Chromosomal Imbalances in Choroid Plexus Tumors

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We studied 49 choroid plexus tumors by comparative genomic hybridization. Chromosomal imbalances were found in 32 of 34 choroid plexus papillomas and 15 of 15 choroid plexus carcinomas. Choroid plexus papillomas frequently showed +7q (65%), +5q (62%), +7p (59%), +5p (56%), +9p (50%), +9q (41%), +12p, +12q (38%), and +8q (35%) as well as -10q (56%), -10p, and -22q (47%); choroid plexus carcinomas mainly showed +12p, +12q, +20p (60%), +1, +4q, +20q (53%), +4p (47%), +8q, +14q (40%), +7q, +9p, +21 (33%) as well as -22q (73%), -5q (40%), -5p, and -18q (33%). Several chromosomal imbalance differences could be found that were characteristic for a tumor entity or age group. In choroid plexus papillomas +5q, +6q, +7q, +9q, +15q, +18q, and -21q were significantly more common whereas choroid plexus carcinomas were characterized by +1, +4q, +10, +14q, +20q, +21q, -5q, -9p, -11, -15q, and -18q. Among choroid plexus papillomas, children more often showed +8q, +14q, +12, and +20q; adults mainly presented with +5q, +6q, +15q, +18q, and -22q. Although the number of aberrations overall as well as of gains and losses on their own bore no significance on survival among choroid plexus tumors, a significantly longer survival among patients with choroid plexus carcinomas was associated with +9p and -10q. Our results show that aberrations differ between choroid plexus papillomas and choroid plexus carcinomas as well as between pediatric and adult choroid plexus papillomas, supporting the notion of different genetic pathways. Furthermore, gain of 9p and loss of 10q seem to be correlated with a more favorable prognosis in choroid plexus carcinomas. (*Am J Pathol* 2002; 160:1105-1113)

Choroid plexus tumors are intraventricular papillary neoplasms derived from choroid plexus epithelium. These rare tumors account for only 0.4 to 0.6% of all intracranial neoplasms¹ and occur most often in childhood in which they constitute 2 to 3% of tumors in children younger than 15 years of age,² 4.3% in children younger than 4-years-old, and 13.1% in the first year of life.³ Investigations on

genetic properties of choroid plexus tumors have so far only been undertaken on ~20 specimens, mainly in the setting of case reports⁴⁻¹² or as singular cases in larger series of brain tumors.¹³⁻¹⁷ So far, only one series of eight choroid plexus papillomas investigated by fluorescence *in situ* hybridization has been reported.¹⁸

Comparative genomic hybridization (CGH) is able to identify chromosomal gains and losses along the entire genome. Its main advantage is that it bypasses the need for laborious cell culture to harvest metaphase spreads, and that it can be applied to archival material. No CGH study has hitherto been undertaken on choroid plexus papillomas and carcinomas. To screen for DNA copy number changes that may be involved in tumorigenesis of choroid plexus tumors, we performed a CGH analysis on 34 choroid plexus papillomas and 15 choroid plexus carcinomas.

Materials and Methods

Patients and Tumors

Formalin-fixed and paraffin-embedded biopsy specimens of 49 primary choroid plexus tumors (26 males, 23 females; mean age, 22.7 years; range, 2 months to 73 years) were investigated (Table 1). These consisted of 34 choroid plexus papillomas World Health Organization (WHO) grade I (19 males, 15 females; mean age, 29.9 years; range, 2 months to 73 years) and 15 choroid plexus carcinomas WHO grade III (7 males, 8 females; mean age, 6.2 years; range, 5 months to 28 years). Of these, 22 tumors were located in the lateral ventricles (12 choroid plexus papillomas, 10 choroid plexus carcinomas), 5 in the third ventricle (3 choroid plexus papillomas, 2 choroid plexus carcinomas), and 22 in the fourth ventricle (19 choroid plexus papillomas, 3 choroid plexus carcinomas). All patients underwent primary surgery. A combination of postoperative radiation therapy plus chemotherapy was applied in 5 patients, whereas 3 patients underwent radiation therapy and 7 patients chemotherapy alone; 34 did not receive any adjuvant therapy (Table 1). Clinical follow-up data were available for all 49 patients.

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Table 1. Summary of Clinical Data

