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Deletions on the long arm of chromosome 17 in pilocytic astrocytoma*

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Summary. Pilocytic astrocytomas are the most common astrocytic tumors of childhood and differ clinically and histopathologically from those astrocytomas that affect adults. Studies of adult astrocytic tumors have revealed allelic losses on chromosomes 10, 17p, 19q and alterations in the epidermal growth factor receptor (EGFR) gene. We have previously examined pilocytic astrocytomas for allelic losses on chromosomes 10 and 19q and for amplification of the EGFR gene, but did not detect genomic alterations at these loci. In the present study we assayed 20 pilocytic astrocytomas for loss of allelic heterozygosity of chromosome17p, including one locus in the p53 tumor suppressor gene. In addition, because pilocytic astrocytomas frequently affect patients with neurofibromatosis type 1 (NF1) and the NF1 gene has been mapped to 17q11.2, we also examined multiple loci on the long arm of chromosome 17. Allelic loss was observed on chromosome 17 in four cases (three sporadic, one NF1); all lost portions of the long arm in chromosome 17, and one tumor lost the short arm as well. One tumor showed an interstitial deletion on the long arm that included the region of the NF1 gene. These data suggest the presence of a tumor suppressor gene on 17q that is associated with pilocytic astrocytomas. A potentiel candidate for this gene is the NF1 tumor suppressor gene.

Key words: Pilocytic astrocytoma – Loss of heterozygosity – Chromosome 17 – Tumor suppressor gene – Neurofibromatosis type 1

Pilocytic astrocytomas are low-grade astrocytic brain tumors that occur predominantly in childhood or adolescence. These tumors arise most commonly in the cerebellar hemispheres and in midline structures of the central nervous system such as the hypothalamus and optic nerves [23]. Pilocytic astrocytomas usually follow a benign course and are frequently cured by surgical resection alone. These tumors occur both sporadically and in association with neurofibromatosis type 1 (NF1, von Recklinghausen's neurofibromatosis). One third of all patients with pilocytic astrocytomas of the optic nerve (optic glioma) have NF1 [10] and 15% of all patients with NF1 develop optic glioma [19]. The histopathological features of pilocytic astrocytomas are identical in the sporadic and NF1 forms and include elongated "piloid" cells, coarse glial fibrillary matrix, microcysts, Rosenthal fibers and granular bodies. The clinical and pathological features readily distinguish pilocytic astrocytomas from adult supratentorial astrocytomas which are often clinically and histologically malignant tumors.

In adult astrocytomas, including the most malignant form, the glioblastoma multiforme, alterations of both oncogenes and tumor suppressor genes have been identified. For instance, loss of portions of chromosome 17 and mutations in the p53 tumor suppressor gene on 17p have been detected in adult astrocytomas of all grades [3, 6, 8, 29]. Loss of chromosome 10 and amplification of the epidermal growth factor receptor (EGFR) gene, however, are common changes only in the highest-grade astrocytomas [1, 13, 30]. Cytogenetic and molecular genetic studies on a small number of pilocytic astrocytomas have shown both normal and extremely aberrant karyotypes [15–17, 27]. To evaluate whether pilocytic astrocytomas are genetically distinct from the adult astrocytomas, we assayed 20 pilocytic astrocytomas for loss of allelic heterozygosity on chromosome 17p, which is a frequent alteration in adult astrocytomas [5, 7, 14, 29].

Hereditary tumor syndromes, such as familial retinoblastoma and Li-Fraumeni syndrome, have been traced to germ-line mutations in tumor suppressor genes. Tumors from patients with these syndromes often

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display allelic losses in the regions of the respective tumor suppressor gene. In the same tumor types that develop on a sporadic basis, allelic losses are also often demonstrable in the region of the same tumor suppressor loci. Because pilocytic astrocytomas are frequent in NF1, and the NF1 gene has been mapped to 17q11.2, we assayed all tumors for allelic loss on the long arm of chromosome 17.

Material and methods

Human tissue samples

Samples of tumors were obtained from patients biopsied at the Massachusetts General Hospital, Boston, USA, the University Hospital, Zurich, Switzerland and from the Southampton General Hospital, England. The clinical data are summarized in the Table 1. The tissues were snap frozen in liquid nitrogen and stored at −80 °C. Constitutional DNA was extracted from peripheral blood cells.

