Human Genomics

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Compound heterozygous structural variants resulting in *CNTNAP2* biallelic loss-of-function: rare mechanisms unveiled by genome sequencing

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Abstract
The *CNTNAP2* gene encodes CASPR2, a transmembrane protein essential for neuronal development and synaptic function. Biallelic pathogenic variants cause Pitt–Hopkins–like syndrome, characterized by intellectual disability, epilepsy, and autistic features. We report two patients with a Pitt–Hopkins–like phenotype carrying compound heterozygous structural variants: an intragenic deletion in *trans* with a paracentric inversion. Short-read genome sequencing detected both variants, and long-read sequencing refined one breakpoint. Non-reccurent deletions involved exon 3, while *CNTNAP2* breakpoints for both inversions were located in intron 1. These findings broaden the mutational spectrum of *CNTNAP2* and underscore the value of genome sequencing in identifying complex structural variants.

Keywords *CNTNAP2*, Structural variants, Genome sequencing, Neurodevelopmental disorders, Pitt–Hopkins–like syndrome

Introduction

The *Contactin-associated protein-like 2* (*CNTNAP2*) gene encodes CASPR2, a transmembrane member of the neurexin family that is highly conserved across species [1]. CASPR2 contributes to the organization of myelinated axons, the regulation of synaptic transmission, and the maintenance of neuronal circuit homeostasis, particularly during brain development [1]. Pathogenic biallelic variants in *CNTNAP2* cause Pitt–Hopkins–like syndrome 1 (MIM #610042), an autosomal recessive neurodevelopmental disorder characterized by intellectual disability (ID), drug-resistant epilepsy frequently associated with focal cortical dysplasia, language impairment, behavioral disturbances, obsessive–compulsive traits, and attention-deficit/hyperactivity disorder (ADHD) [1, 2].

Located on chromosome 7q35, *CNTNAP2* is one of the largest human genes. Although most reported pathogenic variants are single-nucleotide variants, structural variants (SVs), including homozygous deletions, have only rarely been described as disease-causing mechanisms

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affecting *CNTNAP2*. Consequently, the extent to which complex structural variants contribute to the disorder remains unclear [3, 4].

Here, we report two patients with a Pitt-Hopkins-like phenotype, each harboring compound heterozygous structural alterations of *CNTNAP2*: a heterozygous deletion in *trans* with a heterozygous balanced inversion. These observations, made possible by short-read genome sequencing (GS), underscore the capacity of GS to resolve complex SVs and reveal rare mechanisms underlying *CNTNAP2* loss of function.

Materials and methods

Ethical statements

Written informed consent was obtained from the parents. The study was registered with the Data Protection Officer of Lille University Hospital under reference #DEC25-108.

Short read DNA sequencing

GS was performed in trios for both cases, including the probands and their parents. DNA was extracted from blood samples, and sequencing data were aligned to the reference genome GRCh38.92 using BWA-MEM (v0.7.15). Single nucleotide variations (SNVs) and small insertions/deletions (INDEL < 50 bp) were detected using GATK (v4.1.7.0) and annotated with SNPeff (v4.3t). SVs were detected through split-read analysis performed with Lumpy (v0.2.13) [5]. Candidate breakpoints were validated by Sanger sequencing. Primers are available upon request. Repeat-expansion analysis was performed through ExpansionHunter.

Nanopore long-read sequencing

For patient 2, sequencing was then performed according to the standard protocol on an ONT PromethION 2 Integrated sequencer, using a PromethION flow cell (R10.4.1). After sequencing, base calling was performed using the Guppy/Dorado base caller in High Accuracy (HAC) mode. The resulting reads were mapped to the human reference genome GRCh38 using MiniMap2 (version 2.24) [6]. These mapped data were then used for downstream analysis. Visual inspection of structural variants within target regions was performed with the Integrative Genomics Viewer (IGV version 2.19), and Jbrowse 2.[7]

CNV/SV population data and frequency assessment

Population structural variant (SV) data were assessed using the Genome Aggregation Database (gnomAD) SV dataset v4.1. We queried gnomAD to identify copy number variants overlapping the genomic region of interest. Specifically, we extracted deletions overlapping exon 2 and exon 3, or exon 3 alone and inversions with specific breakpoints in the gene. Reported allele frequencies were obtained directly from the gnomAD v4.1 SV dataset (Supplementary Table S1).

