


BRIEF REPORT

Further delineation of putative *ACTB* loss-of-function variants: A 4-patient series

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Abstract

ACTB encodes β -cytoplasmic actin, an essential component of the cytoskeleton. Based on chromosome 7p22.1 deletions that include the *ACTB* locus and on rare truncating *ACTB* variants, a phenotype resulting from *ACTB* haploinsufficiency was recently proposed. We report putative *ACTB* loss-of-function variants in four patients. To the best of our knowledge, we report the first 7p22.1 microdeletion confined to *ACTB* and the second *ACTB* frameshifting mutation that predicts mRNA decay. A de-novo *ACTB* p.(Gly302Ala) mutation affects β -cytoplasmic actin distribution. All four patients share a facial gestalt that is distinct from that of individuals with dominant-negative *ACTB* variants in Baraitser-Winter cerebrofrontofacial syndrome. Two of our patients had strikingly thin and sparse scalp hair. One patient had sagittal craniosynostosis and hypospadias. All three affected male children have attention deficits and mild global developmental delay. Mild intellectual disability was present in only one patient. Heterozygous *ACTB* deletion can allow for normal psychomotor function.

KEYWORDS

ACTB, intellectual disability, loss-of-function, sparse scalp hair, β -cytoplasmic actin

1 | INTRODUCTION

Distinct phenotypes have been reported with distinct classes of heterozygous variants in *ACTB*, which encodes β -actin, an essential component of the cytoskeleton (a) the majority of reported pathogenic variants in *ACTB* are de-novo missense changes in exons 2-4 and were identified in patients with Baraitser-Winter cerebrofrontofacial syndrome (BWCF). These mutations are causing the disorder by gain-of-function or dominant-negative mechanisms (Di Donato et al., 2014; Riviere et al., 2012; Verloes et al., 2015). Among patients with BWCF, seven have been identified with juvenile-onset dystonia and the *ACTB* variant p.Arg183Trp (Procaccio et al., 2006; Skogseid et al., 2018). Whether dystonia represents a

rare nonspecific complication of BWCF or a manifestation specific to the *ACTB* variant p.Arg183Trp remains to be resolved; (b) deletion of *ACTB* was considered responsible for the clinical features observed in patients with rare, nonrecurrent 7p22.1 microdeletions (Cuvertino et al., 2017; Shimojima et al., 2016). Such microdeletions of ≤ 2 Mb in size were identified in patients with developmental delay, intellectual disability, short stature and microcephaly (7p22.1 microdeletion syndrome); a recent compilation of 23 microdeletions had identified a common deleted region of 0.37 Mb involving the *FBXL18*, *ACTB*, *FSCN1*, *RNF216*, and *ZNF815P* genes in such patients (Cuvertino et al., 2017). Recently, a de-novo 60 kb microdeletion encompassing only *FBXL18* and *ACTB* was identified in a 23-month-old child displaying symptoms of 7p22.1 microdeletion syndrome and

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distinctive facial features (Palumbo et al., 2018); (c) most recently, heterozygous variants in exons 5 and 6 of *ACTB* were reported in six patients with thrombocytopenia, minor facial dysmorphism, and microcephaly, with and without mild intellectual disability (Latham et al., 2018).

We report here four patients with putative *ACTB* loss-of-function variants, with intellectual disability being mild or absent, and with three male patients sharing attention deficits and behavioral problems. Strikingly thin scalp hair is present in two patients, and craniosynostosis and hypospadias in one patient. The sharing of patient-related data was facilitated by the GeneMatcher tool (Sobreira, Schiettecatte, Valle, & Hamosh, 2015).