Histopathological evaluation

Histological sections of all tumors were evaluated by two neuropathologists. Tumors were classified and graded according to the WHO guidelines, and were all diagnosed as pilocytic astrocytoma, WHO grade I. None of the cases showed evidence of malignant change. The following histopathological features were assessed and examined for potential associations with the molecular genetic data: abundance of piloid cell processes, microcystic change, presence of Rosenthal fibers, presence of protein droplets, presence of vascular endothelial proliferation, presence of lymphocytic infiltrates and presence of subarachnoid tumor spread.

DNA extraction and Southern blot analysis

Frozen sections were prepared of the tissue samples chosen for DNA extraction. Adjacent tissue without neoplastic change was trimmed. High molecular weight DNA from tumor tissue and leukocytes were obtained as described [24] and resuspended in TRIS/EDTA to a concentration of 500 μg/ml. Approximately 5 μg of tumor and reference DNA were digested to completion with the appropriate restriction endonucleases (New England Biolabs, standard conditions as provided). The DNA was electrophoretically separated in 0.8 % agarose gels and transferred to nylon membranes (Hybond). Prehybridization and hybridization buffers contained 10 % SDS (Sigma), 7 % polyethylene glycol 8000 (Sigma) and 50 μg/ml salmon sperm DNA for normal or human placental DNA for competitive hybridization conditions. Approximately 20 ng DNA were radiolabelled with [^{32}P] dATP by random oligonucleotide primed extension. Following overnight incubation, the membranes were washed three times in 0.1 % SDS and 0.1 × SSC at 65 °C. Autoradiography was performed on X-ray film (Kodak) using intensifying screens and the autoradiographs were analyzed on a densitometer (LKB, Bromma; Ultrascan XL). Membranes were stripped by washing in double-distilled H_2O for 2 hours at 65 °C and rehybridized to other DNA probes.

DNA probes

The following combinations of DNA markers and restriction enzymes were used for the short arm of chromosome 17: p144D6 (D17S34) and *Taq*I; pYNZ22.1 (D17S5) and *Rsa*I; pYNH37.3 (D17S28) and *Taq*I. The following DNA markers and restriction enzymes were used for the long arm of chromosome 17: pbeta8-2 (CRYB1), pEW101 (D17S40), pEW102 (D17S41), pHHH202 (D17S33) and *Rsa*I; NF1-3.0 (no locus designation) and *Taq*I; LEW206 (D17S57) and *Msp*I; pRMU3 (D17S24) and *Pvu*II; pTH17.19 (D17S82), pCMM86 (D17S74) and *Bgl*II; pTHH59 (D17S4) and *Taq*I. All probes except NF1–3.0 were purchased from the American Type Culture Collection (ATCC).

Polymerase chain reaction-restriction fragment length polymorphism analysis

A polymorphic restriction site in the p53 gene on the short arm of chromosome 17 was analyzed by amplifying portions of exon 4 with the primers 5′-GATGCTGTCCGCGGACGATATT and 5′-CGTGCAAGTCACAGACTTGGC [22]. The amplification products from constitutional DNA and tumor DNA were then digested with *BstU*I and separated on a 2 % agarose gel. The DNA fragments corresponding to the different alleles were visualized by ethidium bromide staining.

Table 1. Results of LOH studies in 20 tumors with 13 polymorphic markers on chromosome 17