Results

Patient P1

P1 is a 6-year-old boy, the second child of non-consanguineous parents, with no relevant family history. Prenatal ultrasonography revealed a solitary left kidney. He was born at term without perinatal complications, and early developmental delay was noted. Epilepsy onset occurred at 18 months with status epilepticus. The disease course has been marked by recurrent episodes of status epilepticus and rapid evolution toward drug-resistant, multifocal epilepsy with tonic seizures. Video-EEG monitoring showed alternating lateralization, with seizures arising from either temporal lobe, along with multifocal epileptiform discharges. Sodium channel blockers improved control of prolonged seizures, although brief seizures persist several times per week. The patient presents with severe intellectual disability and an autistic syndrome with stereotypies.

GS identified discordant read mapping and split-read clusters consistent with two distinct structural events at the 7q35 locus (Fig. 1a). A heterozygous 209,219 bp deletion spanning intron 1 to intron 3 of *CNTNAP2* and encompassing exon 2 and 3 was inherited from the unaffected mother (Fig. 1A). Breakpoints were mapped to chr7:146,660,391 and chr7:146,869,613, with analysis of the junction revealing a 3 bp microhomology motif ("CAA") present at chr7:146,660,392–146,660,394 and chr7:146,869,611–146,869,613 (Fig. 1c-d-e). Sanger sequencing confirmed the breakpoint structure, supporting a non-recurrent event consistent with microhomology-driven repair, such as MMEJ or MMBIR/FoSTeS (Supplementary Fig. 1a).

The patient also carried a 1,043,501 bp paracentric inversion inherited from the unaffected father (Fig. 1b). The proximal breakpoint occurred within a LINE L1M4a1 element and was associated with a five–base pair deletion (chr7:145,378,788_145,378,792del) at the junction (Fig. 1d). The distal breakpoint is located in the intron 1 of *CNTNAP2* and included a two–base pair deletion (chr7:146,422,292_146,422,293del) at the junction (Fig. 1e). No microhomology or other sequence features suggestive of templated repair were present, consistent with an inversion generated by two independent double-strand breaks resolved through non-homologous end joining (NHEJ). Variants have been deposited to DECIPHER (#573606).

Patient P2

P2 was born to unrelated parents with no relevant family history. He was delivered at term after an uneventful