Case	Age	Sex	MIB-1	Location	AdjTh	OP	Clinical course	Survival
Choroid plexus papillomas (WHO grade I)								
1	2 m	F	4.3	Lateral ventricle	—	T	Free of disease	>24
2	4 m	M	30.6	Left ventricle	—	T	Recurrence (6 months)	>14
3	7 m	F	3.9	Right ventricle	—	T	Free of disease	>57
4	9 m	F	15.4	Lateral ventricle	—	T	Free of disease	>14
5	11 m	F	16.3	Lateral ventricle	—	T	Free of disease	>70
6	12 m	F	11.1	Lateral ventricle	—	T	Free of disease	>12
7	23 m	F	2.1	Left ventricle	—	T	Free of disease	>20
8	11	M	2.3	4th ventricle	—	T	Free of disease	>42
9	11	M	3.5	4th ventricle	—	ST	Stable disease	>37
10	12	F	0.5	Left ventricle	—	T	Free of disease	>20
11	12	M	0.6	Lateral ventricle	—	T	Free of disease	>97
12	12	M	1.3	Lateral ventricle	—	T	Free of disease	>124
13	23	F	8.6	4th ventricle	—	T	Free of disease	>36
14	25	M	3.6	3rd ventricle	R	ST	Recurrences (48/84 months)	>89
15	26	M	6.7	4th ventricle	R/C	ST	Recurrences (46/71 months)	>72
16	27	F	1.4	4th ventricle	—	T	Free of disease	>7
17	29	M	0.6	4th ventricle	—	T	Free of disease	>44
18	29	M	13.0	3rd ventricle	R	P	Recurrence (12 months)	21
19	31	M	0.5	4th ventricle	—	T	Free of disease	>20
20	34	F	0.8	4th ventricle	—	T	Free of disease	>29
21	36	M	13.2	4th ventricle	—	ST	Recurrences (38/86 months)	132
22	37	M	1.3	4th ventricle	—	T	Free of disease	>36
23	38	M	7.5	4th ventricle	—	T	Free of disease	>24
24	39	M	3.9	4th ventricle	—	T	Free of disease	>42
25	44	M	0.2	Left ventricle	—	T	Free of disease	>69
26	44	M	3.6	4th ventricle	—	T	Free of disease	>50
27	46	F	3.9	4th ventricle	—	T	Free of disease	>17
28	56	M	0.6	3rd ventricle	—	T	Free of disease	>32
29	58	F	7.9	Lateral ventricle	—	T	Recurrence (132 months)	>132
30	59	M	5.2	4th ventricle	—	T	Free of disease	>36
31	62	M	3.8	4th ventricle	—	ST	Stable disease	>48
32	66	F	0.2	4th ventricle	—	P	Stable disease	>25
33	71	F	0.8	4th ventricle	—	T	Free of disease	>127
34	73	F	1.2	4th ventricle	—	T	Free of disease	>43
Choroid plexus carcinomas (WHO grade III)								
35	5 m	M	72.2	Lateral ventricle	C	ST	Recurrences (3/7 months)	>57
36	9 m	F	30.4	Right ventricle	C	ST	Recurrence (4 months)	>4
37	11 m	F	36.5	Right ventricle	C	T	Recurrence (12 months)	13
38	11 m	M	64.8	Lateral ventricle	—	P	Progress	5
39	12 m	F	34.0	4th ventricle	C	T	Recurrence (8 months)	>20
40	20 m	F	59.7	Lateral ventricle	—	ST	Progress	7
41	29 m	F	49.6	Right ventricle	C	T	Recurrences (50/53 months)	>75
42	39 m	M	10.0	Right ventricle	R/C	T	Free of disease	>17
43	39 m	M	21.5	Left ventricle	C	T	Recurrence (14 months)	22
44	8	M	8.6	Right ventricle	R/C	ST	Stable disease	>72
45	9	M	16.3	Left ventricle	—	ST	Recurrence (10 months)	32
46	10	F	20.8	3rd ventricle	R/C	ST	Recurrence (5 months)	>6
47	10	F	23.3	4th ventricle	R/C	ST	Stable disease	>12
48	14	M	15.4	3rd ventricle	C	T	Recurrence (54 months)	>54
49	28	F	6.0	4th ventricle	R	T	Free of disease	>36

Age: in years; m, months.

M, male; F, female; MIB-1, proliferation index in per cent; AdjTh, adjuvant therapy after tumor resection; R, radiotherapy; C, chemotherapy; OP, operation; T, total; ST, subtotal; P, partial.

Survival: survival in months, >: still alive at time of reporting.

Histological criteria for classifying a tumor as choroid plexus papilloma or carcinoma were those of the WHO classification.¹ Briefly, plexus papillomas are characterized by fibrovascular connective tissue fronds covered by a single layer of uniform cuboidal to columnar epithelial cells without conspicuous mitotic activity, brain invasion, and necroses, whereas plexus carcinomas show frank signs of malignancy with nuclear pleomorphism, frequent mitoses, blurring of the papillary pattern with poorly structured sheets of tumor cells, necrotic areas,

and often diffuse brain invasion. Routine hematoxylin and eosin staining and immunohistochemistry using an avidin-biotin complex technique and antibodies against glial fibrillary acid protein, cytokeratins, S-100 protein, vimentin, prealbumin/transferrin, neurofilament, neuron-specific enolase, synaptophysin, epithelial membrane antigen, and smooth-muscle actin as well as the proliferation antigen Ki-67 were performed. Diagnoses were made by two neuropathologists (CHR, WP). In addition, the majority of tumors, in particular the choroid plexus carcinomas,

had been seen and diagnosed by the German Brain Tumor Reference Center (OD Wiestler, Bonn, Germany). Statistical analysis was performed using Student's *t*-test, Fisher's exact test, Kaplan-Meier estimates of survival probabilities and log-rank test where applicable.

