



Cerebellar dysplasia related to *PIK3CA* mutation: a three-case series

Martina Di Stasi¹ · Giana Izzo² · Elisa Cattaneo³ · Vittoria Baraldini⁴ · Chiara Doneda² · Andrea Righini² · Daniela Graziani⁵ · Valentina Toto⁶ · Cecilia Parazzini²

Received: 13 April 2020 / Accepted: 3 September 2020 / Published online: 8 September 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

The term PROS (*PIK3CA*-Related Overgrowth Spectrum) indicates a wide spectrum of overgrowth disorders related to somatic mutations in *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) pathway. We present three cases with *PIK3CA* mutation and clinical characteristics encompassing MCAP (megalencephaly-capillary malformation) condition but lacking all criteria to a certain diagnosis, most of all showing prevalent and peculiar involvement of cerebellar structures at MRI (magnetic resonance imaging) mainly consisting in cortical rim thickening and abnormal orientation of folia axis. These cases expand the spectrum of intracranial MRI features in *PIK3CA* disorders.

Keywords *PIK3CA* · MRI · Cerebellum · Pediatrics · PROS

Introduction

The term PROS (*PIK3CA*-Related Overgrowth Spectrum) has been coined during the Workshop Conference of Bethesda, on September 11 and 12 in 2013, and is used to indicate a wide spectrum of overgrowth disorders related to somatic mutations in *PIK3CA* pathway [1]. This umbrella term refers to diseases with overlapping clinical findings which include the following: macrodactily, fibroadipose hyperplasia or overgrowth (FAO), muscle hemihypertrophy, fibroadipose

infiltrating lipomatosis, vascular malformation, congenital lipomatous overgrowth (CLOVES), hemihyperplasia multiple lipomatosis (HHML), epidermal nevi, scoliosis/skeletal and spinal syndrome, megalencephaly-capillary malformation (MCAP), skin disorders (epidermal nevi, Seborrheic keratosis), and dysplastic megalencephaly (DMEG) [1]. These disorders appear at birth or, alternatively, are characterized by early childhood onset. Diagnostic criteria are divided in two categories: A and B. In the first one, patients must present a spectrum of features with two or more findings among

✉ Martina Di Stasi
martinadistasi@live.it

Giana Izzo
giana.izzo@asst-fbf-sacco.it

Elisa Cattaneo
elisa.cattaneo@asst-fbf-sacco.it

Vittoria Baraldini
vittoria.baraldini@asst-fbf-sacco.it

Chiara Doneda
chiara.doneda@asst-fbf-sacco.it

Andrea Righini
andrea.righini@asst-fbf-sacco.it

Daniela Graziani
daniela.graziani@asst-santipaolocarlo.it

Valentina Toto
valentina.toto@asst-santipaolocarlo.it

Cecilia Parazzini
cecilia.parazzini@asst-fbf-sacco.it

¹ Department of Advanced Biomedical Sciences, University “Federico II”, Via Pansini, 5, 80131 Naples, Italy

² Department of Pediatric Radiology and Neuroradiology, ASST FBF SACCO - V. Buzzi Children’s Hospital, Milan, Italy

³ Clinical Genetics Unit, Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, ASST FBF SACCO - V. Buzzi Children’s Hospital, Milan, Italy

⁴ Pediatric Surgery Unit, Department of Vascular Surgery, ASST FBF SACCO – V. Buzzi Children’s Hospital, Milan, Italy

⁵ Department of Human Pathology, Cytogenetic and Molecular Pathology, ASST Santi Paolo e Carlo, Milan, Italy

⁶ Complex Unit of Pathological Anatomy and Medical Genetics, San Paolo Hospital, University of Milan, Milan, Italy

adipose, muscular, nervous, or skeletal tissue overgrowth, vascular malformation, and epidermal nevus. In the second one, there should be a more specific finding as isolated macrodactyly, splayed feet or hand overgrowth, large lymphatic malformation, hemimegalencephaly, and FCD (focal cortical dysplasia) [2].

