BMC Neurology

RESEARCH

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Diagnostic journey and genetic analysis of a novel homozygous *CYP2U1* mutation causing autosomal recessive spastic paraplegia type 56 (SPG56) in a consanguineous family

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Abstract

Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder, with spastic paraplegia type 56 (SPG56) being an exceptionally rare, autosomal recessive subtype caused by mutations in the *CYP2U1* gene. This study reports a complex case of an adult female from a consanguineous family who presented with cognitive developmental delays, short stature, and progressive neurological symptoms. At age 39, she developed unilateral tremors, which progressed to generalized tremors and leg weakness with a tiptoe gait. The clinical findings included hypertonia in the upper limbs, exaggerated reflexes in the lower limbs, vague speech, and emotional disturbances. Brain MRI revealed corpus callosum thinning, "ears of the Lynx" sign, bilateral globus pallidus calcifications, and mild brain atrophy. Comprehensive genomic analysis, including whole exome sequencing (WES), copy number variation (CNV) assessment, mitochondrial DNA sequencing, variant filtering, and Sanger sequencing, identified a homozygous c.913 C>T (p.His305Tyr) mutation in *CYP2U1* (NM_183075). The heterozygous carriers presented no symptoms. This case contributes to the phenotypic spectrum of SPG56, offering new insights into its diagnosis and genetic underpinnings.

Keywords SPG56, *CYP2U1*, Hereditary spastic paraplegia, Consanguinity, Complex HSP, Ears of the Lynx

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Introduction

Hereditary spastic paraplegia (HSP) is a neurodegenerative disorder characterized by insidious onset and considerable clinical variability. Pathologically, it manifests as length-dependent degeneration of the corticospinal tracts axons, predominantly affecting the thoracic region [1]. Clinically, it is characterized by progressive lower limb spasticity and weakness, often resulting in a characteristic "scissor gait." SPG56 is a rare subtype of HSP caused by biallelic mutations in the *CYP2U1* gene. *CYP2U1*, a member of the cytochrome P450 family, plays a crucial role in mitochondrial function. Mutations in this gene lead to neuronal degeneration, impairing nerve development [2, 3]. HSPs can be classified into simple and complex forms. Simple HSP involves spasticity and other mild pyramidal signs, whereas complex HSP features a broader range of neurological and non-neurological manifestations, including intellectual disability, psychiatric disorders, and various brain MRI abnormalities [4].

Neuro-imaging in HSP often reveals white matter changes, low signals in the globus pallidus, pontocerebellar atrophy, and corpus callosum thinning [5]. The prevalence of HSP ranges from 0.1/100,000 to 9.6/100,000 [6–8]. In this study, the patient was born to parents who were third cousins. Gene sequencing and phenotypic analysis confirmed the diagnosis of complex SPG56, a subtype of HSP characterized by distinctive imaging features. Notable findings included white matter changes, hypointensity of the globus pallidus, ponto-cerebellar atrophy, and a thin corpus callosum. Detailed phenotypic analysis of other family members revealed no similar symptoms or phenotypes. On the basis of these findings, we conducted functional studies on the identified mutation to explore its potential pathogenic effects in greater detail.

Materials and methods

Research subjects

The proband (V8), a 47-year-old female, presented with "left hand tremors persisting for 8 years and whole-body tremors for 1 month." Clinical data including routine blood tests, biochemical profiles, and results of brain MRI, spinal MRI, EEG, EMG, and the Spastic Paraplegia Rating Scale (SPRS) assessments, were collected from the proband and other family members. All procedures were approved by the Medical Ethics Committee of Fujian Provincial Hospital (No. K2023-12-018), and informed consent was obtained from all participants.

