

SNAPC4-related neurodevelopmental disorder defines a stage-dependent spliceosomopathy with heterogeneous clinical trajectories

Marcello Scala

mscala.md@gmail.com

University of Genoa <https://orcid.org/0000-0003-2194-7239>

Mohammad Sadegh Shams Nosrati

Alexandra Ruffus

Alireza Dostmohammadi

University of Duisburg-Essen <https://orcid.org/0000-0003-4311-2056>

Amir Hesam Nemati

Francesca Madia

Chiara Fiorillo

Farooq Ahmed

MUHAMMAD UMAIR

King Abdullah International Medical Research Center (KAIMRC), King Saud Bin Abdulaziz University for Health Sciences

Guillaume Banneau

CHU de Toulouse

Sophie Julia

Inserm and Université de Toulouse 3 Paul Sabatier, UMR 1027

Run Fridriksdottir

Lesley Turner

Cassidy Welsh

Madeleine Franchi

Amy Espinal

Dandan He

Soma Das

University of Chicago

Christopher Gomez

Daniel Calame

daniel.calame@bcm.edu <https://orcid.org/0000-0001-6860-372X>

May Christine Malicdan

National Human Genome Research Institute, NIH, Bethesda, MD <https://orcid.org/0000-0003-1697-6959>

Marie Morimoto

Janna hol

Erasmus University

Cynthia Silveira

UNIVERSITY OF CAMPINAS <https://orcid.org/0000-0002-9034-1217>

França Jr Marcondes Cavalcante

Department of Neurology, School of Medical Sciences, University of Campinas (UNICAMP), 126. Cidade Universitaria <https://orcid.org/0000-0003-0898-2419>

Carelis González-Salavaz

Caleb Bupp

Maddie Donald

Joseph Junewick

Albena Todorova

Sofia Medical University

Mila Sleptsova

Daniela Avdjieva - Tzavella

Radka Maslarska

Vanyo Mitev

David Genevieve

Montpellier University, Inserm U1183, IRMB, Reference center for congenital anomalies, Clinical Genetic Unit, Montpellier University Hospital Center, Montpellier, France. <https://orcid.org/0000-0001-6928-6287>

Jérémie Mortreux

<https://orcid.org/0000-0002-2914-1961>

Mohammed Salah

Salem Alawbathani

Murtadha Ali

Gabriela Oprea

Arcensus <https://orcid.org/0000-0001-5467-5247>

Morteza Doustmohammadi

Zahra Hoseini Tavassol

Monica Traverso

IRCCS Istituto Giannina Gaslini <https://orcid.org/0000-0003-0606-8797>

Michele Iacomino

IRCCS Istituto Giannina Gaslini

Patrizia De Marco

IRCCS Istituto Giannina Gaslini

Mahsa Boogari

Davood Omrani

<https://orcid.org/0000-0002-0673-9717>

Ferruccio Romano

Valeria Capra

<https://orcid.org/0000-0002-3097-0388>

Stephen Scherer

Hospital for Sick Children <https://orcid.org/0000-0002-8326-1999>

Filippo Santorelli

IRCCS Fondazione Stella Maris <https://orcid.org/0000-0002-1359-9062>

Sara Satolli

Amy Crunk

GeneDx

Auragen Consortium

Federico Zara

University of Genoa <https://orcid.org/0000-0001-9744-5222>

Takuya Sakaguchi

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Abstract

Background

The small nuclear RNA-activating complex polypeptide 4 (SNAPC4) encodes the largest scaffolding subunit of the SNAPc complex, essential for transcription of spliceosomal small nuclear RNAs (snRNAs) by RNA polymerase II and III. Although pathogenic variants in *SNAPC4* have been associated with a rare neurodevelopmental disorder, the full phenotypic spectrum and underlying molecular mechanisms remain poorly defined.

Methods

We assembled an international cohort of 14 individuals harboring 18 distinct pathogenic *SNAPC4* variants and one multi-exon deletion, three of which had been previously reported, including frameshift, splice-site, multi-exon deletions, and missense alleles. Detailed clinical and neuroimaging data were integrated with *in silico* structural and protein–protein interaction analyses to assess the impact of these variants on SNAPc architecture and function.

Results

SNAPC4-related disease emerges as a highly heterogeneous clinical continuum rather than a uniform progressive disorder. Core features included global developmental delay and microcephaly, with variable trajectories ranging from static cognitive impairment to adult-onset progressive motor dysfunction, alongside neurobehavioral phenotypes such as autism and ADHD. Neuroimaging revealed abnormalities suggestive of impaired neurogenesis and gliogenesis, including lissencephaly-pachygyria and hypomyelinating leukodystrophy. Structural modeling supports a unifying loss-of-function (LoF) mechanism, whereby variants destabilize the SNAPc complex or disrupt key interfaces required for DNA binding and RNA polymerase recruitment, consistent with impaired SNAPc function and transcriptional dysregulation.

Conclusion

These findings redefine *SNAPC4*-related disease as a stage-dependent neurodevelopmental spliceosomopathy, linking disruption of SNAPC4 to impaired SNAPc function and transcriptional dysregulation, with direct implications for diagnosis and variant interpretation.

Introduction

The small nuclear RNA-activating complex polypeptide 4 (SNAPC4; SNAP190), located at 9q34.3, encodes the largest scaffolding subunit of the small nuclear RNA-activating protein complex (SNAPc). SNAPc directs RNA polymerase II transcription of U1, U2, U4, and U5 snRNAs and RNA polymerase III transcription of U6 snRNA [1]. As catalytic and structural components of the spliceosome, snRNAs are essential for pre-mRNA splicing and eukaryotic gene expression. The 1,469-amino-acid SNAPC4 protein contains a central Myb-like DNA-binding domain that recognizes proximal sequence elements (PSEs) within snRNA promoters and recruits the remaining SNAPc subunits (SNAPC1, SNAPC2, SNAPC3, and SNAPC5) and general transcription factors [1, 2]. Structural studies further show that SNAPC4 winged-helix motifs stabilize interactions with TFIIA, promoting DNA opening and polymerase recruitment [3].

As snRNA biogenesis is required for transcriptomic and splicing homeostasis, SNAPC4 is particularly important in the developing central nervous system, where alternative splicing demands are high. Its depletion reduces snRNA levels and causes widespread splicing dysregulation, affecting transcripts involved in mitotic microtubule regulation, RNA processing, and cytoskeletal organization [1, 4]. Consistent with this essential role, loss-of-function *Snapc4* mutations in zebrafish cause organ-specific defects, including intrahepatic biliary-network degeneration, liver dysfunction, and exocrine pancreas hypoplasia [5, 6].

Biallelic *SNAPC4* loss-of-function and missense variants were first reported in ten individuals from eight families with a rare autosomal-recessive disorder characterized by severe neurodevelopmental delay, psychomotor regression, progressive spastic paraparesis, and oromotor dysfunction (NEDRSO; MIM 620515) [1, 7]. Patient-cell studies showed SNAPc destabilization, reduced snRNA expression, and dysregulated splicing of genes involved in neuronal transport and synaptic maintenance [1]. Progressive cerebral, cerebellar, and basal ganglia atrophy supported the initial view of a uniformly progressive disorder. However, the full clinical and genetic continuum remains unknown, including whether SNAPC4 disease is limited to infantile neurodegeneration and how diverse missense variants perturb SNAPc pre-initiation-complex interfaces.