CGH Analysis

DNA was isolated by phenol-chloroform extraction according to standard protocols. With minor modifications, CGH analysis was performed as described previously.¹⁹ Briefly, tumor DNA was labeled with biotin-16-dUTP (Boehringer Mannheim, Mannheim, Germany) and reference DNA from a healthy male donor with digoxigenin-11-dUTP (Boehringer Mannheim) in a standard nick translation reaction. The DNase concentration in the labeling reaction was adjusted to reveal an average fragment size of 200 to 500 bp. Labeled DNA fragments were purified from remaining nucleotides by column chromatography.

For CGH, 500 ng of tumor DNA, 300 ng of reference DNA, and 30 μ g of human Cot1 DNA (Gibco, Karlsruhe, Germany) were co-precipitated and redissolved in 10 μ l of hybridization buffer. Denaturation of DNA (75°C for 5 minutes) was followed by a preannealing step of 45 minutes at 37°C. Target metaphase spreads (46,XY), which had been prepared following standard procedures, were denatured separately in 70% formamide/2 $\times$ standard saline citrate for 2 minutes at 72°C. Hybridization was allowed to proceed for 3 to 4 days. Posthybridization washes were performed at a stringency of 50% formamide/2 $\times$ standard saline citrate at 45°C and 0.1 $\times$ standard saline citrate at 60°C. Biotinylated and digoxigenated sequences were detected simultaneously, using avidin-fluorescein isothiocyanate (1:200, Boehringer Mannheim) and anti-digoxigenin-rhodamine (1:40, Boehringer Mannheim). The slides were counterstained with 4,6-diamidino-2-phenylindole and mounted in an anti-fade solution (Vectashield, Vector Laboratories, Birmingham, AL, USA).

Microscopy and Digital Image Analysis

Separate digitized gray level images of 4,6-diamidino-2-phenylindole, fluorescein isothiocyanate, and rhodamine fluorescence were taken with a charge-coupled device camera connected to a Leica DMRBE microscope. Image processing was performed using the Applied Imaging Software. Average green-red ratios were calculated for each chromosome in at least 10 metaphases.

Chromosomal regions with CGH ratio profiles surpassing the 50% CGH thresholds (upper threshold, 1.25; lower threshold, 0.75) were defined as loci with copy number gains or losses. Based on experiments with normal control DNA, these thresholds have been shown to eliminate false-positive results. These values have been used in several studies comparing CGH data with results obtained by other cytogenetic methods and have proven to provide robust criteria for the diagnosis of chromosomal gains and losses. Overrepresentations were diag-

nosed as high-level gains when the fluorescence intensity levels exceeded 1.5 or when the fluorescein isothiocyanate fluorescence showed strong focal signals. For the assignment of these high-level gains to chromosomal bands, the signal intensities were compared to the 4,6-diamidino-2-phenylindole banding on individual chromosomes. As tumor specimens and normal DNA were not sex matched, X and Y chromosomes were excluded. Also excluded were centromeric and satellite regions of the acrocentric chromosomes and chromosome 19 because of the abundance of highly repetitive DNA sequences as well as the frequent occurrence of false-positive CGH results as shown by interphase fluorescence *in situ* hybridization using suitable DNA probes.

Results

CGH revealed DNA copy number changes in 32 of 34 choroid plexus papillomas and 15 of 15 choroid plexus carcinomas; six patients showed either only gains or only losses (Table 2). Every chromosome was shown to carry imbalances (Figure 1). The highest number of DNA copy number changes was detected in a choroid plexus carcinoma (case 41, 26 imbalances), the lowest in two choroid plexus papillomas (cases 2 and 18, none) (Table 2). Choroid plexus papillomas showed an average of 7.3 chromosomal changes per tumor (range, 0 to 14; 4.6 gains *versus* 2.7 losses), choroid plexus carcinomas an average of 10.7 imbalances per tumor (not significant; range, 1 to 26; 6.4 gains *versus* 4.3 losses) (Table 1, Figure 1). Choroid plexus papillomas exhibited significantly fewer high-level gains per tumor compared to choroid plexus carcinomas (0.5 *versus* 2.9, $P < 0.05$) (Table 1, Figure 1). Gains were more common than losses in both entities (not significant).

The most common DNA copy number changes overall were gains of 7q (55%), 5q, 7p (47%), 9p, 12p, 12q (45%), 5p (43%), 8q (37%), 9q, 20p, and 20q (33%), as well as of losses of 22q (55%), 10q (47%), and 10p (37%). Choroid plexus papillomas frequently showed +7q (65%), +5q (62%), +7p (59%), +5p (56%), +9p (50%), +9q (41%), +12p, +12q (38%), and +8q (35%) as well as -10q (56%), -10p, and -22q (47%); choroid plexus carcinomas mainly showed +12p, +12q, +20p (60%), +1, +4q, +20q (53%), +4p (47%), +8q, +14q (40%), +7q, +9p, +21 (33%), as well as -22q (73%), -5q (40%), -5p, and -18q (33%). Minimal common regions found in at least five plexus tumors of each entity mapped to +7q21-31.2 (22 tumors), +5q11.2-22 (21 tumors), +7p21-pter (20 tumors), -10q25-qter (19 tumors), +5p12-14 (19 tumors), -22q13 (16 tumors), +9q12-22 (14 tumors), +12q12-15 (13 tumors), +8q13-22 (12 tumors), +15q22-qter (10 tumors), -3p22-pter (6 tumors) in choroid plexus papillomas; and to -22q13 (11 tumors), +12q12-22 (9 tumors), +1p31-pter (8 tumors), +4q12-26 (8 tumors), -5q11.2-32 (6 tumors), +8q21.1-22 (6 tumors), +14q11.2-23 (6 tumors), +21q22 (5 tumors), +7q11.2-32 (5 tumors) in choroid plexus carcinomas. In choroid plexus papillomas high-level gains were located on chromosomes 7 (5 cases), 9