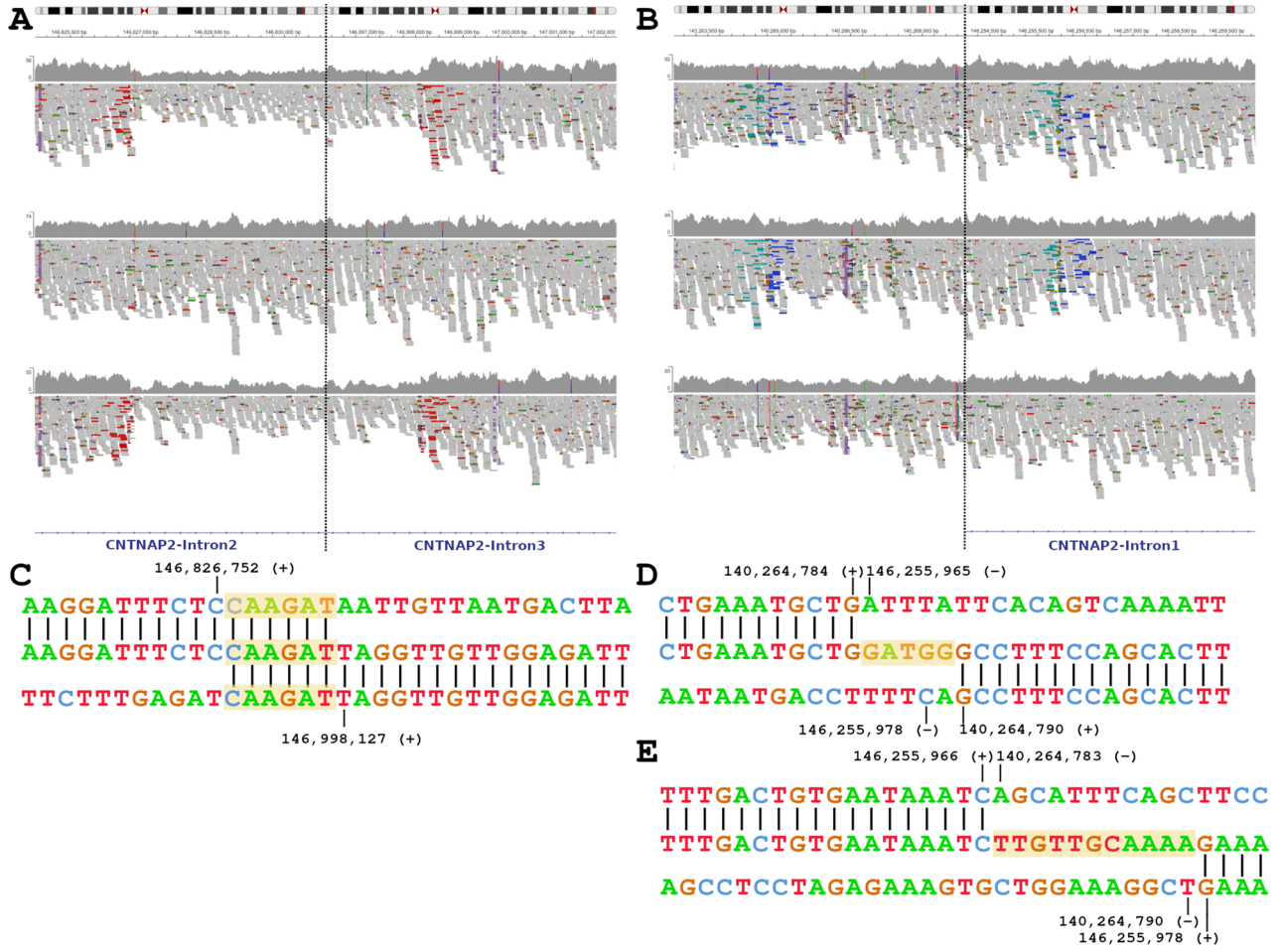

Fig. 1 Structural variants in *CNTNAP2* identified in Patient 1. (A–B) IGV views of genome sequencing data showing chimeric split-read mapping at the distal and proximal breakpoints in the patient and both parents. Panel A displays the heterozygous intragenic deletion, and panel B the heterozygous paracentric inversion. (C–E) Breakpoint-junction sequences derived from sequencing data. Panel C shows the deletion junction with the 3 bp microhomology motif. Panels D and E display the proximal and distal inversion breakpoints, including the duplicated low-complexity motif (D) and the 2 bp deletion at the distal junction (E)

pregnancy, with macrosomia and a head circumference of +2 SD. Early clinical features included congenital ataxia, global developmental delay with marked speech delay, and epilepsy. Independent walking was achieved at approximately 36 months. Behavioral manifestations consisted of frequent temper tantrums with aggression, stereotypies, and intermittent hyperventilation episodes, which subsequently improved. Attention and concentration difficulties persist. Epilepsy onset occurred at 2 years and 9 months, with both generalized tonic–clonic and focal seizures; a status epilepticus episode occurred at 4 years. Seizure control was achieved with carbamazepine. Neurological examination reveals global hypotonia with ataxia. He presents with hypermetropia and right-eye amblyopia, with otherwise normal neuro-ophthalmologic evaluation. Brain MRI at 5 years of age demonstrated superior vermian hypoplasia. The initial EEG showed a slow focus in the right hemisphere. At 6 years, growth parameters are within the normal range (weight + 2 SD, height + 0.5 SD, head circumference 0 SD).