We report an international cohort of 14 individuals from independent families with autosomal recessive *SNAPC4*-related disease, carrying 18 distinct pathogenic variants (three previously reported). Combining deep clinical phenotyping, neuroimaging, *in silico* modelling, and functional assessment in zebrafish, we expand the clinical and molecular spectrum of this disorder. Our findings define *SNAPC4* deficiency as a broad, lifelong neurodevelopmental continuum and provide *in vivo* evidence that disrupted SNAPc function impairs neuromotor activity.

Materials and Methods

Study approval and ethics statement

The study was conducted in accordance with the Declaration of Helsinki and Ethics approval was obtained from the medical ethical committee installed by Gaslini Children's Hospital (Comitato Etico della Regione Liguria, approval code 163/2018). Written informed consent was obtained from parents of all

enrolled subjects for clinical testing and publication of genetic and clinical data, as well as clinical photographs. Zebrafish experiments were conducted under the IACUC protocol approved by the Cleveland Clinic Foundation (Protocol 2812).

Subject enrolment and ascertainment

Affected individuals were recruited through international collaboration involving multiple clinical and research centers, also using match maker platforms such as GeneMatcher [8].

Clinical evaluation and phenotyping

Clinical and phenotypic data for the expanded cohort of 14 affected individuals were gathered retrospectively through an international network of collaborating referring clinicians. To ensure systematic data collection across multiple centers, comprehensive clinical information was extracted from medical records utilizing a standardized phenotyping questionnaire. Assessed domains included prenatal and birth history, neurodevelopmental milestones, longitudinal motor and cognitive function, behavioral/psychiatric manifestations, growth parameters, and systemic physical examinations. Where applicable, clinical features were standardized utilizing Human Phenotype Ontology (HPO) terminology to facilitate accurate cross-cohort comparison. To explicitly delineate the expanding phenotypic spectrum of the disorder, the clinical architecture of our cohort was systematically analyzed and contrasted against the established baseline of previously published *SNAPC4* cases.

Genetic investigation and variant analysis

Exome sequencing (ES) was performed on genomic DNA extracted from peripheral blood of affected individuals and their parents, as previously described [9]. Variants were prioritized according to allele frequency, evolutionary conservation, predicted functional impact, and consistency with recessive inheritance. *In silico* predictions were obtained using established tools (CADD, PolyPhen-2, SIFT), and all candidate variants were validated by Sanger sequencing and segregation analysis when possible. Variant pathogenicity was classified following ACMG/AMP 2015 criteria, with ClinGen 2021 refinements, integrating evidence from gnomAD v4.1, ClinVar, and published literature [10, 11].

Neuroimaging evaluation

Clinical neuroimaging data, primarily brain and spinal magnetic resonance imaging (MRI), were obtained for affected individuals as part of their standard diagnostic workup. Scans were reviewed to evaluate gross cerebral, cerebellar, and brainstem architecture, as well as specific structural anomalies. Assessment of white matter myelination patterns, cortical gyration, and deep gray matter signal integrity was performed utilizing standard multiparametric MRI sequences, including T1-weighted, T2-weighted, and Fluid-Attenuated Inversion Recovery (FLAIR) imaging.

In Silico structural modeling and variant pathogenicity prediction

As no experimentally determined structure is available for the 1,469-amino-acid *SNAPC4* protein, we used the AlphaFold2-predicted model (**Figure S1**) to evaluate the structural impact of identified variants [12]. The model is publicly available through the AlphaFold Protein Structure Database (AF-Q5SXM2-F1-v4) [13].

Variant effects were assessed using a multi-tool in silico approach. ThermoMPNN was used to predict protein stability changes ($\Delta\Delta G$) [14], while SIFT, PolyPhen-2, and Combined Annotation Dependent Depletion (CADD) assessed evolutionary conservation and predicted pathogenicity [15–17]. A CADD PHRED score ≥ 20 was considered supportive of deleteriousness, corresponding to the top 1% of potentially deleterious single-nucleotide variants in the human genome.

Protein-protein interaction (PPI) analysis

To predict the effect of specific variants on *SNAPC4* interactions with its protein partners, we analyzed experimentally determined protein-protein complex structures containing *SNAPC4*. We focused on two complexes with a moderate number of subunits: the *SNAPc*-containing RNA polymerase II pre-initiation complex bound to the U1 snRNA promoter (determined by electron microscopy at 3.5 Å resolution) and the *SNAPc*:TBP–TFIIA–TFIIB sub-complex bound to the U5 snRNA promoter (determined at 3.2 Å resolution). Due to the size and complexity of larger *SNAPC4*-containing assemblies, computational prediction of variant effects on PPI affinity within the resolved regions was performed specifically using mCSM-PPI2^[18] and the graph-based deep learning tool DDMut-PPI [19].

Zebrafish Movement analysis

Zebrafish (*Danio rerio*) larvae were generated by natural crosses of *snapc4*^{s445} heterozygous mutant fish, maintained at 28.5°C, and staged by hours post-fertilization (hpf), as previously described [6]. At 120 hpf, larvae were individually placed in 96-well plates and video-recorded under a dissecting microscope (Leica M125 C). Larvae were subsequently genotyped for the *snapc4*^{s445} mutation as previously reported [5].

Locomotor activity was quantified from 2560 × 1440-pixel, 30-frames-per-second video recordings using automated motion detection in OpenCV. Frames were resized to 40% of their original dimensions and every fifth frame was analyzed, yielding an effective sampling rate of 6 frames per second. Analyzed

frames were converted to grayscale, smoothed using a 5×5 Gaussian blur, and processed with the MOG2 adaptive background subtraction algorithm (variance threshold = 25; history = 200 frames). Foreground masks were refined by morphological opening using a 5×5 elliptical kernel (one iteration), followed by dilation (two iterations). Contours smaller than 500 pixels in downsampled images were excluded as noise.

For each frame, total motion area, motion coverage, and the number of moving regions were recorded. Motion areas were rescaled to original-image pixel units. Per-video mean motion coverage and the proportion of analyzed frames with detected motion were calculated. Group data are presented as mean $\pm$ SEM for wild-type ($n = 10$) and *snpc4*^{s445} mutant ($n = 6$) larvae. $P < 0.05$; $P < 0.01$; N.S., not significant ($P > 0.05$).

Results

Phenotypic spectrum of SNAPC4-related disorders

Core clinical features and expanded phenotypic spectrum

Historically, the clinical spectrum of *SNAPC4*-related disorders has been defined by a highly homogeneous presentation of early-onset developmental delay, subsequent psychomotor regression, progressive spastic paraparesis, oromotor dysfunction, and microcephaly. Analysis of our expanded cohort of 14 individuals confirms that early-onset global developmental delay (GDD), prominent speech and oromotor impairment, and growth restrictions (microcephaly and short stature) constitute the core phenotypic baseline. Lower-limb spasticity, axial hypotonia, and ataxic gait also remain highly prevalent across the cohort (Fig. 1A).

Neurobehavioral and psychiatric manifestations

A notable and previously underrecognized aspect of *SNAPC4*-related disease is the presence of significant neurobehavioral and psychiatric involvement. While earlier reports frequently described relatively preserved social engagement and a generally cheerful disposition despite progressive motor decline, individuals in our cohort exhibited a broader and clinically significant neurobehavioral phenotype.

Severe behavioral dysregulation was observed in multiple individuals, including self-injurious behaviors, repetitive motor stereotypies such as hand-flapping, and pronounced behavioral insomnia (Cases 6 and 9), often requiring clinical management. These features were frequently associated with impaired impulse control and difficulty in behavioral regulation, suggesting involvement of higher-order neurocognitive processes.

