

DETECTION OF CHROMOSOME 1 DELETION BY FISH ON EPENDYMOMA TOUCH IMPRINTS SUGGESTS A REGION OUT OF CHROMOSOME 22 AS IMPORTANT FOR TUMOR RELAPSE

Demetrio Tamiolakis¹, Nikolas Papadopoulos², John Venizelos³, Maria Lambropoulou², Sylva Nikolaidou¹, Sophia Bolioti¹, Artemis Kiziridou⁴, George Alexiadis² and Constantine Simopoulos⁴

¹Department of Cytology, Regional Hospital of Chania; ²Department of Histology-Embryology, Democritus University of Thrace; ³Department of Pathology, Ippokraton General Hospital of Salonica; ⁴Department of Pathology, Regional Hospital of Drama, Greece

SUMMARY – Ependymomas are glial tumors. They constitute approximately 5%-10% of intracranial tumors. Ependymomas are tumors which can recur. Predictive factors of outcome in ependymomas are not well established. Karyotypic studies are relatively scarce and loss of chromosome 22 has been described to correlate with recurrence. We are unaware of any reports involving chromosome 1 aberrations in the malignant progression of ependymomas. Cytogenetic analysis of 4 ependymomas was performed using double-target fluorescent in situ hybridization (FISH) and focusing on chromosomes 1 and 22. One patient had recurrent tumor. FISH was performed on 500 nuclei/tumors. All four cases showed a loss of chromosome 22q, while only one showed an additional loss of chromosome 1p, and it was the one with tumor relapse. We support the presence of tumor suppressor gene on 1p associated with relapse in ependymomas and suggest that the chromosome 1p status by FISH may identify a high risk group of patients harboring this tumor. Additional studies in this direction are needed, as our results refer to a minimal number of individuals analyzed.

Key words: *Brain neoplasms – genetics; Ependymoma – genetics; Ependymoma – pathology; Chromosomes – human – pair 1; Chromosomes – human – pair 22*

Introduction

Ependymal neoplasms account for 5% of all tumors of the central nervous system (CNS). In children, however, they are the third most frequent brain tumor. Ninety percent of these tumors are intracranial (IC) and 10% are intraspinal (IS). They most frequently occur before the age of three years and are more aggressive than in adults. In adults, 60% of ependymomas are tumors of the spinal cord and only 40% are intracranial. Intramedullary spinal ependymomas can be seen in patients with neurofibromatosis type 2 (NF2), a hereditary disease.

Histologically, ependymomas can be benign (myxopapillary, WHO classification grade I), low grade (WHO grade II), or anaplastic (WHO grade III)¹. Ependymomas are tumors that can recur. However, predictive factors of outcome in ependymomas are far from being well established with respect to age, localization, grading, and extent of resection. There is, therefore, a need to identify biological factors to establish correlation with tumor behavior. Some work has been reported on the molecular biology and cytogenetics of these tumors²⁻¹¹.

Allelic losses of chromosome 1 are frequently documented in CNS tumors¹²⁻¹⁵. The existence of certain tumor suppressor gene(s) on this chromosome has been suggested by studies involving neuroblastomas¹² and medulloblastomas¹³, but to our knowledge there are no reports on the association of chromosome 1p abnormalities with the severity of ependymomas.

Correspondence to: *Assoc. Prof. Nikolas Papadopoulos, MD, PhD*, Democritus University of Thrace, Dragana, 68 100 Alexandroupolis, Greece
E-mail: npapad@med.duth.gr

Received January 18, 2005, accepted July 11, 2005

Frequent deletions of chromosome 1p in a series of oligodendroglial tumors using double-target fluorescent in situ hybridization (FISH) have also been recently reported¹⁴. This research has revealed a strikingly high incidence of deletions in the 1p36 region in pure oligodendroglial tumors, and supported the feasibility of FISH for detecting allelic deletions in chromosomes.

In the present study we examined four benign ependymomas for the loss of chromosomes 1 and 22, using double-target FISH. All four ependymomas displayed deletion of chromosome 22 but only one ependymoma displayed additional deletion of chromosome 1; and this deletion was closely related to recurrence of the tumor that had initially been diagnosed as benign by histology.

Materials and Methods

Tumor samples

Four ependymomas were selected from recent patient records. All samples were classified as benign according to the WHO classification of CNS tumors¹⁵. Fresh tumor material was obtained by computed tomography (CT) guided core needle biopsy.

