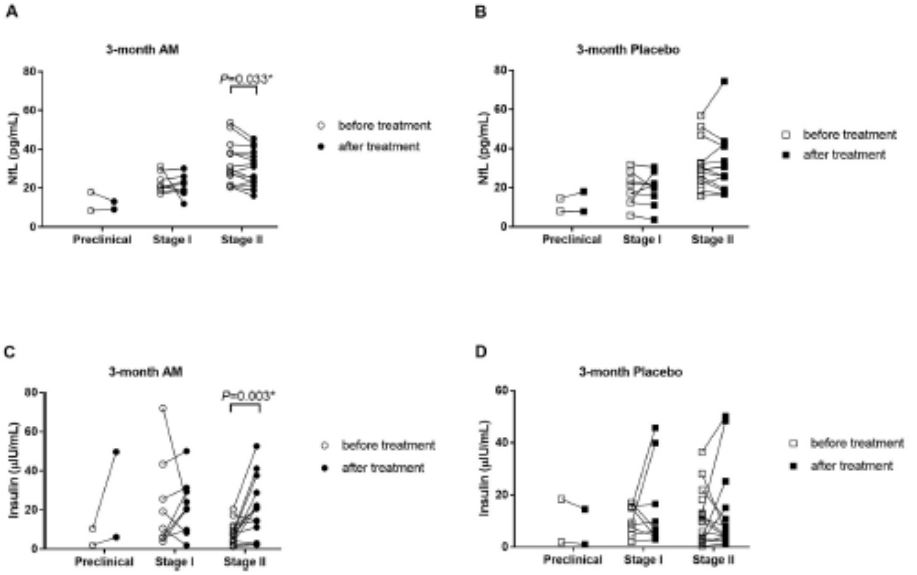

Fig. 3. Subgroup analysis of NfL and insulin before and after three months AM (A and C)/Placebo (B and D) intervention. There were 2 participants in the preclinical stage (SARA <3), 9 in stage I (SARA ≤11), and 15 in stage II (SARA >11). NfL, neurofilament light chain. AM, *Astragalus membranaceus*. SARA, scale for assessment and rating of ataxia.

insulin sensitivity.[49] Taken together, these prior findings provide a rationale for investigating IIS-related effects of AM in SCA3; nevertheless, the current clinical dataset supports only an association with circulating insulin change and does not demonstrate a peripheral-to-central IIS mechanism.

In this study, plasma NfL showed a modest within-treatment fluctuation after AM administration. Considering that NfL reflects axonal injury, this finding may reflect a potential biomarker signal consistent with putative neuroprotective activity. Given that intra-individual biological variation of plasma NfL is in the low single-digit percentage range, the observed −7% reduction may reflect a biological signal but remains within the reported variability range.[50] Although our initial hypothesis emphasized modulation of the IIS, no significant changes were observed in IGF1 or IGFBPs. Therefore, the observed NfL reduction may involve alternative or complementary pathways. Previous research has explored AM's protective effects against neurodegenerative diseases. An initial mechanism involves APS exerting neuroprotective effects by activating the Klotho-Nrf2 antioxidant pathway, leading to elevated expression of HO-1, NQO1, and TrxR1, which mitigates ROS-induced neuronal damage.[51] Additionally, AST demonstrates potent anti-inflammatory and antioxidant effects. In an AD model, it inhibited microglial activation and NADPH oxidase expression, and reduced ROS and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), thereby attenuating neuroinflammation and neuronal injury.[52] Finally, extracts of AM reversed amyloid-induced memory loss and promote axonal regeneration and synaptic reconstruction in mice, further supporting its neuroprotective potential.[53]

Notably, plasma 8-OHdG levels, a marker of oxidative stress, showed a modest decline in stage I patients; however, this finding should be interpreted cautiously given the limited sample size. Preclinical studies indicate that AM and its components reduce oxidative DNA damage. AST alleviated kidney injury by suppressing oxidative stress and lowering 8-OHdG levels,[54] while APS protected cardiomyocytes from high-glucose injury by preserving mitochondria, reducing apoptosis, and decreasing oxidative markers including 8-OHdG.[55] These findings support AM's potential to mitigate oxidative stress-related neurodegeneration in SCA3. However, the detailed mechanisms underlying AM intervention in SCA3 neuropathology warrant further investigation.