GS identified a heterozygous 171,381 bp deletion spanning intron 2 to intron 3 of *CNTNAP2*, inherited from the unaffected father (Fig. 2a). The deletion encompassed exon 3. Breakpoints were mapped to chr7:146,826,752 and chr7:146,998,127. Junction analysis revealed a 6 bp microhomology motif ("CAAGAT") present at chr7:146,826,753–146,826,758 and chr7:146,998,121–146,998,126 (Fig. 2c). The distal breakpoint (chr7:146,998,127) lies within a 2,866 bp LINE element of the L1MA8 subfamily.

GS also detected a maternally inherited paracentric inversion of approximately 5,991,188 bp with a distal breakpoint in the intron 1 of *CNTNAP2* (Fig. 2b, Supplemental Fig. 1). LRS was performed to confirm the breakpoints located in highly repetitive elements. Sniffles2 analysis confirmed both structural variants, calling a

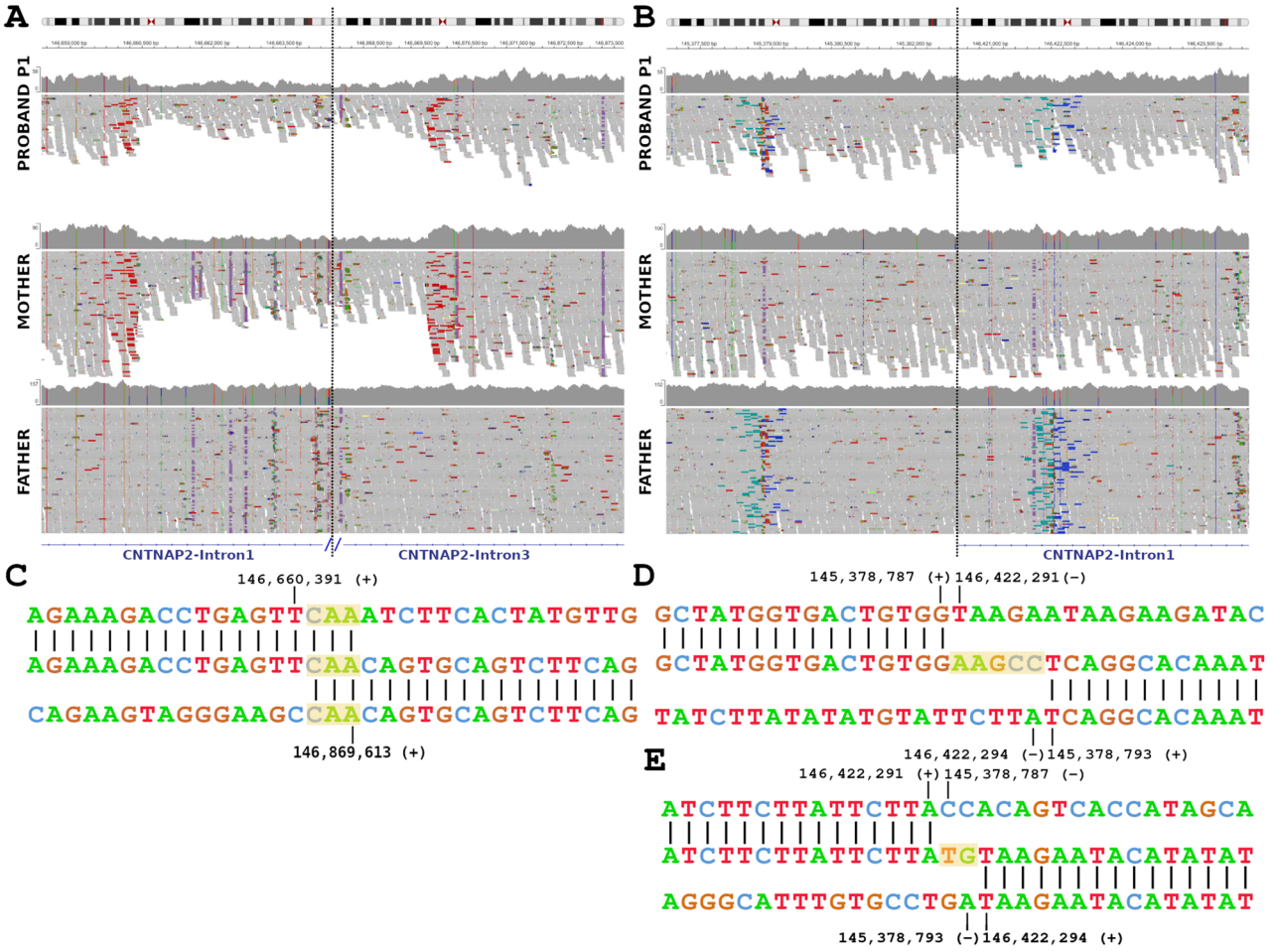

Fig. 2 Structural variants in *CNTNAP2* identified in Patient 2. (A–B) IGV views of genome sequencing data showing chimeric split-read mapping at the distal and proximal breakpoints in the patient and both parents. Panel A depicts the heterozygous intragenic deletion, and panel B the heterozygous paracentric inversion. (C–E) Breakpoint-junction sequences derived from long-read and short-read data. Panel C shows the deletion junction with the 6 bp microhomology motif. Panels D and E show the proximal and distal inversion breakpoints, including the 5 bp deletion (D) and 11 bp insertion (E) at the respective junctions

171,376 bp deletion and a 5,991,191 bp inversion. LRS refined the deletion breakpoint and confirmed the 6 bp microhomology sequence ("CAAGAT"), consistent with a microhomology-mediated mechanism such as MMEJ or MMBIR/FoSTeS. Analysis of the inversion breakpoints revealed a 5 bp deletion ("GATGG") at the proximal junction (chr7:140,264,785_140,264,789del) and an 11 bp insertion ("TTGTTGCAAAA") at the distal junction (chr 7:146,255,967_146,255,977del) (Fig. 2d-e). The absence of microhomology or templated sequence features at these sites suggests that the inversion arose through two independent double-strand breaks followed by rejoining via non-homologous end joining (NHEJ), consistent with the mechanism inferred for patient P1. Variants have been deposited to DECIPHER (#573607).