Table 2. Summary of CGH Findings

Case		Gains (+, respective top lines) and losses (–, respective bottom lines)
Choroid plexus papillomas (WHO grade I)		
1	+	2, 12, 13q, 20
	–	1, 3, 4, 5, 9, 10, 22q
2	+	None
	–	None
3	+	7, 8, 12
	–	10
4	+	8, 12, 13q, 14q, 15q, 20
	–	1, 3, 4, 6, 10
5	+	2, 7, 9, 12, 14q31-qter, 20
	–	3, 6, 10
6	+	7q11.2-31, 8q11.2-22, 12q12-21
	–	1p, 1q42-qter, 3p22-pter, 3q, 4q32-qter, 5p15.1-pter, 5q, 6q22.2-qter, 10, 13q31-qter
7	+	4q12-32, 7, 8, 12p, 12q12-15
	–	10, 17, 22q
8	+	5, 7, 9, 11, 12
	–	21q
9	+	5p12-14, 5q11.2-23, 7, 8q11.2-24.1, 9, 12p, 12q12-22
	–	16, 17p, 20q, 21q, 22q
10	+	5, 7, 8, 9, 12, 14q31-qter, 15q, 18, 20
	–	3, 10, 13q
11	+	5p12-14, 5q11.2-23, 7, 8, 9, 12p, 12q12-22, 18
	–	21q
12	+	7
	–	None
13	+	7, 15q, 17q24-qter, 18, 20q13.1-qter, 22q13
	–	2, 10
14	+	7, 13q
	–	3, 10
15	+	5, 15q22-qter
	–	None
16	+	6, 9, 12
	–	10, 13q, 14q, 15q, 18, 21q, 22q
17	+	5, 9, 13q, 18
	–	2, 10q, 22q
18	+	None
	–	None
19	+	5 (p+q11.2-23), 8
	–	10, 11q, 12q23-qter, 22q12-qter
20	+	4, 5p, 5q11.2-23, 7, 20
	–	22q
21	+	5, 7, 9, 15q, 18
	–	21q, 22q12-qter
22	+	5p12-14, 5q11.2-23, 6, 7p, 7q11.2-31, 8q11.2-22.9p, 9q12-22, 15q, 18p
	–	12q24.1-qter, 16, 17, 20
23	+	4, 5p12-14, 5q11.2-23, 7p, 7q11.2-31.2, 15q
	–	22q
24	+	5, 9p, 9q12-31, 15q, 18
	–	10, 14q, 21q, 22q
25	+	7, 9, 12p, 12q12-22
	–	None
26	+	5, 7p, 17, 20
	–	21q
27	+	5p12-14, 5q11.2-23, 6q13-24, 7p21-pter, 7q21-31, 9p, 12p, 15q
	–	10, 13q, 18, 21q
28	+	4, 5p12-14, 5q11.2-23, 7q11.2-31, 13q, 15q, 18
	–	10
29	+	5p, 5q11.2-21, 6q13-24, 8q13-22
	–	2, 10q, 16, 17, 22q
30	+	5q, 7, 9, 18
	–	1, 6, 10, 16, 22q
31	+	3, 5, 7q, 9, 20
	–	10, 22q12-qter

Table 2. (Continued)