In patients with DM, hyperinsulinemia or insulin supplementation has been shown to stimulate hepatic IGF1 production.[56] In our study, although AM increased insulin levels, it did not lead to elevations in total or free IGF1 in SCA3 patients, nor was there improvement in subjective fatigue. Similarly, IGFBP1, a potential disease biomarker in SCA3, was not significantly altered by AM, despite being regulated by insulin.[12] Previous studies reported that AM or AM-containing formulas can enhance IGF1 or IGFBP3 signaling, thereby facilitating muscle repair and growth in children.[57,58] APS has also been reported to ameliorate insulin resistance in HepG2 cells by restoring glucose uptake and upregulating IGF1/IGF1 receptor expression. This effect was associated with STAT5 and AKT activation, as well as increased IGF1 secretion.[18] The initially elevated free IGF1 levels in our cohort were proposed to represent an endogenous compensatory response to insufficient insulin.[12] The lack of additional IGF1 increase after AM treatment may be attributable to patients' normal baseline IGF1 levels, suggesting that they were not deficient and that AM is unlikely to directly boost IGF1 in this neurodegenerative condition. Overall, the precise mechanisms by which AM modulates insulin and IGF1 warrant further confirmation in future studies.

Routine biochemical analyses indicated that AM was safe with respect to complete blood count as well as renal and hepatic function. Placebo administration was associated with a minor reduction in WBC levels, which remained within the normal range and likely reflected random variation. In contrast, AM led to a modest decrease in creatinine within the normal range, potentially suggesting a renal-protective effect[29] that merits further investigation. AM demonstrated acceptable tolerability, with only a few reports of dry mouth or insomnia, which occurred at similar rates in the placebo group, rendering causality uncertain. Fatigue improvement was likewise observed in both groups, indicating a possible placebo contribution. Prior clinical trials of AM or APS injections reported only mild adverse events such as eczema, febrile

reactions, headache, and constipation, none of which exceeding placebo rates, and all were transient and reversible.[59,60] Preclinical toxicology studies further support a wide safety margin: rats tolerated astragalol at 150 mg/kg/day for 91 days without toxicity, single doses of AM up to 5000 mg/kg caused no mortality, and subchronic studies in rats and dogs showed no distinct toxicity at up to 70- and 35-fold the human equivalent dose, respectively.[27,61] Previous studies suggest that AM extract or its components, such as formononetin, exert dose-dependent effects on neuroprotection and disease modulation, including PI3K/AKT-mediated apoptosis in hepatocellular carcinoma and attenuation of Aβ-induced neuronal toxicity in AD models.[62,63] In contrast, our pilot trial employed a single fixed dose of AM, selected for safety and feasibility considerations in this small-sample clinical setting[28,29]; therefore, potential dose–response effects should be evaluated in future, larger clinical trials. Collectively, these findings indicate that AM is well tolerated, with a safety profile comparable to placebo.

Our findings were considered in the context of previous pharmacological trials in SCA3. An interim analysis of an oral trehalose trial showed no improvement in SARA scores, largely attributed to limited recruitment and underpowering, highlighting the challenges of detecting clinical benefits within short trials.[64] In an open-label IGF1 study, stabilization of ataxia was reported over 12 months, but the absence of a placebo control precluded definitive causal inference.[65] As all pharmacological agents remain investigational, the only currently validated strategies for transiently slowing disease progression are non-pharmacological approaches such as physical rehabilitation and neuromodulation (e.g., TMC and DCS).[5] Importantly, these studies typically do not incorporate plasma biomarkers. In contrast, our randomized triple-blind crossover pilot provides short-term evidence of biomarker changes, and future prolonged follow-up will be valuable to determine whether these biological signals translate into durable clinical benefit.

An apparent discrepancy between biomarker and clinical or molecular readouts is not unexpected in this pilot study. Plasma NfL was selected as the primary outcome to capture short-term biological change, whereas conventional clinical scales such as SARA generally require longer observation periods and larger cohorts to detect treatment effects. Likewise, the increase in insulin without parallel changes in IGF1, IGFBP1, or IGFBP3 suggests that any biological response, if present, may reflect an incomplete or non-uniform response of the entire IIS axis. Therefore, the current datasets should be interpreted as early-phase signals at different levels of evidence, not as convergent proof of a single mechanism.