Formal psychiatric diagnoses were also documented. Case 10 was diagnosed with attention-deficit/hyperactivity disorder (ADHD), disruptive mood dysregulation disorder, and conduct disorder, indicating a complex behavioral profile with both externalizing and affective components. In addition, features consistent with autism spectrum disorder (ASD), including impaired social interaction, limited eye contact, and reduced communicative reciprocity, were explicitly noted in Case 14. Internalizing symptoms were also present, with anxiety and panic disorders reported in adulthood in Case 9.

Importantly, these neurobehavioral manifestations were observed across different stages of disease and were not restricted to individuals with the most severe motor or cognitive impairment, indicating that psychiatric involvement may represent an independent and clinically relevant dimension of the phenotype.

Overall, these findings expand the neuropsychiatric spectrum of *SNAPC4*-related disease and suggest that dysfunction extends beyond motor and developmental domains to include higher-order cognitive and behavioral circuits.

Neuroimaging findings

Neuroimaging revealed a heterogeneous spectrum of structural and signal abnormalities, expanding the recognized radiological profile of *SNAPC4*-related disease. Although previous reports emphasized progressive cerebellar, cerebral, and basal ganglia atrophy, normal or age-appropriate brain MRI was observed in Cases 6, 10, 12, and 14. Notably, Case 10 had clinical microcephaly without detectable structural abnormalities or myelination defects (Fig. 1B).

When present, abnormalities involved distinct neurodevelopmental processes. Case 13 showed severe lissencephaly-pachygyria, with an abnormally broad, smooth cortical ribbon and markedly reduced gyral and sulcal patterns across the bilateral frontoparietal, temporal, and occipital lobes. Mild reduction of underlying white matter volume supported a neuronal migration abnormality. In contrast, Case 2 exhibited a hypomyelinating leukodystrophy-like phenotype, with diffuse hypomyelination predominantly affecting the frontal and temporal lobes and extending through the centrum semiovale, corona radiata, and internal and external capsules. Corpus callosum hypoplasia and a focal left caudate T2 hyperintensity were also present.

Atrophic changes resembling previously reported patterns occurred in Case 3, who showed marked global cerebellar atrophy associated with microcephaly, and in Case 9, who had localized occipital volume loss with prominence of adjacent sulci and asymmetric dilation of the occipital horns and atria of the lateral ventricles. Case 1 showed faint T2/FLAIR hypointensities in the globus pallidus, substantia nigra, red nuclei, and dentate nuclei without cerebral atrophy; spinal imaging revealed attenuation of cervical lordosis and minimal C5–C7 disc protrusions. Case 8 had largely preserved brain architecture, minor nonspecific white matter lesions, a stable left temporal arachnoid cyst, and mild cerebellar atrophy.

Overall, *SNAPC4*-related neuroimaging ranges from normal brain structure to severe cortical malformations, hypomyelination, and atrophic changes, supporting disruption across multiple developmental stages.

Systemic findings

Although *SNAPC4*-related disease is predominantly neurological, our data suggest possible extra-neurological involvement. Congenital cardiac anomalies were identified in two unrelated individuals (Cases 3 and 11), both with septal defects involving the atrial and ventricular septa. Cardiac involvement was not prominent in previous reports; therefore, its recurrence in this cohort should be interpreted cautiously, although it may indicate broader developmental effects of *SNAPC4* dysfunction. No consistent pattern of extracranial organ involvement was observed.

Hepatic abnormalities were identified in Case 6, who harbored p.(Arg1126Serfs*58) and p.(Asp164His) in trans. The patient had constipation and abdominal pain and was followed by pediatric gastroenterology. At 11 years, abdominal ultrasound showed diffuse moderate-to-severe hepatic echogenicity consistent with steatosis, with a normal gallbladder. Transaminases remained elevated (AST, 91 U/L [normal 13–26]; ALT, 71 U/L [normal 8–22]), and marked hyperinsulinemia was documented over multiple years (peak insulin up to 1198 pmol/L), requiring metformin treatment. The patient was borderline overweight (94th percentile).

Together, these findings indicate that, while *SNAPC4* deficiency primarily causes a neurodevelopmental disorder, selected individuals may show systemic manifestations that warrant clinical surveillance.

Variable disease trajectories and expressivity

This expanded cohort challenges the previously assumed uniformity of neurological progression associated with biallelic *SNAPC4* variants. We observed divergent disease trajectories in the reported individuals. Psychomotor regression, previously considered a hallmark feature, was absent in a subset of individuals (4/14; Cases 2, 3, 10, and 12), who instead demonstrated static GDD or mild cognitive impairment persisting into late adolescence. In addition, the temporal onset of disease is substantially broadened. Case 8 achieved age-appropriate developmental milestones before presenting at 21 years with rapidly progressive, left-predominant spastic paraparesis, tremor, and severe bulbar dysfunction (dysphagia and esophageal spasms). Variable expressivity of key neurological features was also evident. Progressive spastic paraparesis was absent in three individuals (Cases 3, 6, and 10), who exhibited normal muscle tone and intact deep tendon reflexes despite severe intellectual disability or marked growth restriction.

Based on clinical course and age at onset, affected individuals can be broadly grouped into three major trajectories, although overlap exists: 1) congenital or early severe neurodevelopmental phenotypes, including early lethal cases (Cases 4 and 5) and individuals with cortical malformations or profound developmental impairment (Cases 3, 11, and 13); 2) static developmental phenotypes characterized by persistent but non-progressive deficits (Cases 2, 10, and 12, and possibly Case 1); 3) progressive phenotypes, ranging from childhood-onset regression (Cases 6, 9, and 14) to adult-onset neurological disease (Case 8). Notably, some individuals (e.g., Case 7) do not fit neatly within this framework, further supporting the continuous and heterogeneous nature of *SNAPC4*-related disease. Notably, individuals within the progressive group include both childhood-onset regression and adult-onset disease, further emphasizing the temporal heterogeneity of *SNAPC4*-related phenotypes. As previously shown (Fig. 1C), the distribution of individual cases across distinct clinical trajectories based on age at onset and disease course highlights the coexistence of heterogeneous clinical trajectories within the same genetic condition.

Mutational landscape of *SNAPC4*

Exome sequencing identified 18 distinct *SNAPC4* variants and one multi-exon deletion across 14 individuals, three of which had been previously reported (Table 1, **Table S1**), including nonsense, frameshift, canonical splice-site, missense variants, and one multi-exon deletion. Variants were distributed across the coding sequence and were consistent with an autosomal recessive inheritance model (Fig. 2A, B). All variants were submitted to the LOVD database with the following accession numbers: #0001075984, #0001075985, #0001075986, #0001075987, #0001075988, #0001075990, #0001075991, #0001075992, #0001075993, #0001075994, #0001075995, #0001075996, #0001075997, #0001075998, #0001075999, #0001076000, #0001076001, #0001076002, #0001076003. The presence of copy number variations (CNVs) was investigated through array comparative genomic hybridization (aCGH) or sequencing-based CNV calling (Supplemental Material).

Table 1
Clinical manifestations of patients harboring *SNAPC4* variants in the study cohort.