The human subjects in this study were tested for diagnostic purposes after obtaining written informed consent. **Patient 1** is an 11-year-old male with mild developmental delay and attention deficit. He attends a mainstream school with 1 year of delay and performance in the lower range. Two episodes of generalized seizures occurred at an age of 5 years, and since then he remained seizure-free without anticonvulsive treatment. From the age of 3 years, he developed an axonal neuropathy that progresses slowly. He has high-arched feet and requires ankle-foot orthoses for ambulation; there is no sensory involvement at age 11 years. The neuropathy is paternally inherited, and present also in a younger brother; there is mild facial dysmorphism (Figure 1a). Routine blood count at age 6 years was normal (no thrombocytopenia). **Patient 2** is the 35-year-old mother of patient 1. She completed lower secondary education. She has strikingly thin and sparse scalp hair (Figure 1b). Facial gestalt shows wavy interrupted eyebrows, dense eyelashes, wide nose, wide mouth, prominent cheeks, and chin. She has hyperopia. Several routine blood counts were normal. **Patient 3** is a 4-year-old male with slow weight gain in the neonatal period. There is a global developmental delay which improves with speech and language therapy. His language skills at the age of 4 years are at the level of 2.5–3 years old. Autism spectrum disorder was diagnosed by Autism Diagnostic Observation Schedule. He does demonstrate a social interest in other children but lacks the skills to engage with them appropriately. He has starrng spells, but EEGs have been normal. Cranial magnetic resonance imaging (MRI) was normal, but a CT of the skull showed sagittal craniosynostosis at an age of 2 years. He had hypospadias with surgical repair, phimosis, penile torsion, and congenital chordee. There is facial dysmorphism with deeply set eyes with prominent brow ridge, a broad nasal tip, large prominent ears and pointed chin, and sparse scalp hair (Figure 1c). He has long toes with 2nd and 5th toe clinodactyly. He has a small patent ductus arteriosus at an age of 4 years, and spells of tachycardia. In several blood counts, no thrombocytopenia has ever been noted. **Patient 4** is a 6-year-old male. Attention deficit disorder was diagnosed. He attends a normal school with support. His comprehension is good but speech is delayed. He has thin scalp hair, deep set eyes, wide nose, deep-set columella, strabismus, and myopia (Figure 1d). Anthropometric patient data are compiled in Table 1.

Whole-exome sequencing (WES) was performed in all patients (Baumann, Steichen-Gersdorf, Krabichler, Muller, & Janecke, 2017)

as no specific clinical diagnosis was suggested. WES variation data were filtered for low allele frequencies corresponding to a rare monogenic disease, using data from two population cohorts (Exome Sequencing Project [ESP] and the Exome Aggregation Consortium, ExAC; <http://exac.broadinstitute.org/>). We identified three distinct *ACTB* variants in these families (submitted to the Leiden open variation database, https://grenada.lumc.nl/LSDB_list/lstdbs/ACTB), that are all predicted to cause *ACTB* loss-of-function; these variants are shown in Figure 1e, together with a compilation of reported *ACTB* variants and their classification.

