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CASE REPORT

ACTB Loss-of-Function Variant and *AUTS2* Duplication in a Patient with Syndromic Intellectual Disability

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Abstract

Background The unbiased detection of multiple genetic hits can aid in the identification of molecular etiologies of congenital anomalies and neurodevelopmental disorders, providing deeper insights into their complex genetic underpinnings.

Purpose To clarify the etiology of a syndromic intellectual disability (ID) case, we performed a reanalysis of exome sequencing data complemented with an optical genome mapping (OGM) assay.

Case Presentation The patient is a 23-year-old Brazilian male presenting with mild ID associated with speech impairment; behavioral alterations, including self-injury and polyphagia; class III obesity; impaired glomerular function; macrocephaly; facial and foot anomalies; and cryptorchidism.

Genetic Findings Two genomic variants were detected, each of which was inherited from a parent: a 7q11.22 4.4 Mb duplication encompassing *AUTS2*, which was inherited from an apparently normal father, and an *ACTB* likely pathogenic variant (NM_001101.5:c.328dup:p. Leu110Pro*fs**16), inherited from a mildly affected mother. OGM analysis revealed that the duplicated 7q11.21q11.22 segment was in tandem and encompassed the entire *AUTS2* sequence; therefore, this gene was not disrupted or inserted into another genomic site.

Conclusion The presence of these two genomic variants highlights the importance of exploring further genetic factors in patients with more complex phenotypes via molecular testing techniques. In addition, OGM allowed precise localization of the duplication in the patient's genome and provided insights into its structure, a capability not offered by previous cytogenomic techniques. This is the first publication supporting the simultaneous involvement of both the *ACTB* loss-of-function (LoF) variant and the *AUTS2* duplication in a patient with a complex ID phenotype, suggesting that *AUTS2* duplications may have a functional impact.

Keywords *ACTB* · *AUTS2* duplication · Optical genome mapping · Intellectual disability

Introduction

Exome sequencing (ES) enables multiple genetic diagnoses in isolated cases, meaning that more than one condition is caused by variations at separate *loci* that segregate independently. Multiple genetic diagnoses have been sporadically documented in individual case reports, and recently, a retrospective analysis of both research and diagnostic data suggested that the prevalence of multiple genetic diagnoses ranges from 1.4% to 7.2% [1–9]. Here, we report a case of syndromic intellectual disability (ID), in which two clinically relevant inherited variants were detected: a 7q11.22 4.4 Mb duplication encompassing *AUTS2* and an *ACTB* likely pathogenic (LP) indel variant, both of which were identified by ES analysis/reanalysis.

Pathogenic loss-of-function (LoF) *ACTB* variants were recently reported to cause autosomal dominant (AD) syndromic ID (*ACTB* haploinsufficiency), which is characterized by cognitive impairment, typical facial dysmorphisms, and an increased frequency of internal organ malformations [10, 11], however, it is not yet categorized as a distinct condition in OMIM. Moreover, *AUTS2* LoF variants are associated with another AD syndromic ID (OMIM#615834), characterized by a highly variable phenotype consisting of global

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developmental delay and/or ID, microcephaly, dysmorphic features, feeding difficulties, hyperactivity, and autism spectrum disorders [12, 13], however, the effects of duplications involving this gene have not been documented. We further investigated the copy number variant (CNV) structure using optical genome mapping (OGM) and investigated the effects of the coexistence of both variants in the patient.

Case Report

Proband Clinical Description

The patient is the firstborn child of nonconsanguineous parents (Fig. 1A). This 23-year-old Brazilian male patient presented with mild ID, aggressive behavior, autism features, sparse hair, facial dysmorphisms, thoracic kyphosis, discrete decreased glomerular function, class III obesity, gait disturbance, insulin-resistant diabetes mellitus, and hypothyroidism. He was first referred for genetic evaluation at the Institute of Biosciences (University of São Paulo) at the age of 15, with the diagnostic hypothesis of Prader–Willi syndrome (OMIM#176270). His parents denied any other family history of genetic disorders or developmental delay. His younger brother suffered neonatal anoxia, resulting in left-sided facial and motor paralysis, but without significant cognitive limitations. His mother reported three miscarriages. The patient also has a healthy paternal younger half-brother (Fig. 1A).