Genetic analysis

The proband underwent next-generation sequencing (NGS) and full-length mitochondrial DNA sequencing. Exon group capture was carried out by using a Naonda NEXome core board and hybridization and washing kit, and then linear PCR amplification and quality inspection were carried out. The coverage rate of the target regions was 99.71%, with an average sequencing depth of 200.51×. After quality control, paired-end (PE) 150 sequencing was conducted on the DNBSEQ-T7 platform. To enrich and screen a plurality of gene fragments, the captured genes included *CYP2U1*, *EIF4G1*, *CASR*, *KMT2C*, *FAT2*, *SERPINC1*, *SERPINI1*, etc. Sequencing fragments were aligned to the UCSC human reference genome (hg19) via BWA software. The alignment data were processed with Picard and SAMtools Markdup software to sort reads and mark duplicates. Single nucleotide variants (SNVs), insertions, and deletions were analyzed with GATK. Exome depth was used to detect exon-level CNVs. Analysis of the NGS data revealed no CNVs associated with the observed phenotype. Subsequently, Exomiser software was used to identify loci related to the phenotype on the basis of mutation data. Primers for the target sequence were designed with Primer Premier 5.0, and the region was amplified for verification through Sanger sequencing. The amplified fragment length of c.913 C>T was 637 bp, with the following primer sequences: F: TTTGCAGGGGTTGTGTTTGC and R: C ATCGGGGTTCAGCGACATA.

Biological information analysis method

The bioinformatics analysis method utilizes the Swiss Model (https://swissmodel.expasy.org/repository/uniprot/P00387) and Chimera software [9] to predict the impact of *CYP2U1* mutations on the protein tertiary structure (Fig. 1).

Result

Clinical phenotype

The proband (V8) is a 47-year-old female who began experiencing paroxysmal tremors at age 39, with no clear trigger, and her symptoms progressively worsened over time. She frequently experienced falls due to lower limb weakness and a tendency to walk on her toes. A month prior, she was hospitalized following a fall down the stairs that resulted in trauma. Reduced activity during her hospital stay led to the development of venous thrombosis in both lower limbs. The patient had a history of developmental abnormalities, repeated urinary tract infections, and basal ganglia tremors, with recent worsening of symptoms suggesting corticospinal tract involvement, as evidenced by decreased limb strength, hyperactive tendon reflexes, and positive pathological signs in the upper limbs. No Kayser-Fleischer ring was observed in the cornea. Neurological examination: The patient exhibited impaired language expression ability. The cognitive assessment indicated reduced abilities in calculation, memory, comprehension, and judgment, with an MMSE