ID	Sex	Age	Localization	D17S34	D17S5	D17S28	p53	D17S33	D17S82	NF1	D17S57	D17S41	D17S74	D17S40	D17S4	D17S24
26	f	7	Mesencephalon	n	–	n	–	n	nd	n	–	nd	n	n	n	nd
68	f	28	Lateral ventricle	n	nd	–	–	–	nd	LOH	–	nd	n	nd	n	nd
74	f	17	Cerebellum vermis	n	nd	n	–	n	–	nd	–	n	n	n	n	–
216	m	10	Temporal	n	nd	n	–	–	–	–	–	–	n	–	–	–
266	f	37	Cerebellum hemispheric	n	n	–	–	–	n	n	–	–	n	–	n	n
314	f	21	Optic glioma	n	n	nd	n	n	nd	n	–	n	n	–	nd	nd
364	f	12	Thalamus, basal ganglia	LOH	LOH	–	LOH	LOH	nd	–	–	nd	LOH	LOH	LOH	nd
366	f	24	Cerebellum hemispheric	n	n	–	–	n	n	n	–	n	n	–	–	n
370	m	16	Thalamus, temporal	n	n	n	n	–	–	–	–	–	n	–	n	n
372	m	9	Cerebellum hemispheric	n	n	n	–	–	nd	n	–	nd	n	–	n	nd
374	m	17	Medial, dorsal, pineal	n	n	nd	–	n	n	n	n	–	n	nd	n	n
376	f	14	Hypothalamus	n	n	n	–	–	nd	LOH	LOH	nd	–	LOH	LOH	nd
400	m	17	Tractus opticus, thalamus	n	n	n	n	–	–	–	–	n	–	n	–	n
452	m	19	Cerebellum, ventricle IV	nd	n	nd	n	n	n	n	–	nd	n	–	n	nd
456	f	22	Parietal posterior	nd	n	nd	–	–	n	n	n	nd	n	n	–	nd
458	m	19	Ventricle III	nd	n	nd	–	n	–	n	–	nd	n	n	–	nd
460	f	9	Optic glioma	n	n	–	–	n	nd	n	n	nd	–	–	n	nd
462	m	13	Cerebellum hemispheric	n	n	n	n	–	nd	n	–	nd	n	–	n	nd
544	m	15	Cerebellum	n	n	n	–	n	nd	n	n	nd	n	nd	n	nd
576	m	23	Cerebellum hemispheric	–	n	n	–	–	nd	–	LOH	–	LOH	LOH	LOH	nd

LOH = loss of heterozygosity; n = maintenance of heterozygosity; – = not informative; nd = not determined. The order of the markers corresponds to the order on chromosome 17

Results and discussion

Of the 20 pilocytic astrocytomas, four (20%) showed loss of heterozygosity (LOH) on chromosome 17. Of these four cases, three (68, 376, and 576) had allelic losses confined to the long arm, and one (364) had loss of the entire chromosome. Analysis with multiple probes on chromosome 17q defined a commonly affected region, extending from the centromere to 17q21 (Fig. 1). The results are catalogued in the Table 1. These allelic losses suggest the involvement of a tumor suppressor gene on the long arm of chromosome 17, located between the centromere and 17q21, in the pathogenesis of pilocytic astrocytoma. Loss of portions of chromosome 17q was not associated with any of the special histopathological features of pilocytic astrocytoma such as Rosenthal fibers, protein droplets or microcystic change and was observed in both supratentorial and infratentorial pilocytic astrocytomas.

Allelic loss of the short arm of chromosome 17 has been noted in 40–50% of adult astrocytomas [5, 7, 13, 29], and has been correlated with mutations in the p53 tumor suppressor gene [29]. Only 1 of the 20 pilocytic astrocytomas in this study, however, displayed LOH on 17p. Although we did not examine the p53 gene for mutations in this series of pilocytic astrocytomas, our data suggest that p53 mutations are not common in pilocytic astrocytomas. Allelic losses on the long arm of chromosome 17, while found in 20% of the pilocytic astrocytomas, have not been detected in our series of adult grade II and III astrocytoma, and have been noted only in rare glioblastomas (data not shown). Furthermore, as we have previously reported, allelic losses on chromosomes 10 and 19q, and amplification of the EGFR gene, are frequent events in adult astrocytomas but are not observed in pilocytic astrocytomas [30, 31]. These molecular genetic findings support the clinicopathological separation of pilocytic astrocytomas from adult astrocytomas. On the other hand, the lack of allelic losses on chromosomes 17p and 10 suggests similarities with another type of benign astrocytoma of childhood, the desmoplastic cerebral astrocytoma of infancy, in which we have been unable to demonstrate allelic losses in two cases [21].

The long arm of chromosome 17 harbors several genes that are involved in tumorigenesis. Among these genes, the NF1 tumor suppressor gene is a particularly interesting candidate since patients afflicted with NF1 are prone to developing pilocytic astrocytomas [19]. The NF1 gene exhibits GAP activity, interacts with the *ras*