The pregnancy was marked by decreased fetal movement, yet prenatal ultrasounds revealed no abnormalities. His mother required insulin therapy to manage her diabetes (diagnosed at the age of 26) during pregnancy. A cesarean section was performed at 35 weeks of gestation (preterm birth) because of maternal diabetes and fetal hypoactivity. At birth, the patient weighed 2.66 kg (7^{th} percentile), measured 45.5 cm in height (4^{th} percentile), and had a head circumference of 34 cm (20^{th} percentile). The Apgar score was 9/9, but he had difficulty sucking (neonatal hypotonia) and required neonatal intensive care for glycemic management; he was discharged from the hospital after three days.

The gonads were not visualized via ultrasonography. The echocardiogram showed normal results. He had seizures without fever in the first months of life. At 1.5 years of age, he was diagnosed with urinary reflux and underwent surgical treatment. He presented with global DD, including motor and speech delays. From 18 months of age onward, the

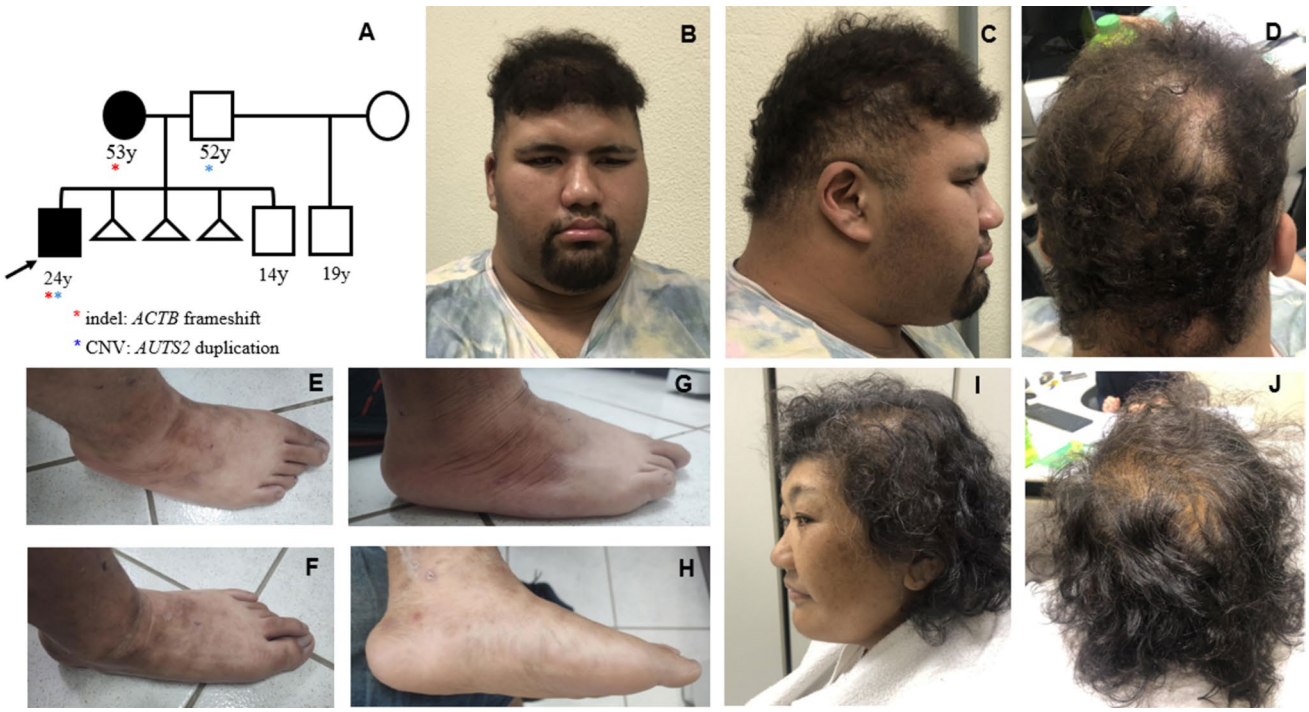

Fig. 1 Patient's pedigree and photographs of him and his mildly affected mother. **A.** Proband pedigree with segregation of the two identified clinically relevant variants and the clinical features of the proband and his mother. Variants are represented by colored asterisks, and the ages of some family members are indicated in years (y). **B-D** Craniofacial features of the proband. **B -** Sparse eyelashes, synophrys, deeply set eyes, broad nose tip, and cupid bow lips. **C -** Ears with thickened upper helices and slight prognathism. **D -** Sparse hair. **E–H** Proband's foot dysmorphisms include flat feet, the second toe overlapping the first, medial deviation of the remaining toes, and small nails on the 5^{th} toe. **I-J** Patient's mother's craniofacial characteristics. **I -** Broad nose tip. **J -** Sparse hair

proband presented with polyphagia and significant weight gain. At 16.5 years of age, he weighed 142 kg and vomited frequently. Currently, his weight and height are 145.20 kg and 176 cm, respectively (23 years old, BMI 46.88—class III obesity). Abdominal ultrasonography revealed grade II hepatic steatosis. Additionally, the patient has hypertension, type II diabetes mellitus, hypothyroidism, hypogonadism, impaired glomerular function, hyperchromic spots on the skin and some lesions suggestive of skin picking.

