

# Genomic Imbalances in Ependymoma by Degenerate Oligonucleotide Primed PCR-Comparative Genomic Hybridization

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**Background :** The most consistent chromosomal abnormality in ependymomas, is loss of 22q (17-75%) and gain of 1q (0-50%). However, significance of this abnormality is uncertain. **Methods :** Genomic imbalances in 27 Korean ependymomas, including 21 low grade ependymomas, 4 anaplastic and 2 myxopapillary ependymomas, were analyzed by degenerate oligonucleotide primed-PCR-comparative genomic hybridization. **Results :** Common gains were found in 17 (63%), 20q (59%), 9q34 (41%), 15q24-qter (33%), 11q13 (30%), 12q23 (26%), 7q23-qter (26%), 16q23-qter (30%), 19 (26%), and 1q32-qter (22%). DNA amplification was identified in 12 tumors (44%). Chromosomal loss was a less common occurrence in our study, but was found in 13q (26%), 6q (19%), and 3 (11%). **Conclusion :** The recurrent gains or losses of the chromosomal regions which were identified in this study provide candidate regions that may be involved in the development and progression of ependymomas.

**Key Words :** Ependymoma-Comparative Genomic Hybridization-Chromosomal aberration-PCR

Ependymomas are neoplasms of ependymal and subependymal cells, and form 4.3-6% of all intracranial gliomas, frequently occurring in the periventricular area of children and young adults.<sup>1</sup> Survival rate is influenced by various factors, including age, site, treatment (including availability of complete excision), and histological grading. Resectability and tumor location are considered to be the most important factors regarding prognosis. Recently, some cytogenetic and molecular studies have been carried out on ependymoma.<sup>2-6</sup> The most consistent chromosomal abnormalities in ependymomas have been found to be monosomy 22, and structural abnormality 22q.<sup>3,7-13</sup> However, the significance of this is as yet unknown. Conflicting results exist regarding the significance of these abnormalities, as with the studies regarding the prognostic significance of histological parameters.

Degenerate oligonucleotide primed-PCR-comparative genom-

ic hybridization (DOP-PCR-CGH) is a recently described technique, which is capable of providing an unbiased screen of the entire genome, for regions of relative genomic imbalance. It operates through the use of PCR, using a small amount of DNA, which can be obtained from microdissected tumor cells.<sup>14</sup>

The aim of this study is to investigate these common chromosomal aberrations in 27 cases of Korean ependymomas, using DOP-PCR-CGH.

## MATERIALS AND METHODS

### Tissue samples

Thirty-one formalin-fixed, paraffin-embedded specimens of

ependymomas were obtained from the archives of the Departments of Pathology of the Seoul National University, Sungkyunkwan University, and Inje University College of Medicine. Twenty-seven out of 31 cases studied evidenced confident results. In the 27 cases, the male to female ratio was 4:5. In 15 cases, samples were isolated from brain tumors, and in 12 cases, spinal cord tumors were the source. There were 21 Low-grade ependymomas, 4 high-grade ependymomas, and 2 myxopapillary ependymomas. Eight cases were supratentorial, and 7 cases were posterior fossa tumors. Four  $\mu\text{m}$  sections from each block were stained with hematoxylin and eosin (H&E), and reviewed by two pathologists to confirm the diagnosis. Three serial 10  $\mu\text{m}$  paraffin-embedded tissue sections were used for the study, and were largely (more than 95%) composed of tumor tissue.

#### Degenerate oligonucleotide-primed (DOP) and comparative genomic hybridization (CGH)

DOP-PCR was performed on a thermocycler, in two separate steps, according to a published protocol.<sup>15</sup> Normal genomic DNA was labeled in a standard Nick Translation assay (Nick Transla-

tion System, Gibco/BRL, Rockville, MD), with digoxigenin-11dUTP (Roche Basel, Switzerland), and tumor DNA was labeled with biotin-16dUTP (Roche, Basel, Switzerland). Equal amounts of labeled normal control and tumor DNA, including 40  $\mu\text{g}$  of Cot-1 DNA (GIBCO BRL), and 1  $\mu\text{g}$  salmon sperm DNA, were precipitated with ethanol. CGH analysis was performed according to the protocol developed by Kallioniemi *et al.* with slight modifications.<sup>16</sup> Metaphase slides were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes, isolated from a healthy donor according to the standard procedures.<sup>17</sup> The CGH experiments were performed using software and protocols supplied by the manufacturer (Vysis inc., Downers Grove, IL, USA). Labeled DNA probes mixed with 7  $\mu\text{L}$  of hybridization buffer (50% formamide, 10% dextran sulfate, 2X SSC, pH7.0) and 3  $\mu\text{L}$  of distilled water were denatured at 75°C for 5 min. Normal metaphase chromosomes were denatured using 70% formamide/2X saline sodium citrate (SSC), pH7.0, at 60°C for 40 s, and dehydrated in ice-cold ethanol. Hybridization was performed at 72°C for 3 days in a humidified chamber. After 3 days of incubation, the slides were washed and treated with avidin-fluorescein isothiocyanate (FITC), 1:400 and mouse anti-digox-

