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Original article

De novo TUBB2B mutation causes fetal akinesia deformation sequence with microlissencephaly: An unusual presentation of tubulinopathy

Annie Laquerriere [a, b], Marie Gonzales [c, d], Yoann Saillour [e, f, g], Mara Cavallin [g, h, i], Nicole Joyē [c, d], Chloé Quēlin [j], Laurent Bidat [k], Marc Dommergues [d, l], Ghislaine Plessis [m], Ferechte Encha-Razavi [g, h, n], Jamel Chelly [o, p], Nadia Bahi-Buisson [g, h, i, *, 1], Karine Poirier [e, f, g, 1]

[a] *Pathology Laboratory, Rouen University Hospital, France*
[b] *Region-Inserm Team NeoVasc ERI28, Laboratory of Microvascular Endothelium and Neonate Brain Lesions, Institute of Research Innovation in Biomedecine, Normandy University, Rouen, France*
[c] *Department of Medical Genetics, Armand Trousseau Hospital, APHP, Paris, France*
[d] *Sorbonne Universities, UPMC, Paris, France*
[e] *Inserm, U1016, Institut Cochin, Paris, France*
[f] *CNRS, UMR8104, Paris, France*
[g] *Paris Descartes - Sorbonne Paris Cité University, Imagine Institute, Paris, France*
[h] *Pediatric Neurology, Necker Enfants Malades University Hospital, Paris, France*
[i] *INSERM UMR-1163, Embryology and Genetics of Congenital Malformation Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine, Université Paris Descartes-Sorbonne Paris Cité, France*
[j] *Department of Clinical Genetics, South University Hospital, Rennes, France*
[k] *Department of Prenatal Diagnosis, Department of Obstetrics and Gynecology, René Dubos Hospital, Pontoise, France*
[l] *Department of Obstetrics and Gynecology, Groupe Hospitalier Pitié Salpêtrière, APHP, Paris, France*
[m] *Department of Genetics, Clinical Genetics, Caen University Hospital, Caen, France*
[n] *Département de Génétique, Necker-Enfants Malades University Hospital, Paris, France*
[o] *Pôle de biologie, Hôpitaux Universitaires de Strasbourg, Strasbourg, France*
[p] *Institut de Génétique et Biologie Moléculaire et Cellulaire - IGBMC, INSERM, CNRS, Université de Strasbourg, Strasbourg, France*

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ABSTRACT

Tubulinopathies are increasingly emerging major causes underlying complex cerebral malformations, particularly in case of microlissencephaly often associated with hypoplastic or absent corticospinal tracts. Fetal akinesia deformation sequence (FADS) refers to a clinically and genetically heterogeneous group of disorders with congenital malformations related to impaired fetal movement.

We report on an early foetal case with FADS and microlissencephaly due to *TUBB2B* mutation. Neuropathological examination disclosed virtually absent cortical lamination, foci of neuronal over-migration into the leptomeningeal spaces, corpus callosum agenesis, cerebellar and brainstem hypoplasia and extremely severe hypoplasia of the spinal cord with no anterior and posterior horns and almost no motoneurons.

At the cellular level, the p.Cys239Phe TUBB2B mutant leads to tubulin heterodimerization impairment, decreased ability to incorporate into the cytoskeleton, microtubule dynamics alteration, with an accelerated rate of depolymerization.

To our knowledge, this is the first case of microlissencephaly to be reported presenting with a so severe and early form of FADS, highlighting the importance of tubulin mutation screening in the context of FADS with microlissencephaly.

1. Introduction

Normal fetal development is dependent on adequate fetal movement, starting at 8 weeks of gestation (WG). Limitation of

* Corresponding author. Université Paris Descartes-Sorbonne Paris Cité, Institut Imagine-INSERM UMR-1163, Embryology and genetics of congenital malformations.
E-mail address: nadia.bahi-buisson@nck.aphp.fr (N. Bahi-Buisson).
[1] Both authors contributed equally to the manuscript.

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movements results in fetal akinesia deformation sequence (FADS; OMIM 208150). FADS was first reported as a syndrome by Pena and Shokeir in 1974 and further delineated as a symptom by Hall in 1981 (Hall, 1981; Haliloglu and Topaloglu, 2013). Its incidence varies among different countries and has been estimated at 1:3000 to 1:5000 by Fahy and Hall (Fahy and Hall, 1990). The clinical presentation is highly variable, ranging from the most severe form called lethal multiple pterygium syndrome characterized by multiple joint contractures and pterygia, lung hypoplasia, short umbilical cord, craniofacial changes consisting of hypertelorism, micrognathism, cleft palate, short neck, low-set ears, along with intrauterine growth retardation and abnormal amniotic fluid volume mainly observed from the first trimester of the pregnancy (Hammond and Donnenfeld, 1995). Less severe phenotypes may present either as distal arthrogryposis or as fetal hypomotility which usually occurs during the third trimester (Pena and Shokeir, 1974).

