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Case Report

A Novel *AP4M1* Variant in an Iranian Child with Spastic Paraplegia 50: A Case Report and Molecular Docking Approach

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What's Known

- Mutations in the *AP4M1* gene cause autosomal recessive spastic paraplegia type 50 (SPG50).
- The core clinical features of SPG50 include spasticity, severe intellectual disability, and delayed or absent speech.

What's New

- This study reported a novel *AP4M1* variant, c.258delG (p.Ala87Profs*44), identified in the proband via whole exome sequencing (WES) and confirmed by Sanger sequencing.
- A review of the literature confirmed the rarity of SPG50, with few documented cases from Iranian families.
- Computational analyses (molecular modeling and docking) were performed to assess the variant's impact on protein structure.

Abstract

Spastic paraplegia 50 (SPG50) is a rare autosomal recessive disorder caused by mutations in the *AP4M1* gene (OMIM 602296). It is characterized by spasticity, severe intellectual disability, and delayed or absent speech. The present study reported a case from consanguineous parents, where the affected child presented with intellectual disability, seizures, muscle weakness, and deep white matter hyperintensity. Whole exome sequencing (WES) of the proband identified a novel, homozygous frameshift variant, c.258delG:p.A87Pfs*44, in the *AP4M1* gene, which was confirmed by Sanger sequencing. This variant is predicted to cause a large truncation of the protein, leading to a loss of function. Molecular modeling and docking analyses further revealed that the loss of a substantial protein segment disrupts proper intramolecular interactions. A review of the literature on Iranian families with SPG50 yielded few reports, consistent with the disease's rarity. This study expanded the knowledge of the clinical and genetic features of SPG50 and underscored the importance of this variant for genetic diagnosis and counseling in affected families.

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Keywords ● Hereditary spastic paraplegia ● Frameshift mutation ● Whole exome sequencing

Introduction

Hereditary spastic paraplegia (HSP) comprises a diverse group of inherited disorders characterized by progressive weakness and spasticity of the lower limbs, resulting from axon degeneration in the corticospinal tract.[1] Among the ultra-rare forms of HSPs are those caused by loss-of-function mutations in the adaptor protein complex 4 (AP-4) genes.[2] Spastic paraplegia 50 (SPG50) is one such severe form, typically presenting with infantile hypotonia that progresses to lower-limb spasticity, and is accompanied by developmental delay, intellectual disability, and microcephaly.

The AP-4 complex consists of four subunits: the large chains (*AP4B1* gene [OMIM 607245] and *AP4E1* gene [OMIM 607244]), and a small chain (*AP4S1* gene [OMIM 607243]), and the medium chain (AP4M1).[3, 4] This complex mediates the export of transmembrane proteins, such as ATG9A, from the trans-Golgi network (TGN), a process vital for autophagosome formation. Mutations in any AP-4 subunits disrupt this process, leading to cargo retention and subsequent neuronal dysfunction.[5]

ADP-ribosylation factor 1 (*ARF1* gene [OMIM 103180]) is a small GTPase that plays a significant role in membrane trafficking

by stimulating the formation of transport vesicles and recruiting coat proteins. ARF1 binds directly to the AP4 complex via its ε and μ4 subunits. The ε subunit specifically binds the GTP-bound form of ARF1, while the μ4 subunit binds both GTP- and GDP-bound forms. This interaction is crucial for recruiting AP4 to the TGN, where it recognizes and sorts transmembrane proteins based on cytosolic signals.[6, 7] Mutations in AP4 subunits impair ATG9A export from the TGN and prevent autophagosome formation, underscoring the importance of the AP4-ARF interaction.[5] Therefore, the ARF1-AP4M1 interaction is essential for the proper function of the AP4 complex in neurons, and its disruption by genetic mutations can lead to SPG50.

We report a new homozygous mutation in the *AP4M1* gene, confirmed by genetic testing in an 8-year-old Iranian girl. This mutation produces a defective protein, resulting in AP-4 dysfunction. Our findings were validated by whole exome sequencing (WES) and Sanger sequencing. Notably, recent advances in gene therapy, such as adeno-associated virus (AAV)-mediated delivery of functional AP4M1, have shown encouraging safety and efficacy in early-phase clinical trials for SPG50, highlighting the translational relevance of ongoing molecular characterizations.[4] Here, we presented a case of an Iranian girl with intellectual disability and related neurological manifestations.