Case	<i>SNAPC4</i> (NM_003086 .4) variant(s) (c.)	<i>SNAPC4</i> (NM_003086 .4) variant(s) (protein)	Inheritance	Disease course	Core neurological phenotype	Neuroimaging	Neurobehavioral features
1	c.1889_1899del; c.1436T > C (PR)	p.(Val630Glyfs*76); p.(Ile479Thr)	Compound het	Static	Spastic paraparesis; distal muscle atrophy	Deep gray matter signal abnormalities; no cerebral atrophy	None reported
2	c.451A > T	p.(Lys151*)	Homozygous	Static	Severe DD/ID; hypotonia; gait impairment	Diffuse hypomyelination; corpus callosum hypoplasia	None reported
3	c.2527 + 1G > A (PR)	p.(Ser835Thrfs*86)	Homozygous	Static	Motor and speech delay; microcephaly; cerebellar involvement	Global cerebellar atrophy	None reported
4	c.3378_3385del	p.(Arg1126Serfs*58)	Homozygous	Very severe (lethal)	DD/ID; neurodegeneration	Not available	None reported
5	c.3378_3385del	p.(Arg1126Serfs*58)	Homozygous	Very severe (lethal)	DD/ID; neurodegeneration	Not available	None reported
6	c.3378_3385del; c.490G > C	p.(Arg1126Serfs*58); p.(Asp164His)	Compound het	Progressive (with regression)	ID; seizures; unsteady gait	Normal MRI	Behavioral dysregulation; insomnia
7	c.3758_3788del; c.4282C > G	p.(Asp1253Glyfs*144); p.(Gln1428Glu)	Compound het	Early developmental	Abnormal movements; seizure-like episodes	Not available	Not reported
8	c.2410G > A; c.2771C > T	p.(Gly804Ser); p.(Ser924Phe)	Compound het	Adult-onset progressive	Spastic paraparesis; dysarthria; ataxia	Mild cerebellar atrophy	Anxiety; insomnia
9	c.1211G > A; c.1244C > T	p.(Trp404Ter); p.(Ala415Val)	Compound het	Progressive (with regression)	Spastic paraparesis; dysmetria; weakness	Occipital volume loss; ventricular enlargement	Anxiety; depression; insomnia
10	c.1073C > T; c.4361T > A	p.(Thr358Met); p.(Leu1454His)	Compound het	Static	Microcephaly; mild ID; speech delay	Normal MRI	ADHD; DMI; conduct disorder
11	c.1306G > A; c.4124C > T	p.(Asp436Asn); p.(Pro1375Leu)	Compound het	Severe developmental	Severe DD; hypotonia; hydrocephalus	Pontocerebellar and corpus callosum hypoplasia	None reported
12	c.914T > A; exons 14–19 deletion (g.136381084_136387365del)	p.(Leu305Gln); -	Compound het	Static	Global DD; hypotonia; spasticity; ataxia	Not available	None reported
13	c.2067 + 1G > T	-	Homozygous	Developmental	Global DD; absent speech; spastic diplegia	Lissencephaly–pachygyria	None reported
14	c.2067 + 1G > T	-	Homozygous	Developmental (with regression)	DD; speech delay	Normal MRI	Autism spectrum disorder

Abbreviations: PR = previously reported by Frost et al., 2023.

Variant spectrum and inheritance

Biallelic variants were identified in all affected individuals, supporting an autosomal recessive mode of inheritance. Homozygous variants included: c.451A > T, p.(Lys151*) in Case 2; c.2527 + 1G > A, p.(Ser835Thrfs*86) in Case 3; c.3378_3385del, p.(Arg1126Serfs*58) in Cases 4 and 5; c.2067 + 1G > T in Cases 13 and 14. The remaining individuals carried compound heterozygous configurations, including combinations of LoF and missense variants, two missense variants, or a missense variant in trans with a multi-exon deletion. Overall, the variant distribution supports a predominantly biallelic LoF or LoF-plus-missense mechanism underlying *SNAPC4*-related disease.

Loss-of-function variants represent the predominant genetic mechanism

LoF variants were predominant and included frameshift variants (c.1889_1899del; p.(Val630Glyfs*76), c.3378_3385del; p.(Arg1126Serfs*58), and c.3758_3788del; p.(Asp1253Glyfs*144)), nonsense variants (p.(Lys151*) and p.(Trp404*)), canonical splice-site variants (c.2067 + 1G > T and c.2527 + 1G > A), and one multi-exon deletion (exons 14–19 in Case 12, in trans with p.(Leu305Gln)). These variants are predicted to result in protein truncation, aberrant splicing, or nonsense-mediated decay, and were classified as pathogenic or likely pathogenic. Collectively, the predominance of biallelic LoF variants strongly supports loss of SNAPC4 function as a major disease mechanism.

Missense variants support functional disruption of SNAPC4

Missense variants affected highly conserved residues and were mostly observed in compound heterozygosity with LoF alleles. Representative variants included p.(Asp164His) (CADD = 27) and p.(Thr358Met) (CADD = 24), both absent from gnomAD and predicted to be deleterious. Additional variants, including p.(Leu305Gln), p.(Ala415Val), p.(Ile479Thr), p.(Gly804Ser), p.(Ser924Phe), p.(Leu1454His), and p.(Asp436Asn), similarly affected conserved residues within functionally relevant or constrained regions of SNAPC4. Most missense variants fulfilled criteria supporting a likely pathogenic classification, while two variants (p.(Pro1375Leu) in Case 11 and p.(Gln1428Glu) in Case 7) remained of uncertain significance due to limited supporting evidence.

Structural and functional consequences of SNAPC4 variants

Predicted Impact on Protein Stability and Pathogenicity

We evaluated the structural and functional impact of twelve missense variants using protein modeling and stability prediction. The majority of variants, including p.(Asp164His), p.(Leu305Gln), p.(Ala415Val), p.(Asp436Asn), p.(Ile479Thr), p.(Gly804Ser), p.(Ser924Phe), p.(Pro1375Leu), and p.(Leu1454His), were predicted to destabilize the SNAPC4 protein, with p.(Leu305Gln) and p.(Ile479Thr) showing the strongest effects ($\Delta\Delta G > 1$ kcal/mol). These predictions were broadly consistent across multiple *in silico* tools (see methods), which classified most variants as deleterious and were consistent with pathogenicity (Fig. 3A).

Domain mapping and functional disruption

Mapping of missense variants to functional domains revealed clustering within regions critical for DNA binding and protein–protein interactions (**Figure S1A**). Variants p.(Leu305Gln), p.(Ile479Thr), and p.(Asp436Asn) localize to helix-turn-helix Myb domains involved in DNA binding, while p.(Ala415Val) also maps to a Myb-type domain (**Figure S1B**). In contrast, p.(Pro1375Leu) lies within the SNAPC2-binding region, suggesting disruption of SNAPc complex assembly. These findings indicate that pathogenic variants are likely to impair SNAPC4 function either by disrupting DNA interaction or by destabilizing essential protein–protein interfaces.

Variant-specific effects and functional divergence

Not all variants were predicted to act through protein destabilization. The p.(Gln1428Glu) variant showed a stabilizing effect and remained classified as a Variant of Uncertain Significance (VUS) according to strict ACMG criteria.; Given its occurrence in trans with a frameshift variant and the limitations of current predictive tools, the contribution of p.(Gln1428Glu) to disease pathogenicity appears limited and remains uncertain.

In contrast, p.(Thr358Met), despite predicted stabilizing effects, was consistently classified as deleterious and localizes to a critical Myb-like domain. This suggests a potential mechanism involving altered protein dynamics or reduced functional flexibility, rather than simple destabilization.