Case		Gains (+, respective top lines) and losses (–, respective bottom lines)
32	+	5q11.2-23, 6q12-24, 7, 8q11.2-24.1, 9p, 12p, 12q12-22
	–	10q25-qter, 17, 22q
33	+	5p12-14, 5q11.2-23, 7, 8, 9
	–	22q12-qter
34	+	5p12-14, 5q11.2-23, 9p
	–	17, 22q13
Choroid plexus carcinomas (WHO grade III)		
35	+	1p12-31, 1q, 2, 5, 7, 10, 12 (p+q12-21), 13q, 14, 18, 20
	–	3, 4, 6, 8, 9, 11, 15, 16, 17, 22q
36	+	1, 4, 20p, 21q
	–	22q13
37	+	None
	–	4, 22q12-qter
38	+	1, 2p, 3q25-qter, 10, 12, 14q, 18, 20, 21q
	–	3p, 4q, 5, 6, 7, 8q, 13q21-qter
39	+	None
	–	22q12-qter
40	+	1, 2p (22-pter), 2q (24-31), 4q, 7 (q11.2-32), 10p, 12, 14q, 20q
	–	5, 6, 8, 9, 11, 15q, 16, 17p, 18, 22q
41	+	1, 2p (22-pter), 2q (24-31), 3p22-pter, 4p, 7q, 8q (21.1-qter), 10, 12p, 12q12-23, 14q11.2-23, 15q22-qter, 20p, 20q, 21q22
	–	3q, 5, 6, 7p, 9, 11, 13q, 16, 17, 18, 22q
42	+	1, 4, 7q11.2-32, 9, 20, 21q
	–	5, 15q, 17, 18
43	+	1, 5p, 8q, 10q23-qter, 12, 14q, 20
	–	5q11.2-32, 9p, 18q, 22q
44	+	4q12-28, 5q11.2-23, 7, 8q11.2-22, 9p
	–	2, 3, 10, 15q, 18q
45	+	1p31-pter, 1q (21-25), 4p, 4q12-26, 8, 12, 14q, 17q, 20, 21q
	–	2, 3, 4q31.1-qter, 5, 11
46	+	4, 8q11.2-24.1, 9p, 12p, 12q12-23
	–	10, 22q
47	+	None
	–	22q12-qter
48	+	4, 8, 9, 11, 12p, 12q12-22, 17, 20
	–	10, 13q, 22q
49	+	4, 6p, 8, 9p, 11, 12, 20p
	–	10, 22q

Underlined: high level gains.

(4 cases), 15q (3 cases), 5, 8 (2 cases), 12, and 20 (1 case); in carcinomas on 20q (6 cases), 1 (5 cases), 2p, 12, 20p (4 cases), 2q, 14q (3 cases) 4q, 8, 10q, 18, 21q (2 cases), 3q, 4p, 5p, 7q, 9p, 10p, 13q, and 17p (1 case).

Comparing the distribution and frequency of specific chromosomal imbalances between choroid plexus papillomas and carcinomas, and between pediatric and adult papillomas to establish chromosomal imbalances characteristic for a tumor entity or age group, the following significant differences could be found (relative frequencies and *P* value are in parentheses): 1) In choroid plexus papillomas, +5q (62% versus 7%, *P* < 0.0005), +6q (15% versus 0%, *P* < 0.05), +7q (65% versus 33%, *P* < 0.03), +9q (41% versus 13%, *P* < 0.03), +15q (29% versus 7%, *P* < 0.05), +18q (24% versus 13%, *P* < 0.05), and –21q (24% versus 0%, *P* < 0.005) were significantly more common whereas choroid plexus carcinomas were characterized by +1 (53% versus 0%, *P* < 0.0005), +4q

Figure 1. CGH ideogram summarizing gains and losses of DNA sequences in choroid plexus papillomas (**A**) and choroid plexus carcinomas (**B**). Pediatric cases are marked in gray, adult cases in black. Gains are shown as bars on the right of the chromosome ideogram and losses on the left. High-level gains are marked as thick lines. Each vertical line represents the affected chromosomal region seen in a single tumor specimen. For clarity, in **A** not all changes have been drawn for chromosomes 7, 9, and 10. Figures underneath give frequency of respective imbalances.

(53% versus 12%, $P < 0.003$), +10 (33% versus 0%, $P < 0.005$), +14q (40% versus 9%, $P < 0.005$), +20q (53% versus 24%, $P < 0.003$), +21q (33% versus 0%, $P < 0.005$), -5q (40% versus 6%, $P < 0.005$), -9p (27% versus 3%, $P < 0.01$), -11 (27% versus 3%, $P < 0.01$), -15q (27% versus 3%, $P < 0.01$), and -18q (33% versus 3%, $P < 0.005$). 2) Among choroid plexus papillomas, children (patients younger than 18 years of age) significantly more often showed +8q (58% versus 23%, $P < 0.001$), +14q (25% versus 0%, $P < 0.01$), +12 (83% versus 18%, $P < 0.0005$), and +20q (33% versus 18%, $P < 0.01$) whereas adults mainly presented with +5q (77% versus 33%, $P < 0.005$), +6q (23% versus 0%, $P < 0.01$), +15q (36% versus 17%, $P < 0.005$), +18q (27% versus 17%, $P < 0.01$), and -22q (59% versus 25%, $P < 0.001$).

The mean MIB-1 proliferation indices were significantly different between choroid plexus papillomas (mean, 5.3%; SD, 6.4%) and choroid plexus carcinomas (mean, 31.3%; SD, 21.3%; $P < 0.0005$). Among the choroid plexus carcinomas, the mean MIB-1 proliferation indices