Case Presentation

An 8-year-old girl from a consanguineous family from Lorestan, Iran, was referred to the Medical Genetics Laboratory in Khorramabad (Iran) due to symptoms suggestive of SPG50. Her clinical presentation included intellectual disability, seizures, muscle weakness, and hyperintensity in deep white matter on magnetic resonance imaging (MRI). Written informed consent was obtained from the patient's parents, and the study protocol was approved by the Ethics Committee of Lorestan University of Medical Sciences (IR.LUMS.REC.1403.347).

Following ethical approval, WES and Sanger sequencing were performed. To this end, genomic DNA was extracted from the patient's peripheral blood sample using the standard salting-out method (SinaClon, Iran). WES was conducted using the SureSelect Human All Exon V7 Enrichment Kit (Agilent Technologies, USA), and the resulting libraries were sequenced on the Illumina NovaSeq 6000 platform (Illumina Inc., USA) to achieve approximately 100x coverage. The quality of the raw reads was assessed with FastQC (Babraham Institute, UK), and preprocessing was performed using Trimmomatic (USA), with particular attention paid to GC content (about 50%) and Phred quality score (≥20). The reads were aligned to the human reference genome (GRCh37/hg19) using BWA-MEM (Burrows-Wheeler Aligner). Subsequent processing of alignment files was carried out using SAMtools and GATK HaplotypeCaller (Broad Institute, USA) to generate a variant call format (VCF) file. Variant annotation was performed using Ensembl's Variant Effect Predictor (VEP), and benign/common variants were filtered out based on population frequencies in the Exome Aggregation Consortium (ExAC) v1 (Broad Institute, USA), the Genome Aggregation Database (gnomAD) v3 (Broad Institute, USA), and the Iranome databases (www.iranome.ir).

A novel homozygous frameshift variant, c.258delG (p.A87Pfs*44), was identified in exon 4 of the *AP4M1* gene (OMIM 602296) in the proband. This variant has not been previously reported in the National Center for Biotechnology Information (NCBI) database of single-nucleotide polymorphisms (dbSNP) or in the literature. According to the American College of Medical Genetics (ACMG) guidelines,[8] the c.258delG (p.A87Pfs44) variant was classified as pathogenic. The variant was confirmed by Sanger sequencing after amplification of the patient's DNA by polymerase chain reaction (PCR) using the primers GACTAGTAAGAGGCATCG (forward) and CTTCCGTCTGGATGAAAT (reverse). Sequencing was performed on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, USA), and the resulting chromatograms were compared to the reference genome (hg19, NCBI Build 37) (figure 1).

Following the analysis of sequencing results, computational tools were employed to predict the pathogenicity of the identified variant. To this purpose, the protein sequences of AP4M1 and ARF1 were retrieved from the UniProt database (https://www.uniprot.org/, SIB Swiss Institute of Bioinformatics, Switzerland). Analysis with the Mutalyzer server (https://mutalyzer.nl/) confirmed that the mutation causes a frameshift and premature translation termination (figure 2A). Homology modeling of both wild-type and mutated *AP4M1* sequences, along with the ARF1 sequence, was performed using the SWISS-MODEL server (https://swissmodel.expasy.org/). The wild-type AP4M1 model showed acceptable quality (GMQE score: 0.86; QMEAN score: 0.81). The generated protein models were energy-optimized using Chimera software (https://www.cgl.ucsf.edu/chimera/docs/credits.html).

