Could Dissimilar Phenotypic Effects of *ACTB* Missense Mutations Reflect the Actin Conformational Change? Two Novel Mutations and Literature Review

Anna Sandestig^a Anna Green^a Jon Jonasson^a Hartmut Vogt^b
Johan Wahlström^b Alexander Pepler^c Katarina Ellnebo^a Saskia Biskup^c
Margarita Stefanova^a

Departments of ^aClinical Genetics and ^bPediatrics, Linköping University Hospital, Linköping, Sweden;
^cCeGaT GmbH, Tübingen, Germany

Keywords

ACTB gene · Actin · Baraitser-Winter syndrome ·
Missense mutation · Protein conformational change ·
Residue position

Abstract

The beta-actin gene encodes 1 of 6 different actin proteins. De novo heterozygous missense mutations in *ACTB* have been identified in patients with Baraitser-Winter syndrome (BRWS) and also in patients with developmental disorders other than BRWS, such as deafness, dystonia, and neutrophil dysfunction. We describe 2 different novel de novo missense *ACTB* mutations, c.208C>G (p.Pro70Ala) and c.511C>T (p.Leu171Phe), found by trio exome sequencing analysis of 2 unrelated patients: an 8-year-old boy with a suspected BRWS and a 4-year-old girl with unclear developmental disorder. The mutated residue in the first case is situated in the actin H-loop, which is involved in actin polymerization. The mutated residue in the second case (p.Leu171Phe) is found

at the actin barbed end in the W-loop, important for binding to profilin and other actin-binding molecules. While the boy presented with a typical BRWS facial appearance, the girl showed facial features not recognizable as a BRWS gestalt as well as ventricular arrhythmia, cleft palate, thrombocytopenia, and gray matter heterotopia. We reviewed previously published *ACTB* missense mutations and ascertained that a number of them do not cause typical BRWS. By comparing clinical and molecular data, we speculate that the phenotypic differences found in *ACTB* missense mutation carriers might supposedly be dependent on the conformational change of *ACTB*.

© 2018 S. Karger AG, Basel

The beta (β)-actin gene (*ACTB*; OMIM 102630) encodes 1 of 6 different actin isoforms. *ACTB* is one of the most highly-conserved proteins known, and it is ubiquitously expressed as a cytoplasmic protein. It has 4 different subdomains with diverse functions. The only 2

cytoplasmic actins, β - and γ -actin, are essential for cell migration, cell shape maintenance, mitosis, and intracellular transport processes. The driving force behind these cellular activities is the ability of actin for the transition between monomeric globular G-actin and polymeric filamentous F-actin states under the control of nucleotide hydrolysis, ions, and actin-binding proteins. This involves a cyclic process of G-actin polymerization to F-actin and depolymerization of the F-actin to actin-monomer. The actin filament assembly/disassembly requires careful regulation, both temporally and spatially. In the cell, this regulation is controlled by a series of actin-binding proteins such as the mammalian profilin and gelsolin [Fujii et al., 2010; Dominiguez and Holmes, 2011; Porta and Borgstahl, 2012; Kühn and Mannherz, 2017]. Each of the actin 3-dimensional structures (4 different subdomains, a nucleotide-binding site, actin loops, pointed and barbed end, and C- and N-terminal) has a specific, but not yet fully understood role in this process.

Rivière et al. [2012] showed that mutations in the *ACTB* gene cause Baraitser-Winter syndrome (BRWS; OMIM 243310). They found de novo mutations of *ACTB* in 10 patients with BRWS. Mutation clustering suggested a gain-of-function effect. Verloes et al. [2015] presented detailed phenotypic descriptions and neuroimaging of 36 patients and reviewed 6 cases from the literature with a molecularly proven actinopathy. Recently, Yates et al. [2017] provided an overview of the clinical characteristics (including some novel findings), diagnosis, management, mutation spectrum, and genetic counseling.

Heterozygous disease-causing missense mutations of *ACTB* have also been reported in patients with a phenotype that cannot be recognized as BRWS [Nunoi et al., 1999; Gearing et al., 2002; Procaccio et al., 2006; Johnston et al., 2013; Conboy et al., 2017].

A distinct but “non-BRWS” phenotype has been recently described by Cuvertino et al. [2017] in a large cohort of patients with loss-of-function mutations (deletion, frameshift, and nonsense mutations) in *ACTB*.