were significantly higher in patients younger than 3 years of age (mean, 49.6%) compared to the age group older than 3 years (mean, 15.2%; $P < 0.001$). Furthermore, the mean ages of patients were significantly different between choroid plexus papillomas and choroid plexus carcinomas (mean, 29.9 years versus 6.2 years; $P < 0.000005$). As to the tumor site, there was prevalence for the fourth ventricle among choroid plexus papillomas (56% versus 20% in carcinomas) and for the lateral ventricles among choroid plexus carcinomas (67% versus 35% in papillomas). Among choroid plexus papillomas, tumor location was age-dependent: the lateral ventricles were significantly more often involved in younger patients (mean, 12.0 years) compared with the fourth ventricle (mean, 40.2 years; $P < 0.0005$). Additionally, there was a female prevalence for the lateral (8 versus 4 patients) and a male prevalence for the fourth ventricle (12 versus 7) among choroid plexus papillomas whereas the opposite was the case for choroid plexus carcinomas (6 versus 4 and 0 versus 3, respectively). MIB-1 proliferation indices were higher, albeit not significantly, among lateral ventricle tumors compared with fourth ventricle tumors, regardless of tumor entity (choroid plexus papillomas, 7.9% versus 3.6%; choroid plexus carcinomas, 37.0% versus 21.1%, respectively). Among all plexus tumors, patient age increased and proliferation decreased with more caudal location: 7.9 years and 21.1% in the lateral ventricles, 26.8 years and 10.7% in the third ventricle, and 36.5 years and 6.0% in the fourth ventricle.

Clinical follow-up data were available for all 49 patients. At the time of reporting, 2 of 34 patients with choroid plexus papillomas (5.9%) and 5 of 15 patients with choroid plexus carcinomas had died of their disease (33.0%). The respective 1-, 3-, and 5-year survival rates were 100%, 95%, and 90% for choroid plexus papillomas and 85%, 44%, and 28% for choroid plexus carcinomas. Although the number of aberrations overall as well as of gains and losses bore no significance on survival among choroid plexus tumors, a significantly longer survival among patients with choroid plexus carcinomas was associated with +9p and -10q: comparing the five patients who had succumbed to their disease with the five long-term survivors (>24 months), Fisher's exact test for small sample sizes revealed the significance level to be $P = 0.048$ for each of the above aberrations, whereas Kaplan-Meier estimates of survival probabilities and consecutive log-rank tests calculated the significance level to be $P = 0.0186$ each. Furthermore, deceased carcinoma patients tended to be younger at tumor diagnosis than long-term survivors (3.2 years versus 10.6 years, not significant) and their tumors showed higher MIB1 proliferation indices (39.8% versus 30.4%, not significant).

Discussion

Molecular genetic data about choroid plexus tumors are scarce. Only ~20 papillomas and carcinomas have so far been investigated.⁴⁻¹⁸ Some of these occurred in the setting of Li-Fraumeni syndrome or neurofibromatosis type 2 as well as in patients suffering from Aicardi's

syndrome, Down's syndrome, and von Hippel-Lindau disease.²⁰

Molecular Genetics

Previous cytogenetic studies reported changes predominantly consisting of hyperploidy and gains of chromosomal material^{5,7-9,16,18} whereas losses were less frequently encountered.^{4,12,15} Singular cases of choroid plexus papillomas published in the past either showed no changes,^{13,16} as in our case 2 and case 18, or gains on chromosomes 7, 12, 15, 17, and 18.^{5,6,18} In our series, gains of 7q and 7p were the most and third most common imbalances among papillomas, occurring in 65% and 59% of cases, respectively, whereas gains on chromosomes 12, 15, 17, and 18 were rarer and only accounted for 38%, 29%, 6%, and 24% of cases, respectively (Table 2, Figure 1a). An association with duplication of 9p has previously been reported in one carcinoma and one papilloma⁷ whereas +9p was present in 50% of papillomas and 33% of carcinomas in our cohort. Furthermore, losses of chromosome 22¹⁵ and 3⁴ have been documented in one choroid plexus papilloma each; whereas the former was seen in 47%, the latter in just 18% of our series. Interestingly, the papilloma with loss of chromosome 3 was reportedly associated with von Hippel-Lindau disease⁴ that is characterized by a mutation of the VHL tumor suppressor gene on 3p25-26. This region maps to the minimal common region of -3p22-pter that was found in 6 of 34 choroid plexus papillomas in our study. However, no history of this disease has been reported among our cases. Furthermore, previous investigations of choroid plexus carcinomas showed them to have gains of chromosomes 7, 12, and 20,¹⁶ in particular 12p and 20q,⁸ that were encountered in our study in 33% (7q), 60% (12p, 12q, and 20p), and 53% (20q), the latter imbalance being particularly frequent. Additionally, loss of heterozygosity of chromosome 22q11 secondary to a germ line mutation of the tumor suppressor gene hSNF5/INI1, which has also been linked to the development of CNS atypical teratoid/rhabdoid tumors²¹ and which may define a new hereditary syndrome predisposing to a variety of tumors,²² was reported in one family with plexus carcinomas and one sporadic carcinoma.^{12,17} In our study, loss of material from chromosome 22q was by far the most common change found in 73% of choroid plexus carcinomas and 47% of choroid plexus papillomas. Loss of material on chromosome 22q has also been encountered frequently in ependymomas,²² meningiomas,²³ neurinomas,²⁴ atypical teratoid/rhabdoid tumors,²¹ pediatric high-grade astrocytomas,¹⁹ and pineoblastomas.²⁵ Furthermore, three of four familial cases of choroid plexus tumors in the setting of Li-Fraumeni syndrome have been documented to carry a TP53 germline mutation in codon 248^{11,26} whereas no TP53 mutations were reported in sporadic cases.²⁷ In our study, chromosome 17 was affected in a significant minority of tumors: losses on 17p were encountered in 4 of 15 carcinomas (27%) and 6 of 34 papillomas (18%), thus indicating a potentially interesting involvement of TP53.