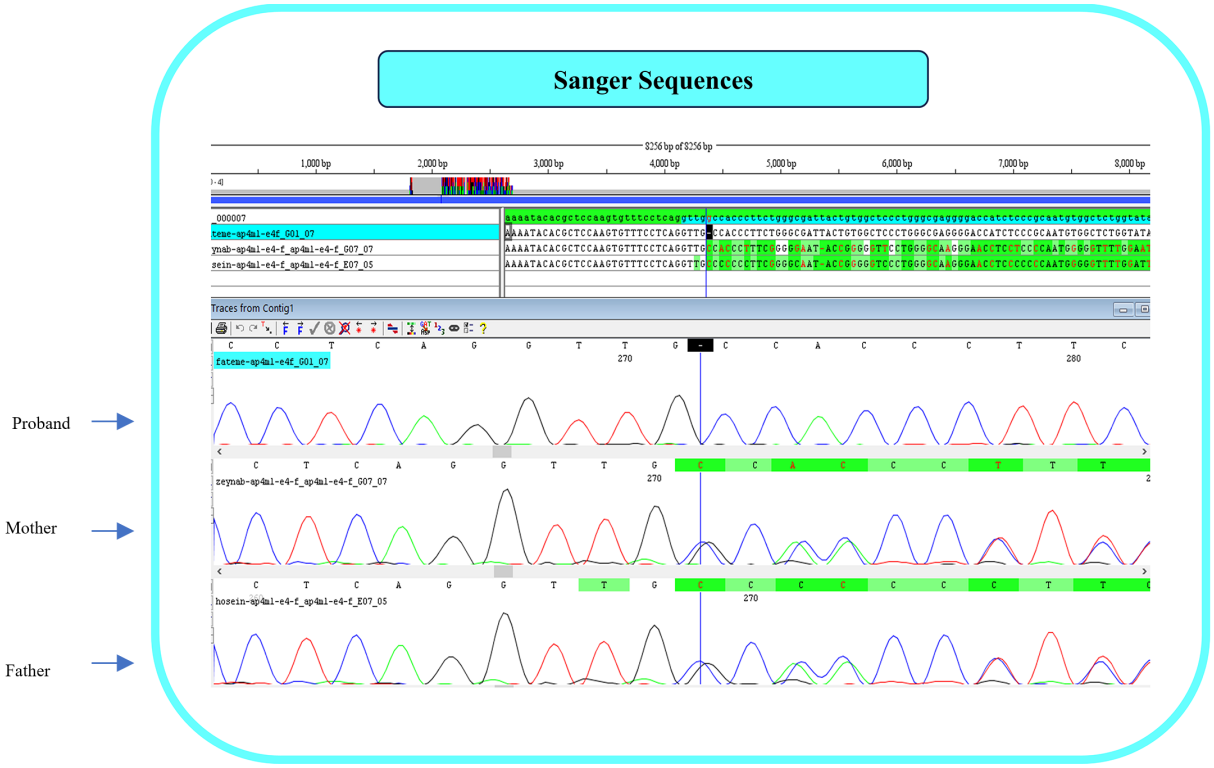

Figure 1: Sanger sequencing chromatogram confirmed the homozygous c.258delG variant in the proband. Both parents are heterozygous carriers.

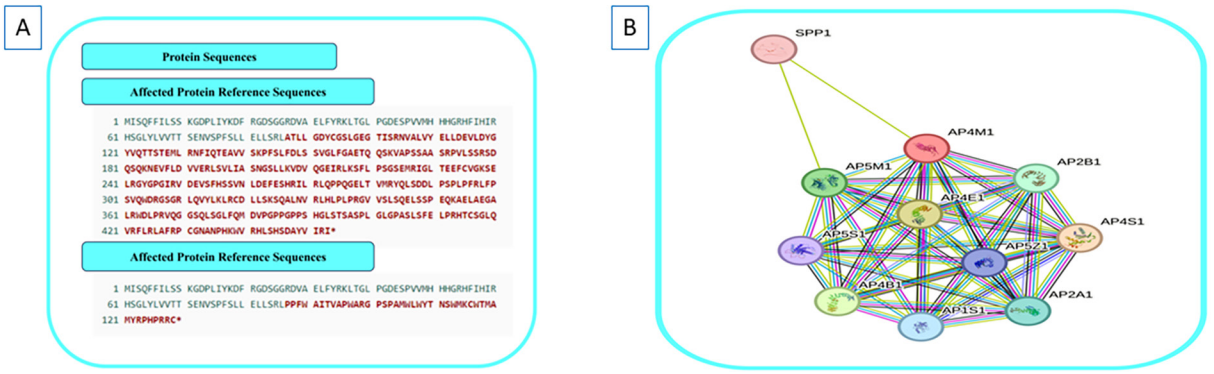

Figure 2: A: Sequence analysis of the c.258delG variant in the *AP4M1* gene using the Mutalyzer3 server shows the resulting frameshift and premature stop codon. B: The protein–protein interaction network for *AP4M1*, generated by the STRING database, indicates potential biological partners and affected pathways.