Impaired protein-protein interactions

To further assess the impact on SNAPc complex assembly, selected variants (p.(Asp164His), p.(Leu305Gln), and p.(Thr358Met)) were computationally analyzed within resolved pre-initiation complexes (Figs. 3B, C). All three variants showed reduced protein–protein interaction affinity, indicating disruption of SNAPc complex formation. Together, these findings support a unifying mechanism in which both protein destabilization and disruption of critical interaction interfaces converge to impair SNAPc assembly and transcriptional function.

Zebrafish *snapc4*^{s445} mutant larvae show reduced spontaneous locomotion without overt morphological abnormalities.

The *snapc4*^{s445} allele introduces a premature stop codon (p.Gln937*), predicted to truncate the protein and remove the C-terminal SNAPC2-binding domain while preserving upstream domains (Fig. 4A). Synteny analysis further demonstrated conservation of the genomic region surrounding zebrafish *snapc4* and human SNAPC4, supporting orthology between the two loci (Fig. 4B).

We previously reported that *snapc4*^{s445} mutant zebrafish display hepatopancreatic developmental defects. We therefore investigated whether these mutants also exhibit locomotor abnormalities. At 120 hours post-fertilization (hpf), *snapc4*^{s445} mutant larvae did not show overt gross morphological abnormalities compared with wild-type (WT) siblings (Fig. 4C, D).

To assess neuromotor function, we recorded spontaneous locomotor activity at 5 days post-fertilization (dpf) and observed a marked reduction in movement in *snapc4*^{s445} mutant larvae compared with WT siblings (Fig. 4E, F; **Supplementary Movies 1 and 2**). Quantitative analysis confirmed a significant decrease in both average motion coverage and the proportion of active frames in mutants (Fig. 4G, H). This reduction was consistent across multiple independent locomotor metrics (Fig. 4E–H). Notably, these functional deficits occur in the absence of overt morphological abnormalities at this stage. Together, these findings indicate that loss of *snapc4* function results in early neuromotor impairment in zebrafish larvae.

Discussion

We report 14 individuals harboring 18 pathogenic SNAPC4 variants, including three previously reported substitutions, substantially expanding the genetic and clinical spectrum of SNAPC4-related disease. Rather than an exclusively early-onset progressive disorder, SNAPC4 deficiency emerges as a lifelong neurodevelopmental continuum. Integrating clinical, neuroimaging, structural, and zebrafish data, we propose that impaired SNAPC-mediated snRNA transcription affects embryonic corticogenesis, myelinogenesis, postnatal neuronal maintenance, and higher-order network maturation. SNAPC4 therefore links basal transcriptional machinery to selective neuronal vulnerability (Fig. 1C, D). The coexistence of static and progressive phenotypes also supports a role in long-term neuronal homeostasis, consistent with the broad consequences of RNA-processing and spliceosomal dysfunction in neurodevelopmental disorders [20–24].

Our data expands the previously defined phenotypic boundaries. Although developmental delay and microcephaly remain core features, the absence of classical regression in Cases 2, 3, 10, and 12 demonstrates that impaired snRNA transcription can produce stable impairment rather than obligate neurodegeneration. Conversely, Case 8 attained age-appropriate milestones before onset at 21 years, suggesting that hypomorphic alleles may maintain sufficient early activity but fail when transcriptional demands increase [3, 25, 26]. Severe self-injurious behavior, ADHD, and autism spectrum disorder further indicate that impaired SNAPC-mediated transcription affects cognitive, behavioral, and synaptic networks beyond neuronal survival [1, 27, 28].

Neuroimaging supports temporally distinct vulnerabilities. In addition to the reported progressive cerebral, cerebellar, and basal ganglia atrophy [1], we identify lissencephaly-pachygyria in Case 13, suggesting disturbed neuronal proliferation or migration, and hypomyelinating leukodystrophy in Case 2, indicating impaired gliogenesis and white matter maturation [29–32]. These developmental findings coexist with unremarkable conventional MRI in severely affected Cases 6, 10, and 12, emphasizing the stage-specific and cell-type-specific consequences of SNAPC4 dysfunction [33–35].

Diverse variants are predicted to converge on impaired SNAPC stability and function. Truncating and splice-site variants are compatible with loss of function, potentially through nonsense-mediated decay, whereas missense variants likely cause domain-specific disruption. Myb-domain variants may impair binding to the proximal sequence element (PSE), while p.(Asp164His), p.(Leu305Gln), and p.(Thr358Met), located in resolved SNAPC4-containing complexes, are predicted to weaken protein–protein interactions and pre-initiation-complex stabilization [3, 36, 37]. This heterogeneity is reconciled by SNAPC4 (SNAP190) modular architecture (Fig. 2B, C): the amino terminus nucleates the core complex with SNAPC3 and SNAPC1, the Myb domain recognizes the PSE, and the carboxy terminus recruits SNAPC2 and enables RNA polymerase II/III engagement [2, 3]. Thus, variants may affect distinct steps of one assembly pathway. In particular, C-terminal p.(Arg1126Serfs*58) and p.(Pro1375Leu) may disrupt the SNAPC2 interface while preserving Myb-mediated DNA binding, yielding promoter-bound but assembly- or activation-deficient complexes with reduced snRNA output.

This model explains severe neurological dysfunction despite preserved gross morphology or non-specific MRI. Partial promoter binding may permit early developmental programs, but insufficient regulated transcription may compromise mature neuronal circuits. The *snapc4*^{s445} zebrafish allele, p.(Gln937*), removes the C-terminal SNAPC2-binding region while preserving upstream domains, including the Myb DNA-binding region. Mutant larvae showed markedly reduced spontaneous locomotion without overt gross abnormalities, supporting a requirement for this interface in neuromotor function in vivo and underscoring limitations of minimal SNAPC reconstitution systems.

Possible systemic involvement merits consideration. Case 6, carrying p.(Arg1126Serfs*58) in trans with p.(Asp164His), had unremarkable brain MRI but hepatic steatosis, persistent transaminase elevation, and marked hyperinsulinemia requiring treatment. Although metabolic contributors cannot be excluded, this parallels hepatopancreatic abnormalities in *snapc4* mutant zebrafish and earlier animal models [5, 6]. The same truncation was homozygous in early-lethal Cases 4 and 5, raising the possibility that disruption of the SNAPC2 interface contributes to a partially separable multisystem phenotype.

A severity gradient may unify the spectrum. Variants severely impairing complex stability, promoter engagement, or assembly could cause early snRNA and splicing defects, disrupting corticogenesis and migration, as in lissencephaly-pachygyria. Milder perturbations may permit early development but fail in mature neurons, causing later neurodegeneration, spasticity, or psychiatric manifestations. Reported dysregulation of mitotic microtubule regulators [1] provides a plausible link to migration defects, whereas hypomyelination in Case 2 may reflect the high splicing demands of differentiating oligodendrocytes during myelinogenesis [38]. These findings place SNAPC4 deficiency among neurodevelopmental and neurodegenerative spliceosomopathies, alongside GEMIN5, WBP4, and primary U2 snRNA disorders [39–41].

In conclusion, SNAPC4-related disease spans congenital cortical malformations, both stable and progressive neurodevelopmental impairment, psychiatric features, and, in some individuals, systemic involvement. Stage- and domain-dependent disruption of promoter recognition, SNAPC assembly, and neuromotor function offers a unifying framework for this phenotypic spectrum. Patient-derived iPSCs, cerebral organoids, and complementary in vivo models will be needed to resolve the temporal dynamics of snRNA depletion and its downstream splicing consequences.