With respect to craniofacial characteristics (see Fig. 1B-D), at 23 years of age, he exhibited macrocephaly (head circumference = 59.5 cm; > 98 th percentile), sparse hair and eyelashes, synophrys, deeply set eyes, a broad nose tip, ears with thickened upper helices, cupid bow lips and slight prognathism. The patient presented with thoracic kyphosis, and anomalies of the hands and feet (Fig. 1E-H), such as large hands (21.5 cm; > 97 percentile), a 9 cm middle finger (> 97 percentile), deviations of the 3rd finger, mild camptodactyly of the 5th finger, flat feet with the second toe overlapping the first, a medial deviation of the remaining toes, and small nails on the 5th toe.

The proband's mother, a 53-year-old Brazilian female, was evaluated after ES analysis. She presented with mild ID, sparse scalp hair, mild facial dysmorphisms (hypertelorism, broad nose tip—Fig. 1I-J), cataracts, decreased finger mobility, discrete decreased glomerular function, premature ovarian insufficiency, recurrent spontaneous abortion, gait disturbance, type II diabetes mellitus and impaired personality functioning. She recently experienced acute myocardial infarction, requiring coronary artery bypass graft surgery.

Genetic Tests

Previously, the following genetic tests were performed: G-banding karyotype, multiplex ligation-dependent probe amplification (MLPA SALSA P070 - 82 and p245_81-MRl-MRC-H0LLAND), Fragile X syndrome test (AmplideX PCR/CE FMR1–Asuragen), and methylation test of SNURF-SNRPN (Prader−Willi syndrome region), all of which returned negative results. Trio ES was performed for the analysis of single nucleotide variants (SNVs), indels, and CNVs, as previously described [14].

Four years after the initial ES analysis, we performed a reanalysis via the Franklin platform (http://franklin.genoox.com), applying the following filtering criteria: (1) allele frequency < 2% in gnomAD, (2) variants in coding regions or within 10 bp of splice junctions, (3) protein-altering variants (missense or LoF), and (4) high or medium aggregated quality score. Compound heterozygous variants were also considered. Variants were prioritized by Franklin on the basis of their degree of pathogenicity and clinical information transcribed into Human Phenotype Ontology (HPO) terms (https://hpo.jax.org/). Pathogenicity classification followed the American College of Medical Genetics and Genomics (ACMG) guidelines [15], using Varsome [16] and Franklin with manual review. The nomenclature adopted is recommended by the Human Genome Variation Society (HGVS) [17].

