

## Clinico-pathological correlation in case of *BRAT1* mutation

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### Abstract

*The clinical picture of BRCA1-associated protein required for ATM activation-1 (BRAT1) comprises retractable early-onset epileptic encephalopathy, progressive microcephaly, and early demise. Both, inter- and intrafamilial variations of features of BRAT1-associated disease have been described. Here, the familial case of a brother and sister with homozygous pathogenic variants in BRAT1 is presented with special emphasis on differences in seizure type/onset and central nervous system lesions. The neuropathology is extensively discussed and hypotheses put forward that may shed light on etiology of brain symptomatology within the context of BRAT1 mutations.*

**Key words:** *BRAT1 mutation, epileptic encephalopathy, seizures, central nervous system lesions.*

### Introduction

Development of the central nervous system requires proper expression of a number of genes involved in a variety of pathways. Early neuronal apoptosis and microcephaly may result from *BRCA1* gene defects [20]. Downstream targets of *BRCA1* include Akt/mTOR proteins [1]. Indirectly, both seemingly distant pathways are linked by *BRAT1* gene (*BRCA1*-associated protein required for ATM activation-1) (since 2013, the name *BRAT1* has been used for the gene and for the protein) [1,31].

Homozygous pathogenic variants in the *BRAT1* gene lead to “Rigidity and multifocal seizure syndrome, Lethal neonatal – RMFSL” with intractable

epileptic encephalopathy as the prominent feature [OMIM#614498]. The syndrome is characterized by a very early onset, severe course, microcephaly, rigidity, refractory epilepsy with myoclonic jerks/multifocal seizures and early demise [3,22,25,29,35,36]. Apart from the severe, lethal form of *BRAT1*-related disorders, less severe forms exist [8,12,17,33,34]. Familial cases of the condition have been described [30,33].

Herein, we present a further familial case of intractable epileptic encephalopathy caused by mutations in *BRAT1* in siblings. We extensively discuss the possible etiology of neuropathological lesions present in one of the patients.

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## Case presentation

The patient's family history is significant.

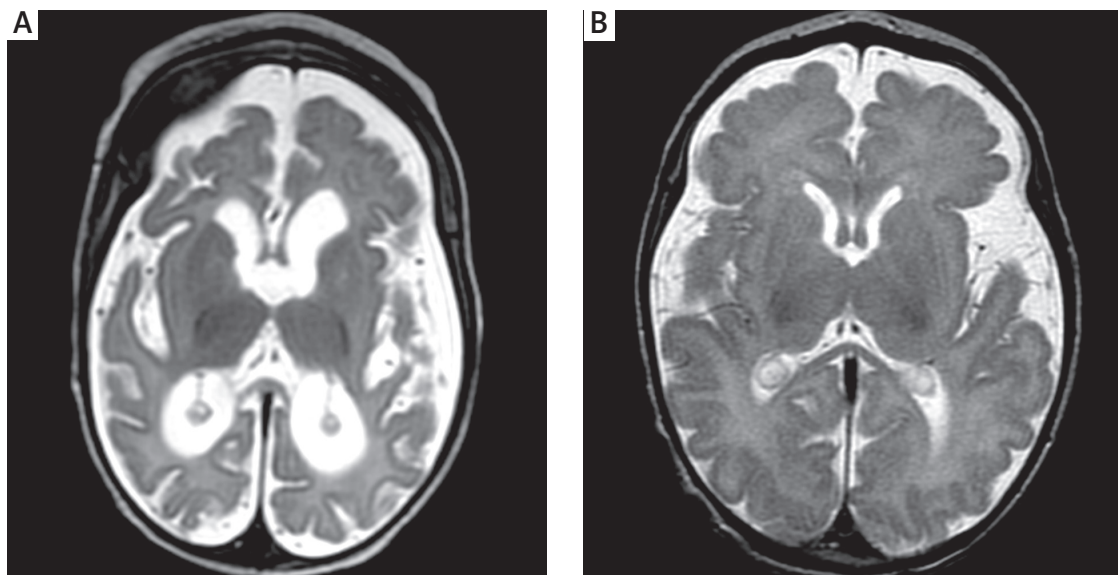
### Patient 1

G-II sister, with developmental epileptic encephalopathy and seizures from the first day of life. The mother experienced infectious complications during pregnancy, normal vaginal delivery at 40 weeks of gestation (tightly wrapped umbilical cord around the neck, difficulty during shoulder delivery), asphyxia. Upon Apgar score evaluation: 4 points at 1 minute; 4 points at 3 minutes; 7 points at 5 minutes. The girl was born with a 3070 g birth weight and she was ventilated with an Ambu bag. Myoclonic convulsions and globally increased muscle tone were observed. Dysmorphic features were also present (hypertelorism, epicanthic folds, low-set structure of the ear, high-arched palate.) The girl had a forced body posture with extended and crossed lower limbs, flexed and adducted upper limbs, flexure and internal rotation of the fists. Hyperactivity to stimuli was present, along with coarse tremors. Wide-range diagnostics were performed, directed both at congenital and metabolic diseases, and at examining the karyogram – the results were normal.