Declarations

Author contributions

Conceptualization: M.S.S.N., M.S. and T.F.S. **Study coordination and supervision:** M.S.S.N., F.Z., M.S. and T.F.S. **Clinical and genetic data curation:** M.S.S.N., with input from all contributing clinical and diagnostic centers. **Clinical phenotyping, diagnostic evaluation, case-level data collection and/or variant interpretation:** F.M., C.F., F.A., M.U., G.B., S.J., R.F., L.T., C.W., M.F., A.E.E., D.H., S.D., C.M.G., D.C., M.C.M., M.M., J.H., C.S., M.C.F.J., C.G.-S., C.P.B., M.D., J.J.J., A.T., M.S.I., D.A.-T., R.M., V.M., D.G., J.M., M.A.S., S.A., M.A., G.O., M.Dou., Z.H.T., M.T., M.I., P.D.M., M.B., M.D.O., F.R., V.C., S.W.S., F.M.S., S.S. and A.C. **Consortium contribution:** Members of the Auragen Consortium contributed relevant clinical, genetic, diagnostic and/or case-level information, as applicable. **Zebrafish investigation and locomotor analysis:** A.S.R. and T.F.S. **Structural modeling and protein–protein interaction analysis:** A.D. and A.H.N. **Data interpretation:** M.S.S.N., A.D., A.H.N., A.S.R., T.F.S. and M.S. **Writing – original draft:** M.S.S.N. and M.S., with input from A.D., A.H.N., A.S.R. and T.F.S. **Writing –**

review and editing: All authors. **Corresponding authors and overall supervision:** M.S. and T.F.S. All authors reviewed and approved the final manuscript and agree to be accountable for the work.

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Competing interests

A.C. is employees of GeneDx and may own stock in the mentioned comany. The remaining authors declare no conflicts of interest.

Data Availability

Data supporting the findings of this study are available from the corresponding authors upon reasonable request, in accordance with ethical and privacy regulations.

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Figures

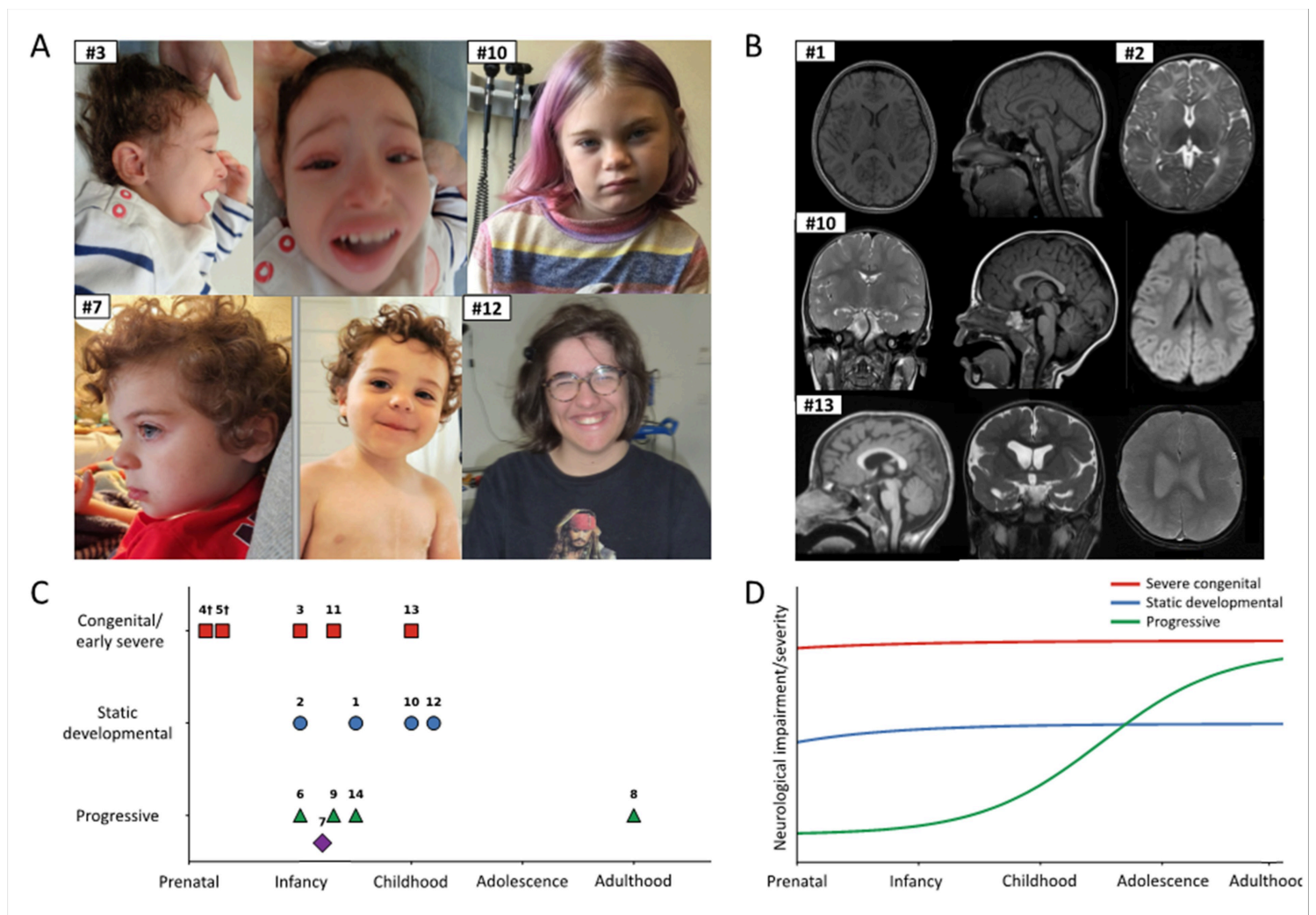


Figure 1

Clinical and neuroimaging spectrum of *SNAPC4*-related disease and associated disease trajectories. (A) Representative clinical photographs of individuals with *SNAPC4*-related disease, illustrating the phenotypic variability across the cohort. Case identifiers are indicated. (B) Brain MRI findings in selected individuals. Cases #1 and #10 show no major structural abnormalities, whereas Case #13 demonstrates cortical malformation consistent with lissencephaly–pachygyria. These findings highlight the marked variability in neuroimaging features, ranging from normal MRI to severe structural brain abnormalities. (C) Distribution of affected individuals according to age at onset and clinical course. Cases cluster into three main groups: congenital/early severe phenotypes (red squares; including early lethal cases #4 and #5, and individuals with major developmental impairment or structural brain abnormalities such as #3, #11, and #13), static developmental phenotypes (blue circles; cases #2, #1, #10, and #12), and progressive phenotypes (green triangles; cases #6, #9, and #14 with childhood-onset regression, and case #8 with adult-onset disease). Case #7 (purple diamond) does not fit neatly within these categories and is classified as having an uncertain trajectory. This representation underscores the clinical heterogeneity and continuum of disease presentation. (D) Conceptual model of disease trajectories in *SNAPC4*-related disorder. Three major patterns are illustrated: severe congenital phenotypes characterized by early and sustained impairment, static developmental phenotypes with stable deficits over time, and progressive phenotypes with delayed onset and worsening neurological impairment. These trajectories reflect stage-dependent vulnerability to *SNAPC4* dysfunction across neurodevelopment.

