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Ganglioglioma associated with alterations of *NBN* gene. A case report

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Abstract

We report a case of a 13-year-old girl with a tumour of the right fronto-parietal region of the brain. The tumour consisted of two components: a well-differentiated astroglial component with Rosenthal fibres and a neoplastic neuronal component. The final histopathology established diagnosis of ganglioglioma WHO grade I. The patient was selected from a group of children with central nervous system (CNS) tumours screened for the most common molecular variants in the NBN *gene (exons 5 and 6). Molecular analysis revealed the presence of c.511A>G (p.Ile171Val) substitution on one allele. This is the first patient with ganglioglioma and confirmed mutation in the* NBN *gene.*

Key words: *ganglioglioma, paediatric brain tumours, Nijmegen breakage syndrome, nibrin, DNA repair genes, NBN gene.*

Introduction

Gangliogliomas (GGs) are mixed glio-neuronal neoplasms, composed of neoplastic ganglion cells in combination with a neoplastic glial element. GGs represent about 1.3% of all brain tumours [3]. Most of them correspond to WHO grade I or II [3]. Some gangliogliomas exhibit anaplastic glial components and are considered to be grade III according to WHO criteria. Low-grade gangliogliomas are more frequently recognized in children and adolescents. These tumours are well-differentiated, slowly growing neoplasms causing intractable epilepsy. The majority of them are localized in the temporal lobe, but other locations such as frontal or parietal lobes, optic nerves, cerebellum and spinal cord have also been encountered. Most supratentorial GGS involving the temporal lobe are usually associated with medically intractable epilepsy in children and young adults [3,14]. Computed tomography (CT) may demonstrate a circumscribed solid mass or cyst with a mural nodule. MRI shows a T1-weighted hypointense, T2-weighted hyperintense circumscribed solid or cystic mass [18]. Microscopically gangliogliomas are composed of two histologically different elements: glial and neuronal [3,10]. The glial component usually exhibits astroglial origin and might reveal features of pilocytic astrocytoma, fibrillary astrocytoma, pleomorphic xanthoastrocytoma or anaplastic astrocytoma. The most common glial element of paediatric GGs is pilocytic

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astrocytoma with Rosenthal fibres and eosinophilic granular bodies. The neuronal component consists of large, sometimes bi- or multinucleated ganglion cells. Additional histopathological features of GGs include calcifications, extensive perivascular lymphoid infiltrates, and an abundant capillary network. Mitotic figures are rare. Reported Ki-67/MIB-1 labelling index, involving only the glial component, has ranged from 1.1 to 2.7% [3,14].

Ganglioglioma of the optic nerve has been noted in a patient with neurofibromatosis type 1 [15]. The most frequent cytogenetic alterations of GGs is the gain of chromosome 7 and partial loss of chromosome 9p [26]. *CDKN2A* deletion was observed in 2/3 of anaplastic gangliogliomas [26]. The glial component of some gangliogliomas demonstrates alterations in the *TSC2* gene, including polymorphisms in intron 4 and exon 41 and a somatic mutation in intron 32 [1,2] Low-grade GGs with recurrences revealed *TP53* mutation [1-3,26]. New genes potentially involved in pathogenesis of ganglioglioma are still being sought. One of them appeared to be the *NBN* (previously named *NBS1*) gene [5,11,13,27]. Mutations in this gene result in Nijmegen breakage syndrome (NBS) – a rare, autosomal recessive chromosomal instability disorder [6,22,25]. NBS is clinically characterized by microcephaly, dysmorphic features, immunodeficiency, radiosensitivity, and increased risk for cancer (mainly lymphomas and other neoplasms, including brain tumours). The most common *NBN* mutation (c.657_661del5) is found in over 90% of NBS patients (founder effect). The *NBN* gene (8p21) encodes nibrin, one of the nuclear Mre11/Rad50/NBN (MRN) complex components, playing an important role in the detection of double-strand breaks [5,13,19,30]. It is well documented that relatives of NBS patients carrying a mutation on one *NBN* allele may also develop diverse neoplasms [22,24]. Many studies have shown an increased risk in heterozygous carriers of *NBN* gene mutations of malignant melanoma, non-Hodgkin's lymphomas, acute lymphoblastic leukaemia, stomach and colorectal cancer, breast and ovarian cancers, rhabdomyosarcoma, and medulloblastoma [6-8,11,16,17,20,21,24].