Brain magnetic resonance imaging (MRI) is often necessary to diagnose most PROS patients. Many anomalies have been described at MRI: Jansen et al. [3] studied 33 children with mutation in *PI3KCA* pathway genes and found different brain signs, such as abnormalities of the cortex (lissencephaly or polymicrogyria), of the white matter (increased T2-weighted signal, calcifications, and cystic changes), and other minor anomalies as ventricular and cortical liquor space widening. However, all these findings are not specific, and MRI features of these conditions are not completely clear. In particular, among PROS, MCAP disorders have complex features, which have been reviewed by Mirzaa and Conway et al. in 2012 [4]: progressive megalencephaly with segmental overgrowth dysregulation, face and/or body vascular anomalies (mainly capillary malformations), and syndactyly/polydactyly.

Here, we present three cases, with *PIK3CA* proven mutation and with clinical characteristics widely encompassing MCAP condition, but lacking all criteria to a certain diagnosis, and most of all showing prevalent involvement of cerebellar structures at MRI.

Material and methods

Imaging technique

The MRI studies were performed on 1.5 Tesla (Achieva, Philips Medical Systems) by using a phased array head coil, with a standard pediatric protocol including T1-weighted volumetric spoiled gradient echo (3D- GRE) images, T2-weighted fast spin-echo (FSE) multiplanar images, fluid-attenuated inversion recovery (FLAIR) axial and coronal images, diffusion-weighted axial (DWI) images, and T2 gradient echo (FEE) or susceptibility-weighted axial sequences. In the third case, the intra-uterine MRI examination was performed with a phased array abdominal coil and a protocol including T2-weighted single-shot FSE multiplanar sections and balanced steady-state (B-TFE) multiplanar sections. The post-mortem protocol, performed with a knee coil, included high-resolution T2-weighted FSE multiplanar sections, T1-weighted SE multiplanar sections, and DWI sections.

DNA sequencing

DNA was quantified with Qubit fluorometer and underwent sequencing with Next Generation Sequencing Illumina MiSeq

platform with a custom kit containing oligos for coding regions of genes involved in vascular anomalies including the gene *PIK3CA*. TruSeq Custom Amplicon procedure was performed according to Illumina reference TSCA protocol. Mutations were confirmed with Sanger sequencing.

Case 1

This 18-month-old female patient was the first child of healthy, non-consanguineous parents with no history of genetic disorders. She was born at term by vaginal delivery. Since birth, she presented macrosomia, macrodactyly, and foot dysmorphism. Later she was diagnosed with scoliosis, complex truncal vascular capillary malformation, dyschromic facial features, and segmental overgrowth of left leg. At last follow-up, she had a HC (head circumference) of 51,5 cm (99th percentile) and normal neurological development. Brain MRI showed asymmetric enlargement of left cerebellum, with dysplastic characteristics, mainly consisting in cortical rim thickening and abnormal orientation of its folia axis (Fig. 1). No other cerebral findings were noticed. Spinal MRI showed a filum lipoma and confirmed scoliosis. Next Generation Analysis (NGS) on DNA from a cutis bioptic sample at the level of truncal capillary malformation found a mosaic somatic mutation (allele frequency 8%) in *PIK3CA* gene: NM_006218.2: c.1633G>A; p.Glu545Lys, not detected on DNA from peripheral blood.

Case 2

This 18-month-old male patient was born at term by vaginal delivery, from healthy non-consanguineous parents. No history of genetic diseases was reported in familial anamnesis. Pregnancy course was referred to be normal until the 20th gestational week, when ultrasound demonstrated lymphangiomatous malformation in axillary region. Prenatal invasive diagnosis was refused. At birth, facial asymmetry due to cheek/jaw vascular anomalies and lipoma, segmental overgrowth of the left arm, and complex vascular malformation in both inferior limbs, right arm, and left hemithorax were noticed. Last medical evaluation showed a mild neurodevelopment retardation and head circumference of 55 cm (97th percentile). Brain MRI (Fig. 2) showed diffuse enlargement of the cerebellum, asymmetrical for the left hemisphere prevalent as demonstrated by the ipsilateral tonsillar ectopia; the vermis was clearly recognizable only in its cranial portion, and anomalous orientation of folia axis and dysmorphism of fourth ventricle was also evident. Supratentorial findings consisted only in a mild asymmetry of the frontal lobes and lateral ventricles with prevalence of the left side: no cortical malformations or white matter abnormalities were present. Next Generation Analysis (NGS) on DNA from a cutis bioptic sample at the level of lower limb vascular malformation found a mosaic somatic mutation (allele frequency 15%)



Fig. 1 Case 1. Coronal T2-weighted (a), sagittal T1-weighted (b), and axial T2-weighted (c) sections showing markedly abnormal folia orientation and morphology, especially in the left cerebellum hemisphere (arrows) that appears moderately enlarged

in *PIK3CA* gene: NM_006218.2: c.1636C>A; p.Gln546Lys, not detected on DNA from peripheral blood.