Magnetic resonance imaging (MRI) at the age of 5 months consisted of T1- and T2-weighted imag-

ing as well as FLAIR imaging. The dominant feature was brain atrophy in the supratentorial region with a pronounced white matter volume loss, widening of the ventricles and widening of the subarachnoid spaces (including brain sulci) (Fig. 1A). Other features included: thinning of the corpus callosum, inferior cerebellar peduncles, middle cerebellar peduncles and pons. Thinning of the brain cortex could be observed in T2-weighted images. Myelination pattern abnormalities were present, probably due to delayed myelination and/or damage to myelin. There was insufficient myelination within the semioval centers and perirolandic areas. Thinning of posterior limbs of internal capsules; the anterior limbs were non-myelinated. Myelination of the brainstem and cerebellum was minimally affected.

The girl suffered from polymorphic seizures resistant to treatment with topiramate, phenobarbital, phenytoin, clonazepam, carbamazepine, clobazam, levetiracetam, tetracosactide or pyridoxine. The etiology of the girl's symptoms could not be determined based on the aforementioned diagnostics. She died at the age of 6 months and she was diagnosed with early-childhood myoclonic encephalopathy. Autopsy was not performed as it was recognized that a hypoxic-ischemic process may have been the cause of the clinical and neuroradiological picture.



**Fig. 1.** MRI with T2-weighted imaging. **A)** Patient 1: Brain atrophy in the supratentorial region with pronounced white matter volume loss, widening of the ventricles and widening of the subarachnoid spaces (including brain sulci). **B)** Patient 2: The myelination pattern was appropriate for the patient's age, ventricles were normal and subarachnoid space was slightly widened.

## Patient 2

The brother is described as: G-III, CC-delivery, 3300 g birth weight, length 53 cm, head circumference 34 cm, 7 points on the Apgar scale. The boy has had polymorphic seizures and significantly increased muscle tension since his first days of life. On physical examination at the age of 3 months he manifested features of dysmorphia, head circumference of 36 cm, dysphagia, lack of cough reflex, generalized muscle stiffness, no response to visual stimuli. Permanent myoclonus of the facial muscles, migratory myoclonus, clonic seizures, tonic seizures (usually right-sided) and axillary myoclonus were observed. There was significant hypersensitivity to tactile and auditory stimuli characterized by the occurrence of the above seizures. The EEG displayed generalized and focal sharp and spike waves.

The seizures did not improve after treatment with topiramate, phenobarbital, clonazepam, clobazam, pyridoxal phosphate, vigabatrin, or levetiracetam. Transient improvement was observed after treatment with Valproic acid, with a serum concentration of 104 µg/ml. However, there was an increase in ammonia concentration up to 200, which forced the discontinuation of treatment. The boy suffered from recurrent generalized infections. Genetically determined metabolic disease was suspected, thus diagnostic tests were performed – the results were within the norm. The karyotype was normal. A cerebrospinal fluid examination for metabolic disorders of biogenic amines and an aminogram of fluid and plasma were performed. Biogenic amines in the CSF showed decreased HVA (dopamine metabolite) concentration (253 nmol/l; reference value: 300-1000 nmol/l), decreased 5-HIAA (serotonin metabolite) concentration (143 nmol/l; reference value: 300-1000 nmol/l).

At 2 weeks of age, MRI with T1- and T2-weighted imaging as well as FLAIR imaging was performed. The myelination pattern was appropriate for the patient's age. The ventricles were normal. The subarachnoid space was slightly widened (Fig. 1B).

Pale optic discs were observed upon ophthalmological examination.

The boy died at the age of 12 months.

## Genetic examination

The next generation sequencing (NGS) was performed in proband using TruSight One™ Sequencing Panel Kit (Illumina, San Diego, CA, USA) allowing to