We further investigated structural variants (SVs) via OGM analysis (Bionano, San Diego, CA, USA). OGM was conducted using ultrahigh-molecular-weight DNA samples (> 150 kb) extracted from the peripheral blood cells of the proband with the Bionano Prep SP Blood and Cell DNA Isolation kit. The DNA was labeled with the DLS DNA Labeling Kit to add fluorophores to the CTTAAG motif. The sample was run on the Saphyr chip to collect data on the Saphyr System at 100 × coverage. OGM data were analyzed using the *de novo* assembly pipeline, followed by the CNV and SV pipelines, and visualized using the Bionano Access v8 software. We adopted the nomenclature recommended by the publication of Moore et al. [18] on behalf of the International System for Human Cytogenomic Nomenclature—ISCN—Standing Committee.

Results

In the ES data analysis, we identified the variant seq[GRCh38] dup(7)(q11.21-q11.22)NC_000007.14:g.66647744_71077845 dup in the proband, which was inherited from his apparently unaffected father [14] – Fig. 2A). This 4.43 Mb duplication spans the entire *AUTS2* and *SBDS* genes, along with other non-OMIM genes. This CNV was classified as a variant of unknown significance (VUS), as the effects of *AUTS2* and *SBDS* complete duplication cannot be evaluated (criterion 4L—case−control and population evidence). Additionally, it was not possible to determine the exact location of this duplicated segment in the genome using ES data alone. Therefore, we evaluated SV via OGM, which confirmed the entire duplication of the *AUTS2* gene and revealed that this CNV occurred in tandem: ogm[GRCh38]dup(7)(q11.21q11.22)(66646774_71056001) (4.409 Mb; Fig. 2B).

In an ES reanalysis, an LP *ACTB* variant (NM_001101.5:c.328 dup:p. Leu110Pro*fs** 16), leading to a frameshift and consequent premature stop codon (PVS1_very strong, PM2_supporting), was detected, which was inherited from the patient's mother (Supplementary Fig. 1 A-B). Further clinical analysis was performed on the proband and his mother to evaluate the consistency of their clinical condition with the detected LoF *ACTB* variant; the mother was also shown to be mildly affected.

Discussion

ACTB Variants

The *ACTB* gene maps to 7p22.1, encodes one of six different actin proteins and is expressed constitutively as an