Tissue preparation

Tumor touch imprints were prepared by lightly touching a piece of tissue against a precleaned slide. The slides were air-dried, fixed in methanol-acetic acid (3:1) at -20 °C for 20 min, and at room temperature for an additional 20 min before FISH was performed.

FISH

For detection of deletions of chromosome 22 we used cosmid probes G9 centromeric and F7 telomeric. The probes were labeled by nick translation with either biotin-11-dATP (GIBCO BRL, Gaithersburg, MD) or digoxigenin-11-dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN).

For detection of deletions of chromosome 1, we used the repetitive DNA probe pUC1.77¹⁶, which is specific for the pericentromeric region (1q12), and the cosmid probe CI1-5335¹⁷, which is specific for the subtelomeric region (1p36). pUC1.77 was labeled with biotin (bio)-16-dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN) and CI1-5335 was labeled with digoxigenin (dig)-11-dUTP (Boehringer Mannheim) by nick translation. Bio- and dig-labeled probe solutions were mixed in a ratio of 7:2 (volume/volume) (pUC1.77 and CI1-

5335) and 1.0 µL of Cot-DNA (5mg/mL; GIBCO BRL, Gaithersburg, MD) was added to 9 µL of the mixed probe solution. Then, double-target FISH was performed as described elsewhere^{14,18}.

Scoring of interphase nuclei

Under an Olympus BX-FLA Reflected Light Fluorescence Microscope (Olympus, Tokyo, Japan) interphase nuclei were screened through a UV filter; only intact nuclei, no torn or overlapping ones, were evaluated. Signals of all probes were visualized simultaneously through a double or triple band-pass filter. Hybridization signals were counted in 500 interphase nuclei, and the numbers of signals were recorded for each nucleus.

Results

Imprint cytology

All cases exhibited moderately cellular tumors with dual epithelial and fibrillated qualities. Typical perivascular pseudorosettes had fibrillated cellular processes radiating from thin-walled vessels (Fig. 1). The cells were elongated, with bipolar, eosinophilic processes and fine nuclear chromatin (Fig. 2). Mitoses were absent. Other areas showed epithelium-like features, with polygonal cells with fine nuclear chromatin arranged in an epithelial mosaic pattern and faintly discernible cytoplasmic borders. Poorly formed acinar structures and short cellular cords were also seen (Fig. 3). Blepharoblasts were not observed. Isolated areas exhibited highly fibrillated tumor cells in a haphazard configuration. Finally, globoids of myxoid material were seen juxtaposed against slender, fibrillated cells.

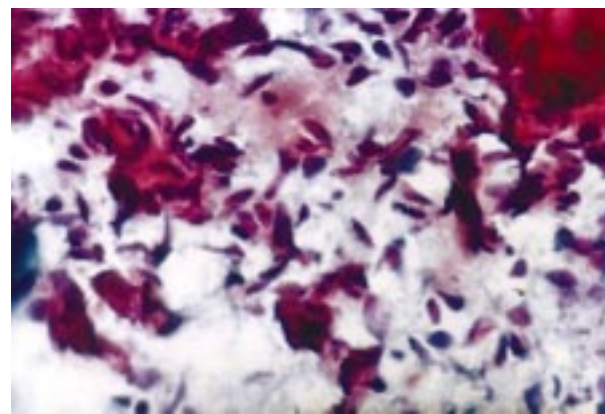


Fig. 1. Myxopapillary ependymoma. Pseudorosette formation. Touch imprint preparation. (Papanicolaou stain, X400)

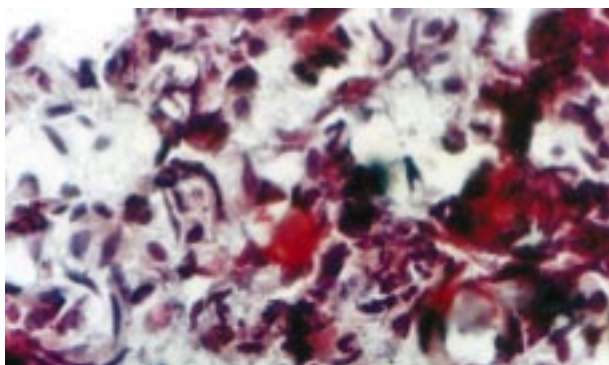


Fig. 2. Myxopapillary ependymoma. Elongated cells with bipolar processes. Touch imprint preparation. (Papanicolaou stain, X400)

Histology

Microscopic examination revealed a cellular tumor consisting of uniform, ovoid cells in a papillary fashion with ill defined borders. These cells were embedded in a myxoid degenerated, fibrovascular matrix and mostly arranged around small hyalinized blood vessels, forming pseudorosettes (Fig. 4).