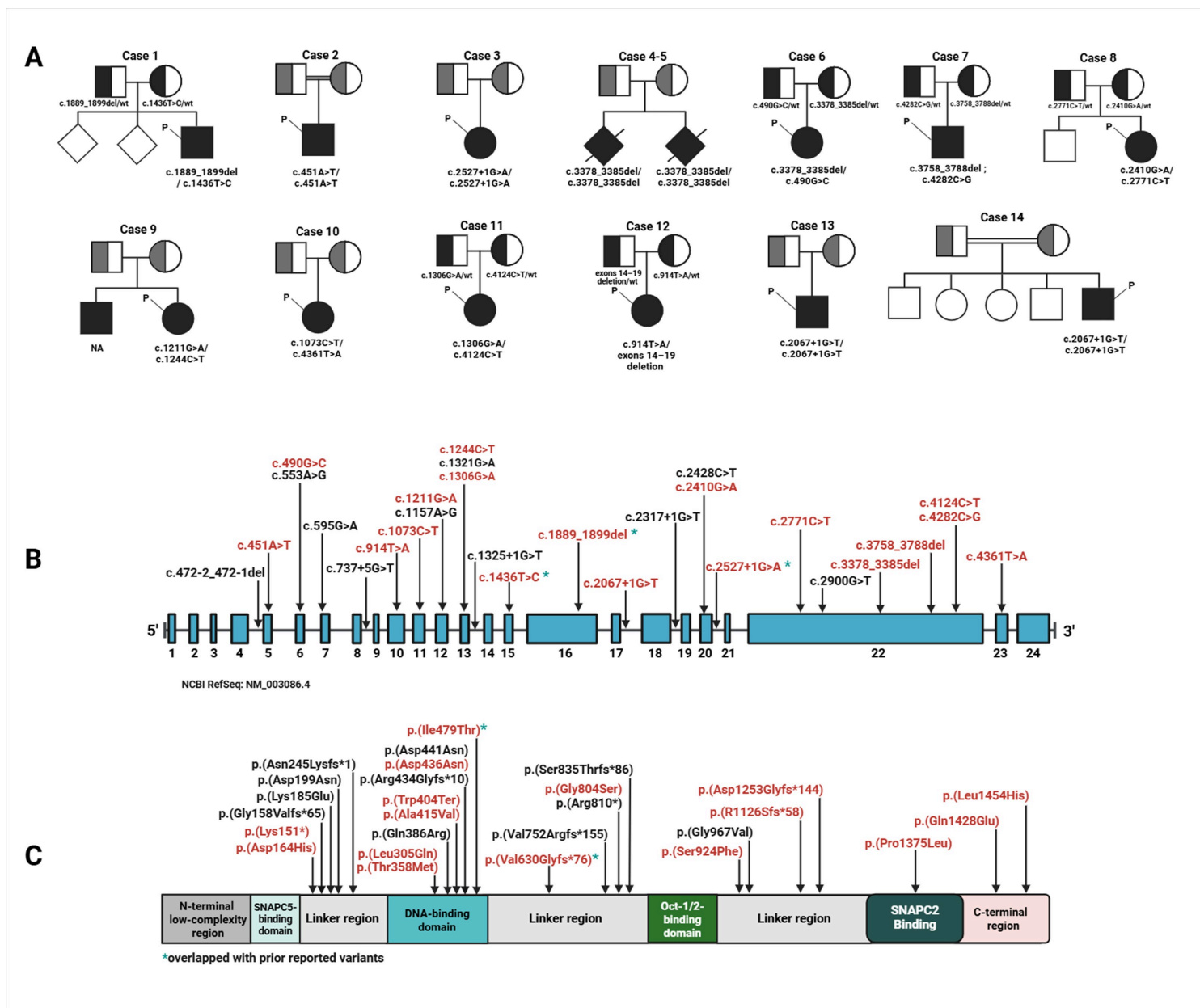


Figure 2

SNAPC4 cohort: variant mapping at the genomic and protein levels. (A) Pedigrees showing familial segregation of *SNAPC4* variants in the cohort. Affected individuals are filled, unaffected individuals are open, heterozygous carriers are half-filled, and gray symbols indicate presumed carriers. Squares indicate males, circles females, and diamonds unspecified sex; deceased individuals are marked by a diagonal slash. The proband is indicated by an arrow, and double lines indicate consanguinity. "wt" indicates the wild-type/reference allele, "n.t." not tested, and "n.d." not determined. Distribution of *SNAPC4* variants identified in the present cohort mapped across the gene (B) and protein (C). Newly identified variants are shown in red, whereas previously reported variants are shown in black.