We studied 2 unrelated patients with 2 novel de novo heterozygous missense mutations in the *ACTB* gene: an 8-year-old boy with c.208C>G (p.Pro70Ala) and a 4-year-old girl with c.511C>T (p.Leu171Phe). While the diagnosis BRWS was clinically suspected for the boy, the girl showed a facial gestalt that would not be recognized as BRWS as well as ventricular arrhythmia and thrombocytopenia.

Clinical Reports

Patient 1

The 8-year-old boy is the second child to young healthy nonconsanguineous parents of Arabic origin and unremarkable family history. He is a product of a term gestation, born after an uneventful pregnancy and delivery with a birth weight of 3,300 g (–1 SD), and a birth length of 53 cm (+1 SD). Notable craniofacial features included distinct hypertelorism, epicanthus, a prominent nasal bridge, ptosis, anteverted nares, smooth philtrum, a thin upper lip (Fig. 1a), small ears with increased posterior angulation and a prominent helix, short neck, broad and short hands, short fingers, single palmar crease (Fig. 1b, c), multiple café-au-lait spots, low-set nipples, retention testis, and an overriding scrotum. His weight at the age of 7.5 years was 22 kg (–1 SD), height 115 cm (–2 SD), and his head circumference at 5 years of age was 48.8 cm (–1.2 SD). The boy showed moderate intellectual disability; he started walking at the age of 15 months and presented with a delayed speech development. Brain MRI showed a Chiari malformation, grade 1, but no changes indicating pachygyria or heterotopia. Ophthalmologic investigation revealed astigmatism and hyperopia, no colobomas were found. Ultrasound of the heart and kidney showed no changes. Previous genetic testing included SNP-array analysis which was normal.

Patient 2

The 4-year-old girl is the second child to nonconsanguineous healthy parents of Swedish origin. The mother was 35 years old and the father 42 years old at conception. The family history was unremarkable in respect to the patients’ phenotype. During the pregnancy, polyhydramnios and fetal ventricular arrhythmia (extrasystoles) were noted at 24 weeks of gestation, and the delivery was induced at 38 weeks of gestation (Apgar score 4–8–10). Her birth weight was 3,100 g (–1 SD), length 51 cm (+0.5 SD), and her head circumference was 33 cm (–1.8 SD). The girl presented with a midline cleft soft palate and ventricular arrhythmia, diagnosed prenatally, which required regular beta-blocker medication. Echocardiography showed no changes. Isolated thrombocytopenia was observed soon after birth and followed up until present. Bone marrow exam showed no morphological abnormalities and no signs of malignancy. Notable dysmorphic features included a metopic ridge, deep-set eyes, mild telecanthus, midface hypoplasia, a broad nose, small jaw, and posteriorly rotated ears with prominent helices and thick lobuli (Fig. 1d, e). Additionally, she had long and slender hypermobile fingers, overriding toes (Fig. 1f, g), and umbilical hernia. At the age of 4 years, her weight was 15.7 kg (0.5 SD) and height 103 cm ($\pm$ 0 SD). Her head circumference at the age of 2.5 years was 47.8 cm (–1 SD). The girl’s development was delayed; she sat steadily without support at the age of 11 months and started walking at the age of 2 years. She exhibits jerk movements and weakness of the arms. Brain MRI showed mild gray matter heterotopia and periventricular leukodystrophy. An ophthalmologic exam revealed astigmatism and hyperopia, no colobomas were detected. Kidney ultrasound showed no changes.

Previous genetic testing included SNP-array analysis and *KCNJ2* mutation screening in order to rule out a suspected diagnosis of Andersen syndrome. Both results were normal.

Although the patient was a subject of extensive diagnosis-solving discussion held at the Swedish Dysmorphology Meeting with a participation of experienced European syndromologists, BRWS was not suggested.

Fig. 1. a–c Patient 1 phenotype at the age of 5 years showing typical BRWS gestalt: palpebral fissures, downslanting arched eyebrows, hypertelorism, ptosis, prominent nasal bridge, anteverted nares, long smooth philtrum, a thin upper lip (**a**), small ears with a prominent helix and uplifted lobule, a short neck (**b**), and broad and short hands and fingers (**c**). **d–g** Patient 2 phenotype at the age of 3 years (**d**) and at the age of 1 year (**e–g**) with dysmorphic features including arched eyebrows, deep-set eyes, midface hypoplasia, a broad nose, prominent philtrum, small jaw (**d**), posteriorly rotated ears with thick lobuli (**e**), tapering fingers with hypermobile interphalangeal joints, clinodactyly of the 5th finger (**f**), and overriding toes (**g**).