Case 3

This case was referred from prenatal ultrasound study for suspected hemimegalencephaly and somatic hemihypertrophy. Intra-uterine MRI at 21th gestational week showed increased head biometry and asymmetry with hemimegalencephalic brain due to an overdevelopment of the right hemisphere. On the same side, some irregularity of cortical profile was present, and the ventricle was enlarged and dysmorphic. Right cerebellar hemisphere was also noted to be enlarged and dysplastic (Fig. 3). After termination of pregnancy, post-mortem MRI confirmed these features, better demonstrating the anomalous lamination of hemimegalencephalic parenchyma corresponding to altered cell migration, poor

myelination, cystic change, and gliosis. The ipsilateral hemimegalo-cerebellum also displayed areas of signal alterations compatible with neuronal ectopia. Pathology macroscopic evaluation revealed macrosomia, capillary malformation of the overgrown leg, and a more widely capillary dysplasia involving superficial and visceral tissues, foot dysmorphism, right kidney hypertrophy, and right hemimegalencephaly with polymicrogyric cortex associated and asymmetric enlargement of the right cerebellum with anomalous folia orientation. In the giant hemisphere, the microscopic evaluation demonstrated derangement of the embryonal parenchymal layers, large ortho- and heterotopic neural cells, blurred boundaries of the gray and white matter, and cortical thickening with diffuse polymicrogyric pattern and lack of lamination. Similar findings were present in the dysplastic hypertrophic right cerebellum (Fig. 4). Next Generation Analysis (NGS) on DNA from a bioptic sample at the

Fig. 2 Case 2. Axial T2-weighted (a and b) sections showing markedly abnormal cerebellar folia orientation and morphology, especially in the left cerebellar hemisphere (white arrows). Black arrow shows asymmetric facial cutaneous fat hypertrophy. In (c), arm region angiomatous anomaly is depicted (white arrow)

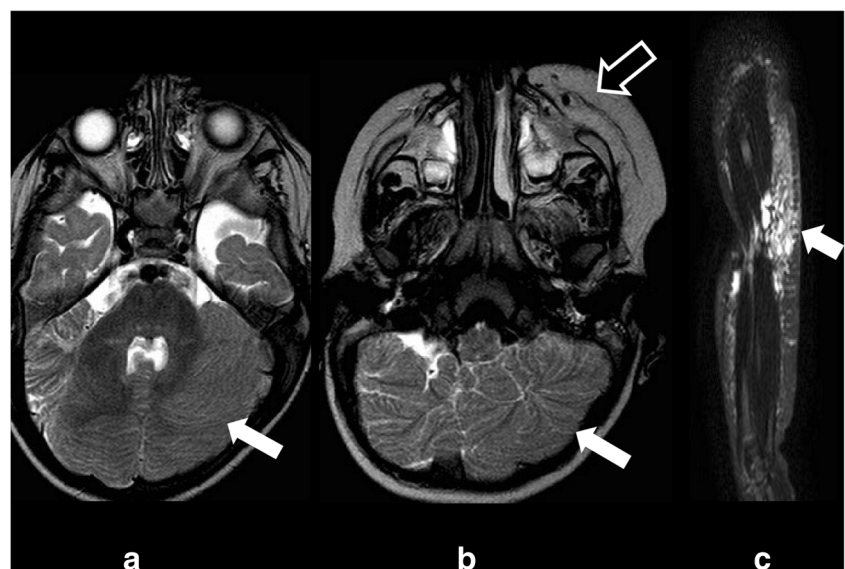
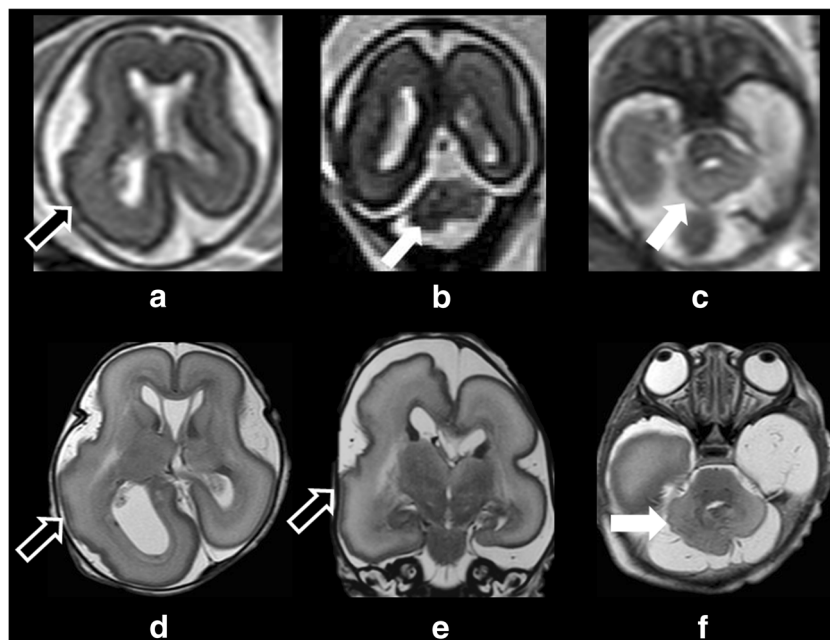