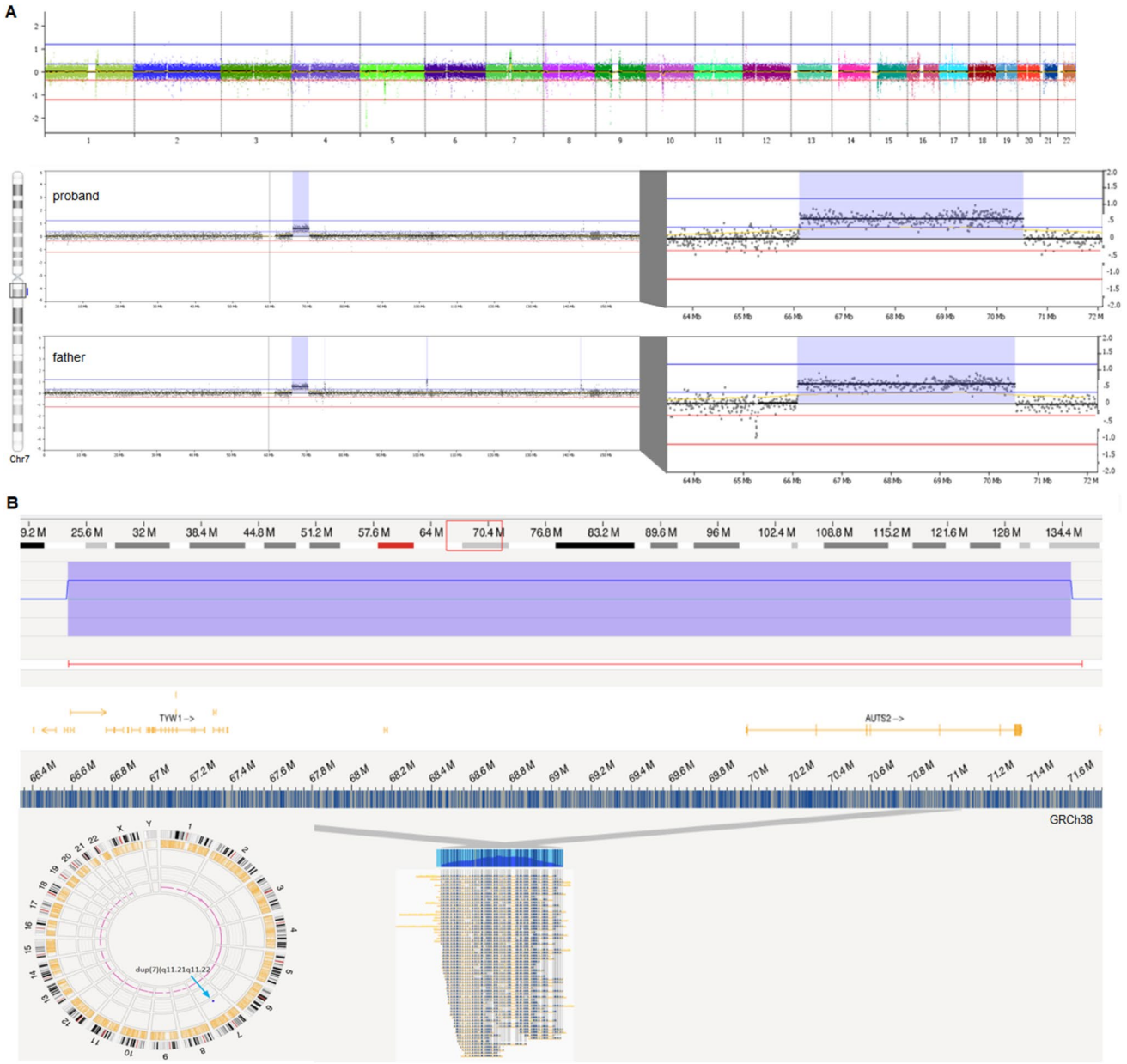

Fig. 2 A 7q11.21q11.22 4.4 Mb duplication including the *AUTS2* gene was identified in the CNV analysis of the family ES data (A), and its investigation was further refined through OGM (B). **A** – Profile overview of the proband's autosomal chromosomes (top) and a 4.43 Mb duplication at 7q11.21q11.22 detected in both the proband and his father (bottom). Each color represents a chromosome, with indications on the X axis of the upper graph. In the three graphs, the Y axis represents the $\log_2$ scale of the copy number ratio (values close to 0 indicate regions with similar copy numbers to the reference, positive values represent gains, and negative values represent losses). The location of the mutation is highlighted in purple on an ideogram of chromosome 7 and in the Log R ratio graphs. On the right, the duplicated region is shown in greater detail (zoom in). Graphs extracted from Nexus Copy Number software (Bionano). **B** OGM analysis revealed an in tandem 7q11.21q11.22 duplication encompassing the entire *AUTS2* gene. Bottom left corner: genome-wide circle plot showing the presence of the duplication. The genome map against the reference reveals that the SV structure is in tandem. The SV is also not disruptive to the *AUTS2* gene (represented in orange). This image is a compilation of charts extracted from Bionano Access software

endogenous housekeeping gene [19], playing a vital role in various cellular functions [20, 21]. Actin filaments are known to play a crucial role in the structure of synapses and are essential for synaptic plasticity [22], which has a recognized impact on neurodevelopment. Given the functional relevance of the ACTB protein and its ubiquitous presence in various organ systems, *ACTB* pathogenic variants exhibit pleiotropic effects.