These observations prompted us to study the potential involvement of the most frequently identified *NBN* gene mutations (c.657_661del5, c.511A>G, and c.643C>T) in pathogenesis of paediatric brain tumours. The presented patient was selected from a group of children with brain tumours screened for the most common molecular variants in the *NBN* gene (exons 5 and 6). The study was part of a project assessing the frequency of heterozygote carriers of *NBS1* gene mutations in Polish children with defined malignancy, particularly central nervous system tumours.

Case report

A 13-year-old girl presented in 2002 with grand mal seizures. Brain MRI scan showed a lesion in the frontal/parietal right lobes, with weak enhancement after contrast administration. The patient underwent surgery in the Department of Neurosurgery, The Children's Memorial Health Institute. The lesion was only partially resected and biopsy material was scarce. Pathological examination was inconclusive and revealed abnormal neuronal cells, and glial tissue with no evidence of malignancy. The patient was subsequently followed with serial brain MRI scans. Two years later (2004) MRI showed an increase in size of the residual right frontal lesion. The second surgery and pathological examination were performed in another centre. The lesion was partially resected and histopathological diagnosis of diffuse astrocytoma was suggested. No further treatment was advised. Nine months later she deteriorated neurologically. The number of seizures increased and left hemiparesis was observed. Brain MRI scan revealed tumour progression, suggesting the development of malignant glioma. At this time she was consulted in the Department of Oncology, The Children's Memorial Health Institute. Due to the size of the tumour and involvement of more than one lobe neurosurgical treatment was not recommended. Pathology specimens from two surgeries were reassessed and the final diagnosis of ganglioglioma WHO grade I was established in the Department of Pathology, The Children's Memorial Health Institute. Chemotherapy consisting of cisplatinum and temozolomide was implemented but due to tumour progression it was discontinued after 4 courses. Radiotherapy was then administered. She received a total dose of 54 cGy to the tumour with margins. Radiotherapy was followed by maintenance chemotherapy resulting in disease stabilization.

Pathological findings

Microscopically, the tumour exhibited complex structure and consisted of glial and neuronal elements. The glial component was predominantly composed of elongated astroglial cells with oval nuclei.

The neoplastic tissue showed the presence of numerous Rosenthal fibres (Fig. 1) and eosinophilic granular bodies. Mitoses and necrosis were absent. The majority of the glial element revealed features of pilocytic astrocytoma. The neuronal component comprised large, atypical neurons with multiple processes and bi- or multi-nucleated ganglion cells (Fig. 2). Neuronal nuclei were large and round with prominent nucleoli. Perivascular lymphocytic infiltrates were occasionally observed.

Immunohistochemical examination revealed immunoreactivity for glial fibrillary acid protein (GFAP) and S-100 protein in neoplastic astrocytic cells (Fig. 3). Neoplastic neuronal cells were positive for synaptophysin (Fig. 4), neurofilament protein (NF), neuronal specific enolase (NSE), and chromogranin A. Labelling index of Ki67 was about 2%. The histopathological features and immunohistochemistry allowed the diagnosis of ganglioglioma WHO grade I to be established. The immunoexpression of nibrin was assessed due to alteration of the *NBN* gene in this patient. The neoplastic cells of both glial and neuronal components were positive for nibrin. These cells showed nuclear and cytoplasmic staining. Strong cytoplasmic staining was especially found in neuronal elements (Fig. 5). In control tissue samples taken from paediatric ependymoma without alteration of the *NBN* gene neoplastic cells revealed strong nuclear staining of nibrin (Fig. 6).