Patient 1 and his mother (patient 2) harbor an intrachromosomal deletion of 36.6 kb (NC_000007.14:g.5522788_5559357del) on their shared chromosome 7, which completely removes the *ACTB* gene, and leaves approximately 9 and 36 kb of intergenic sequences intact with respect to its neighboring genes, *FBXL18* and *FSCN1*. To the best of our knowledge, this represents the first 7p22.1 microdeletion that is confined to *ACTB*. The boundaries of this intrachromosomal 7p22.1 deletion, which was detected in WES data with the panelcn.MOPS software (Povysil et al., 2017), were narrowed down by quantitative polymerase chain reaction (qPCR) to ultimately facilitate breakpoint sequencing in patients 1 and 2. *ACTB* PCR, qPCR, and sequencing primer sequences were based on the NCBI reference sequence for mRNA (NM_001101.4), and genomic DNA (NG_007992.1). Primer sequences and PCR conditions are available from the authors upon request. Nucleotide numbering reflects complementary DNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence. Patient 3 carries a heterozygous *ACTB* c.890_891delCA frameshift variant in exon 5 (p.Thr297Serfs*37), which is predicted to trigger nonsense-mediated mRNA decay (NMD; Lykke-Andersen & Jensen, 2015). Patient 4 carries a de-novo p.(Gly302Ala) variant in *ACTB*; the introduction of the larger alanine for glycine is predicted to bring the alanine residue in hydrogen-bonding distance to ADP as modeled on pdb structure 6FM2 (Kotila et al., 2018; Figure 1f,g). The effect of this missense variant was predicted to be deleterious by three of four different in silico prediction programs, Provean (Choi, Sims, Murphy, Miller, & Chan, 2012), MutationTaster (Schwarz, Rodelsperger, Schuelke, & Seelow, 2010), and CADD (Rentzsch, Witten, Cooper, Shendure, & Kircher, 2019) and to be benign by Polyphen2 (Adzhubei et al., 2010; Table 1). The expression of *ACTB* was analyzed in mononuclear cells isolated from patient 4 and healthy donor blood samples; we found that that the p.(Gly302Ala) mutation neither affects *ACTB* mRNA nor total *ACTB* levels (Figure 1h,i). Fractionation of cell lysates into detergent-soluble (s) and insoluble (ins) fractions, however, revealed a substantial decrease in the amount of cytoskeleton-associated *ACTB* in P4 lymphocytes (Figure 1j). We also detected an elevated level of an α -tubulin form with a lower molecular weight in the patient (Figure 1i,j). This form showed a high degree of acetylation in the soluble fraction and was not detected in control samples (Figure 1k). Our findings indicate that the expression of *ACTB* p.(Gly302Ala) variant is likely to affect the lymphocyte cytoskeleton organization by changing the cellular distribution and