This study was limited by a 28% dropout rate, primarily related to comorbid conditions and transportation difficulties; however, no participants withdrew during the three months of AM in both groups. Baseline characteristics were comparable between completers and non-completers, suggesting that attrition was unlikely to introduce substantial bias. Although no statistical carryover effect was detected, residual biological effects cannot be completely excluded due to the chronic progressive nature of SCA3 and the relatively short washout period in this crossover design. To mitigate the impact of missing data, we applied LOCF analysis, which confirmed the preliminary signal of AM-associated NfL reduction. Alternative imputation methods (e.g., multiple imputation) could be considered in additional research to further strengthen robustness. Subgroup analyses by disease stage were underpowered, particularly in the preclinical group (n = 2) and stage I (n = 9), and should therefore be regarded as exploratory and hypothesis-generating. Given the pilot nature of this study and the relatively small sample size, the overall statistical power to detect modest treatment effects was restricted, particularly in subgroup analyses; thus, non-significant findings should be interpreted with caution and not as evidence of absence. Moreover, the relatively small sample size and multiple biomarker assessments mean that the possibility of statistical fluctuation or type I error cannot be fully excluded. In our multiple regression analysis, the reduction in NfL with AM was not significantly greater than with placebo. However, this association yielded a lower *P*-value than other potential confounders such as CAG repeats or BMI, indicating a potential signal that warrants confirmation in larger studies. The intervention period in this study lasted three months, which was likely insufficient to detect changes in SARA scores.[26] In addition, the detailed mechanism by which AM influences plasma NfL, including the interplay of insulin or other systems, remains unclear. Furthermore, potential residual bias related to sample size and subgroup stratification should be considered when interpreting the findings. In the subsequent studies, employing purified compounds derived from AM, such as APS or AST, may provide a more targeted means of enhancing IIS compared to the crude extract used in this study.

5. Conclusion

In conclusion, this randomized, triple-blind, crossover pilot study suggests that AM administration was associated with measurable short-term biomarker fluctuations in patients with SCA3. A within-treatment reduction in plasma NfL and an increase in circulating insulin were observed; however, the adjusted between-treatment difference in NfL was not statistically significant, and changes in NfL did not correlate with those in insulin. Given the exploratory pilot nature, limited sample size, and attenuation of significance after adjusted modeling, the observed NfL reduction should be interpreted cautiously and not as confirmatory evidence of efficacy.

Importantly, this study represents the first clinical intervention in SCA3 to incorporate plasma NfL as an outcome measure and the first triple-blind randomized crossover trial evaluating AM in cerebellar ataxias. These features highlight both the methodological innovation and the translational relevance of the present work. The feasibility of using plasma NfL as a biomarker supports a more efficient and biologically grounded framework for early-phase therapeutic evaluation in rare neurodegenerative diseases.

Future studies with larger cohorts, extended follow-up periods, and adequately powered designs are needed to determine whether the observed biomarker changes translate into sustained clinical benefits. Integration of multimodal outcome measures-including quantitative motor assessments, longitudinal functional scales, and neuroimaging biomarkers-may further clarify therapeutic impact. Additional mechanistic studies exploring insulin-related and alternative neuroprotective pathways will be essential to refine therapeutic strategies. Collectively, these results provide a foundation for continued biomarker-guided investigation of AM in SCA3.

CRediT authorship contribution statement

Chungmin Chiu: Project administration, Conceptualization, Data curation, Writing - original draft. **Wenling Cheng:** Writing - review & editing, Data curation, Methodology. **Tatung Lin:** Data curation, Methodology. **Huiju Chang:** Data curation, Methodology. **Minhui Tseng:** Project administration, Methodology. **Yujun Chang:** Resources, Software. **Shihli Su:** Validation, Writing - review & editing. **Chiaju Lee:** Validation. **Henhong Chang:** Project administration, Funding acquisition, Writing – review. **Chinsan Liu:** Project administration, Funding acquisition, Writing - review & editing.

Ethics statement

This randomized, triple-blind, placebo-controlled crossover trial study was conducted with the approval of the Independent Ethics Committee (CCH-IRB approval No:200703) at Changhua Christian Hospital. All participants provided written informed consent. The study was performed according to ethical principles outlined by Belmont Report, Declaration of Helsinki, and the journal's ethical publication guidelines.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT to assist with English language editing and rephrasing. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. Sun Ten Pharmaceutical Co., Ltd. assisted in preparation of the randomization list but had no role in study design, data collection, data analysis, manuscript preparation, or the decision to publish.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2026.06.002.

Data availability

In adherence to transparency and reproducibility principles, we acknowledge the importance of data availability statements. However, due to patient privacy and confidentiality concerns, we are unable to share the dataset publicly. We are committed to scientific collaboration and will make the data underlying our findings available to qualified researchers upon request. This access will be subject to ethical and legal considerations, along with a data access agreement to ensure participant privacy. Interested researchers can contact Chin-San Liu (liu48111@gmail.com) and Chung-Min Chiu (zhongdiah@gmail.com) for further information.

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