The change in Gibbs free energy (ΔΔG) values was calculated using the FoldX v4 plugin in YASARA (version 25.1.13) (https://www.yasara.org/), and ΔΔG was computed as the difference in free energy between the mutated and wild-type proteins. The results indicated that the mutation caused a decrease in protein stability, with the stability of the protein decreased from 113.72 to 79.55, corresponding to a ΔΔG change of 34.17 Kcal/mol. A ΔΔG value greater than 2 Kcal/mol typically signifies substantial protein destabilization, suggesting that the mutant version of AP4M1 has impaired structural integrity and reduced functional interaction.

Protein-protein docking of the optimized models was performed using the ClusPro server (https://cluspro.org/), and protein interactions were analyzed with the PDBsum server (https://www.ebi.ac.uk/thornton-srv/databases/pdbsum). Docking analyses demonstrated that the mutated AP4M1 binded to ARF1 through different amino acids compared to the wild-type protein, indicating improper binding that is likely to disrupt function (figure 3). The effects of the mutation of c.258delG on the *AP4M1* sequence were evaluated using the Mutalyzer3 server to simulate the impact of the mutation and generate the mutated sequence (figure 2A). Additionally, the interactions of AP4M1 with other proteins, obtained from the STRING database (https://string-db.org/), suggested that this mutation might disrupt additional biological pathways (figure 2B).