CGH in Plexus Papillomas Versus Plexus Carcinomas

Apart from these previously recognized DNA copy number changes, we found numerous novel and nonrandom chromosomal imbalances, in particular frequent gains of 5q (62%), 5p (56%), 9q (41%), and 8q (35%) and losses of 10q (56%) and 10p (47%) among choroid plexus papillomas, as well as gains of 1, 4q (53%), 4p (47%), 8q, 14q (40%), and 21q (33%), and losses of 5q (40%), 5p, and 18q (33%) among choroid plexus carcinomas. Target oncogenes and tumor suppressor genes potentially involved in the tumorigenesis of choroid plexus tumors and mapping within the minimal common regions found in our investigation are numerous and listed below (Table 3). The only gene so far demonstrated in connection with choroid plexus tumors is the tumor suppressor gene hSNF5/INI1 that in two recent studies showed loss of heterozygosity in familial as well as one sporadic carcinoma.^{12,17} The product of the gene is a component of the SW/SNF complex that seems to facilitate transcription factor access.²² The occasional development of choroid plexus neoplasms in hereditary tumor syndromes such as Down's, neurofibromatosis type 2, and von Hippel-Lindau disease²⁰ may point toward an involvement of chromosome 21 as well as the NF2 and VHL tumor suppressor genes on 22q12 and 3p25-26, respectively. However, no unequivocal demonstration of deletions or mutations of the respective genes has been presented. Another interesting chromosomal region is 10q where losses have been demonstrated by CGH in pediatric high-grade astrocytomas,¹⁹ meningiomas,²³ and ependymomas,²⁸ and where the tumor suppressor genes PTEN and MXI1 as well as DMBT1 are located. These genes have previously been implicated in a number of central nervous system malignancies.²⁹⁻³¹ Furthermore, gains of 9q and 20q have frequently been encountered in ependymomas²⁸ and atypical meningiomas,²³ of which the latter also showed gains of 12q in 43% of cases whereas gains of 12p were frequently found in cerebral germ cell tumors.³² Regarding a link with chromosome 21, 33% of choroid plexus carcinomas but none of the choroid plexus papillomas in our series showed +21 among our patients without clinical evidence of an underlying Down syndrome. Thus, the majority of the genes mapping to the minimal common regions found by us has hitherto not been linked to or implicated in the development of choroid plexus tumors and will have to be investigated in future studies.

When comparing the distribution and frequency of specific chromosomal imbalances between choroid plexus papillomas and carcinomas to establish chromosomal imbalances that are characteristic for either tumor entity, significant differences could be observed. In choroid plexus papillomas, gains of 5q (62% *versus* 7%), 6q (15% *versus* 0%), 7q (65% *versus* 33%), 9q (41% *versus* 13%), and 15q (29% *versus* 7%), as well as loss of 21q (24% *versus* 0%) were more common than in choroid plexus carcinomas. The latter, on the other hand, typically showed gains of chromosome 1 (53% *versus* 0%), 4q (53% *versus* 12%), 10 (33% *versus* 0%), 14q (40%

Table 3. Target Oncogenes and Tumor Suppressor Genes Potentially Involved in the Pathogenesis of Choroid Plexus Tumors

Chromosomal region	Gene [function]
Choroid plexus papillomas	
-3p22-pter	VHL [tumor suppressor gene]
+5p12-14	TRE [transcription factor]
+5q11.2-22	FER [tyrosine kinase], NPM, IL3 [oncogenes], IRF1 [transcription factor]
+7p21-pter	EGFR [tyrosine kinase], ETV1 [oncogene]
+7q21-31.2	MET [tyrosine kinase], WNT2 [growth factor]
+8q13-22	MOS, LYN [tyrosine kinases], MYBL1, MYC [transcription factors], ETO/MTG8 [oncogene]
+9q12-22	AF9 [oncogene], TRKB [tyrosine kinase]
-10q25-qter	MXI1 [tumor suppressor gene]
+12q12-15	CDK4 [cell cycle], CHOP [oncogene], HER3 [tyrosine kinase], WNT1 [growth factor], GLI1 [transcription factor]
+15q22-qter	FPS/FES [tyrosine kinase]
-22q	NF2, BAM22, hSNF5/INI1 [tumor suppressor genes]
Choroid plexus carcinomas	
+1p31-pter	AF1P, PAX7, BLYM [oncogenes], MYCL1, TAL1, E2F2, JUN [transcription factors], FGR and LCK [tyrosine kinase]
+4q12-26	KIT [tyrosine kinase], AF4, IL2 [oncogenes]
-5q11.2-32	MSH3, MCC, APC [tumor suppressor genes]
+7q11.2-32	MET [tyrosine kinase], WNT2 [growth factor]
+8q21.1-22	LYN [tyrosine kinase], MYBL1, MYC [transcription factors], ETO/MTG8 [oncogene]
+12q12-22	CDK4 [cell cycle], CHOP, BTG1 [oncogenes], HER3, ELK3 [tyrosine kinases], WNT1 [growth factor], GLI1 [transcription factor]
+14q11.2-23	FKH2/QIN, FOS [transcription factors]
+21q22	AML1, ERG [oncogenes], ETS2 [transcription factor]
-22q	NF2, BAM22, hSNF5/INI1 [tumor suppressor genes]