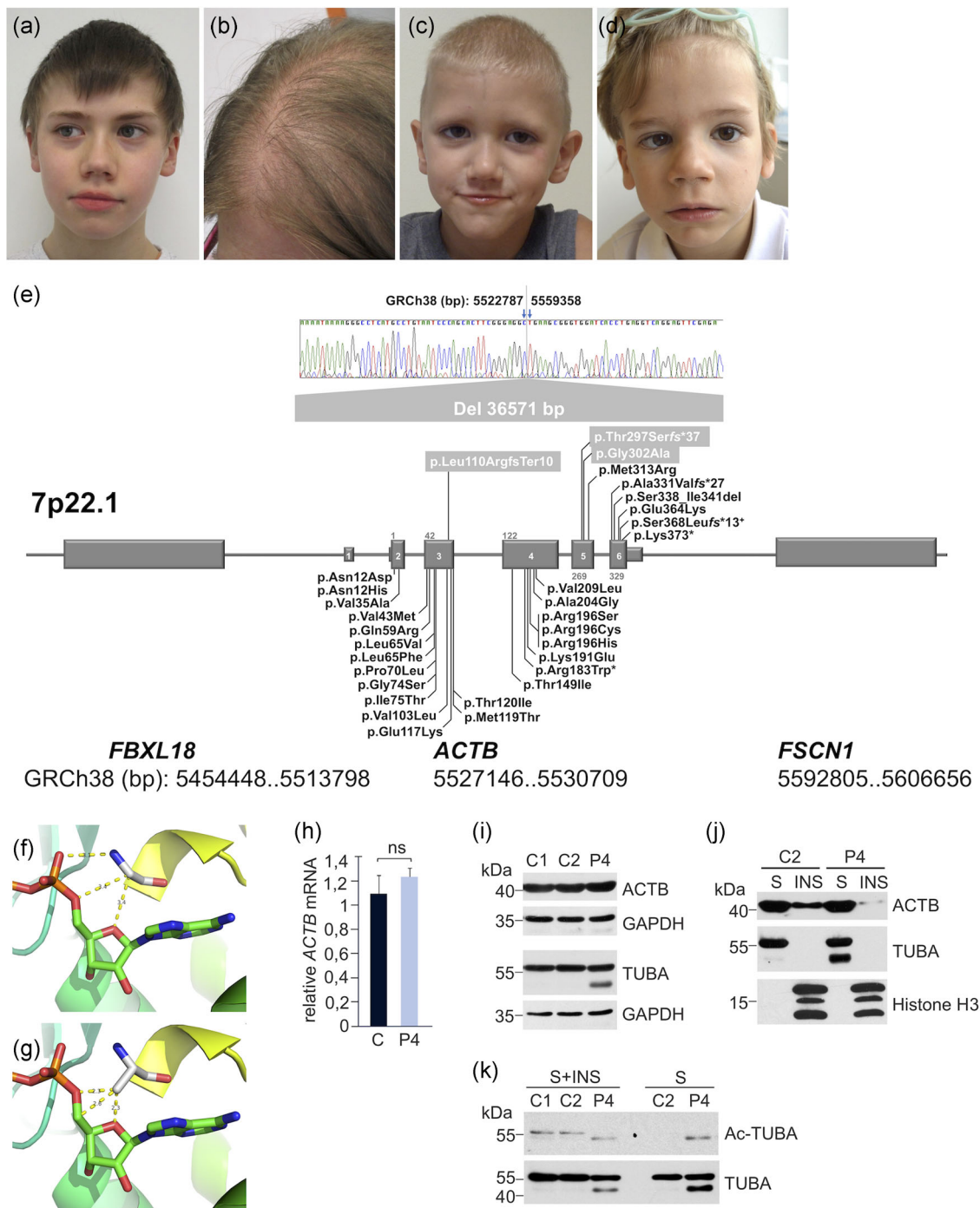


FIGURE 1 Clinical findings, *ACTB* variant compilation, and effects of the *ACTB* p.(Gly302Ala) variant. (a) Patient 1 at the age of 11 years. (b) Patient 2 at the age of 35 years. (c) Patient 3 at the age of 6 years. (d) Patient 4 at the age of 4 years. Shared facial dysmorphism consists of wavy interrupted eyebrows, dense eyelashes, wide nose, wide mouth, prominent cheeks, and chin. Sparse scalp hair in patients 2 and 4. (e) Compilation of novel and reported *ACTB* variants associated with BWCF, putative *ACTB* loss-of-function and *ACTB*-AST (chromosomal order of genes and exons not drawn to scale). Modeling of *ACTB* residue Gly-302 (f) and variant Ala-302 (g). (h) The relative *ACTB* messenger RNA expression in a healthy donor (C) and Patient 4 (P4) was assessed by quantitative real-time polymerase chain reaction using the $\Delta\Delta C_t$ method. Values were normalized to the amount of human ribosomal protein L32 mRNA. Immunoblot analysis of *ACTB* and α -tubulin (TUBA) in total lysates, NP-40 soluble (S) and insoluble (INS) fractions of blood mononuclear cells from healthy individuals (C1 and C2) and Patient 4 (P4). GAPDH and histone H3 were used as loading controls (i,j). (k) TUBA acetylation was analyzed in total lysates (S+INS) and NP-40 soluble (S) fraction obtained from control and Patient 4 blood mononuclear cells using anti-Ac-TUBA and anti-TUBA antibodies. BWCF, Baraitser-Winter cerebrofrontofacial syndrome

TABLE 1 Clinical history and findings in four patients with putative ATCB loss-of-function variants