Non-genetic factors may cause FADS, such as environmental limitation of fetal movements, maternal infection, drugs and immune mechanisms (maternal autoimmune myasthenia). The FADS phenotype is observed in a number of known genetic syndromes. Non syndromic or isolated FADS is genetically heterogeneous and encompass multiple neurogenic processes affecting the central or the peripheral nervous system, the neuromuscular junction and the skeletal muscle (Bamshad et al., 2009; Navti et al., 2010; Laquerriere et al., 2014). Until recently, the neurogenic form characterized by spinal cord motoneuron paucity, either isolated or associated with pontocerebellar hypoplasia was considered as the most frequent cause (Banker, 1986; Vuopala et al., 1995a, 1995b). Conversely, brain malformations are very infrequently observed in association with FADS, and mainly described in lissencephalies type I and II as deformations of the extremities (Devisme et al., 2012; Witters et al., 2002). To our knowledge, FADS has never been reported in association with tubulin related cortical malformations. Here, we report on the most severe presentation of tubulinopathy in a fetus harboring a *de novo* missense mutation in the β-tubulin gene *TUBB2B* gene (MIM 615101), along with neuropathology and molecular data focusing on the consequences of the mutation, that could explain at least partly the severity of the lesions and early fetal presentation.

2. Patient and methods

2.1. Case history

A 32-year-old woman, gravida 4, para 3, underwent routine ultrasonography (US) at 12 WG, which revealed severe fetal akinesia. Control ultrasound examination performed at 14 WG confirmed total lack of movements, retrognathia and dilatation of the third and fourth cerebral ventricles (Supplementary Fig. 1). A medical termination of the pregnancy was achieved at 15 WG, in accordance with French law. A complete autopsy was performed with informed written consent from both parents. Brain lesions identified at autopsy suggested a possible Walker Warburg syndrome (WWS) despite absent eye lesions, so that a first-line screening of WWS genes was performed, but was negative. Indeed, known environmental causes of FADS were excluded, as well as syndromic causes. Chromosomal analysis performed on trophoblast biopsy revealed a normal male karyotype, 46, XY. The parents were non consanguineous and there was no relevant personal or family history. Three children born to a previous marriage were in good health.

After having obtained written informed consent from the parents, DNAs were purified from fetal lung tissues, and from peripheral blood cells in both parents by using a standard phenol/chloroform method. Mutation analysis was performed by PCR amplification and direct SANGER sequencing of all coding exons and splice sites of the *TUBB2B* gene revealed a *de novo* missense mutation in exon 4, c.716G > T determining a p.Cys239Phe substitution (previously reported in (Fallet-Bianco et al., 2014)). No other variant was identified after sequencing of the other genes involved in cortical malformations.

2.2. Neuropathological evaluation

Tissues including the brain, eyes and spinal cord were fixed in a 10% formalin-zinc buffer solution. Seven-micrometer sections obtained from paraffin-embedded tissues were stained using Haematoxylin-Eosin. Adjacent brain and spinal cord sections were assessed for routine immunohistochemistry, using antibodies directed against vimentin (diluted 1:100; Dakopatts, Trappes, France), calretinin (1:200; Zymed Clinisciences, Montrouge, France), and MAP2 (diluted 1:50, Sigma, St Louis, MO). Immunohistochemical procedures included a microwave pre-treatment protocol to aid antigen retrieval (pretreatment CC1 kit, Ventana Medical Systems Inc, Tucson AZ). Incubations were performed for 32 min at room temperature using the Ventana Benchmark XT system. After incubation, slides were processed by the Ultraview Universal DAB detection kit (Ventana). All immunolabellings were compared with an age matched control case examined after a spontaneous abortion for premature rupture of the membranes, and whose brain was histologically normal.

2.3. Functional analyses

2.3.1. Protein modeling

A model of human β-tubulin was built by homology modeling using available structures (Research Collaboratory for Structural Bioinformatics PDB code 1TUB) from Nogales et al. (Nogales et al., 1998). The images in Fig. 4C were rendered using PyMOL software (http://www.pymol.org).

2.4. Cloning and in vitro translation

TUBB2B sequence was generated by PCR using a template from the human brain cDNA library (Clontech, Mountain View, CA). The PCR product was cloned into the pcDNA 3.1-V5-His vector (Invitrogen, Carlsbad, CA) and checked by DNA sequencing. These products were cloned both into the cDNA3.1-V5-his-TOPO-TA cloning vector (Invitrogen) and pET vector. An in-frame tag encoding the FLAG epitope (DYKDDDDK) was incorporated by PCR along with the C-terminus of the TUBB2B wild-type sequence allowing for the distinction of the transgene from other highly homologous endogenously expressed β-tubulin polypeptides. The p.Cys239Phe mutation was introduced by site-directed mutagenesis using a QuikChange II kit (Stratagene, La Jolla, CA) and verified by DNA sequencing. Transcription/translation reactions were performed at 30 °C for 90 min in 25 μl of rabbit reticulocyte lysate (TNT; Promega, Madison, WI) containing ^{35}S-methionine (specific activity, 1000 Ci/μmol; 10 μCi/μl). For the generation of labeled β-tubulin heterodimers, transcription/translation reactions were chased for a further 2 h at 30 °C by the addition of 0.375 mg/ml of native bovine brain tubulin. Aliquots (2 μl) were withdrawn from the reaction, diluted into 10 μl of gel-loading buffer (gel running buffer supplemented with 10% glycerol and 0.1% bromophenol blue) and stored on ice prior to resolution on a non-denaturing gel. Labeled reaction products were detected by autoradiography after resolution on either SDS–PAGE or on native polyacrylamide gels as described (Tian et al., 1996, 1997).