Materials and Methods

In order to screen for disease-causing genetic changes, diagnostic trio exome sequencing analyses were performed in the settings of a research project.

Trio exome sequencing of patient 1 and his parents was performed at the Oxford Gene Technology (OGT, England) by the Illumina HiSeq2000 platform using TruSeqv3 chemistry. Read files were mapped to human reference genome (hg19) using the Burrows Wheeler Aligner (BWA) package, version 0.6.2. Local realignment of the mapped reads around potential insertion/deletion sites was carried out with the Genome Analysis Tool Kit (GATK) version 1.6. Duplicate reads were marked using Picard version 1.107. Additional BAM file manipulations were performed with Samtools 0.1.18. SNP and indel variants were called using the GATK Unified Genotyper. SNP novelty was determined against dbSNP release 137. Variants were annotated with gene and gene function data from Ensembl. Known variants from dbSNP are annotated within the dataset.

Trio exome sequencing of patient 2 and her parents was performed by the Center for Genomics and Transcriptomics (CeGaT, Germany). The coding regions and flanking intronic regions were enriched using Agilent SureSelect XT All Exon V6 in-solution technology and were sequenced using the Illumina HiSeq4000 system. Illumina CASAVA was used to demultiplex sequencing reads. The reads were mapped to the human reference genome (hg19) using the BWA, and variants were called using Samtools and varscan. Variants in the coding region and the flanking intronic regions (± 8 bp) with a minor allele frequency (MAF) $< 1.5\%$ were

evaluated. Known disease-causing variants (according to HGMD) within ± 30 bp of flanking regions and up to 5% MAF were also evaluated. MAFs are taken from the following databases: 1000 Genomes, dbSNP, Exome Variant Server, ExAC, and an in-house database.

Results

In patient 1, trio exome sequencing analyses revealed a novel *de novo* heterozygous missense variant of the *ACTB* gene NM_001101.3:c.208C>G, resulting in a proline-to-alanine substitution at amino acid residue 70 (p.Pro70Ala) in the H-loop, residue 70–78.

In patient 2, trio exome sequencing analyses revealed a novel *de novo* heterozygous missense variant of the *ACTB* gene NM_001101.3:c.511C>T, resulting in a leucine-to-phenylalanine change at amino acid residue 171 (p.Leu171Phe) in the W-loop, residue 165–172.

Both gene variants were verified by Sanger sequencing. Parentage was confirmed by the thousands of variants in common between child and parent in the exome trio analysis. No other *de novo* or rare biallelic variants have been found in the patients.

Discussion

We report on 2 unrelated patients where exome sequencing analysis has revealed 2 novel de novo missense mutations in the *ACTB* gene. The affected individuals, an 8-year-old boy (patient 1) and a 4-year-old girl (patient 2), exhibited different clinical features. While the BRWS gestalt was recognizable in patient 1 (Fig. 1a), it could not be seen in patient 2 (Fig. 1d) – not even by a group of experienced dysmorphologists and not even after actinopathy had been molecularly confirmed. Additionally, patient 2 showed features that are not typically found in persons with *ACTB* mutations, such as ventricular arrhythmia with prenatal debut and thrombocytopenia. Individuals with *ACTB* mutations have been reported with a broad phenotypic variability [Verloes et al., 2015] and, in some cases, described as distinct clinical entities [Johnston et al., 2013]. Verloes et al. [2015] studied 36 patients with a molecularly proven actinopathy and described 6 additional cases from the literature (33 *ACTB* and 9 *ACTG1*). They suggested that there could be an important mutation-specific variability in the phenotype of actinopathies. Johnston et al. [2013] hypothesized that the distinct disease phenotypes associated with the various β -actin mutations reflect the sensitive allosteric regulation associated with normal actin cytoskeletal dynamics and the sensitivity that developmental processes have to alterations in cytoskeletal behavior. The phenotype associated with a particular mutation will be affected by the extent to which a particular mutation affects the allosteric behavior of this peptide, resulting in either differential effects on filament dynamics or the ability of actin to be controlled by regulatory proteins that interact with this allosteric system.