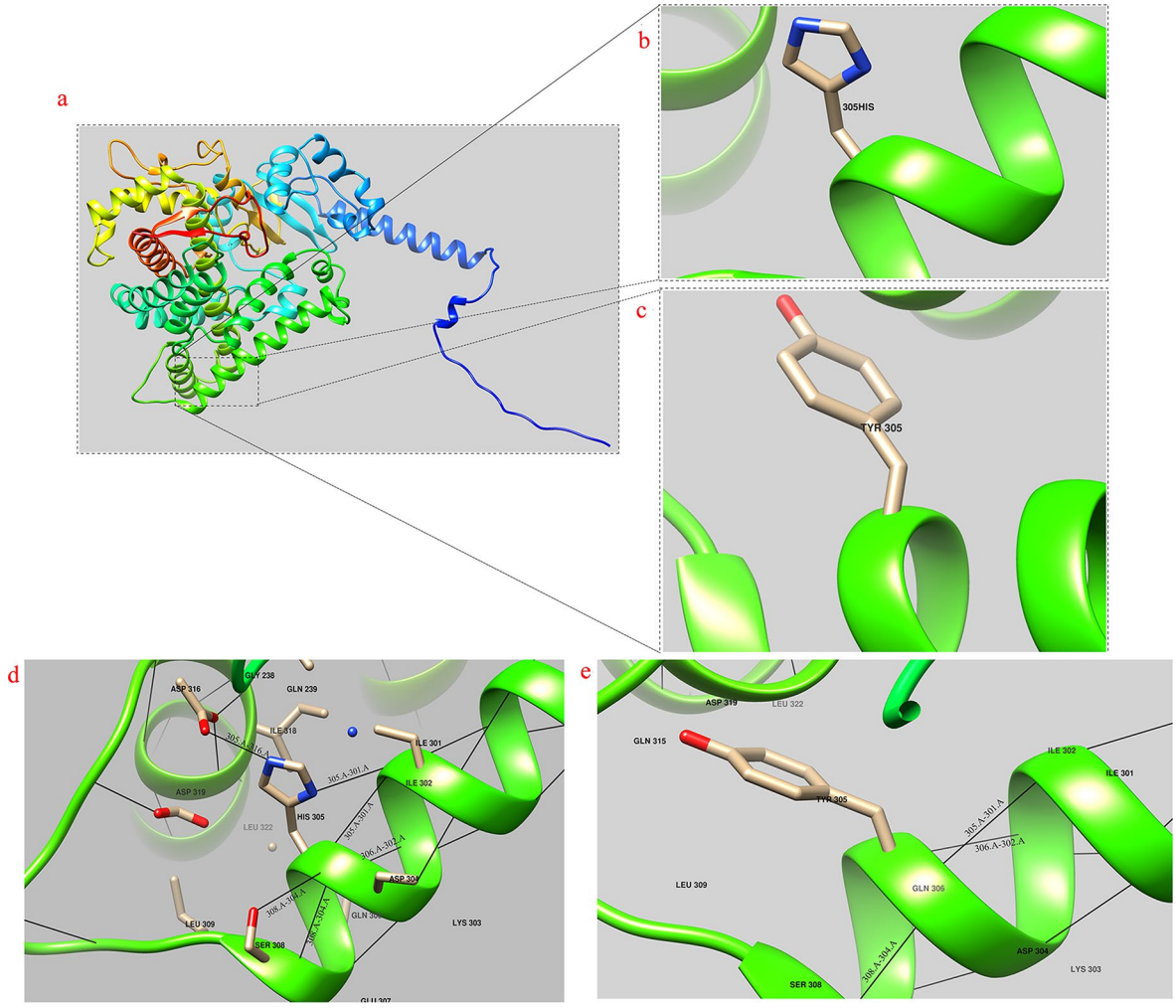

Fig. 1 Prediction diagram of the *CYP2U1* protein structure. (**a**) The overall structure of the *CYP2U1* protein; (**b**) Local structural diagram of the histidine residue at position 305; (**c**) Local structural diagram of the tyrosine residue at position 305; (**d**) Hydrogen bonding between histidine at position 305 and adjacent amino acids; (**e**) Hydrogen bonding between tyrosine at position 305 and adjacent amino acids; Black represents hydrogen bonds

score of 13 and a MoCA score of 7 (consistent with the primary school education level). The patient exhibited involuntary tremors in the head, neck, and limbs, with increased muscle tone, particularly in the upper limbs. Muscle strength was graded as 4 in the upper limbs and 3 in the lower limbs. Reflex examination revealed an extensor plantar reflex, left biceps and triceps reflexes graded as (++), right biceps and triceps reflexes (+++), a bilateral radial membrane reflex (++), a knee reflex (++), and an Achilles tendon reflex (+++). Both the palm-chin test and Hoffman sign were positive. The results of the finger-to-nose test were unstable and imprecise, whereas those of the rotation test were coordinated; however, the heel-knee-shin test and Romberg sign test could not be performed. The complete blood count was normal, with vitamin E, B1, and B2 levels within the normal range, as well as normal folate and homocysteine levels. The patient's serum folate level was 1.9 ng/mL (reference range: 3.1–17.5 ng/mL), CoQ10 was 3.4 μg/mL (reference range: 0.5–1.5 μg/mL), and neopterin was 18.6 nmol/L (reference range: 6.0–15.0 nmol/L), indicating reduced folate levels and elevated CoQ10 and neopterin levels.

Brain MRI revealed patchy short T1 and long T1/T2 signals in the cerebral hemispheres and basal ganglia, with no obvious high signal on DWI. Some lesions, particularly those in the central left hemisphere and left frontal lobe, presented mildly elevated T2 signals. The bilateral globus pallidus displayed diffuse, slightly shortened T1 signals, indicative of calcium salt deposition. Mild brain atrophy was suggested by slight widening of some sulci and cisterns, particularly in the bilateral cerebellar hemispheres. The midline brain structure was symmetrical, with a thinner corpus callosum displaying a characteristic "Ears of the Lynx" appearance (Fig. 2). Electromyography (EMG) revealed decreased electrical activity in the left tibialis anterior, abductor pollicis