**Table 1.** Summary of the chromosomal changes found in 27 ependymomas

No.	Age/Dx	Gain	Loss
1	65/LE	None	None
2	21/LE	None	None
3	55/LE	17, 19	13q
4	23/LE	1q31~qter, 5q31~qter, 11p11.2~q13, 12q23~qter, 15q22~q26.1, 20	3,6
5	59/LE	5, 7, 8, 9, 10p12~pter, 10q25~qter, 12q23~qter, 13q, 15q, 18, 20, 21q	3,6p12~qter
6	7/LE	1q, 5p, 14q24~qter, 15q22~qter, 17, 19, 20, 22q	None
7	56/LE	1q32~qter, 6p21.3, 7q32~qter, 11q13, 15q24~q26.1, 16p, 17	13
8	23/LE	1q31~qter, 2q11.2~q13, 9q32~qter, 12q23~qter, 15q22~qter, 16, 17, 20, 22q13	1p31~1p10, 5pter-q31, 11p
9	38/LE	6p21, 7q11.1~q21, 9q34, 11q13, 12q23~qter, 14q32, 15q24, 16, 17, 20q, 22q	4q22~q31.1, 13q21~q32
10	36/LE	7, 20, 17, 20q12~qter, Xp21~pter,	13q11~q21
11	25/LE	7, 9q34, 11q11.2~q13.1, 12, 17, X	13q21~q31
12	25/LE	7, 9q34, 11q11~q13.2, 16, 17, 18, 20q	12p12.1~12p10, 14q
13	12/LE	19	None
14	27/LE	6p21, 16p11.2~p10, 16q22~qter, 17p~q22, 19, 20q	9p21~pter
15	30/LE	1q, 11q13, 14q, 15q22~qter, 17, 18, 19, 20	2q21~q32.3, 6q, 7p
16	59/LE	1q41~qter, 4, 5, 7q32~qter, 9q32~qter, 12q23~qter, 14q24~qter, 17, 20	2q21~q34, 3p14~qter, 6q,
17	52/LE	6p21~pter, 17, 18q21~qter, 20q,	None
18	19/LE	3p24~pter, 4p15~pter, 7, 9, 11q, 15q, 17q21~qter, 18, 19, 20	10
19	29/LE	None	None
20	59/LE	5q31~qter, 7p~q11, 8p22~pter, 9, 12, 15q24~qter, 16, 17q, 18p11.3, 20	3, 6p11.2~qter, 11, 13q13~q32
21	63/LE	9q34, 11q13, 16q23~qter, 17, 20	13q10~q31
22	2/HE	6p21, 9q34, 16q22~qter, 17, 20q	None
23	6/HE	None	None
24	3/HE	None	None
25	26/HE	18q12, 20p12~pter	None
26	44/ME	8p12~q23, 14q13~q22, 17q23~qter, 19q	None
27	16/ME	9p21~qter, 17, 18, 19, 20q13.2~qter, 21q21, X	None

LE, low-grade ependymoma; HE, high-grade (anaplastic) ependymoma; ME, myxopapillary ependymoma; none, no chromosomal loss or gain.

igenin, 1:100. Metaphase slides were counterstained with DAPI (Oncor, Gaithersburg, MD) for chromosomal identification. Images of 10 to 15 metaphases were captured with a cold charge-coupled device camera (Photometrics, Tucson, AZ), which was connected to a ZEISS Axioskop fluorescence microscope. Chromosomal identification was performed on the reverse DAPI banding images. Average ratio profiles were calculated based on the analysis of selected metaphases. Thresholds for gains and losses were defined as the theoretical values of 1.25 and 0.75, respectively. DNA amplification was defined as degree of amplification greater than 1.5. The centromeric and telomeric regions of the chromosomes were excluded from CGH analysis.