There are three AD conditions recognized in OMIM: Baraitser-Winter syndrome 1 (BRWS1; OMIM#243310), dystonia-deafness syndrome 1 (DDS1; OMIM#607371), and thrombocytopenia 8 with dysmorphic features and DD (THC8; OMIM#620475); the distribution of causal variants for these conditions in the *ACTB* gene is shown in Fig. 3. Most reported pathogenic *ACTB* variants are *de novo* missense variants located in exons 2 to 4 and are associated with the BRWS1 phenotype [23], which is characterized predominantly by frontal pachygyria, ID, wasting of the shoulder girdle muscles, sensory impairment due to iris or retinal coloboma and/or sensorineural deafness, and distinctive craniofacial features (hypertelorism, a broad nose with a large tip and prominent root, and ptosis) [24]. DDS1 is caused by a specific *ACTB* variant [NM_001101.3: c.547 C > T: p.(Arg183 Trp)] and is characterized by sensorineural deafness with later onset of progressive dystonia [25–27]. THC8 patients present features distinct from those of BRWS1 patients, including mild DD and thrombocytopenia with platelet anisotropy,the causal THC8 variants tend to cluster in *ACTB* exons 5 and 6 and seem to inhibit the final stages of platelet maturation [23].

A 7p22.1 microdeletion syndrome was previously described [28, 29] and Cuvertino et al. [10] pinpointed *ACTB* as the critical gene among several studied cases of non-recurrent microdeletions. Baumann et al. [11] described a microdeletion of 36.6 kb (NC_000007.14:g.5522788_5559357 del) involving only *ACTB*, which was identified in a patient and his mother. These patients presented with developmental delay, ID; an increased frequency of internal organ malformations — particularly affecting the heart and renal tract — growth delay; and distinctive facial features, including wavy interrupted eyebrows, dense eyelashes, a broad nose, a wide mouth, and a prominent chin. Cuvertino et al. [10] and Baumann et al. [11] also reported cases of *ACTB* LoF non-CNV variants (Fig. 3), reinforcing LoF as a mechanism of disease. Indeed, the *ACTB* gene is highly intolerant to LoF variants [gnomAD data: pLi = 1; o/e = 0.06 (0.02–0.19)] [30]. Fibroblasts subjected to *ACTB* siRNA knockdown and patient-derived fibroblasts with 7p22.1 microdeletion presented findings consistent with the physiological role of *ACTB* disruption [10].

Thus, *ACTB* LoF variants cause AD syndromic ID through a haploinsufficiency mechanism characterized by cognitive impairment, typical facial dysmorphisms, and an increased frequency of internal organ malformations [10, 11], not yet categorized as a distinct condition in OMIM. Nevertheless, additional patients with intragenic *ACTB* variants are needed to establish the clinical profile of this new haploinsufficiency syndrome, and other genes with 7p22.1 microdeletions may also influence the phenotype.

Notably, different *ACTB* variant types can cause diverse phenotypes (Fig. 3). More C-terminal truncating variants may not trigger the nonsense-mediated mRNA decay (NMD) mechanism, which likely impacts phenotypic manifestations [10, 11]. The phenotypic impact of a genetic variant is influenced by a complex network of molecular pathways. The BRWS1 phenotype is thought to be associated with a gain-of-function or dominant-negative mechanism, as the causal variants are predominantly missense [10, 11, 24, 31–33]. Another argument for recognizing *ACTB* haploinsufficiency as a distinct syndrome from BRWS1 is the difference in clinical profiles. Although there is some overlap between the two syndromes, such as a wide mouth and hypertelorism, agyria/pachygyria and coloboma are common in BRWS1 but not in *ACTB* haploinsufficiency [10].