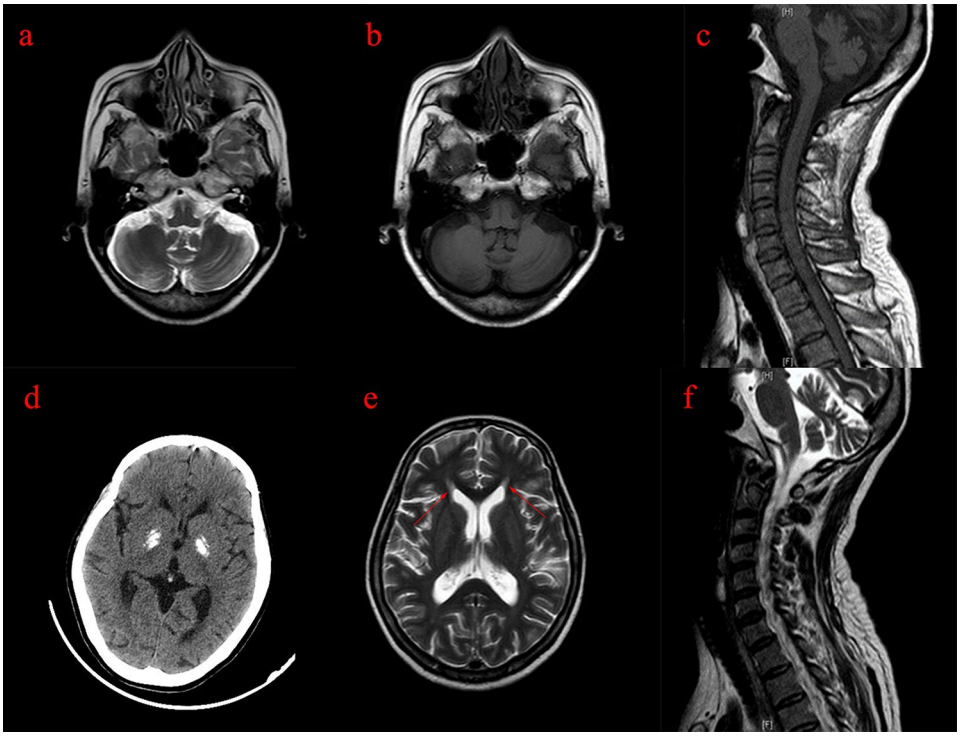

Fig. 2 Brain and cervical MRI images of proband V8. (**a**, **b**, **e**) Brain MRI image showing patchy, hyperintense T1 and T2 signals within the cerebral hemisphere and basal ganglia. T2-FLAIR images revealed mildly elevated signals in certain lesions, notably within the central left hemisphere and left frontal lobe. The bilateral globus pallidus presented with scattered, slightly hypointense T1 signals. Mild widening of the sulci and cisterns was observed, with atrophy noted in the bilateral cerebellar hemispheres. The midline structures are symmetrical. The red arrow points to the characteristic "lynx ears" sign. (**c**, **f**) Cervical spine imaging revealed marginal hyperosteophytes at the edges of the vertebral bodies, with slight flattening observed at C6, and no significant signal abnormalities were detected. Reduced T2 signal intensity is noted in certain cervical intervertebral discs, with mild posterior protrusions at C3–4, C5–6, and C6–7, causing minimal compression of the local dural sac. The cervical spinal cord morphology and signal remained normal, with no evidence of lesions within the spinal canal. (**d**) CT image showing bilateral basal ganglia calcification

brevis, dorsal interosseous muscle, and quadriceps femoris, with the involvement of motor and sensory nerve fibers in the limbs, suggesting axonal injury (detailed EMG results are available in Table S1).

Upon admission, the patient's SPRS score [10] (Supplementary Material 2) was 37/52, which improved to 29/52 following treatment with olanzapine, levodopa and benserazide hydrochloride tablets, and clonazepam. This regimen significantly controlled tremors and slightly improved muscle strength, allowing the patient to walk independently, although with persistent gait instability and marked incoordination on bilateral finger-to-nose testing. Our patient demonstrated typical neurological symptoms, including tremors, progressive impairment, and cognitive decline.