## RESULTS

27 ependymomas, out of the 31 cases studied, were successfully analyzed using DOP-PCR CGH. Table 1 summarizes the clinical and chromosomal findings for all 27 cases. The aforementioned chromosomal changes were detected in 22 tumors. 5 cases showed no chromosomal imbalances. A schematic summary of copy number changes detected by CGH analysis in each of the 22 abnormal cases is shown in Fig. 1. A number of prominent sites of genomic imbalances were observed. Common chromosomal gain was found in 17 (63%), 20q (59%), 9q34 (41%), 15q24-qter (33%), 11q13 (30%), 12q23 (26%), 7q32-qter (26%), 16q23-qter (30%), 19 (26%), and 1q32-qter (22%). DNA amplification ( $\geq 1.5$ ) was identified in 12 tumors (44%). Gains were found at various chromosomal regions, especially chromosomes 7 and 17. Non-random amplification was detect-

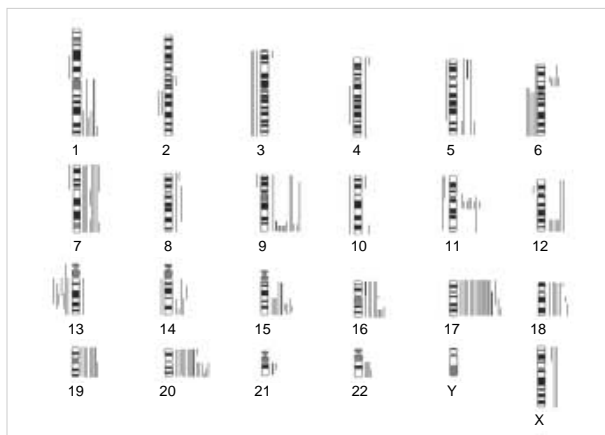


Fig. 1. Summary for gains and losses of chromosomal regions in 27 ependymomas. Gains are shown on the right side of the chromosome ideograms and losses on the left. Each line illustrates the affected region of the chromosome in a single tumor sample.

ed chromosomes 17 (4 cases), 20 (2 cases), and 9q34 (2 cases). Examples of such gains and DNA amplifications are shown in Fig. 2. Chromosomal loss was less common in the studied ependymomas, but was still found in 13q (26%), 6q (19%), and 3 (11%).

## DISCUSSION

In Previous CGH studies, the most commonly reported chromosomal changes in ependymomas were the gain of 1q and loss of 22q.<sup>7-11,18-20</sup> However, the incidence of these abnormalities in ependymomas was quite variable, i.e., a gain of 1q occurred in 0% to 50% of cases, and loss of 22q in 17% to 75% (Table 2). Heterogeneous gain and loss of variable chromosomes have also been reported, such as gains of 2, 6q, 7, 9, 12, 14q, 17, and 18<sup>3,8,9,11</sup> and losses of 1, 2, 3p, 6, 10, 13q, 16q, 19, 20q, and 21.<sup>18,21</sup> Constitutional translocation (1;22) (p22;q11.2) have also been reported in ependymomas.<sup>22</sup> (Table 2). Ward *et al.* reported that in recurrent ependymomas, gains of 7q and 9p and losses of 17 and 22 tended to be more common.<sup>11</sup>

We obtained DOP-PCR data in 27 out of a total of 31 cases of studied ependymomas, and 5 (18.5%) cases showed no chromosomal imbalance, which is consistent with the results of previous reports, i.e., 0% to 45%.<sup>8,11,13,19,22</sup> The chromosomal aberrations found in our 27 ependymomas were also observed in previous reports, but gains of 11q13 and 15q24-qter were new findings. No statistical differences were found regarding chromosomal imbalances between low- and high-grade ependymomas, between conventional and myxopapillary subtypes, or between adult and childhood ependymomas. This is partially due to the small number of high grade, myxopapillary and childhood ependymomas in our study.

In our studies no loss of 22q was detected, but the frequency of 1q gain was consistent with that of other reports. We thought

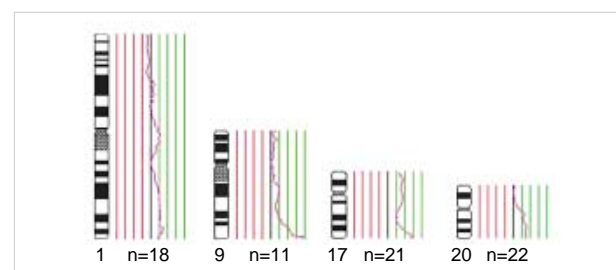


Fig. 2. Examples of the CGH profiles of chromosomal aberrations in ependymomas. Red and green lines represent thresholds for chromosomal losses (0.75) and gains (1.25), respectively. Gain of 1q31-qter, 9q21-qter, 17, and 20q were observed. \*n=number of evaluated chromosomes in each case.