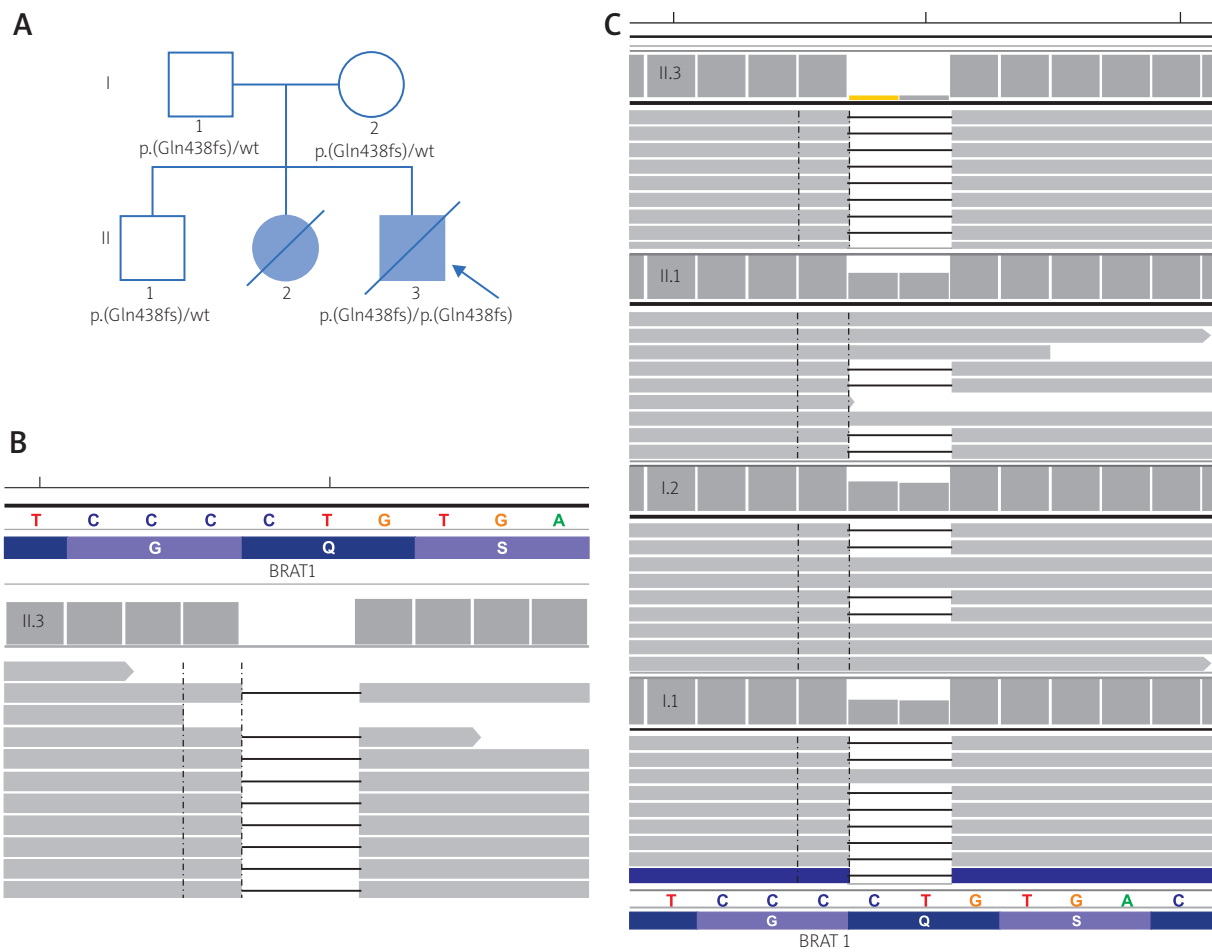
study the coding sequence and splice sites of 4811 *loci* associated with known clinical phenotypes. Library was prepared according to the manufacturer's instructions and pair-end sequenced (2 × 100 bp) on an Illumina HiSeq1500. 96% of target bases was covered at a minimum of 20× whereas 99% had coverage of min. 10×. Raw data were analyzed as previously described [21] with Hg19 genomic build used for alignments. The following databases were searched for previously identified variants in the general population: the GnomAD database (including ExAC), the Single Nucleotide Polymorphisms Database dbSNP, 1000 Genomes and our own collection of > 2000 Polish individuals screened by whole exome sequencing or Illumina TruSight One™ Sequencing Panel.

NGS sequencing results were verified by amplicon deep sequencing (ADS) method. 474bp long PCR amplicons were generated using specific primers (forward: ccacatgcctcgaagtc, reverse: ctaggacctccccatcaac). The amplicons were prepared for sequencing using Nextera XT kit (Illumina) according to the manufacturer's instructions. Libraries were pair-end (2 × 100 bp) sequenced on an Illumina HiSeq1500. The results were visualized on the Integrative Genomics Viewer (<http://software.broadinstitute.org/software/igv/>) and analyzed for the presence of the particular variant in proband and family members.

## Results

NGS sequencing revealed in patient 2 the presence a homozygous variant in BRAT1 gene (Hg19: chr7: 002580938-CCT>C, NM\_152743.3:c.1313\_1314delAG, p.(Gln438fs)) located in exon 9. This variant is predicted to result in frameshift and to cause loss of the full-length protein (821 amino acids) due to truncation after first 487 residues. The p.(Gln438fs) is predicted to be pathogenic (VarSome, <https://varsome.com/>, ClinVar <https://www.ncbi.nlm.nih.gov/clinvar/>). The p.(Gln438fs) was previously reported in GnomAD database with allele frequency 0.00007139, however homozygotes were not reported (<http://gnomad.broadinstitute.org/>).