CYP2U1 mutation analysis

Heterozygous carriers in the proband's family—including her parents, uncle, two sisters, niece, and son—show no phenotypic expression associated with the mutation (Fig. 3a). A homozygous c.913 C > T (p.His305Tyr) mutation in exon 2 of the *CYP2U1* gene (NM_183075) was identified in proband V8 via second-generation sequencing and exon capture technology. This mutation causes a histidine-to-tyrosine substitution at position 305, altering the protein's structure (Fig. 3 bcd). According to the guidelines published by the American College of Medical Genetics and Genomics (ACMG) in 2015 [11], this variant was considered likely pathogenic (PS2 + PP2 + PP3). This classification supports the potential pathogenicity and deleterious impact of this mutation.

Bioinformatics prediction

The *CYP2U1* protein structure was obtained from the Alpha Fold protein structure database and visualized with Chimera software [9]. In this study, we identified a histidine-to-tyrosine mutation at position 305 in *CYP2U1*. Using chimera, we observed that, prior to mutation, His305 forms hydrogen bonds with Asp316 and Ile301. Following the mutation to Tyr305, hydrogen bonding was observed with Ile301 only, indicating a change in the hydrogen bonding pattern surrounding the mutation site (Fig. 1).

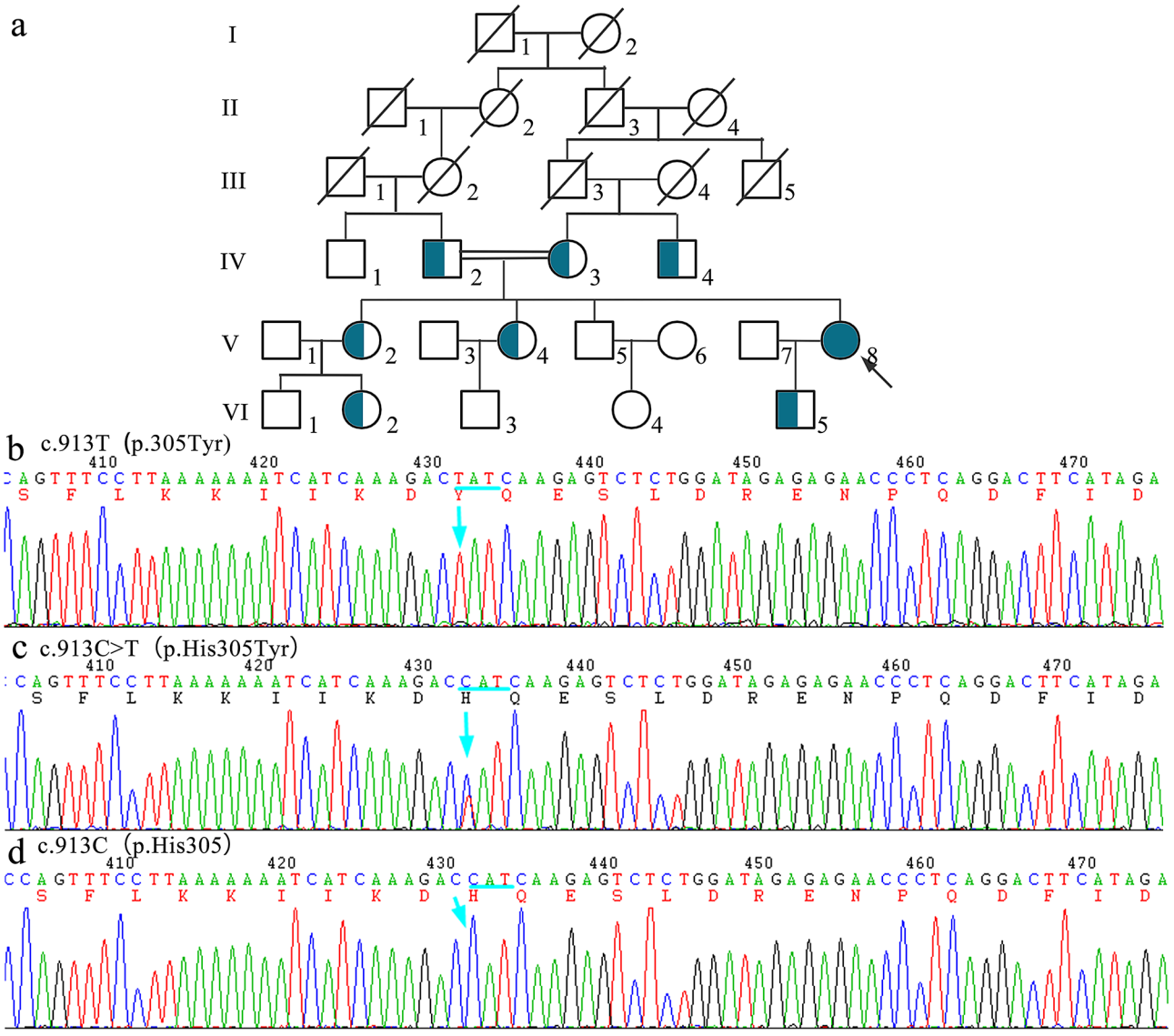

Fig.3 Family diagram and the Sanger sequencing map. (**a**) Pedigree illustrating autosomal recessive inheritance of spastic paraplegia type 56 (SPG56) due to a homozygous mutation in *CYP2U1* (c.913 C > T, p.His305Tyr) within a consanguineous family. Half-shaded symbols indicate carriers of the heterozygous *CYP2U1* variant (c.913 C > T, p.His305Tyr), and the fully shaded symbol denotes the proband (V8), who exhibits HSP with the homozygous variant (c.913T, p.305Tyr). (**b**) Sanger sequencing map of the homozygous variation of c.913T (p.305Tyr) in exon 2 of *CYP2U1* (NM_183075). (**c**) Sanger sequence diagram of the carrier of the heterozygote c.913 C > T (p.His305Tyr). (**d**) Wild-type sequencing map of c.913C (p.His305)

Discussion