Fig. 3 Case 3. (a–c) T2-weighted sections from fetal MR imaging study showing right hemimegalencephaly (black arrow) and hemihypertrophy of the right cerebellar hemisphere (white arrows); (d–f) T2-weighted sections from MR autopsy study confirming prenatal findings and better demonstrating the dysplastic characteristics of the overgrown ipsilateral cerebral (black arrows) and cerebellar (white arrow) hemisphere



level of overgrown leg found a mosaic somatic mutation (allele frequency 24%) in *PIK3CA* gene: NM_006218.2: c.1633G>A; p.Glu545Lys, not detected on DNA from peripheral blood.

Discussion

These three cases expand the spectrum of intracranial MRI features detectable in *PIK3CA* disorders by showing peculiar prevalent involvement of cerebellum, with features of overgrowth, dysplastic cortical rim and aberrant folia orientation. *PIK3CA* gene encodes for the alpha catalytic subunit (p110 α) of phosphatidylinositol-4,5 bisphosphatase 3-kinase that catalyzes the phosphorylation of PtdIns-4,5-P2 to generate PtdIns-3,4,5-

P3, a second messenger that regulates cellular processes, including cell growth, proliferation, and migration [5]. Most common *PIK3CA* mutations are gain of function mutations seen in 80% of human cancer tissues. In a recent study by Niesen et al., it has been demonstrated that in medulloblastoma-murine models, a mutated *PIK3CA* has a poor oncogene function on its own, as also shown in PROS patients, since no tumor formation was observed in mice when activating specific *PIK3CA* (H1407R) mutation alone. Conversely, in transgenic mouse models carrying the double mutation status *PIK3CA* and *SmoM2*, as well as *PIK3CA* and *Ptch1*, a 100% tumor growth and faster progression occurred than when just inducing *SmoM2* or *Ptch1* mutation alone, suggesting a *PIK3CA* role in tumor progression and therapy resistance, leading to a more aggressive phenotype [6]. However, other types of mutations with different level of gain

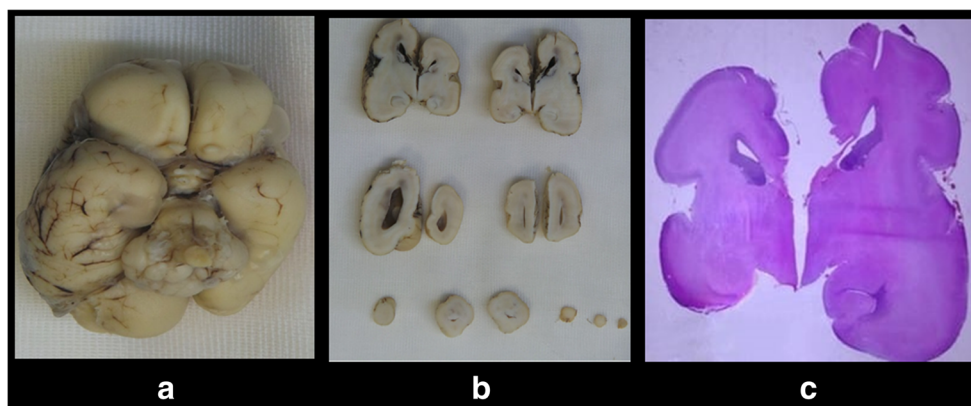


Fig. 4 Case 3. (a and b) Macroscopic samples revealing right hemimegalencephaly, polymicrogyria, and asymmetric enlargement of right cerebellum with anomalous folia orientation. (c) Coronal