ADS verification resulted in obtaining the coverage > 4000× in all cases and confirmed the homozygous variant in proband and revealed a heterozygous p.(Gln438fs) in each of the examined family members – both in the parents and in healthy brother



**Fig. 2.** Genetic examination and family study. **A)** The pedigree of examined family. **B)** NGS sequencing results identified a homozygous p.(Gln438fs)/c.1313\_1314delAG variant in *BRAT1* gene. **C)** ADS verification of family members. NGS results were visualized on the Integrative Genomics Viewer (<http://software.broad-institute.org/software/igv/>), wt – wild type.

(Fig. 2). No other plausible causative candidate variants were found.

### Neuropathological evaluation of Patient 2

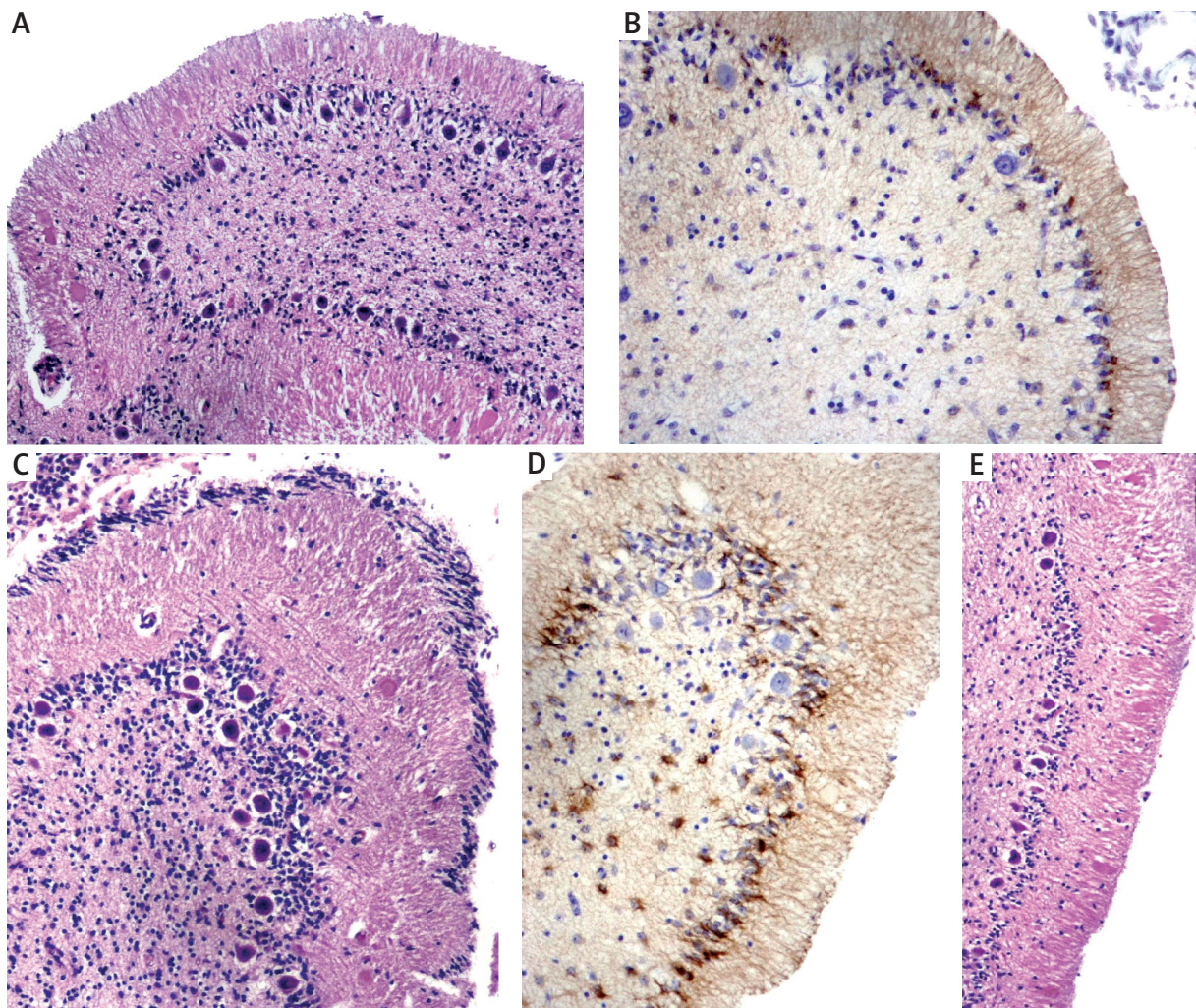
The brain was fixed in formalin. The specimens from the cerebral hemispheres, the brain stem and the cerebellum were taken and embedded in paraffin. The sections were stained with hematoxylin-eosin (H-E), cresyl violet, Klüver-Barrera methods, and GFAP immunoreaction was done.

Gross neuropathological evaluation showed hypoplastic brain hemispheres, especially frontal and temporal lobes. The brain stem and the cerebellum were also small. The spinal cord was normal in shape and size. On the frontal brain sections thin

commissural system, in particular corpus callosum was observed. There was narrow white matter with widening of the lateral ventricles.