Our patient, in whom we detected an LP *ACTB* variant via exome reanalysis, presented with a clinical overlap with *ACTB* haploinsufficiency syndrome, including ID, behavioral alterations, sparse scalp hair, a wide nose, a

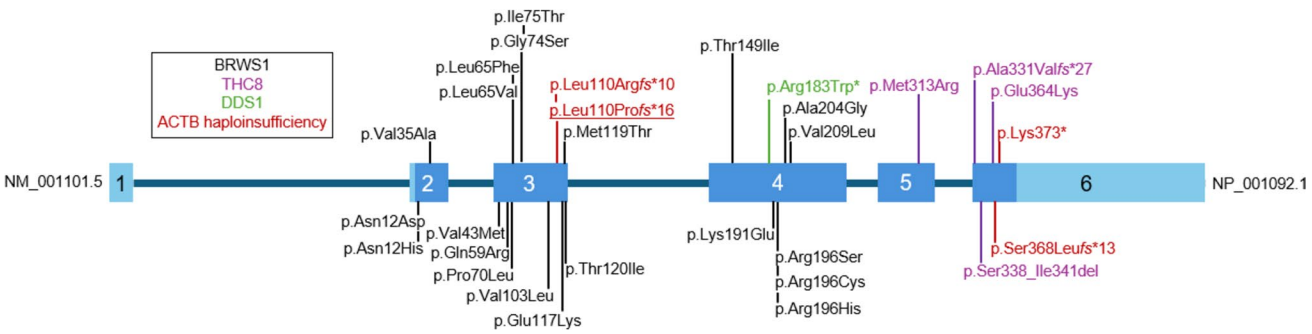

Fig. 3 Pathogenic *ACTB* variants and related phenotypic categories. The exons of the *ACTB* gene are represented by blue boxes; light blue represents untranslated regions (UTRs), and darker blue represents coding sequences. Variants are depicted along the coding portion of the *ACTB* gene via the Human Genome Variation Society (HGVS) nomenclature for proteins (p.) [17]. BRWS1 phenotype variants are shown in black, THC8 in purple, DDS1 in green, and those causing the *ACTB* haploinsufficiency-related phenotype in red. The variant identified in this case report is underlined. This diagram is based on data from the publications of Cuvertino et al. [10], Baumann et al. [11], and Latham et al. [23]

prominent chin, small nails, and overlapping toes. Some of the patients also presented with renal tract abnormalities, similar to our patient, who required surgery for vesicoureteral reflux and exhibited impaired glomerular function. Other abnormalities reported in some patients by Cuvertino et al. [10] and present in our patient included hearing loss, cryptorchidism and spinal abnormalities (scoliosis/kyphosis). The additional clinical features observed in the patient reported here are macrocephaly, grade III obesity, hepatic steatosis, hypertension, type II diabetes mellitus, hypothyroidism, sparse eyelashes, ears with thickened upper helices, cupid bow lips, and gait disturbance. Obesity is more prevalent among individuals with cognitive impairment and those with psychiatric disorders [34]. Hepatic steatosis and hypertension may be consequences of our patient's obesity and other metabolic issues, diabetes may also affect his renal function. There are reports of patients with *ACTB* haploinsufficiency syndrome having microcephaly [10], but our patient, in contrast, has macrocephaly.

The patient's *ACTB* (NM_001101.5):c.328 dup (p. Leu110Pro*fs** 16) variant was inherited from his mother, who was found to be mildly affected in a retrospective clinical evaluation, mainly with mild ID, sparse hair, hypertelorism, and a wide nose. Other clinical features seemingly unrelated to the *ACTB* variant include premature ovarian insufficiency, recurrent spontaneous abortion, gait disturbances, early-onset type II diabetes mellitus, and an episode of acute myocardial infarction.

AUTS2 Structural Variant

In addition, a paternal 4.4 Mb duplication encompassing the *AUTS2* gene was previously detected in this patient [14], but the precise genomic location and structure of the duplicated segment were unknown. *AUTS2* duplications, such as the case reported here, have not been published previously. We now employ an OGM assay, which is increasingly used in genetic diagnosis and research [18] because of its ability to provide information not detectable by chromosome microarray analysis or short-read sequencing, such as the genomic location and orientation of segments/insertions [35]. The 4.4 Mb 7q11.21q11.22 duplication encompassing the *AUTS2* gene was detected by OGM, with increased precision in determining the breakpoints and elucidation of its location and orientation in the patient's genome. OGM revealed a tandem structure of this SV, with adjacent duplicated segments, and therefore did not disrupt another genomic region or the *AUTS2* gene sequence.