Both patient 1 of this study (p.Pro70Ala) and patient B15 (p.Pro70Leu) in Verloes et al. [2015], originally described by Bitton et al. [2012], had a recognizable BRWS phenotype. The mutated amino acid residue is a part of the actin H-loop, denoted as sensor loop [Graceffa and Dominguez, 2003], which is involved in a conformational change regulating actin filament dynamics, i.e., in actin polymerization/depolymerization [Porta and Borgstahl, 2012; Johnston et al., 2013] (Table 1). The affected residues of the typical BRWS patients B23, B29, and B20 in Verloes et al. [2015] lie also in the H-loop (Table 1). Similarly, the most common recurrent missense change at residue 196, shown to cause clinically recognizable BRWS phenotype in 14 patients [Rivière et al., 2012; Verloes et al., 2015], is placed at the large actin domain that is thought to help alignment of actins in the polymerization

process [Porta and Borgstahl, 2012] (Table 1). Additionally, patients with BRWS phenotype were shown to present with mutations affecting residue 12 [Rivière et al., 2012; Verloes et al., 2015] in the actin S-loop (residues 11–16), which exhibits a pronounced H-bonding (Table 1).

Accordingly, the BRWS phenotype seems likely to be dependent on anomalous actin polymerization due to a structural conformational change of the actin G-monomer. The *ACTB* gene is known to exhibit haploinsufficiency but loss-of-function mutations do not cause BRWS [Cuvertino et al., 2017].

We also reviewed the heterozygous missense *ACTB* mutations in individuals who do not show a recognizable BRWS phenotype, as in our patient 2 (Table 1). The residue 171 affected by the missense change (p.Leu171Phe) lies in the actin W-loop (residues 165–172), which is located at the actin barbed end and makes contact to stabilize the profilin binding to actin as well as actin binding to other actin subunits in F-actin. The W-loop has been described as a nucleotide state sensor [Porta and Borgstahl, 2012]. Structural changes in the W-loop will primarily affect the interactions with actin-binding proteins. Thus, it is conceivable that one should expect a more pleiotropic effect of W-loop structural variation than that observed for H-loops. Nunoi et al. [1999] described a 12-year-old female with a heterozygous de novo mutation in the *ACTB* gene, c.1174G>A (p.Glu364Lys), and revealed that the patient's mutant actin showed an impaired binding to profilin in vitro and intact actin polymerization (Table 1). The affected residue 364 lies at the C-terminal domain, also important for binding to profilin and other actin-regulatory molecules [Porta and Borgstahl, 2012]. The patient had moderate intellectual impairment, repeated infections, thrombocytopenia, and impaired neutrophil functions. She was not recognized as BRWS; a description of facial features or photos were not available. Interestingly, this patient and our patient 2 are the only known *ACTB* mutation carriers reported to have thrombocytopenia. Contradictory results regarding the interaction of p.E364K actin with profilin have been published [Hundt et al., 2017]. Additionally, Johnston et al. [2013] described a female patient diagnosed originally as Dubowitz syndrome and shown to have an *ACTB* mutation, c.349G>A (p.Glu117Lys) (Table 1). The authors state that her diagnosis was changed to BRWS, based on the molecular findings, with absence of some of the characteristic findings of this diagnosis. Residue 117 found mutated in this patient is a part of a helix (residues 113–118), which appears to interact across the strand-strand

Table 1. Clinical manifestations of patients with *ACTB* missense mutations listed by the respective actin region and its function