Patient	Sex	Age (years)	Prenatal and neonatal history, birth measures		Postnatal growth retardation	Microcephaly	Motor delay	Speech delay	Intellectual disability	Behavioral, psychiatric and neurological features		Facial gestalt	Malformation and physical anomalies	ACTB Variant	Inheritance
			Normal At term	SGA						Attention deficit, seizures at age 5 years, additional paternally inherited axonal neuropathy	None				
1	M	11	Normal 40 weeks	SGA 40 weeks	N (3rd-10th centile)	N (10th-25th centile)	Y	Y	None	Attention deficit, seizures at age 5 years, additional paternally inherited axonal neuropathy	Wavy interrupted eyebrows, dense eyelashes, wide nose, wide mouth, prominent cheeks and chin	Anisometropia, unilateral high-grade hyperopia and astigmatism	Complete heterozygous ACTB gene deletion	Maternal (son of patient 2)	
2	F	35	Normal At term	SGA 40 weeks	N (25th centile)	N (25th centile)	N	N	None	None	Sparse scalp hair, wavy interrupted eyebrows, dense eyelashes, wide nose, wide mouth, prominent cheeks and chin	Hyperopia	Complete heterozygous ACTB gene deletion	Unknown	
3	M	4	Normal At term	SGA 40 weeks	N (10th-25th centile)	N (10th-25th centile)	Y	Y	None	Autism, staring spells with normal EEG, possible attention deficit and impulse control disorder	Sparse scalp hair, deeply set eyes with prominent brow ridge, broad nasal tip, pointed chin, large prominent ears	Long toes with 2nd and 5th toe clinodactyly, sagittal craniosynostosis, hypospadias, patent ductus arteriosus	Heterozygous c.890.891delCA; p.Thr297Serfs*37	De novo? - no access to paternal sample	
4	M	6	Normal 40 weeks	SGA 40 weeks	N (25th-50th centile)	N (3rd-10th centile)	Y	Y	Mild, WISC: FSIQ 66	Attention deficit, visuospatial deficits, speech delay	Thin hair, deep set eyes, wide nose, deep set columella	Strabismus, myopia	Heterozygous c.905G>C; p.(Gly302Ala) MutationTaster: pathogenic (0.999; range 0-1) CADD score: pathogenic (24.0; range 0-100) PolyPhen2: benign (0.281; range 0-1) Provean: deleterious (-4.177, range -40-12.5, cutoff: <-2.5)	De novo	

Abbreviations: CADD, combined annotation dependent depletion; F, female; FSIQ, full scale intelligence quotient; ID, intellectual disability; M, male; N, no or none known; SGA, small for gestational age; U, unknown; WISC, Wechsler intelligence scale for children; Y, yes; Y, years.

properties of microfilament and microtubule specific proteins, β -actin and α -tubulin, respectively.

We performed WES in three unrelated boys with mildly delayed motor and language development, and deficits in attention span and social communication skills. These patients share the facial features with patients described by Latham et al. (2018), which, however, might not be sufficiently specific to clinically diagnose a disease entity. The identified *ACTB* deletion in patients 1 and 2, and the frameshift mutation in patient 3 are supposed to abrogate *ACTB* protein production from these alleles; unfortunately, no material was available from these patients to study their *ACTB* transcript and protein levels, and address the question of *ACTB* haploinsufficiency. A recent study found no consistent differences in β -cytoplasmic actin levels in cells from four individuals with *ACTB* microdeletions versus controls; nevertheless, these authors proposed that *ACTB* haploinsufficiency causes the symptoms of 7p22.1 microdeletion syndrome, where the core features were intellectual disability, developmental delay and mild facial dysmorphism (Cuvertino et al., 2017). In that study, 30 of 33 individuals had microdeletions in 7p22.1 with at least 3 further genes deleted in addition to *ACTB*. The sizes of the different intrachromosomal deletions were very variable, as were the degree of intellectual disability and developmental problems in these patients (Cuvertino et al., 2017). Three patients in that study had heterozygous truncating *ACTB* variants of which only one was likely to trigger NMD of *ACTB*; this patient had mild developmental delay and slight social inhibition, but had no other features of 7p22.1 microdeletion syndrome; this patient also had compound-heterozygous variants in *DPYD* (Cuvertino et al., 2017). Our study thus emphasizes that the deletion of one *ACTB* copy is not associated with intellectual disability per se, and can go without developmental delay and behavioral problems as seen in our patient 2. However, mild developmental delay and attention deficits with behavioral problems might emerge as typical although inconstant features of putative *ACTB* loss-of-function variants. Most recently, six individuals from four unrelated families carrying de-novo or cosegregating heterozygous variants in exons 5 and 6 of *ACTB* were described. Common features amongst this cohort of patients with 3'-*ACTB* variants included developmental delay, microcephaly, and transient or permanent thrombocytopenia with platelet anisotropy and enlarged platelets. Five of six patients presented with microcephaly and two of six patients had a mild intellectual disability. The authors named this condition *ACTB*-associated syndromic thrombocytopenia (*ACTB*-AST; Latham et al., 2018). Two late-truncating variants identified in *ACTB*-AST, p.(Ala331-Val_{fs}*27) and p.(Ser368Leu_{fs}*13), are not expected to trigger NMD. The p.(Ser368Leu_{fs}*13) was identified in a 5-year-old female with a history of recurrent thrombocytopenia during the first year of life, microcephaly, periventricular nodular heterotopias, and a low normal IQ. The same variant had been reported before in a patient with feeding difficulties, moderate intellectual disability, microcephaly, hyperactivity, dystonia and tracheoesophageal fistula, esophageal atresia, overlapping toes, a short foot, and tapered fingers and pectus excavatum (Cuvertino et al., 2017). This represents an observation of variable involvement in patients with the same *ACTB* variant, and