Immunohistochemistry and FISH analysis

Immunohistochemical staining showed strong expression of glial fibrillary acid protein (GFAP) and vimentin, and lack of staining for cytokeratin, S-100 protein, neurospecific enolase, and chromogranin. FISH analysis (Fig. 5) showed deletions 1p36 in 62% of the cells in only one of our cases. All four cases showed deletion 22q13 in 56.5%, 64.1%, 67.2%, and 70.0% of the cells, respectively. Table 1 summarizes tumor details and FISH analysis in all four cases.

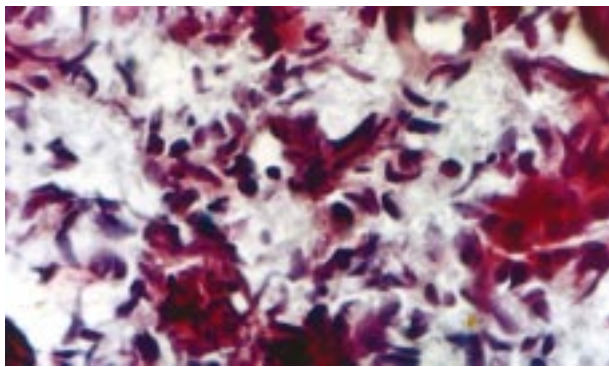


Fig. 3. Myxopapillary ependymoma. Poorly formed acinar structures and cords. Touch imprint preparation. (Papanicolaou stain, X400)

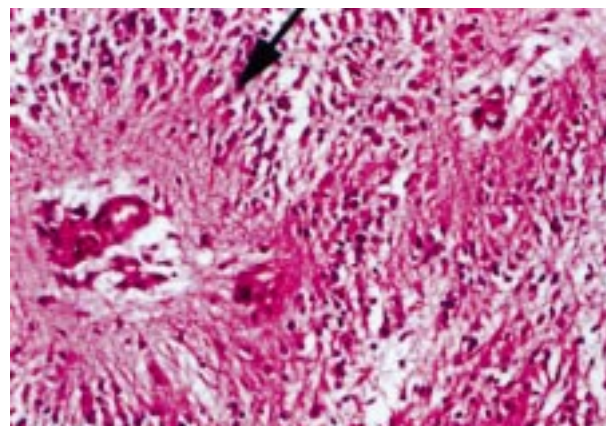


Fig. 4. Myxopapillary ependymoma. Pseudorosette formation of spindle-shaped cells arranged around blood vessels. Tissue section. (H&E stain, X400)

Discussion

The cytogenetics of ependymomas has been infrequently reported compared with other brain tumors. About 100 ependymomas have been cytogenetically analyzed^{3-11,16,17,19,20}. In the literature, abnormalities of chromosome 22 have been reported in 30% of cases^{3,6,7,17,20}. This frequency of monosomy 22 was the same in pediatric and adult ependymomas. The existence of

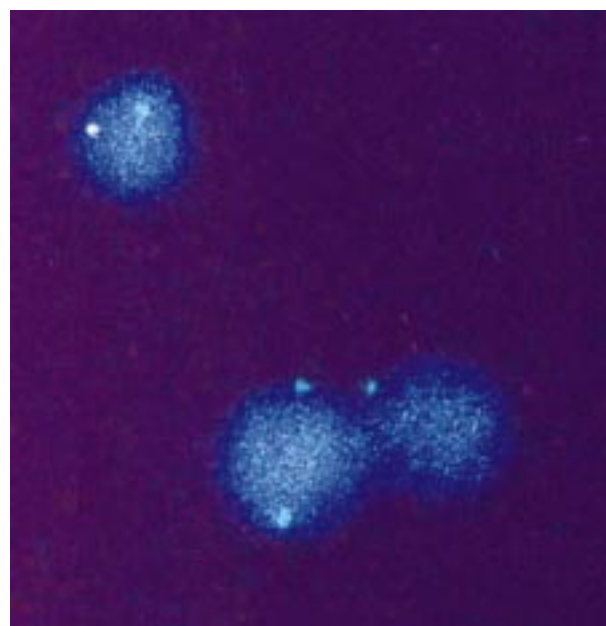


Fig. 5. Results of double-target FISH using probes specific for 1q12 to interphase nuclei prepared from ependymoma samples.