versus 9%), 20q (53% versus 24%), and 21q (33% versus 0%) as well as losses of 5q (40% versus 6%) and 18q (33% versus 3%). Additionally, we also noticed distinct differences between choroid plexus papillomas occurring in children and adults. Papillomas in children significantly more often showed gains of chromosome 12 (83% versus 18%) whereas adult tumors mainly presented with gains of 5q (77% versus 33%) and 6q (23% versus 0%). Somewhat surprising for this low-grade tumor is the high number of chromosomal imbalances found in choroid plexus papillomas (mean, 7.3 per case). Previous CGH data of low-grade cerebral neoplasms found pineocytomas, pilocytic astrocytomas, meningiomas of WHO grade I, oligodendrogliomas of WHO grade II and astrocytomas of WHO grade II to show 0,²⁵ 0.3,³³ 2.9,²³ 3.8,³⁴ and 4.0 imbalances,³⁵ respectively. Among ependymomas, however, the myxopapillary variant (WHO grade I) demonstrated 9.1 aberrations per tumor compared with 5.3 and 1.8 in WHO grades II and III, respectively.²⁸ Thus, low-grade tumors can harbor a substantial number of DNA copy number changes; at the same time, it has to be kept in mind that CGH cannot detect balanced alterations that may occur in some tumors.

Tumor Proliferation and Prognosis

In accordance with data from a previous study of 14 papillomas and 5 carcinomas,³⁶ the mean MIB-1 proliferation indices in our cohort were significantly higher in choroid plexus carcinomas than in papillomas (31.3% versus 5.3%; $P < 0.0005$). However, whereas Vajtai and colleagues³⁶ found no correlation between patient age and tumor proliferation, we demonstrate a significantly higher, albeit not prognosis-associated proliferation index in choroid plexus carcinoma patients younger than 3

years of age compared with older than 3 years old (49.6% versus 15.2%; $P < 0.001$). Furthermore, in contrast to a previous study,³⁷ no correlation between proliferation and risk of recurrence could be found for either tumor entity. Choroid plexus papillomas are known to have a survival rate of close to 100% whereas carcinomas tend to present with a less favorable outcome resulting in 5-year survival rates of between 26 to 40%;^{38,39} this is corroborated in our cohort by figures of 90% and 28%, respectively. Gross total resection seems to be the treatment of choice for choroid plexus carcinomas and is a relevant prognostic factor with patients undergoing total or gross total resection showing a 5-year survival rate of 86%.³⁸ Among our carcinoma patients, five of the seven patients whose tumors had been removed totally according to the neurosurgeons were still alive after between 17 and 75 months; however, five patients had also suffered from recurrences despite total removal and additional adjuvant therapy in all cases (Table 1). Brain invasion may determine the poor clinical outcome in some of these patients.⁴⁰ Surprisingly, two of our patients died of their choroid plexus papillomas (cases 18 and 21, Table 1) of which both had unusually high proliferation indices. However, in both instances, the tumor could not be completely removed and recurred emphasizing the prognostic importance of total surgical removal even among WHO grade I tumors. Although the number of aberrations overall as well as of gains and losses bore no significance on survival among choroid plexus tumors, a significantly longer survival among patients with choroid plexus carcinomas was associated with +9p and -10q; no such correlation could be found for choroid plexus papillomas. However, it has to be kept in mind that the former group consisted of only 15 patients who underwent a variable extent of tumor removal and adjuvant

therapy, whose status of recurrent disease could not always be established clearly and whose follow-up time was relatively short in some cases. When comparing deceased carcinoma patients with patients surviving for more than 24 months, the former tended to be younger at tumor diagnosis than the long-term survivors (3.2 years *versus* 10.6 years) while their respective tumors showed higher proliferation indices (39.8% *versus* 30.4%). However, no significant correlation emerged between prognosis and patient age or proliferation in our cohort.

In conclusion, we present a systematic CGH analysis in a large cohort of choroid plexus tumors. Our results show that aberrations differ significantly between choroid plexus papillomas and choroid plexus carcinomas as well as between pediatric and adult choroid plexus papillomas, indicating that distinct genetic pathways may be involved in the development of these entities. This is in accordance with the clinical observation that both choroid plexus papillomas and choroid plexus carcinomas develop *de novo* and rarely, if ever, show malignant progression from WHO grade I to grade III. Furthermore, gain of 9p and loss of 10q seem to be correlated with a more favorable prognosis in choroid plexus carcinomas.

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