**Table 2.** The most consistent cytogenetic abnormalities of ependymomas in the previous reports

References	22q (-)	1q (+)	Other gain	Other loss	No imbalance	Method
1 <sup>9</sup>	17/56 (30%)	10/56 (17%)	5q21, 7q, 9, 12, 18p	6q, 16, 17, 19, 20q		CGH
2 <sup>18</sup>	22/86 (26%)	+	-	6q, 10q/10, 13, 4q/14		CGH
3 <sup>8</sup>	15/20 (75%)	None (0%)	4q	1p, 2p, 3p, 6, 10q, 16p, 19, 21	0/20 (0%)	CGH
4 <sup>19</sup>	14/44 (35%)	7/24 (29%)	1q, 7, 9	6q, 9, 13	4/44 (9%)	CGH
5 <sup>21</sup>	9/26 (36%)	13/26 (50%)	9q, 17, 20q, 22q	2q, 4q, 6q, 10, 13q	1/26 (3.8%)	CGH & FISH
6 <sup>11</sup>	10/40 (25%)	8/40 (20%)	7q, 8q, 9p	17	18/40 (45%)	CGH
7 <sup>13</sup>	20/28 (71%)	None (0%)	4q, 5q, 7q, 9q, 12q	1p, 6, 16, 17, 19q, 20q	0/28 (0%)	CGH
8 <sup>20</sup>	4/23 (17%)	5/23 (22%)	9	6q, X	9/23 (39%)	CGH
Present study	0/27 (0%)	6/27 (22%)	7, 9q, 11q, 12q, 15q, 16q, 17, 18, 19, 20q	3, 6q, 13q	5/27 (19%)	CGH

No, Number of cases (chromosomal imbalance/total).

that there might be some factors affecting our CGH study. Firstly, case variance may have affected our results, we studied only 27 cases. The second factor could be specimen variance. Our study used paraffin-embedded tissue. Since the paraffin-embedded tissue is good for selection of pure neoplastic areas by microdissection, it has been widely used since 2000, after the introduction of DOP-PCR-CGH. However, it is true that fresh frozen tissue is much better for CGH studies, and if we had used fresh frozen tissue, a relatively small amount of DNA would have been enough for analysis, and we might have gained better results. The third factor might be primer variance, since the results may have been affected by the primer type. We used UNI primer, but there are various primers. The fourth factor is involved with discrimination of chromosome loss. Chromosome gain can be detected at 2Mb change, but loss of 10Mb is required for the detection of chromosome loss. Any contamination of normal cells might reduce chromosomal loss. All of these might have affected our CGH investigation.

Gains of 1q32-qter and 9q34 were a common finding in this study. 1q32 contains *RBBP5* (Retinoblastoma-binding protein 2, homolog 1A), *ELK4*, *SAP1* (ETS-domain protein, SRF accessory protein 1), and *GAC1* (Glioma amplification on chromosome 1)<sup>23</sup> (Fig. 2). Among the genes mapped to 9q34, *NOTCH1* is encoded, which is necessary to maintain the neoplastic phenotype in Ras-transformed human cells in vitro and in vivo.<sup>24</sup> Cultured ependymoma cell lines expressed potential oncological markers related to the immature state of tumoral cells (nestin and Notch-1).<sup>25</sup>

Over-representation of 17 and 20q were the most frequent findings in this study (Fig. 2). Chromosome band 17q21 contains several oncogenes, such as papillary serous carcinoma of the peritoneum (*PSCP*), Fibroblast growth factor-11 (*FGF11*), mitogen activating protein 3 kinase 14 (*MAP3K14*), *WNT3*, and *WNT15* (Wingless-type MMTV) (28-33). On 20q13, tumor

necrotic factor receptor superfamily 6B (*TNFRSF6B*) and *breast carcinoma amplified sequence 1* (BCAS1) are reported.<sup>26</sup>

11q13.2 is another frequently amplified band, which encodes Cyclin D1, *CCND1*, and *Mitogen-activated protein 3 kinase 11* (*MAP3K11*).<sup>27</sup> On 12q23, apoptotic protease-activating factor 1 (*APAF1*), and dual-specificity phosphatase-6 (*DUSP6*) were encoded.<sup>28</sup> On 15q24, BCL2-related protein A1 (*BCL2A1*), c-src tyrosine kinase (*CSK*), and cellular retinoic acid binding protein (*CRABP1*) were encoded.<sup>29</sup>

Among above-mentioned tumor-related genes, the cyclin D1<sup>30</sup> and mos (mitogen-activated protein kinase)<sup>31</sup> were studied in ependymomas. Cyclin D1 did not reliably correlate with clinical outcome or recurrence, but overexpression of mos significantly correlated with a biologically aggressive subgroup of ependymal tumors, suggesting their involvement in neoplastic progressions. However, most of the above listed tumor suppressor genes and oncogenes were not detected in ependymomas. The recurrent gains or losses of chromosomal regions identified in this study provide candidate regions that may be involved in the development and progression of ependymoma. We do not know the reason for absence of the loss of 22q in our studied ependymomas. Further studies in more Korean cases and with more specific molecular tools, such as FISH or LOH, are required.

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