Discussion

We report two patients carrying compound heterozygous SVs in the *CNTNAP2* gene. Each patient has a heterozygous deletion and a heterozygous balanced paracentric inversion, which they inherited from unaffected parents. Although pathogenic single-nucleotide variants, small INDELs, and heterozygous deletions in *CNTNAP2* are well-established causes of Pitt-Hopkins like syndrome, SV, particularly compound heterozygous SVs, remain exceedingly rare [3, 4]. The structural complexity of the *CNTNAP2 locus* and the limited sensitivity of conventional diagnostic pipelines likely contribute to these events being under-recognized. Our findings broaden the mutational spectrum of *CNTNAP2* and underscore the importance of considering the complete range of structural rearrangement combinations in autosomal recessive neurodevelopmental disorders [1, 2].

These structural associations illustrate the importance of GS approaches for detecting SVs. Short-read GS identified both the heterozygous deletions and the balanced paracentric inversions. LRS provided breakpoint-level resolution for patient P1. Although exome sequencing can identify multi-exonic deletions through depth-of-coverage analysis, even when breakpoints are located in intronic regions, it would not have detected the balanced inversion involving intronic junctions. The GS approach significantly improves the diagnostic yield of unresolved neurodevelopmental disorders by using short reads supplemented by long reads [7]. The deletions in both patients displayed short microhomology sequences (3 bp in P1 and 6 bp in P2), supporting replication-based mechanisms such as microhomology-mediated end joining (MMEJ) or microhomology-mediated break-induced replication/fork stalling and template switching (MMBIR/FoSTeS). The inversion breakpoints were associated with minimal or absent microhomology and no evidence of templated insertions, consistent with two independent double-strand breaks followed by non-homologous end joining (NHEJ) [8].