Table 1. Tumor details and FISH analysis

Case No.	Age (yrs)	Tumor	Biopsy origin	% deletions 22q13	% deletions 1 p 36
1	40	Spinal	Needle/fresh	70.0	62.0
2	46	Spinal	Needle/fresh	67.2	–
3	51	Spinal	Needle/fresh	64.1	–
4	56	Spinal	Needle/fresh	56.5	–

intramedullary spinal ependymoma in *NF2* is known but is less frequent than meningiomas. It was speculated that the gene “*MERLIN*” located at 22q12 in *NF2* could play a role in ependymomas²¹⁻²⁴. However, ependymomas in non-*NF2* familial cases have been reported²⁵. Vagner-Capodano *et al.* have reported that recurrent tumors for which a karyotype analysis of initial tumors was performed showed monosomy 22 in primitive tumors²⁶. It seems that a putative ependymoma tumor suppressor gene located on chromosome 22, independent of the *NF2* gene, may play a role in the progression of ependymomas^{6,8,27,28}.

Cytogenetic deletions and/or loss of allelic heterozygosity on chromosome 22 are also a consistent feature of other CNS tumors (e.g., schwannomas and meningiomas). In these tumors, the locus of the gene is different from the *NF2* locus²⁹. A gain of chromosome 7 has been frequently reported in other glial tumors, and its specificity has been discussed elsewhere³⁰. This abnormality was reported in only nine cases of ependymomas; four of them were anaplastic^{3,4,7,9,16}. Vagner-Capodano *et al.* have also reported trisomy 7 in all anaplastic tumor cases analyzed²⁶.

In ependymomas, abnormalities of chromosome 11 have been described; monosomy 11 was found in six cases^{3,6,7,9,17} and trisomy 11 has been reported once²⁶. Rearrangements involving 11q13 have been described in four pediatric ependymomas^{10,11,19}. The locus q13 of chromosome 11 contains the oncogenes *BCT1*, *HST*, and *INT2*, which are amplified in some human cancers¹⁰. One of these genes is perhaps implicated in ependymomas. In the literature, six cases of abnormalities of chromosome 6 have been described in ependymomas^{7,16,17,19,26}, six cases with monosomy 6 and one case with a translocation involving 6q11.

Chromosome 22q and 1p FISH analysis of our ependymomas series showed losses of 22q in 56.5%, 64.1%, 67.2% and 70.0% of the cells, respectively, and loss of

1p in 62% of the cells in only one patient in whom tumor recurred 2.5 years after the surgery. The sample from the recurrent tumor was diagnosed pathologically as a grade 2 (atypical) ependymoma. These findings strongly indicate the existence of one or more tumor suppressor genes on chromosome 1p, which may contribute to progression of grade 1 (benign) ependymoma to grade 2 (atypical) ependymoma. This theory is valid only assuming that 1p deletions are found in grade 2 ependymomas and not in grade 1 ependymomas. It would be important to determine how grade 1 ependymoma progresses to grade 2 in individual cases. In the recurrent case the histologically benign ependymoma showed signs of atypia in the recurrent tumor. In this case the histologically benign ependymoma showed deletions of 1p as early as the first surgery. Considering the fact that the recurrent tumor demonstrated a histologically atypical phenotype, the results can be interpreted as suggesting that the intrinsic tumor had been detectable by means of FISH before the pathologic changes occurred. It would be reasonable to assume that genetic changes would precede the morphologic changes. We suggest that 1p FISH analysis may aid in assessing the risk of recurrence in histologically benign ependymomas.

References

1. KLEIHUES P, BURGER P, SCHEITAUER B. The new WHO classification of brain tumors. *Brain Pathol* 1993;3:255-68.
2. HAMILTON RL, POLLACK IE. The molecular biology of ependymomas. *Brain Pathol* 1997;7:807-22.
3. RANSOM DT, RITLAND SR, KIMMEL DW, MOERTEL CA, DAHL RJ, SCHEITAUER BW, KELLY PJ, JENKINS RB. Cytogenetic and loss of heterozygosity studies in ependymomas, pilocytic astrocytomas, and oligodendrogliomas. *Genes Chromosom Cancer* 1992;5:348-56.
4. ROGATTO SR, CASARTELLI C, RAINHO CA, BARBIERINETO J. Chromosomes in the genesis and progression of ependymomas. *Cancer Genet Cytogenet* 1993;69:146-52.
5. STRATTON MR, DARLING J, LANTOS PL, COOPER CS, REEVES BR. Cytogenetic abnormalities in human ependymomas. *Int J Cancer* 1989;44:579-81.
6. WEREMOWICZ S, KUPSYWJ, MORTON CC, FLETCHER JA. Cytogenetic evidence for a chromosome 22 tumor suppressor gene in ependymoma. *Cancer Genet Cytogenet* 1992;61:193-6.
7. WERNICKE C, THIEL G, LOZANOVA T, VOGEL S, KINTZEL D, JANISCH W, LEHMANN K, WITKOWSKI R. Involvement of chromosome 22 in ependymomas. *Cancer Genet Cytogenet* 1995;79:173-6.
8. BIJLSMA EK, VOESTEN AM, BIJLEVED EH, TROOST D, WESTERVELD A, MEREL P, THOMAS G, HULSEBOS TJ.