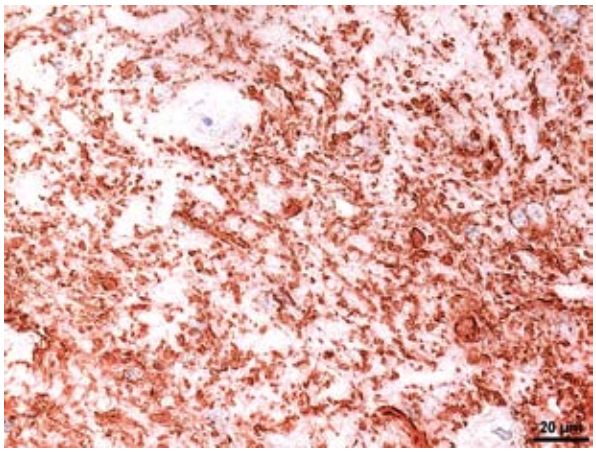

Fig. 3. Ganglioglioma – expression of GFAP.

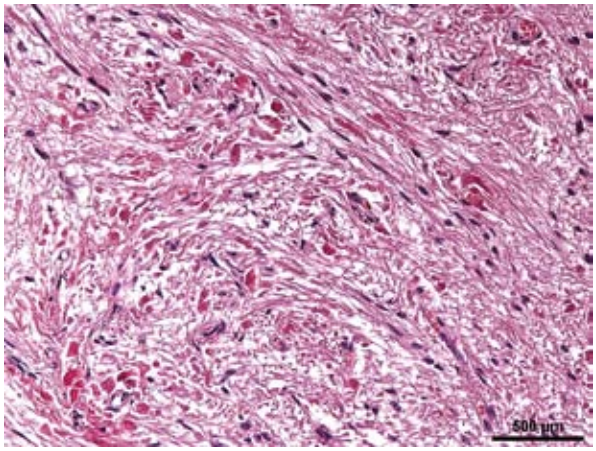

Fig. 1. Ganglioglioma – glial component with Rosenthal fibres. H&E.

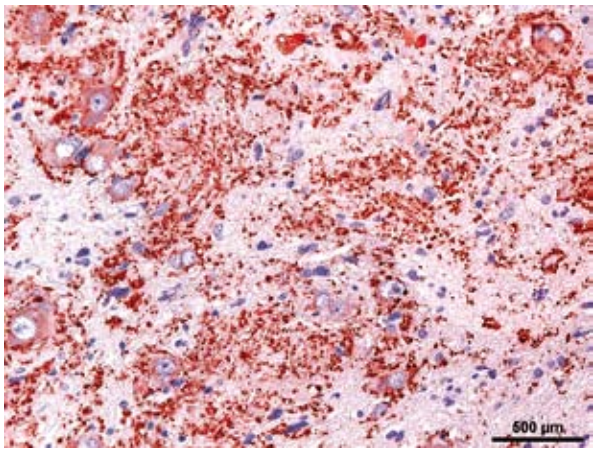

Fig. 4. Ganglioglioma – expression of synaptophysin in ganglion cells.

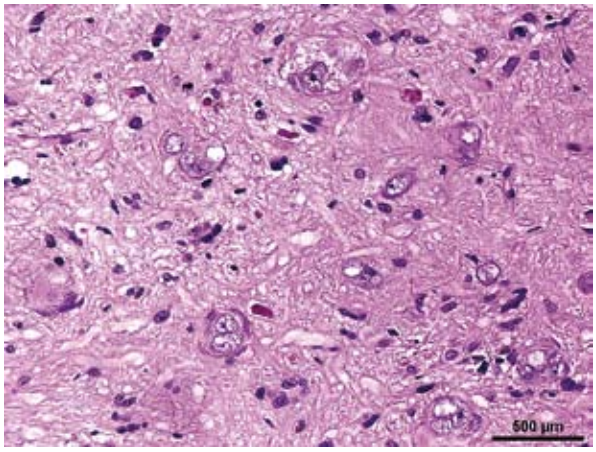

Fig. 2. Ganglioglioma – neuronal component with bi- and multi-nucleated ganglion cells. H&E.