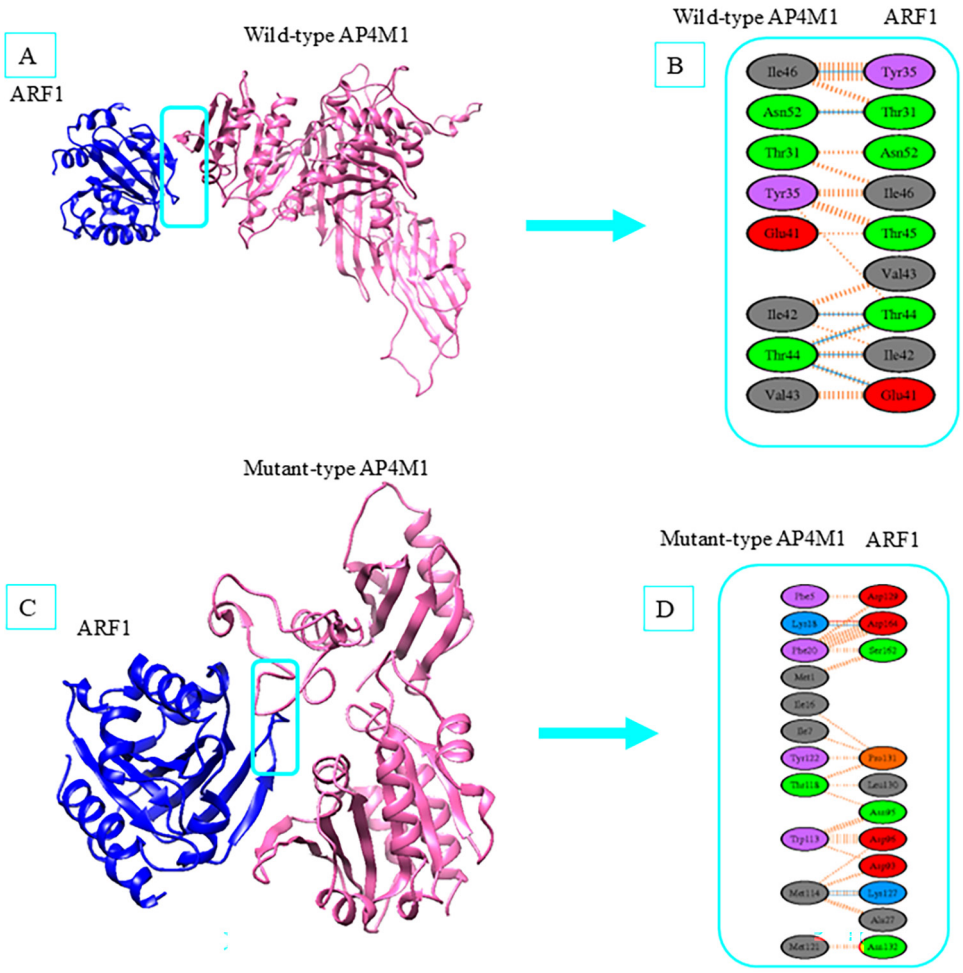

Figure 3: A: Docking result of the wild-type AP4M1 and ARF1 proteins shows a normal interaction. B: Interaction analysis of wild-type AP4M1 and ARF1, using the PDBsum server, illustrates standard contact residues. C: Docking result of the mutant AP4M1 with ARF1 (carrying the c.258delG variant) demonstrates altered binding patterns. D: Interaction analysis of the mutant AP4M1 and ARF1 using the PDBsum server reveals disrupted or shifted binding residues compared to the wild-type.

The AP4M1 variant (c.258delG:p.A87Pfs*44) was classified as pathogenic according to the ACMG guidelines. As a frameshift variant leading to a premature stop codon, it was predicted to result in a truncated protein and loss-of-function (LOF), fulfilling the pathogenic very strong 1 (PVS1) criterion. This classification is further supported by the patient's clinical features—including intellectual disability, hyperintensity in deep white matter, seizures, and motor dysfunction, which are highly specific for AP4M1-related SPG50. The pathogenic classification was also confirmed using the VarSome clinical platform (v13.10.0, March 2025).

Discussion

HSP is a genetically and clinically heterogeneous disorder, posing significant diagnostic challenges.[9] Autosomal recessive mutations in the AP4M1 subunit, which cause SPG50, represent an ultra-rare form of HSP, with only a limited number of cases reported globally.[10]

The present study reported a novel homozygous frameshift variant, c.258delG (p.Ala87Profs*44), in the *AP4M1* gene in an Iranian patient with SPG50. This variant, identified via WES data analysis, has not been previously reported. It occurred at a conserved position in the N-terminal region and is predicted to cause a large loss of the protein product due to premature termination. This domain plays an important role in regulating membrane trafficking via protein-protein interactions.[11] Consequently, we investigated the structural impact of this mutation through protein modeling and docking. The analyses demonstrated that the mutation altered the amino acids involved in the ARF1 and AP4M1 binding interface, resulting in an incorrectly formed structure that disrupts this crucial interaction.

We reviewed previously reported AP4M1 variants in Iranian families. Table 1 summarizes 12 affected individuals from diverse ethnic backgrounds (Persian, Kurdish, Lor, Turkmen, and Arab) with missense, nonsense, or frameshift mutations.

Table 1: Distribution of all *AP4M1* variants reported in the literature, including this report in Iranian families

Family number	Ethnicity	*AP4M1* genotype	Consequence	Zygosity	Classification	Diagnosis	Author, Year, Reference
1	Arab	c.1012C>T (p.Arg338)	Nonsense	Homo	Pathogenic	Spastic paraplegia	Becker et al., (2023)[13]
2	Kurd	g.99701748G > A	Missense	Homo	Pathogenic	Spastic paraplegia	Ashrafi et al., (2023)[14]
3	Persian	g.99703887A > C	Missense	Homo	Pathogenic	Spastic paraplegia	Ashrafi et al., (2023)[14]
4	Persian	g.99700491del[a]	Nonsense	Homo	Pathogenic	Spastic paraplegia	Ashrafi et al., (2023)[14]
5	Turkmen	g.99703627G > A	Missense	Homo	Pathogenic	Spastic paraplegia	Ashrafi et al., (2023)[14]
6	Persian	g.99701748G > A	Missense	Homo	Pathogenic	Spastic paraplegia	Ashrafi et al., (2023)[14]
7	Persian	g.99701748G > A	Missense	Homo	Likely pathogenic	Spastic paraplegia	Ashrafi et al., (2023)[14]
8	Persian	c.802C>T (p.R268X)	Stop codon	Homo	Likely pathogenic	Spastic paraplegia	Miryounesi et al., (2018)[15]
9	Persian	c.1225T>C(p. F409L)	Missense	Homo	Likely pathogenic	Spastic paraplegia	Miryounesi et al., (2018)[15]
10	Lur	c.1339G>A(p. Val447Met)	Missense	Homo	Pathogenic	Spastic paraplegia	Iranome database
11	Lur	c.1363G>A(p. Ala455Thr)	Missense	Homo	Pathogenic	Spastic paraplegia	Iranome database
12	Arab	c.1363G>A(p. Ala455Thr)	Missense	Homo	Pathogenic	Spastic paraplegia	Iranome database
13	Lur	c.258delG(p. A87Pfs44)	Missense	Homo	Likely Pathogenic	Spastic paraplegia (SPG50)	Present study