This study describes an adult female from a consanguineous family with a homozygous *CYP2U1* c.913 C > T (p.His305Tyr) mutation underlying SPG56. The proband exhibited a unique onset, with no other family members showing clinical manifestations. The heterozygous carriers remained asymptomatic. Bioinformatics analysis indicated that the p.His305Tyr substitution disrupts protein stability and may impair neuronal function through altered hydrogen bonding [12]. Pathogenicity assessment of this variant aligns with existing data supporting the pathogenic role of homozygous *CYP2U1* mutations in neurodegeneration [13]. Our findings highlight a rare variant, broadening the understanding of the involvement of *CYP2U1* in neurodegenerative processes and contributing valuable insight into the genetic and clinical characteristics of SPG56.

Notably, the clinical manifestations of *CYP2U1* mutations exhibit considerable heterogeneity across individuals and involve both the neurological and non-neurological systems [14]. Neurologic presentations may include intellectual disability, dystonia, dysphagia, seizures, and corticospinal tract involvement primarily characterized by increased spasticity in the lower limbs. Non-neurological manifestations may include visual impairment, macular degeneration, and recurrent

urinary tract infections. Huang et al. documented two Chinese brothers with early-onset SPG56 who benefited from over 15 years of folinic acid supplementation and maintained normal cerebrospinal fluid 5-MTHF levels. The younger brother, who was diagnosed early, displayed near-normal functioning, contrasting with the progression in his elder sibling [15]. Similarly, Luca Leonardi et al. reported three patients from a consanguineous family with a novel *CYP2U1* mutation (c.1168 C > T, p.R390*) who presented with pigmented maculopathy alongside progressive paraplegia [16]. The numerous subtypes of HSP, complex clinical phenotypes, and diverse genetic etiologies underscore the value of detailed genetic and clinical characterization, which can guide targeted treatment strategies. Given the rapid identification of new HSP-related genes, substantial disease heterogeneity, and gradual neurological progression, defining universal therapeutic targets for HSP remains a challenge [8]. Early disease detection and identification of sensitive biomarkers are critical for managing disease progression and enhancing quality of life through symptom management, emphasizing the importance of early biomarker identification in the clinical workup of HSP.