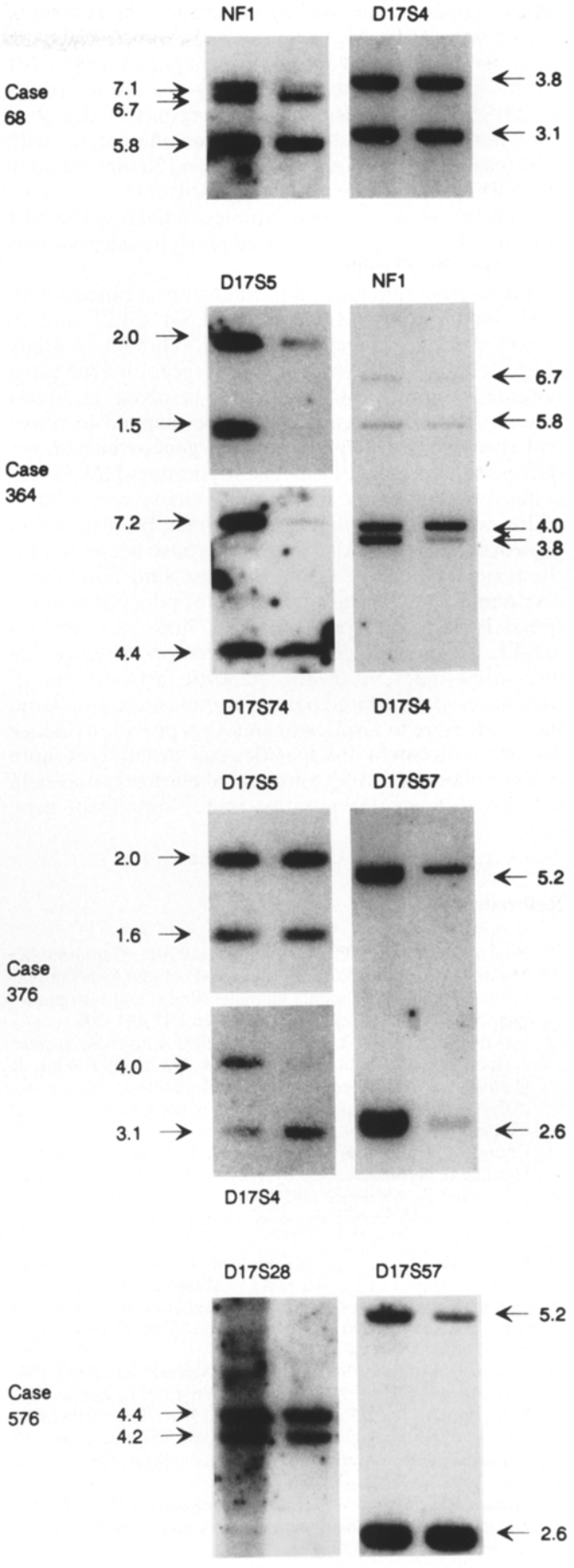

Fig. 1. Autoradiographs of four pilocytic astrocytomas with loss of heterozygosity (LOH) on chromosome 17q. *Left side of each panel* = reference DNA; *right side* = tumor DNA. D17S5 and D17S28 are located on the short arm of chromosome 17, NF1, D17S57, D17S74 and C17S4 are located on the long arm of chromosome 17. Numbers indicate band sizes in kb. Cases 68, 376 and 576 show LOH on chromosome 17q. Case 364 shows LOH of the entire chromosome 17. The signal in the tumor DNA in case 576 probed with D17S57 is reduced by 50%

protooncogenes, and may be involved in the control of cell growth [4, 12, 34]. Alterations in the NF1 gene in patients with NF1 and in tumors from patients with NF1 have been detected in only a few cases [2, 20, 25, 28, 32], although large regions of the gene have not been studied in detail. Our one patient with NF1 (case 376) demonstrated LOH on 17q that included the NF1 locus, but the remaining copy of the NF1 gene has not yet been examined. Studies to analyze the NF1 gene in sporadic and NF1-related pilocytic astrocytomas are currently in progress.

Early-onset familial and sporadic breast cancers have been linked to the D17S74 locus on 17q21 and to proximal 17q, respectively [9, 11]. The latter study suggested that the thyroid hormone receptor gene was a potential tumor suppressor gene involved in breast cancer. Another tumor-related gene mapped to proximal chromosome 17q is the nm23 gene, which shows decreased expression in metastatic tumors [26]. Nm23 codes for a nucleoside diphosphate kinase whose interaction with GTP-binding proteins may be involved in growth control [33]. Allelic deletions have been found in the region of the nm23 gene in breast, lung and kidney carcinomas [18]. Metastatic spread of pilocytic astrocytomas is extremely rare; this may make the nm23 a less-likely candidate gene in these tumors. Because the interstitial deletion in the pilocytic astrocytoma of patient 68 spans a large part of chromosome 17q, from the centromere to 17q21, we cannot yet provide evidence for the involvement of a specific gene. Analysis of more cases of pilocytic astrocytomas for allelic losses may help to narrow down this putative tumor suppressor gene locus.

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