Despite this substantial genetic and ethnic heterogeneity, all variants were classified as pathogenic or likely pathogenic, confirming a strong disease association for *AP4M1*. While affected individuals consistently present with core features such as spasticity and developmental delay, phenotypic variability exists. For instance, increased white matter density, as observed in our patient, was not usually reported in other cases. This highlighted considerable phenotype diversity even among patients with similar genotypes. The rarity of SPG50 and the limited number of reported cases underscored the value of each new case in expanding the understanding of genotype-phenotype correlations for *AP4M1*-related disorders. The identification of our novel frameshift mutation in a Lur family further broadens this spectrum and emphasizes that *AP4M1*-related disorders are not confined to a specific ethnic group within Iran. Such diversity highlights the importance of comprehensive molecular testing across the entire population when evaluating patients with hereditary spastic paraplegia.

This novel mutation is predicted to disrupt AP4M1's interactions with other proteins through structural or functional alterations, which may subsequently impair associated cellular signaling pathways. In HSP, pathogenic variants can occur not only in the *AP4M1* gene but also in other AP4 complex subunits, including *AP4B1*, *AP4E1*, and *AP4S1*.[12] Supporting our findings, Becker and colleagues reported a homozygous nonsense variant in the *AP4M1* gene in three patients from unrelated families with spastic paraplegia.[13]

As a case report, this study had inherent limitations. Segregation analysis within the family was not possible due to the unavailability of DNA samples from other family members. Furthermore, the extreme rarity of SPG50 precluded a comparison with larger, ethnically matched cohorts.

The identification of pathogenic mutations is crucial for genetic counseling in families with a history of inherited disorders. It enables carrier testing and prenatal diagnosis through both non-invasive methods, such as the analysis of cell-free fetal DNA in maternal blood, and invasive techniques such as chorionic villus sampling. In addition, the discovery and characterization of novel mutations are fundamental to improving the prognosis and developing effective treatments for rare genetic diseases.

Conclusion

The identification of novel variants in the *AP4M1* gene is critical for improving the clinical and molecular diagnosis of SPG50 disease and enabling the provision of essential services such as genetic counseling and accurate

prognostication to affected families. In the present study, we reported for the first time an individual from a consanguineous family who presented with symptoms of intellectual disability, seizures, muscle weakness, and increased signal intensity in the deep white matter. Clinical investigations identified a novel homozygous *AP4M1* mutation (c.258delG:p.A87Pfs*44) via WES, which was confirmed through Sanger sequencing. Computational analyses further confirmed that this frameshift variant is expected to cause a substantial loss of protein function.

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Authors' Contribution

H.E.L: Supervision, study conception and design, critical revision of the manuscript; H.KH: Supervision, study conception and design, critical revision of the manuscript; M.J: Supervision, study conception and design, critical revision of the manuscript; L.A, M.Z, A.J, F.K: Data acquisition and analysis, drafting or critical revision of the manuscript. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of AI

The authors declare that no AI tools were used in the preparation of this manuscript.

Conflict of Interest: None declared.