The microscopical evaluation showed most prominent lesions in the cerebellum (Fig. 3). Generalized damage of cerebellar granular internal layer was observed. In the majority of vermin and hemispheric gyri there was a complete lack of granule neurons. In a few hemispheric gyri, a single layer of external granule cells was seen. At these places there were located some granule cells under Purkinje neurons. A few glial fibrillary protein (GFAP)-positive glial cells were dispersed in the granular layer beneath the Purkinje layer. Purkinje neurons were much better preserved. They





**Fig. 3.** Cerebellar damage. Patient 2. Lack of granule cells in the internal granular layer. **A, C, D**) Purkinje neurons well preserved in the Purkinje cell layer. **C**) A single layer of external granule cells. **D**) GFAP-positive glial cells dispersed in the granular layer. **A, C, E**) Eosinophilic homogenous spheroids in the molecular layer. **A, C, E**) Hematoxylin-eosin staining (orig. magn. 10×), **B, D**) GFAP immunoreactivity (orig. magn. 20×).

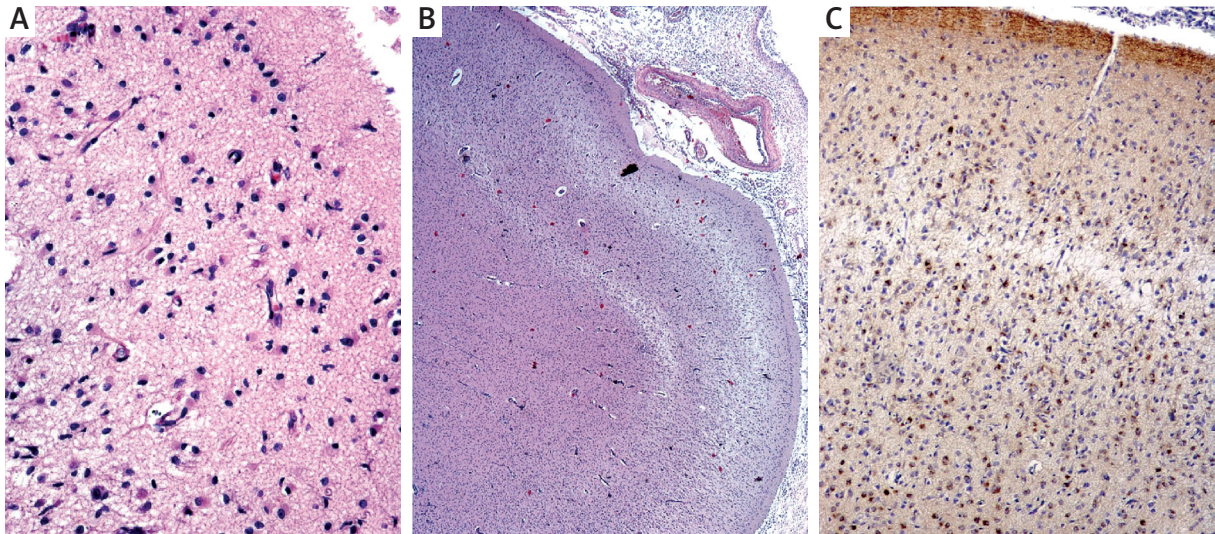
formed a layer in which Purkinje cells were tightly packed in many cerebellar cortical segments. Loss of Purkinje neurons was moderate, mainly focal at the bottom of some sulci with mild Bergmann glia reaction. In some locations small disorganization of the cerebellar cortex was revealed. There was disarrangement of a few Purkinje cells and a cluster of heterotopic granule cells in the molecular layer. In the molecular layer slight eosinophilic homogenous spheroids were observed.

In the cerebral hemispheres, disseminated cortical lesions were stated (Fig. 4). Widespread necrosis within layers II-V with moderate to severe glia reaction