Mutation	Actin region	Main functional involvement	Phenotype	Reference
c.208C>G; p.Pro70Ala	H-loop (SD1)	P/DP by DNaseI binding	BRWS	Patient 1 in this report
c.209C>T; p.Pro70Leu	H-loop (SD1)	P/DP by DNaseI binding	BRWS	Bitton et al., 2012; B15 in Verloes et al., 2015
c.220G>A; p.Gly74Ser	H-loop (SD1)	P/DP by DNaseI binding	BRWS	Patient 2 in Di Donato et al., 2013; B23 in Verloes et al., 2015
c.220G>A; p.Gly74Ser	H-loop (SD1)	P/DP by DNaseI binding	BRWS	B29 in Verloes et al., 2015
c.224T>C; p.Ile75Thr	H-loop (SD1)	P/DP by DNaseI binding	BRWS	B20 in Verloes et al., 2015
c.34A>G; p.Asn12Asp	S-loop (SD1)	P/DP by DNaseI binding	BRWS	LP98-085 in Rivière et al., 2012; B2 in Verloes et al., 2015
?, p.Val43Met	D-loop (SD2)	P/DP by DNaseI binding	BRWS	Patients 1 and 2 (mother and daughter) in Yates et al., 2017
?, p.Met47Thr	D-loop (SD2)	P/DP by DNaseI binding	BRWS	Patient 4 in Yates et al., 2017
c.193C>G; p.Leu65Val	Residue of an interstrand contact area (SD2)	P/DP by providing an interstrand contact area to for the hydrophobic plug loop	BRWS	Patient 2 in Ramer et al., 1995; LP90-050 in Rivière et al., 2012; B3 in Verloes et al., 2015
c.193C>T; p.Leu65Phe	Residue of an interstrand contact area (SD2)	P/DP by providing an interstrand contact area to for the hydrophobic plug loop	BRWS	B33 in Verloes et al., 2015
c.586C>T; p.Arg196Cys	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	61456 in Rivière et al., 2012; B6 in Verloes et al., 2015
c.586C>T; p.Arg196Cys	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	Der Kaloustian et al., 2001; Patient 3 in Di Donato et al., 2013; B24 in Verloes et al., 2015
c.586C>T; p.Arg196Cys	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	B1 in Verloes et al., 2015
c.586C>T; p.Arg196Cys	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	B18 in Verloes et al., 2015
c.586C>T; p.Arg196Cys	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	B19 in Verloes et al., 2015
c.586C>T; p.Arg196Cys	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	Eker et al., 2014; B30 in Verloes et al., 2015
c.586C>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	B21 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	58248 in Rivière et al., 2012; B4 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	59169 in Rivière et al., 2012; B5 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	LR04-173 in Rivière et al., 2012; B7 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	LR09-079 in Rivière et al., 2012; B8 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	59169 in Rivière et al., 2012; B9 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	Patient 1 in Fryns and Aftimos, 2000; 11-11287 in Rivière et al., 2012; B10 in Verloes et al., 2015
c.587G>A; p.Arg196His	Loop 195-198 (SD4)	P/DP by transverse interaction across the helix axis	BRWS	11-10211 in Rivière et al., 2012; B13 in Verloes et al., 2015

Table 1 (continued)

Mutation	Actin region	Main functional involvement	Phenotype	Reference
c.611C>G; p.Ala204Gly	Helix Glu207 (SD4)	P/DP by flattening of the actin molecule by a propeller-like twist of the 2 major actin domains as well as by providing an interstrand contact area	BRWS	B14 in Verloes et al., 2015
c.625G>A; p.Val209Leu	Helix Glu207 (SD4)	P/DP by flattening of the actin molecule by a propeller-like twist of the 2 major actin domains as well as by providing an interstrand contact area	BRWS	B25 in Verloes et al., 2015
c.733G>A; p.G245S	Residue of an interstrand contact area (SD4)	P/DP by providing an interstrand contact area for the actin subunits along the long-pitch strands	BRWS	Patient 1 in Okamoto et al., 2015
c.511C>T; p.Leu171Phe	W-loop, barbed end (SD3)	ABP interaction by providing a stable contact of the profilin binding to actin	Non-BRWS	Patient 2 in this report
c.349G>A; p.Glu117Lys	α -helix of residues 113–118 (SD1)	ABP interaction by strand-strand interface interaction of the actin filament	Non-BRWS	Patient 1 in Johnston et al., 2013; B31 in Verloes et al., 2015
c.547C>T; p.Arg183Trp	Close to the nucleotide ATP (SD4)	ABP interaction by influencing opening of the nucleotide cleft between the SD1–SD2 and SD3–SD4 domains	Non-BRWS	Gearing et al., 2002; Procaccio et al., 2006; B26 and B27 (twins) in Verloes et al., 2015
c.547C>T; p.Arg183Trp	Close to the nucleotide ATP (SD4)	ABP interaction by influencing opening of the nucleotide cleft between the SD1–SD2 and SD3–SD4 domains	Non-BRWS	Conboy et al., 2017
c.1174G>A; p.Glu364Lys	C-termini, barbed end (SD1)	ABP interaction by providing a stable contact of the profilin binding to actin	Non-BRWS	Nunoi et al., 1999

ABP, actin-binding proteins; P/DP, polymerization/depolymerization; SD1–SD4, subdomains 1–4; ?, unknown position.