References

1 Blackstone C. Hereditary spastic paraplegia. Handb Clin Neurol. 2018;148:633-52. doi: 10.1016/B978-0-444-64076-5.00041-7. PubMed PMID: 29478605.
2 Francisco S, Lamacchia L, Turco A, Ermondi G, Caron G, Rossi Sebastiano M. Restoring adapter protein complex 4 function with small molecules: an in silico approach to spastic paraplegia 50. Protein Sci. 2025;34:e70006. doi: 10.1002/pro.70006. PubMed PMID: 39723768; PubMed Central PMCID: PMCPMC11670165.
3 Brent JR, Deng HX. Paving a way to treat spastic paraplegia 50. J Clin Invest. 2023;133. doi: 10.1172/JCI170226. PubMed PMID: 37183815; PubMed Central PMCID: PMCPMC10178831
4 Dowling JJ, Pirovolakis T, Devakandan K, Stosic A, Pidsadny M, Nigro E, et al. AAV gene therapy for hereditary spastic paraplegia type 50: a phase 1 trial in a single patient. Nat Med. 2024;30:1882-7. doi: 10.1038/s41591-024-03078-4. PubMed PMID: 38942994; PubMed Central PMCID: PMCPMC11271397
5 Mattera R, De Pace R, Bonifacino JS. The role of AP-4 in cargo export from the trans-Golgi network and hereditary spastic paraplegia. Biochem Soc Trans. 2020;48:1877-88. doi: 10.1042/BST20190664. PubMed PMID: 33084855.
6 Boehm M, Aguilar RC, Bonifacino JS. Functional and physical interactions of the adaptor protein complex AP-4 with ADP-ribosylation factors (ARFs). EMBO J. 2001;20:6265-76. doi: 10.1093/emboj/20.22.6265. PubMed PMID: 11707398; PubMed Central PMCID: PMCPMC125733.
7 Donaldson JG, Honda A, Weigert R. Multiple activities for Arf1 at the Golgi complex. Biochim Biophys Acta. 2005;1744:364-73. doi: 10.1016/j.bbamcr.2005.03.001. PubMed PMID: 15979507.
8 American College of Medical Genetics and Genomics [Internet]. Practice Guidelines. C2025. Available from: www.acmg.net/ACMG/Medical-Genetics-Practice-Resources/Practice-Guidelines.aspx
9 de Souza PVS, de Rezende Pinto WBV, de Rezende Batistella GN, Bortholin T, Oliveira ASB. Hereditary Spastic Paraplegia: Clinical and Genetic Hallmarks. Cerebellum. 2017;16:525-51. doi: 10.1007/s12311-016-0803-z. PubMed PMID: 27271711.
10 Bettencourt C, Salpietro V, Efthymiou S, Chelban V, Hughes D, Pittman AM, et al. Genotype-phenotype correlations and expansion of the molecular spectrum of AP4M1-related hereditary spastic paraplegia. Orphanet J Rare Dis. 2017;12:172. doi: 10.1186/s13023-017-0721-2. PubMed PMID: 29096665; PubMed Central PMCID: PMCPMC5669016.
11 Rossi V, Banfield DK, Vacca M, Dietrich LE, Ungermann C, D'Esposito M, et al. Longins and their longin domains: regulated SNAREs and multifunctional SNARE regulators. Trends Biochem Sci. 2004;29:682-8. doi: 10.1016/j.tibs.2004.10.002. PubMed PMID: 15544955.
12 Alecu J, Schierbaum L, Ebrahimi-Fakhari D. AP-4-Associated Hereditary Spastic Paraplegia. In: Adam MP, Feldman J, editors.

GeneReviews (R). Washington: Seattle; 1993. PubMed PMID: 30543385.

13 Becker A, Felici C, Lambert L, de Saint Martin A, Abi-Warde MT, Schaefer E, et al. Putative founder effect of Arg338* AP4M1 (SPG50) variant causing severe intellectual disability, epilepsy and spastic paraplegia: Report of three families. Clin Genet. 2023;103:346-51. doi: 10.1111/cge.14264. PubMed PMID: 36371792; PubMed Central PMCID: PMCPMC10108251.

14 Zare Ashrafi F, Akhtarkhavari T, Fattahi Z, Asadnezhad M, Beheshtian M, Arzhangi S, et al. Emerging Epidemiological Data on Rare Intellectual Disability Syndromes from Analyzing the Data of a Large Iranian Cohort. Arch Iran Med. 2023;26:186-97. doi: 10.34172/aim.2023.29. PubMed PMID: 38301078; PubMed Central PMCID: PMCPMC10685746.

15 Miryounesi M, Tabei SMB, Dianatpour M, Fardaei M, Ghafouri-Fard S. Report of three cases with hereditary spastic paraplegia and investigation of the mutations. Meta Gene. 2018;16:105-7. doi: 10.1016/j.mgene.2018.02.009.