- Molecular analysis of genetic changes in ependymomas. *Genes Chromosom Cancer* 1995;13:272-7.
9. SAWYER JR, SANMARTINO G, HUSAIN M, BOOP FA, CHADDUCK WM. Chromosome aberrations in four ependymomas. *Cancer Genet Cytogenet* 1994;74:132-8.
 10. DAL CIN P, SANDBERG AA. Cytogenetic findings in a supratentorial ependymoma. *Cancer Genet Cytogenet* 1988;30:289-93.
 11. SAINATI L, MONTALDI A, PUTTI MC, GIANGASPERO F, RIGOBELLO L, STELLA M, ZANESCO L, BASSO G. Cytogenetic t(11;17)(q13;q21) in a pediatric ependymoma. Is 11q13 a recurring breakpoint in ependymoma? *Cancer Genet Cytogenet* 1992;59:213-6.
 12. BRODEUR GM, FONG CT. Molecular biology and genetics of human neuroblastoma. *Cancer Genet Cytogenet* 1989;41:153-74.
 13. BIGNER SH, MARK J, FRIEDMAN HS, BIEGEL JA, BIGNER DD. Structural chromosomal abnormalities in human medulloblastoma. *Cancer Genet Cytogenet* 1988;30:91-101.
 14. HASHIMOTO N, ICHIKAWA D, ARAKAWA Y, DATE K, UEDA S, NAKAGAWA Y, *et al.* Frequent deletions of material from chromosome arm 1p in oligodendroglial tumors revealed by double-target fluorescence in situ hybridization and microsatellite analysis. *Genes Chromosom Cancer* 1995;14:295-300.
 15. BELLO MJ, LEONE PE, NEBREDAP, de CAMPOS JM, KUSAK HE, VAQUERO J, *et al.* Allelic status of chromosome 1 in neoplasms of the nervous system. *Cancer Genet Cytogenet* 1995;83:160-4.
 16. GRIFFIN CA, LONG PP, CARSON BS, BREM H. Chromosome abnormalities in low-grade central nervous system tumors. *Cancer Genet Cytogenet* 1992;60:67-73.
 17. JENKINS RB, KIMMEL DW, MOERTEL CA, SCHULTZ CG, SCHEITAUER BW, KELLY PJ, DEWALD GWA. A cytogenetic study of 53 human gliomas. *Cancer Genet Cytogenet* 1989;39:253-79.
 18. INAZAWA J, ARIYAMA T, ABE T. Physical ordering of three polymorphic DNA markers spanning the regions containing a tumor suppressor gene of renal cell carcinoma by three color fluorescence in situ hybridization. *Jpn J Cancer Res* 1992;83:1248-52.
 19. NEUMANN E, KALOUSEK DK, NORMAN MG, STEINBOK P, COCHRANE DD, GODOARD K. Cytogenetic analysis of 109 pediatric central nervous tumors. *Cancer Genet Cytogenet* 1993;71:40-9.
 20. NIJSSEN PC, DEPREZ RH, TIJSSEN CC, HAGEMEIJER A, ARNOLDUS EP, TEEPEN JL, HOLL R, NIERMEYER MF. Familial anaplastic ependymoma: evidence of loss of chromosome 22 in tumor cells. *J Neurol Neurosurg Psychiatry* 1994;57:1245-8.
 21. EGELHOFF JC, BATES DJ, ROSS JS. Spinal MR findings in neurofibromatosis types 1 and 2. *Am J Neuroradiol* 1992;13:1071-7.
 22. LEE M, REZAI AR, FREED D, EPSTEIN FJ. Intramedullary spinal cord tumors in neurofibromatosis. *Neurosurgery* 1996;38:32-7.
 23. LOUIS D, RAMESH V, GUSELLA J. Neuropathology and molecular genetics of neurofibromatosis 2 and related tumors. *Brain Pathol* 1995;5:163-72.
 24. ROULEAU G, MEREL P, LUTCHMAN M, SANSON M, ZUCMAN J, MARINEAU C, HOANG X, DEMCZUX S, DESMAZE C, PLOUGASTEL B, PULST S, LENOIR G, BILJSMA E, FASHOLD R, DUMANSKI J, de JONG P, PARRY D, ELDRIDGE R, AURIAS A, DELATTRE O. Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. *Nature* 1993;363(6429):515-21.
 25. PARK JP, CHAFFEES, NOLL WW, RHODES CH. Constitutional *de novo* t(1;22)(p22;q11-2) and ependymoma. *Cancer Genet Cytogenet* 1996;86:150-2.
 26. VAGNER-CAPODANO AM, ZATTARA-CANNONI H, GAMBARELLI D, FIGARELLA-BRANGER D, LENA G, DUFOUR H, GRISOLI F, CHOUX M. Cytogenetic study of 33 ependymomas. *Cancer Genet Cytogenet* 1999;115:96-9.
 27. HOANG-XUAN K, MEREL P, VEGA F, HUGOT JP, CORNU P, DELATTRE JY, POISSON M, THOMAS G, DELATTRE O. Analysis of the NF₂ tumor suppressor gene and of chromosome 22 deletions in gliomas. *Int J Cancer* 1995;60:478-81.
 28. SLAVC I, Mac COLLIN MM, DUNN M, JONES S, SUTTON L, GUSELLA JF, BIEGEL JA. Exon scanning for mutations of the NF2 gene in pediatric ependymomas, rhabdoid tumors and meningiomas. *Int J Cancer* 1995;64:243-7.
 29. DUMANSKI JP, ROULEAU GA, NORDENSKJOLD M, COLLINS VP. Molecular genetic analysis of chromosome 22 in 81 cases of meningioma. *Cancer Res* 1990;50:5863-7.
 30. HEIM S, MANDAHL N, JIN Y, STROMBALD S, LINDSTROM E, SALFORD LG, MITELMANN F. Trisomy 7 and sex chromosome loss in human brain tissue. *Cytogenet Cell Genet* 1989;52:136-8.