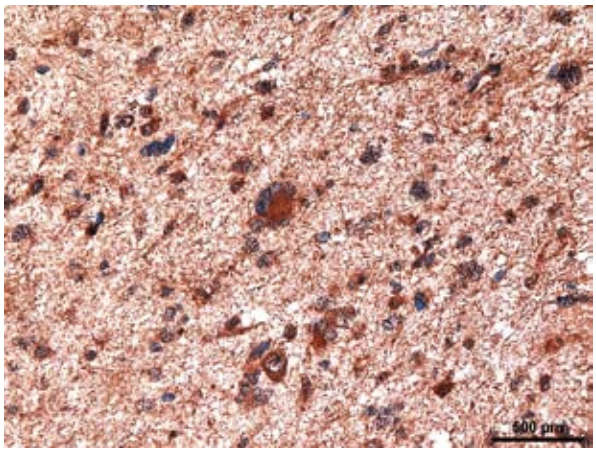

Fig. 5. Strong cytoplasmic immunoexpression of nibrin in neoplastic ganglion cell.

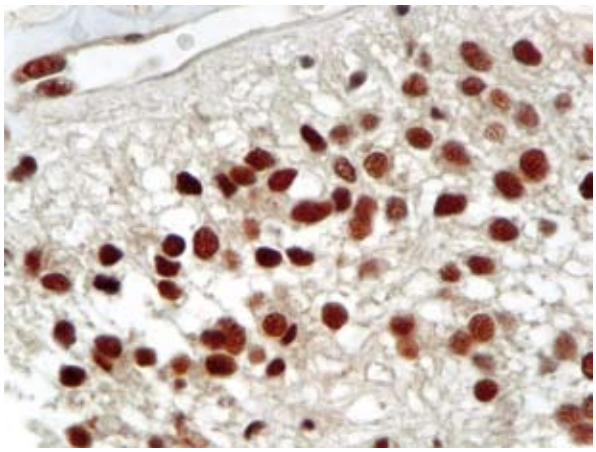

Fig. 6. Strong nuclear immunoexpression of nibrin in cells of ependymoma without alteration of *NBN* gene.

Molecular study

Molecular studies were conducted on genomic DNA extracted from the patient's dried blood spot. Searching for the most common molecular variants in *NBN* gene exons 5 and 6 (c.511A>G, c.657_661del5, and c.643C>T) revealed the presence of c.511A>G (p.Ile171Val) substitution on one allele (Fig. 7). Loss of heterozygosity (LOH) analysis was not performed due to lack of a frozen tumour sample from the patient.

Discussion

Gangliogliomas are well-differentiated, slowly growing biphasic neuroepithelial tumours composed of neoplastic ganglion cells and a neoplastic glial component. In children, various glioneuronal tumours and cortical dysplastic abnormalities are a frequent cause of intractable epilepsy [3,9,12]. Surgery is the best method of treatment of GGs, and in most cases, residual tissue grows slowly [3]. The role of adjuvant therapy remains controversial. Malignant transformation of gangliogliomas as in other glioneuronal tumours is rarely observed [4,14]. One can speculate that the unfortunate clinical course of our patient might have been associated with the presence of alteration in the *NBN* gene.

Cytogenetic abnormalities of GGs have appeared inconsistent. The most frequently diagnosed cytogenetic alterations were the gain of chromosome 7 and partial loss of chromosome 9p [26]. Approximately 65% of anaplastic gangliogliomas presented with *CDKN2A* deletion [26]. The glial component of GGs demonstrated various alterations, including splice-site specific polymorphisms in intron 4 and exon 41, and a somatic mutation in intron 32 of the *TSC2* gene [1-3,23,26]. The *TP53* mutation was reported in the recurrence of a low-grade ganglioglioma [26] .