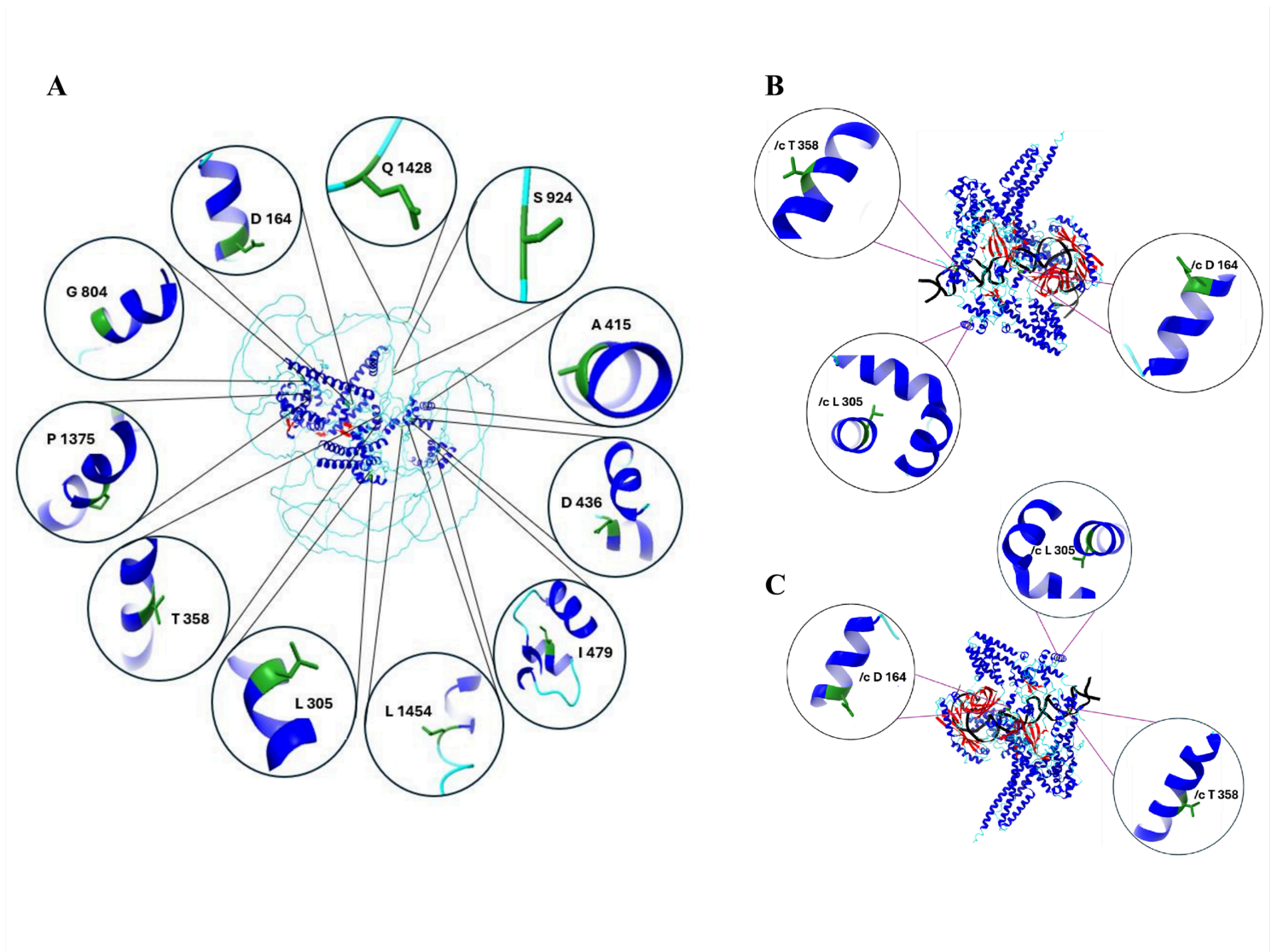


Figure 3

Structural localization and predicted impact of *SNAPC4* variants, from the full-length protein to the SNAPc transcription pre-initiation complexes. (A) Predicted three-dimensional structure of the full-length wild-type SNAPC4 protein (AlphaFold model AF-Q5SXM2-F1-v4), showing the positions affected by the variants identified in this study. Each affected residue is shown in forest green in ball-and-stick representation and labeled (insets). The structure was visualized using ChimeraX [42, 43]. (B) Structure of the SNAPc-containing RNA polymerase II pre-initiation complex bound to the U1 snRNA promoter (PDB 7ZXE; cryo-EM, 3.5 Å) [3]. Of the variants shown in panel A, only D164, L305, and T358 fall within the SNAPC4 region resolved in this structure (residues 81–398); these positions are shown in forest green in ball-and-stick representation and labeled, with DNA in black. (C) Structure of the SNAPc:TBP–TFIIA–TFIIB sub-complex bound to the U5 snRNA promoter (PDB 7ZWC; cryo-EM, 3.2 Å) [3]. The same positions (D164, L305, and T358) are highlighted and labeled as in panel B, with DNA in black.

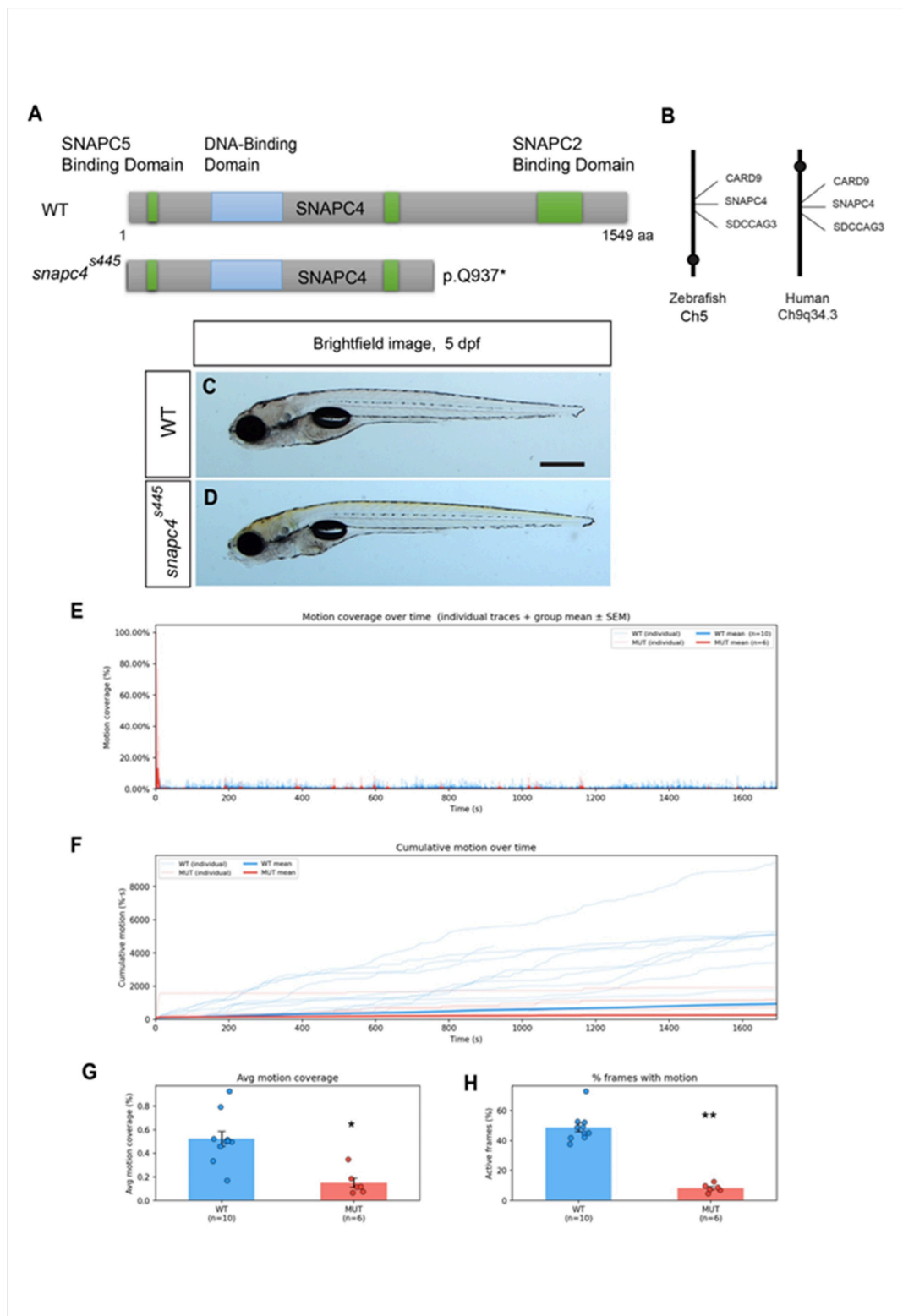


Figure 4

SNAPC4 loss of function causes neuromotor impairment without overt morphological abnormalities in zebrafish.

(A) Schematic representation of the zebrafish Snapc4 protein. The *snapc4*^{s445} mutation introduces a premature stop codon (p.Q937*), resulting in a truncated protein lacking the SNAPC2-binding domain. (B) Synteny analysis showing conservation between zebrafish chromosome 5 and human chromosome 9q34.3, supporting orthology of SNAPC4. (C–D) Representative brightfield images of zebrafish larvae at 5 days post-fertilization (dpf): (C) wild-type (WT) and (D) *snapc4*^{s445} mutants. Mutant larvae do not display overt morphological abnormalities. Scale bar as indicated. (E) Motion coverage over time (% of frame area with detected movement). Light traces represent individual larvae (blue, WT; red, mutant); bold lines indicate group means with shaded regions denoting ± SEM. (F) Cumulative motion over time, showing total locomotor activity for individual larvae (light traces) and group means (bold lines). (G) Average motion coverage per larva. (H) Percentage of frames with detected motion. Bars represent group means with error bars indicating SEM;

individual data points are shown as dots. WT larvae (n = 10) exhibit significantly greater locomotor activity compared to *snapc4^{s445}* mutants (n = 6). *p < 0.05; **p < 0.01; N.S., not significant (p > 0.05).

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