Sažetak

OTKRIVANJE DELECIJE KROMOSOMA 1 POMOĆU TEHNIKE FISH NA OTISCIMA EPENDIMOMA UKAZUJE NA REGIJU IZVAN KROMOSOMA 22 VAŽNU ZA RECIDIV TUMORA

*D. Tamiolakis, N. Papadopoulos, J. Venizelos, M. Lambropoulou, S. Nikolaidou, S. Bolioti, A. Kiziridou, G. Alexiadis
i C. Simopoulos*

Ependimomi su glialni tumori. Oni čine otprilike 5%-10% intrakranijskih tumora. Ependimomi su tumori koji se mogu ponavljati. Čimbenici koji bi ukazivali na ishod ependimoma nisu dobro utvrđeni. Kariotipska ispitivanja su relativno rijetka, a opisano je kako gubitak kromosoma 22 korelira s ponovnom pojavom tumora. Nisu nam poznata izvješća o upletenosti aberacija kromosoma 1 u malignoj progresiji ependimoma. U ovoj studiji je citogenetska analiza 4 ependimoma provedena pomoću dvociljne fluorescentne in situ hibridizacije (FISH) i fokusiranja na kromosomima 1 i 22. U jednog se bolesnika radilo o ponovnoj pojavi tumora. FISH je izvedena na 500 jezgara/tumora. Sva četiri slučaja pokazala su gubitak kromosoma 22q, dok je samo jedan, i to onaj s ponovnim razvojem tumora, pokazao dodatni gubitak kromosoma 1p. Priklanjamo se mišljenju kako je prisutnost gena tumorske supresije na 1p udružena s recidivom kod ependimoma i ukazujemo na to da bi status kromosoma 1p utvrđen pomoću FISH mogao identificirati visoko rizičnu skupinu bolesnika koji nose ovaj tumor. Potrebne su daljnje studije u ovom smjeru, jer se naši rezultati odnose na mali broj analiziranih osoba.

Ključne riječi: *Neoplazme mozga – genetika; Ependimom – genetika; Ependimom – patologija; Kromosomi – ljudski – par 1; Kromosomi – ljudski – par 22*