Ganglioglioma has been reported in neurofibromatosis type 1 and 2, Turcot syndrome, and in

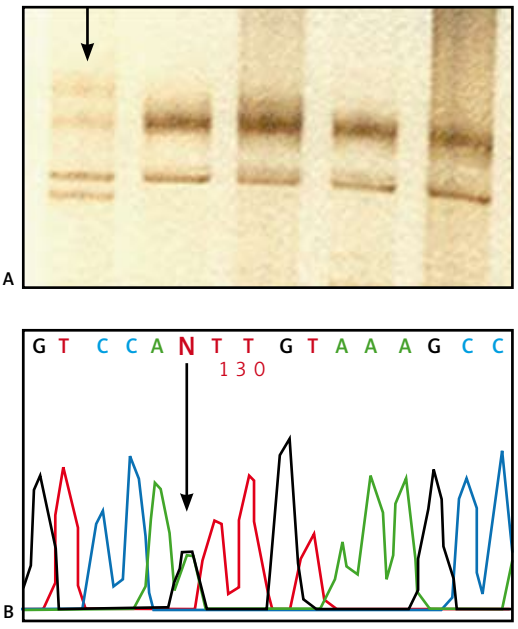

Fig. 7A-B. A>G substitution in position 511 of the *NBN* gene identified in the patient with ganglioglioma. **A.** SSCP analysis. **B.** Sequencing. The arrows show heterozygous mutation.

a Peutz-Jeghers patient with a germline mutation of the cyclin-dependent kinase *CDK5* [1,3,15].

Our screening for the common *NBN* gene molecular variants revealed the presence of c.511A>G (p.Ile171Val) substitution on one allele. The mutation p.Ile171Val disturbs the structure and activity of nibrin. It is assumed to affect its BRCA1 carboxy-terminal (BRCT) domain, which is highly conserved in eukaryotic nuclear proteins engaged in cell cycle, gene regulation, and DNA repair pathways [5,13,19,25,30]. An increased incidence of tumourigenesis, associated with the presence of the germline p.Ile171Val mutation in the *NBN* gene, has been described among patients with lymphoid malignancies (acute lymphoblastic leukaemia, ALL), breast and ovarian cancers, and larynx cancers [16,17,20-22,24,28,29]. The above-mentioned findings could suggest the probability that this molecular variant of the *NBN* gene could be a general susceptibility factor for malignancies. Thus, we could not exclude the role of *NBN* gene mutation (probably in association with other genes) in pathogenesis of ganglioglioma in our patient. Recent studies have shown the co-presence of mutations in the *NBN* gene and *TP53* gene in medulloblastoma tissue [11]. It has been suggested that mutation of the *NBN* gene may have caused genomic instability and subsequent molecular alterations of the *TP53* gene [11,27]. Our present finding of alteration in the *NBN* gene in ganglioglioma requires further study associated with molecular alterations of other genes, including the *TP53* gene.

Immunohistochemical analysis of the presented ganglioglioma revealed expression of nibrin in the glial and neuronal component. Astrocytic cells showed weak nuclear and cytoplasmic positivity for nibrin, whereas neoplastic ganglion cells revealed mainly cytoplasmic immunostaining. In contrast, the cells of ependymoma without abnormalities in the *NBN* gene demonstrated a very strong nuclear reaction. Recently, some studies have indicated a role of nibrin overexpression as a marker of aggressive head and neck cancer [28]. Analysis of nibrin immunoexpression in our case showed a slightly different pattern in comparison with control brain tumour tissue, probably due to identified alteration in the *NBN* gene. The correlation between the immunohistochemistry results of nibrin expression with mRNA and protein levels should be ascertained.

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