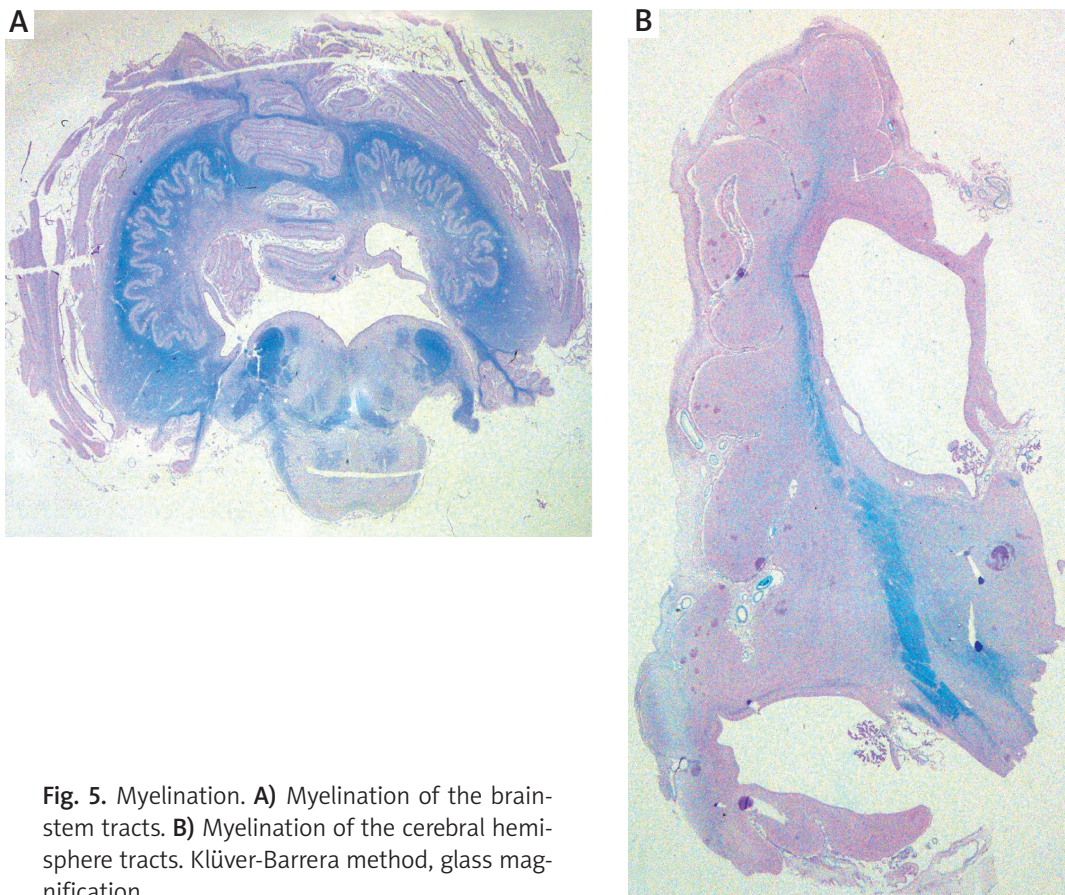
was visible in many gyri of frontal and temporal lobes. Degeneration with microvacuolization in the middle layers (layer III of the frontal superior gyrus and layer IV of the gyrus temporalis superior) and moderate loss of nerve cells were found. Numerous GFAP positive glial cells were scattered throughout the cerebral cortex layers. The marginal zone was formed with irregular wideness, dispersion of cells and was rich in the glial fibers. Corpus striatum was atrophic, especially caudate nucleus. The white matter of all cerebral lobes was narrow, with moderate glial reaction.

The generalized retardation of myelination of cerebral tracts was stated (Fig. 5). Myelination was





**Fig. 4.** Cerebral cortex lesions. Patient 2. **A)** Widespread necrosis within layers II-V. **B)** Degeneration with microvacuolization in the middle layers and moderate loss of nerve cells. **C)** Numerous GFAP positive glial cells scattered throughout the cerebral cortical layers. Hematoxylin-eosin staining (**A** – orig. magn. 20×, **B** – orig. magn. 4×), GFAP immunoreactivity (**C** – orig. magn. 4×).



**Fig. 5.** Myelination. **A)** Myelination of the brain-stem tracts. **B)** Myelination of the cerebral hemisphere tracts. Klüver-Barrera method, glass magnification.

appropriate to myelination of a mature newborn. The pattern of progression of myelination was normal, an overall delay of myelination was about several months. In the brainstem well myelinated were inferior cerebellar peduncles, medial lemnisci, slightly olivocerebellar tract and very weakly, almost none at all pyramidal tracts (Fig. 5A). In the cerebral hemispheres myelin fibers were stainable in internal capsule, fasciculus and ansa lenticularis, some fibers of corona radiata along lateral ventricle in the frontal lobe (Fig. 5B). The optic radiation was very poorly stained. Corpus callosum and the remaining white matter of the cerebral hemispheres were unmyelinated.

Features of bacterial meningitis were seen in the meninges.

## Discussion

In 2006, Aglipay *et al.* isolated a new protein that interacted with the C-terminal of BRCA1 (breast cancer 1) and cloned the gene [1]. They found that the protein also binds to ATM (ataxia-telangiectasia mutated) serine/threonine kinase and named this protein BAAT1 (BRCA1-associated protein required for ATM activation-1) (since 2013, the name BRAT1 has been used for the gene and for the protein). They suggested that the interaction of BRCA1 with BAAT1 (BRAT1) is crucial for the tumor-suppressing activity of BRCA1. The study also showed that loss of BRAT1 resulted in activation of the p53-mediated cell cycle checkpoint, leading to apoptosis [1]. Further studies have shown that BRAT1 also interacts with DNA-PKs and mTOR (as part of the PI3K/Akt/mTOR signaling cascade), thus stabilizing these proteins [19,31].

BRAT1 plays also an important role in regulating mitochondrial functions.