CNTNAP2, spanning more than 2.3 Mb on chromosome 7q35, is among the largest human genes [1]. In previously *CNTNAP2* descriptions, compound SVs typically involved two deletions or a deletion combined with a duplication. Mefford et al. described a patient carrying both an exon 2–4 deletion and a 370 kb deletion overlapping *CNTNAP2*, and D'Onofrio et al. reported two intragenic deletions [3, 4].

Population SV data from gnomAD v4.1 show that deletions overlapping exon 2 and exon 3, or exon 3 alone, are present in the general population, with 10 such deletions reported and a cumulative allele frequency of 1.68×10^{-4}. Therefore, the occurrence of compound heterozygous deletions affecting these exons is not unexpected. However, the deletion with a balanced inversion that we observed in our patients is more unusual, especially considering that no inversion with a predicted pathogenic impact on the allele has been reported in gnomAD. Both deletions encompassed exon 3 or exon 2 and 3. Since exons 1 or 2 and 4 are not in phase, an equivalent frameshift could be associated with them. Both inversions disrupt the first coding exon, including the ATG start codon, as well as the native promoter and enhancer elements, raising the possibility not only of loss of function independent of coding disruption but also of impaired transcription of the gene. Together, these changes result in biallelic loss of function, which is consistent with the observed clinical phenotypes [2]. Biallelic structural alterations are rarely described. For example, a recent description of the *PRKN* gene (~ 1.3 Mb) revealed that compound heterozygous structural variants (SVs), including copy loss and copy gain, contribute to early-onset Parkinson's disease (MIM #600116) [9].

Both patients displayed core features of *CNTNAP2*-related Pitt–Hopkins–like syndrome, including global developmental delay, ID, epilepsy, and behavioral disturbances [2]. Their early-onset seizures, at 18 months for P1 and 2 years 9 months for P2, fit the typical presentation, as most reported individuals develop epilepsy within the first two years of life and often evolve toward pharmacoresistance [2]. P1 presented with multifocal drug-resistant epilepsy with alternating temporal onset, consistent with the predominance of temporal lobe involvement previously described [10]. P2 showed a broader phenotype with congenital ataxia and superior vermian hypoplasia, observed in a subset of patients and frequently associated with cerebellar involvement [1]. His behavioral disturbances, attention deficits, and speech delay are consistent with the varied but characteristic symptoms of the disorder. It is noteworthy that, although uncommon, partial seizure control with carbamazepine has been documented in a small number of individuals with *CNTNAP2*-related focal epilepsy [2].

These findings demonstrate that compound heterozygous structural variants in *CNTNAP2* can cause Pitt-Hopkins-like neurodevelopmental phenotypes and may be more common than previously thought. The application of GS strategies for SV detection was essential for revealing these compound rearrangements. As genome-wide SV detection becomes more accessible, a broader range of balanced pathogenic rearrangements will likely be uncovered. This will ultimately improve diagnostic rates and our understanding of the genomic architecture underlying neurodevelopmental disorders.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40246-026-00952-9.

Supplementary Material 1. Long Read Visualisation of Balanced Inversion from Patient 2. (A) Long-read sequencing visualization of the deletion in Patient 2 with Jbrowse2. Multi-panel display of chimeric reads showing the proximal breakpoint located within an AT(n) repeat (*CNTNAP2* intron 2) (top panel) and the distal breakpoint located within a LINE L1MAB element (*CNTNAP2* intron 3) (bottom panel). (B) Long-read sequencing visualization of the inversion in Patient 2 with Jbrowse2 [11]. Multi-panel display of chimeric reads showing the proximal breakpoint located within an *MLT1B* element (intergenic region) (top panel) and the distal breakpoint located in *CNTNAP2* intron 1 (bottom panel).

Supplementary Material 2. Table S1. Copy number variants overlapping the genomic region of interest in gnomAD v4.1 SV. Table S2. Applied ACMG/ClinGen SV Criteria for structural rearrangments.

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Author contributions

JF and TS wrote the manuscript, and performed the experiments. JF, PPB, MT, CT, and TS performed the data interpretation. RC, CC, AT, PC, and JG collected

and evaluated the clinical and genetic data. All authors discussed the results, commented on the manuscript, and approved the final manuscript.

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Data availability
The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

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