References list all publications where the respective patient has either been reported or reviewed.

The following references support the data in the columns 2 and 3: Otterbein et al. [2001]; Dominguez and Holmes [2011]; Porta and Borgstahl [2012]; Johnston et al. [2013]; Hundt et al. [2014]; Kühn and Mannherz [2017].

Previously reported patients with missense mutation affecting residues Gln59, Val103, Met119, Thr120, and Thr149 [Ramer et al., 1995; Di Donato et al., 2013; Verloes et al., 2015] are not included in the table since no clear information about the function of the respective region had been reported so far, to the best of our knowledge.

interface of the actin filament (Table 1). The authors discuss that most mutations they have previously studied in the 113–118 helix seem to affect the interaction between actin and the regulatory proteins with lesser effects on the actin's inherent polymerization properties (Table 1). We reviewed 3 additional patients with non-BRWS phenotype, 2 related (twins) [Gearing et al., 2002; Procaccio et al., 2006; Verloes et al., 2015] and one unrelated [Conboy et al., 2017], all 3 showing missense mutations affecting residue Arg183 (Table 1). All 3 patients shared common features such as mild intellectual disability, refractory dystonia with an especially severe bulbar component, and hearing loss, but none of them exhibited BRWS facial features. It was shown that p.R183W mutation directly interferes with nucleotide cleft between the SD1–SD2 and SD3–SD4 domains [Hundt et al., 2014].

In our literature review, most of the reported patients with missense mutations of *ACTB* and even *ACTG1* presented with a BRWS phenotype. This could be due to a bias of ascertainment. Requests for sequencing analysis of the *ACTB* gene will primarily concern patients with suspected BRWS. Wider use of exome sequencing analysis could help identify the genetic etiology, even behind atypical disease.

In summary, because the 2 novel mutations studied here showed 2 distinct phenotypes, we reviewed the previously reported *ACTB* missense mutations with regard to the residue position and correlated the phenotype with the expected effect of the predicted 3D conformational change on G-actin interactions. We suggest that 2 main groups of *ACTB* missense mutations emerge, represented by the 2 patients studied here:

(1) Variants, such as p.P70A in patient 1, resulting in anomalous actin polymerization/depolymerization dynamics, which would result in a recognizable BRWS phenotype.

(2) Variants, such as p.L171Phe in patient 2, mainly affecting binding and regulation of actin by profilin and other regulatory proteins, which would result in a pleiotropic phenotype not readily associated with typical BRWS. A separate entity might be more appropriate for notifying both this phenotype and the phenotype described by Cuvertino et al. [2017], due to deletion, frameshift and nonsense mutations of *ACTB*, for example a non-BRWS actinopathy.

In all reviewed cases, we were able to show a correlation between the phenotype and the functional specificity of the actin structure. Functional studies will be needed in order to prove this conjecture.