The dysfunction of mitochondria results in decreased ATP production, increased glucose metabolism and increased mitochondrial ROS [32]. The overproduction of mitochondrial ROS leads to cell death and eventually to neurodegeneration. The clinical course of BRAT1 depletion is probably secondary to the role of three phosphatidylinositol 3-kinase-related kinases (PIKKs) – ATM, DNA-PKs, mTOR and BRCA1 as well as mitochondrial dysfunctions [7,31,32].

Early descriptions of BRAT1-associated syndrome of “Rigidity and multifocal seizure syndrome, Lethal neonatal – RMFSL” (OMIM#614498) were those of single occurrences within families, where variants were found through extensive whole-exome sequenc-

ing efforts [22,29]. The clinical picture was consistent with intractable epilepsy, progressive microcephaly and early death [22,25,29,35].

However, subsequent reports presented both the variability of clinical picture between families and within one family [34]. Families in which children presented a minor progress of motor development were described. They survived significantly longer, up to 10 years [11,12,17,30,33]. Two of the four children described by Srivastava *et al.* did not suffer from epilepsy. In their clinical picture, ataxia dominated with cerebral atrophy in magnetic resonance imaging (MRI). One of them experienced epilepsy at a later age.

The clinical course of disease in both siblings we described was similar. It corresponds to a less severe clinical course reported originally with RMFSL. Since their first days of life, increased muscle tone, tonic and clonic seizures and migratory myoclonus were observed.

Regardless of the clinical course, all patients with *BRAT1* mutation that were described in the literature presented an inadequate rise in head circumference, leading to progressive microcephaly. Likewise, we confirmed a minor rise in head circumference of Patient 2 from the very beginning of life, even though he was born with correct head circumference. This was likely a consequence of loss of neurons and secondary neuropil loss.

Neuroimaging in Patient 1 was done at the age of 5 months, in Patient 2 at 2 weeks, which might be the reason for the difference between the radiological images.

In neuroimaging studies of other individuals with *BRAT1* mutation, cerebral atrophy, cerebellar hypoplasia/atrophy, decreased myelination/dysmyelination, thin corpus callosum, brainstem atrophy have been described [19,33].

Of the *BRAT1* mutation cases described so far, only two children who underwent these diagnostic tests presented cerebrospinal fluid neurotransmitter and pterin profiles. These children’s results were within normal limits [11,30].

In the remaining patients, this diagnostic study was probably not performed.

In our patient, HVA and 5-HIAA, both biochemical parameters, were below the low normal range. There have been described cases of secondary disorders of biogenic amine metabolism, among others in the mitochondrial DNA depletion [16]. Considering both the test results of biogenic amine metabolites in the cases described by Smith *et al.* and Hanes *et al.* as well as our results it can only be stated that



*BRAT1* mutation may lead to secondary disorders of biogenic amine metabolism suggesting a deficiency of tetrahydrobiopterin (BH4).

The central nervous system damage in the presented case is comparable to the neuropathological lesions described in the literature: microcephaly, severe neuronal loss and gliosis [19,22,25,30,36].

The main feature observed in our case is the cerebellar destruction. Cerebellar atrophy in cases of *BRAT1* mutation was described in neuroimaging studies [11,17,22,34,36] as well as in neuropathological descriptions. The neuropathological evaluation of cerebellar damage was reported by Smith, Saitsu and van de Pol [25,30,36]. Saitsu presented moderate Purkinje cell depletion and some dendritic expansions in the cerebellar cortical layer [25]. Smith described atrophic cerebellar folia with marked reduction in neurons within the granular layer and reduction of white matter volume [30]. In his description, van de Pol indicated severe atrophy of cerebellar hemispheres with loss of granular neurons and Purkinje cells, as well as reactive Bergmann glia [36].

The picture of pathological cerebellar changes presented in this paper confirms van de Pol's and Smith's descriptions. The dominant change was the total loss of neurons in the internal granular layer of vermis and cerebellar hemispheres with relatively well preserved Purkinje cells.

Most cerebellar degenerative diseases are characterized by predominant Purkinje neurons loss. The cases with damage of internal layer granule neurons with sparing of the Purkinje cells are less often described. The term "granular cell aplasia" reflects diffuse loss of external and internal layers. The granule cell aplasia can be a primary impairment or a secondary one to epigenetic factors like X irradiation, antimetabolic agents. Primary degeneration of the granular layer as unusual form of atrophy was described by Margaret Norman in 1940 [18]. Vodovnik reported cerebellar granular aplasia coexisting with congenital hydrocephalus [37] and del Bigio detailed multifocal atrophy of granular neurons in Lesh-Nyhan disease [6]. Sarnat and Alcalá presented global cerebral hypoplasia as a syndrome with diverse causes [27,28]. Friede in his monography paid attention to the role of granule cells precursors in the superficial granular layer [10]. This view was supported by Sarnat who stated that global cerebellar hypoplasia may occur due to lack of differentiation of granule cells because of a faulty genetic program for their formation [26]. Granule cells originate in the rhom-

bic lip and go through the double migration process. In the first stage granule cells precursors migrate along the cerebellar surface and form the external granular layer. In the second stage, postmitotic neurons expand the parallel fibers and move along them following the Bergmann glia processes towards the internal granule layer [4]. Migration of neurons from the superficial external layer to the internal granule layer takes place until the first year of life. Developmental defects affecting the granule cells precursors in the rhombic lip, their migration, proliferation and differentiation into matured granule cells may result in cerebellar hypoplasia [2].