hematoxylin-eosin stainings of the giant hemisphere showing blurred boundaries of the gray and white matter, cortical thickening with diffuse polymicrogyria and absence of normal lamination

of function, from strong to weak, have been described. Mirzaa et al. [7] demonstrated that the mutational spectrum among these pathologies is quite different: nevertheless, currently, the specific causative event determining the relative phenotype still remain unclear. As previously mentioned, macrocerebellum is a rare neuroimaging finding occurring in isolation or in complex neurodevelopmental disorders. In patients with de novo mutations in *PIK3R2* and *PIK3CA* genes, it is still unknown whether the cerebellar overgrowth is an element of generalized brain overgrowth or whether there are distinct underlying mechanisms: however, generic cerebellar enlargement progressively leading to a Chiari type 1 condition or cerebellar asymmetry has been reported in some PROS patients. Beyond the overgrowth features, our three cases have in common some peculiar cerebellar abnormalities (dysplastic cerebellum with abnormal folia orientation) not previously reported as characteristic of patients with *PIK3CA* mutations. Only in another patient with megalencephaly and a *PIK3CA* hotspot mutation a form of vermis dysplasia was described [8]. Hemimegalencephaly or unilateral megalencephaly is a disorder characterized by hamartomatous overgrowth of whole or part of a cerebral hemisphere that usually occurs ipsilaterally to the somatic overgrowth. The affected hemisphere may have focal or diffuse neuronal migration defects, with areas of polymicrogyria, pachygyria, heterotopias, and dysplasia. Not surprisingly, the altered cellular proliferation/migration processes could also involve the cerebellum alone, and it could be related to a specific subgroup of mutations in *PIK3CA* pathway. In this regard, other genetic-based conditions with peculiar cortical cerebellar abnormalities should be mentioned for purpose of differential diagnosis. Poretti-Boltshauser syndrome is determined by loss of function of *LAMA1*: patients present myopia/retinopathy and multiple cerebellar findings such as cysts, cerebellar cortical dysplasia, and abnormal shape of IV ventricle [9].

Cerebellar cortical dysplasia and multiple small cysts can either be found in *COL3A* and *GPR56* gene-related mutations underlying abnormal fibril organization within disorders of neuronal migration. *GPR56* allelic variants also lead to defects in basement membrane with cerebellar polymicrogyria, fused adjacent folia, and cerebral cortical anomalies ranging from polymicrogyria to cobblestone-like cortex [10]. Peculiar cerebellar anomalies involving also cortical morphology are found in tubulinopathies: Romaniello et al. [11] reported a cohort of patients with loss of function related to *TUBA1A*, *TUBB2B*, and *TUBB3* genes, which play a role in migration of granular cells. Finally, different cerebellar findings involving the cortex are described in dystroglycanopathies [12]. However, even if the above mentioned diseases have to be taken into account, most of them are usually associated with cerebellar hypoplasia and cysts, which were not present in our cases.

Major difficulty in approaching cerebellar dysplasias is the lack of an universally accepted classification: while cerebral hemisphere malformations represent a well-studied group of diseases, the cerebellar counterpart has not been clearly

elucidated. For this reason, Patel and Barkovich proposed to first differentiate cerebellar malformations with hypoplasia from those with dysplasia and then consider if a disorganized architecture and/or altered signal intensity affects an isolated area or represents a generalized condition: the purpose is to allow the grouping of similar malformations and the underlying genetic causes [13].