other observations are those of patient 1 and 2 in our study here, and patients 1 and 2 in Latham et al. (2018), where patient 1 presented with mild developmental delay, incomplete cleft lip, heart defect, microcephaly, and thrombocytopenia. His father, patient 2, only displayed thrombocytopenia. Father and child carried a p.(Met313Arg) variant in exon 5 (Latham et al., 2018), a rare instance of an *ACTB* missense variant not associated with *BWCFF*. The child's phenotype resembles the first patient described with an *ACTB* variant, a de novo p.(Glu364Lys) variant in exon 6 associated with moderate intellectual disability, abnormal white blood cell counts, and thrombocytopenia (Nunoi et al., 1999).

In the absence of functional and mechanistic studies, we hypothesize that the patients in our report, together with those described by Latham et al. (2018) represent a phenotypic spectrum with mild ID, facial dysmorphism, and variable effects on platelet morphology and function. This phenotypic spectrum is, however, widely different from *BWCFF* syndrome. *BWCFF* is a well-defined syndrome with recognizable facial features, developmental disability, neuronal migration defects, hearing loss, ocular colobomas, heart and renal defects, and progressive muscle wasting (Verloes et al., 2015) due to heterozygous missense variants in *ACTB*, that exert a gain-of-function effect.

Two of our patients had strikingly thin and sparse scalp hair, which was also seen in a number of patients reported with 7p22.1 microdeletion syndrome, which might argue for the presence of some degree of *ACTB* haploinsufficiency. The same holds for urogenital malformations, which were seen frequently in the cohort of patients with larger 7p22.1 deletions, including an instance of hypospadias and an instance of a micropenis. Hypospadias was present in our patient 3, suggesting that *ACTB* haploinsufficiency might contribute to this malformation. In contrast, craniosynostosis was not reported in patients with 7p22.1 deletions, suggesting that the craniosynostosis seen in patient 3 might be unrelated to *ACTB* haploinsufficiency. Among ten individuals with 7p22.1 microdeletion syndrome investigated, eight were found to have some structural abnormality detected by brain MRI. In our study, a single patient undergoing brain MRI had normal results, showing that structural brain defects are not a mandatory feature associated with putative *ACTB* loss-of-function variants or haploinsufficiency.

Coexamination of actin and microtubule cytoskeleton constituents in megakaryocytes and thrombocytes from patients with 3' *ACTB* variants indicated that these variants inhibited the final stages of platelet maturation by compromising microtubule organization, and variably brought about qualitative changes to β -actin filaments (Latham et al., 2018). In fibroblasts from patients with 7p22.1 microdeletion syndrome, altered cell shape, and migration, reduced cell proliferation, altered expression of cell-cycle genes, and decreased amounts of nuclear, but not β -cytoplasmic actin were observed. Reduced cell attachment surface area, volume and migratory capacity of *ACTB*-AST fibroblasts were also seen; variants affected polarization, movement, and morphology at the single-cell level, whilst the wild-type phenotype was rescued with cell-cell contact formation (Latham et al., 2018). *ACTB*-AST variants also

caused altered expression ratios of different actins and the highly disordered nature of β -tubulin in patient-derived platelets was demonstrated. Along these lines, we hypothesize that the novel p.(Gly302Ala) variant disturbs the fine balance between actin and microtubule cytoskeletal organization.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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