In the presented case another neurodegenerative feature should be emphasized. The spheroid-shaped elements were evident in the cerebellar molecular layer. Asteroid bodies in the molecular layer were shown in the cerebellar hypoplasia described by Sarnat and Alcalá [26,28]. Authors presented bulbous swellings occurring in Purkinje cells dendrites and abnormal fibrils within the molecular layer. Normally, axons of the granular neurons form the parallel fibers that pass through the molecular layer and connect to dendrites of Purkinje cells. Loss of granular neurons and their axons induces the deficiency of synapses with Purkinje dendrites, which leads to dendritic asteroid formations. Spheroid bodies can be also a sign of dystrophic changes of granular neurons axons as a result of neuron degeneration.

In our case, neuropathological examination presented a significant delay in myelination. Myelination of the brainstem and cerebellar pathways corresponded to the degree of myelination in a newborn [24]. Myelination of the pyramids and of the hemispheric pathway passing through the internal capsule was very weak. There was no myelin sheath in the occipital lobe (with the exception of traces of myelination in the distal optic radiation) which physiologically should be myelinated at the end of the 3rd month of life [13,14,23].

Myelination disorder in cases of *BRAT1* mutation was previously described in neuroimaging studies. Van de Pol described hardly any myelination in a 1-year-old child upon MRI examination [36]. Mundy described decreased myelination in such cases [17]. Yu described unspecific hypomyelination in a 3-year-old child [38]. Ommeron described delayed myelination at a few weeks of age [19]. Myelination disorders were diagnosed upon neuropathological examination in some other cases. In a post-mortem examination of a 3-month-old child, Saitsu observed



no myelination of the cerebral white matter in the frontal lobe, though myelination of the brainstem and spinal cord was well developed [25]. Ommeron described delayed and patchy myelination throughout white matter, as well as the absence of myelination in the corpus callosum (the age of the child at the time of death was 2.5 months) [19]. Many genetic and epigenetic disorders are characterized by delayed myelination. Retardation of myelination can be observed in FAS disorder, hypothyroidism, chromosomopathies, and many organic acidurias [27]. Normal myelination is a long-lasting complex process involving multiple transcription factors that impact oligodendrocyte development and myelin generation. Oligodendrocyte precursors Olig1 and Olig2 are crucial for oligodendrocyte proliferation. Olig1 is crucial for oligodendrocyte progenitor cells maturation, Olig2 is important for oligodendrocyte lineage specification [15]. It is known that oligodendrocyte precursors have increased susceptibility to oxidative and free radical-mediated injury [9]. The susceptibility of oligodendrocyte precursors can have an impact on the myelination delay. The impairment of myelination progress can be a primary pathology affecting oligodendrocyte precursors which occurs during the early postnatal period.

In our case, cortical damage appeared with a range of lesions: from moderate loss of neurons, especially in layers II and III with preservation of the pyramidal neurons, to total necrosis covering all layers of the cortex with compensatory gliosis. In our case, cortical damage is rather a secondary lesion.

It is difficult to determine what morphological changes can be attributed to the cause of epilepsy and what its results or consequences are. Lobular atrophy of the cerebellum, diffuse cortical atrophy, lesions of the Ammon's horn and subpial gliosis can be observed in long-lasting epilepsy. However, it appears that the disruption of the myelination process, as well as neurodegenerative changes of the granule cells in the granular layer, may be associated with the gene's function. At the same time the effect of persistent myoclonic seizures, tonic and clonic epileptic states cannot be ruled out.

In the previously described cases of mutations in the *BRAT1* gene, both the variable clinical course and changes in neuroimaging and neuropathological studies were observed. The diversity in phenotypes of *BRAT1* cases may be due to a specific damaged region of BRAT1 protein and its effect on the protein activi-

ty as well as due to mutation zygosity. It is postulated that truncation/loss of function homozygous variants located in conserved N-terminal CIDE-N (cell death-inducing DFF45-like effector) domain of BRAT1 protein have a disruptive effect on protein function and produce more severe phenotype. In addition to the severe, lethal form of BRAT1-related disorders, both mild and moderate forms may exist [33,34].

Moreover, severe phenotypes in patients with *BRAT1* mutations may be due to its connection with epileptic seizures [5,7].

Sometimes, only when the second child of healthy parents is born with the same symptomatology of a given neurodevelopmental disorder, high suspicion of a genetically determined autosomal recessive condition can be put forward and appropriate testing initiated. Informed genetic counselling can be provided to such families once the cause of the disease is known.

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## Disclosure

The authors report no conflict of interest.

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