Conclusions

In conclusion, the dysplastic cerebellum may constitute a new core finding in PROS patients, especially in those who present some features overlapping MCAP condition. Thus, in the clinical setting of patients with capillary malformation, segmental overgrowth, and distal limb anomalies, brain MRI should also focus on cerebellar structures, because their dysplastic changes may be suggestive of this condition so to prompt proper genetic testing.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Keppler-Noreuil KM, Rios JJ, Parker VER, Semple RK, Lindhurst MJ, Sapp JC, Alomari A, Ezaki M, Dobyns W, Biesecker LG (2015) *PIK3CA*-related overgrowth spectrum (PROS): diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. *Am J Med Genet A* 167:287–295
2. Kang HC, Baek ST, Song S, Gleeson JG (2015) Clinical and genetic aspects of the segmental overgrowth spectrum due to somatic mutations in *PIK3CA*. *The Journal of Pediatrics* 167:957–962
3. Jansen LA, Mirzaa GM, Ishak GE, O’Roak BJ, Hiatt JB, Roden WH, Gunter SA, Christian SL, Collins S, Adams C, Rivière JB, St-Onge J, Ojemann JG, Shendure J, Hevner RF, Dobyns WB (2015) *PI3K/AKT* pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. *Brain* 138:1613–1628
4. Mirzaa GM, Conway RL, Gripp KW, Lerman-Sagie T, Siegel DH, deVries LS, Lev D, Kramer N, Hopkins E, Graham JM Jr, Dobyns WB (2012) Megalencephaly-capillary malformation (MCAP) and megalencephaly-polydactyly-polymicrogyria-hydrocephalus (MPPH) syndromes: two closely related disorders of brain overgrowth and abnormal brain and body morphogenesis. *Am J Med Genet* 158A:269–291
5. Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT (2017) The *PI3K* pathway in human disease. *Cell* 170:605–635
6. Niesen J, Ohli J, Sedlacik J et al (2020) *Pik3ca* mutations significantly enhance the growth of SHH medulloblastoma and lead to metastatic tumor growth in a novel mouse model. *Cancer Letters* 477:10–18
7. Mirzaa GM, Rivière J-B, Dobyns WB (2013) Megalencephaly syndromes and activating mutations in the *PI3K-AKT* pathway: MPPH and MCAP. *Am J Med Genet* 163c:122–130

8. Mirzaa G, Timms AE, Conti V et al (2016) PIK3CA-associated developmental disorders exhibit distinct classes of mutations with variable expression and tissue distribution. *J Clin Invest Insight* 1(9)
9. Micalizzi A, Poretti A, Romani M, Ginevrino M, Mazza T, Aiello C, Zanni G, Baumgartner B, Borgatti R, Brockmann K, Camacho A, Cantalupo G, Haeusler M, Hikel C, Klein A, Mandrile G, Mercuri E, Rating D, Romaniello R, Santorelli FM, Schimmel M, Spaccini L, Teber S, von Moers A, Wentz S, Ziegler A, Zonta A, Bertini E, Boltshauser E, Valente EM (2016) Clinical, neuroradiological and molecular characterization of cerebellar dysplasia with cysts. (Poretti–Boltshauser syndrome). *Eur J Human Genet* 24: 1262–1267
10. Vandervore L, Stouffs K, Tanyalçin BI et al (2016) Bi-allelic variants in COL3A1 encoding the ligand to GPR56 are associated with cobblestone-like cortical malformation, white matter changes and cerebellar cysts. *J Med Genet* 54(6):432–440
11. Romaniello R, Arrigoni F, Panzeri E, Poretti A, Micalizzi A, Citterio A, Bedeschi MF, Berardinelli A, Cusmai R, D'Arrigo S, Ferraris A, Hackenberg A, Kuechler A, Mancardi M, Nuovo S, Oehl-Jaschkowitz B, Rossi A, Signorini S, Tüttelmann F, Wahl D, Hehr U, Boltshauser E, Bassi MT, Valente EM, Borgatti R (2017) Tubulin-related cerebellar dysplasia: definition of a distinct pattern of cerebellar malformation. *Eur Radiol* 27:5080–5092
12. Astrea G, Pezzini I, Picillo E, Pasquariello R, Moro F, Ergoli M, D'Ambrosio P, D'Amico A, Politano L, Santorelli FM (2016) *TMEM5*-associated dystroglycanopathy presenting with CMD and mild limb-girdle muscle involvement. *Neuromuscul Disord* 26: 459–461
13. Patel S, Barkovich AJ (2002) Analysis and classification of cerebellar malformations. *AJNR* 23(1074):1087

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.