References

- Bitton N, Alexander S, Ruggiero S, Parameswaran A, Russo A, Ferguson F: Case report: Noonan-like multiple central giant cell granuloma syndrome. *Pediatr Dent* 34:144–147 (2012).
- Conboy E, Vairo F, Waggoner D, Ober C, Das S, et al: Pathogenic variant in *ACTB*, p.Arg183Trp, causes juvenile-onset dystonia, hearing loss, and developmental delay without midline malformation. *Case Rep Genet* 2017:9184265 (2017).
- Cuvertino S, Stuart HM, Chandler KE, Roberts NA, Armstrong R, et al: *ACTB* loss-of-function mutations result in a pleiotropic developmental disorder. *Am J Hum Genet* 101:1021–1033 (2017).
- Der Kaloustian VM, Pelletier M, Costa T, Blackston DR, Oudjhane K: A new syndrome with craniofacial and skeletal dysmorphisms and developmental delay. *Clin Dysmorphol* 10:87–93 (2001).
- Di Donato N, Rump A, Koenig R, Der Kaloustian VM, Halal F, et al: Severe forms of Baraitser-Winter syndrome are caused by *ACTB* mutations rather than *ACTG1* mutations. *Eur J Hum Genet* 22:179–183 (2014).
- Dominguez R, Holmes KC: Actin structure and function. *Annu Rev Biophys* 40:169–186 (2011).
- Eker HK, Derinkuyu BE, Ünal S, Masliah-Planchon J, Drunat S, Verloes A: Cerebro-frontofacial syndrome type 3 with polymicrogyria: a clinical presentation of Baraitser-Winter syndrome. *Eur J Med Genet* 57:32–36 (2014).
- Fryns JP, Aftimos S: New MR/MCA syndrome with distinct facial appearance and general habitus, broad and webbed neck, hypoplastic inverted nipples, epilepsy and pachygyria of the frontal lobes. *J Med Genet* 37:460–462 (2000).
- Fujii T, Iwane AH, Yanagida T, Namba K: Direct visualization of secondary structures of F-actin by electron cryomicroscopy. *Nature* 467:724–728 (2010).
- Gearing M, Juncos JL, Procaccio V, Gutekunst CA, Marino-Rodriguez EM, et al: Aggregation of actin and cofilin in identical twins with juvenile-onset dystonia. *Ann Neurol* 52:465–476 (2002).
- Graceffa P, Dominguez R: Crystal structure of monomeric actin in the ATP state. Structural basis of nucleotide-dependent actin dynamics. *J Biol Chem* 278:34172–34180 (2003).
- Hundt N, Preller M, Swolski O, Ang AM, Mannherz HG, et al: Molecular mechanisms of disease-related human β -actin mutations p.R183W and p.E364K. *FEBS J* 281:5279–5291 (2014).
- Johnston JJ, Wen KK, Keppler-Noreuil K, McKane M, Maier J, et al: Functional analysis of a de novo *ACTB* mutation in a patient with atypical Baraitser-Winter syndrome. *Hum Mutat* 34:1242–1249 (2013).
- Kühn S, Mannherz HG: Actin: structure, function, dynamics, and interactions with bacterial toxins. *Curr Top Microbiol Immunol* 399:1–34 (2017).
- Nunoi H, Yamazaki T, Tsuchiya H, Kato S, Malech HL, et al: A heterozygous mutation of beta-actin associated with neutrophil dysfunction and recurrent infection. *Proc Natl Acad Sci USA* 96:8693–8698 (1999).
- Okamoto N, Miya F, Tsunoda T, Kato M, Saitoh S, et al: Targeted next-generation sequencing in the diagnosis of neurodevelopmental disorders. *Clin Genet* 88:288–292 (2015).
- Otterbein LR, Graceffa P, Dominguez R: The crystal structure of uncomplexed actin in the ADP state. *Science* 293:708–711 (2001).
- Porta JC, Borgstahl GE: Structural basis for profilin-mediated actin nucleotide exchange. *J Mol Biol* 418:103–116 (2012).
- Procaccio V, Salazar G, Ono S, Stiers ML, Gearing M, et al: A mutation of beta-actin that alters depolymerization dynamics is associated with autosomal dominant developmental malformations, deafness, and dystonia. *Am J Hum Genet* 78:947–960 (2006).
- Ramer JC, Lin AE, Dobyns WB, Winter R, Aymé S, et al: Previously apparently undescribed syndrome: shallow orbits, ptosis, coloboma, trigonocephaly, gyral malformations, and mental and growth retardation. *Am J Med Genet* 57:403–409 (1995).
- Rivière JB, van Bon BW, Hoischen A, Kholmanskikh SS, O’Roak BJ, et al: De novo mutations in the actin genes *ACTB* and *ACTG1* cause Baraitser-Winter syndrome. *Nat Genet* 44:440–444 (2012).
- Verloes A, Di Donato N, Masliah-Planchon J, Jongmans M, Abdul-Raman OA, et al: Baraitser-Winter cerebrofrontofacial syndrome: delineation of the spectrum in 42 cases. *Eur J Hum Genet* 23:292–301 (2015).
- Yates TM, Turner CL, Firth HV, Berg J, Pilz DT: Baraitser-Winter cerebrofrontofacial syndrome. *Clin Genet* 92:3–9 (2017).

Acknowledgments

We gratefully acknowledge the families for participating in this study. Special thanks and acknowledgments are also due to John Cousin, BSc, Inside Sales Executive, Oxford Gene Technology, for his valuable assistance. This work was funded by the Forskningsrådet Sydöstra Sverige (FORSS) as well as by ALF Grants, Region Östergötland (LIO-440331).

Statement of Ethics

Ethics approval was provided by the Linköping University Ethics Committee, and written consent was obtained at the time of sample collection.

Disclosure Statement

The authors have no conflict of interest to declare.