LoF *AUTS2* variants cause intellectual developmental disorder 26 with an AD pattern of inheritance (OMIM #615834). However, the impact of the entire duplication of the *AUTS2* gene is unknown, although a deleterious effect cannot be ruled out. Rare CNVs are associated with the risk of neurodevelopmental disorders (NDDs), which are characterized by varying degrees of cognitive impairment [36, 37]. *AUTS2* is a gene with a high predicted probability of triplosensitivity (pTriplo 0.97; [38]), suggesting that it is more likely to be dosage sensitive to whole-gene duplication. Furthermore, in the DECIPHER database (freeze on September 2024), there are 25 cases of duplications ranging from 100 kb- 10 Mb encompassing *AUTS2,* among other genes, mostly with diverse cognitive phenotypes described in association, such as global developmental delay, autism, attention deficit hyperactivity disorder, delayed speech and language development, and learning disability. Notably, almost half of these DECIPHER cases with available information were inherited from apparently normal parents. In particular, the 7q11.21q11.22 duplication detected in the case here reported was inherited from an apparently unaffected father, and we cannot exclude a mild or even subtle cognitive deficit in the carrier father. Kendall et al. [39] suggested that the outcomes of carrying a CNV can be variable,this variability may indicate the presence of additional genetic and environmental factors modifying the penetrance and severity of the neurodevelopmental phenotype. In the same vein, family background plays a role in influencing neurodevelopmental outcomes in CNV carriers [40], causing a "shift" from expected functioning, depending on the parental starting point for quantitative traits such as intelligence and social abilities, the threshold for an NDD diagnosis can be reached or not reached. Thus, CNVs implicated in NDD are associated with cognitive deficits, even among unaffected individuals, which are generally subtle in these cases.

Conclusion

In summary, this case substantiates the role of *ACTB* LoF variants as a cause of a syndromic form of ID distinct from BRWS1 via a haploinsufficiency mechanism, while also broadening the associated clinical spectrum. The impact of *AUTS2* duplication on the patient's phenotype remains to be evaluated, as it is a rare, very large CNV, that affects a gene that is likely to be dosage sensitive to duplication. Therefore, we propose the possible joint effect of the coexistence of both variants, likely contributing to the full phenotype exhibited by the patient. We acknowledge that conclusions regarding the impact of the patient's genetic background on the phenotype must be drawn cautiously given the limited data. However, our data highlight the importance of searching for additional genetic hits in patients with complex phenotypes via multiple techniques, including the OGM as a tool to fully characterize SVs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40142-025-00213-6.

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Author Contributions AK and CR conceived and designed the study. LMLC performed NGS initial analysis. ACDM performed the NGS reanalysis and was responsible for the initial writing of the manuscript. LMLC and ACDM produced the figures under the guidance of AK. AK performed the OGM analysis. DRB clinically evaluated the patient and his mother and contributed to the interpretation of the results. LMLC, ACDM and FTRV contributed to obtaining additional clinical data and biological samples. All authors have read, edited, and approved the final manuscript.

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Data Availability The frameshift variant *ACTB*(NM_001101.5:c.328 dup:p.Leu110Profs* 16) and the in tandem 7q11.21q11.22 duplication encompassing the entire *AUTS2* gene have been submitted to DECIPHER under patient ID 539578 (https://www.deciphergenomics.org/patient/539578/overview/general).

Declarations

Ethical Compliance and Informed Consent This research is in accordance with ethical standards established in the Declaration of Helsinki (1964), its subsequent revisions, and Resolution 466/2012 of the Brazilian National Health Council. The project was approved by the Research Ethics Committee of the Biosciences Institute of the University of São Paulo. Informed consent was obtained from the patient's mother, who also authorized the publication of photographs.

Conflicts of interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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