In the clinical diagnostic and treatment process, the patient's initial misdiagnosis was due, in part, to the receiving physician's limited familiarity with this rare disease, coupled with the patient's presentation as a sporadic case without familial clustering. The patient's family reported a previously stable disease course, with this admission marking an acute exacerbation. MRI findings revealed calcification in the basal ganglia, which, alongside the patient's motor and cognitive symptoms, led the physician to initially consider degenerative diseases, particularly Huntington's disease, which often presents in middle age with motor, cognitive, and psychiatric symptoms similar to this case. Additionally, the patient's initial inability to participate in a gait examination, due to significant muscle weakness, masked the hallmark scissor gait often seen in HSP, and ataxic gait was noted only posttreatment. Consequently, distinct imaging features of HSP, such as thinning of the corpus callosum and "ears of the Lynx" sign on MR images, as well as phenotypic signs such as sphincter dysfunction and recurrent urinary tract infections, were initially overlooked. This led to the initial diagnosis of the patient as having Fahr's disease, Parkinson's syndrome, multiple system atrophy, and other conditions. Genetic testing ruled out Huntington's disease (Supplementary Material 3), and the diagnosis of HSP was ultimately confirmed. This case highlights the need for enhanced knowledge of rare diseases, emphasizing careful condition analysis and differential diagnosis to prevent misdiagnosis and inappropriate treatment of HSP. Future research should focus on elucidating the effects of *CYP2U1* mutations in HSP, exploring targeted therapies, and continuing to accumulate and study related cases.

Conclusions

HSP is a genetically heterogeneous neurodegenerative disorder with significant clinical and phenotypic variability. Diagnosis relies on clinical presentation, family history, and genetic testing, with no curative treatment currently available. Management focuses on improving quality of life and slowing disease progression through medication, physical therapy, and psychological support. This case highlights the importance of early diagnosis, accurate genetic testing, and careful differential diagnosis in the management of SPG56. The patient is currently stable and under regular follow-up care.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12883-025-04211-7.

Supplementary Material 1: Table. Detailed results of electromyography of the proband (V8)

Supplementary Material 2: Spastic Paraplegia Rating Scale before and after treatment

Supplementary Material 3: Exclusion of the Huntington gene

Acknowledgements

None.

Author contributions

LC, XLJ, YFL, and JWL conceived and designed the study. DDR, XLJ, QC, and WW collected the data. QC, YC, JHZ and XLR conducted the data analysis and interpretation. HPY, JZ, XC and YC wrote the manuscript. XLJ, YFL, and JWL made critical revisions to the important contents of the manuscript.All authors reviewed the manuscript.

Funding

This work was supported by the Fujian Province Natural Science Fund Project (2022J01409, 2022J01996, 2023J011846, 2024Y0033, 2024J011017), Traditional Chinese Medicine Science and Technology Project of Fujian Provincial Health Commission (2021zyjc06), the Fujian Provincial Health Commission Science and Technology Program (2024GGA090, 2024QNA002), Joint Funds for the Innovation of Science and Technology in Fujian Province (2023Y9284), Fujian Province Medical Innovation Foundation (2022CXB002, 2022CXA001), the Special Research Foundation of Fujian Provincial Department of Finance (2024-881#), and National famous and old Chinese medicine experts (Zhang Xuemei, Yan Xiaohua, Lv Shaoguang, Yi Chunjin) inheritance studio construction project, China.

Data availability

The data underlying the results presented in the study are available from National Center for Biotechnology Information: SCV005077966 (https://www.ncbi.nlm.nih.gov/clinvar/variation/3255488/).

Declarations

Ethics approval and consent to participate

This study was approved by the Bioethics Committee of Fujian Provincial Hospital, Fuzhou, China (Approval Number: No. K2023-12-018). All participants provided written informed consent prior to their inclusion in the study, in accordance with the Declaration of Helsinki.

Consent for publication
Written informed consent was obtained from the patient for the publication of any potentially identifiable images or data included in this article.

Competing interests
The authors declare no competing interests.

Received: 16 December 2024 / Accepted: 28 April 2025
Published online: 15 May 2025

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