Loss of NF1 Alleles Distinguish Sporadic from NF1-Associated Pilocytic Astrocytomas

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Abstract. Pilocytic astrocytomas classified as WHO grade I typically arise in childhood and upon complete surgical removal carry a favorable prognosis. Children with neurofibromatosis 1 (NF1) have a vastly increased risk for pilocytic astrocytomas, especially for those of the optic nerve. Using 4 intragenic NF1 microsatellite markers, we examined losses of NF1 alleles on the long arm of chromosome 17 in 12 NF1-associated and 25 sporadic pilocytic astrocytomas. The TP53 gene region on the short arm of chromosome 17 was also examined in these tumors using 3 markers. Loss of 1 NF1 allele was detected in 11 of 12 (92%) informative NF1-associated pilocytic astrocytomas. In contrast, only 1 of 24 informative (4%) sporadic pilocytic astrocytomas exhibited allelic loss in the NF1 region. Among the 11 NF1-associated tumors with NF1 loss, 5 had also lost alleles on 17p. The high rate of NF1 allele loss in NF1-associated pilocytic astrocytomas suggests a tumor initiating or promoting action of the NF1 gene in these patients. On the other hand, the much lower rate of NF1-allele loss in sporadic pilocytic astrocytomas argues for only minor importance of NF1 in that patient group. The present data support different mechanisms in the formation of NF1-associated and sporadic pilocytic astrocytomas.

Key Words: Glioma; LOH; NF1: Optic; Pilocytic astrocytoma.

INTRODUCTION

Pilocytic astrocytomas corresponding to WHO grade I are benign childhood tumors (1). Optic gliomas are considered a subgroup of pilocytic astrocytomas. Neurofibromatosis type 1 (NF1) is a common autosomal dominant disease with an incidence of 1 in 4,000 and is caused by genetic alterations in the NF1 gene on the chromosomal arm 17q. Children with NF1 have increased risk for pilocytic astrocytomas, especially for optic gliomas (2, 3).

Allelic losses of the NF1 gene have been reported in neurofibromas (4, 5) and plexiform neurofibromas (6), supporting the tumor suppressor function of this gene. A recent publication reported on allelic losses in 2 of 4 of these tumors and on absent neurofibromin expression in 8 tumors, supporting the role of NF1 deficiency in the development of NF1-associated optic gliomas (7). On the other hand, chromosomal losses on 17q in sporadic pilocytic astrocytomas were detected only at low rate (8), and the expression of neurofibromin has been demonstrated in sporadic pilocytic astrocytomas (9, 10).

In order to explore the role of the NF1 gene in the development of both NF1-associated and sporadic pilocytic astrocytomas, we examined 12 NF1-associated and 25 sporadic pilocytic astrocytomas for allelic losses on chromosome 17 with special focus on the NF1 region.

MATERIALS AND METHODS

NF1-patients were diagnosed according to the NIH criteria (11). The study design was approved by the Institutional Review Board and all participants provided informed consent to the analyses performed on blood and tumor tissues. The tumors were obtained from the neurosurgical departments of the University Hospital Eppendorf in Hamburg, the Hospital Nordstadt in Hannover, the University Hospital Bonn in Bonn, and the Charité Hospital in Berlin. Of 12 WHO grade I NF1-associated pilocytic astrocytomas from 12 unrelated patients, 5 were removed from the chiasm, 5 from the optic nerve, 1 from cerebellum, and 1 was resected from the spinal cord. Of 25 WHO grade I sporadic pilocytic astrocytomas from 25 unrelated patients, 1 was removed from the chiasm, 1 from the optic nerve, 2 from the optic tract, 10 from the cerebellum, and 11 from the temporal lobe. Eleven of the 25 sporadic pilocytic astrocytomas have been examined in a previous study (8). Localization and genetics of the tumors included are summarized in Table 1. All tumors were verified by histological examination.

DNA was extracted from blood and tumors using QIAamp Blood and QIAamp Tissue Kits from Qiagen (Hilden, Germany) or phenolic extraction. Analysis of allelic loss in tumors was performed using 4 microsatellite markers within the NF1 gene (5): IVS27CA28.4 (intron 27), IVS27TG24.8 (intron 27), IVS38GT53 (intron 38), and M98509 (intron 27), as well as three 17p markers: D17S520, D17S796, and D17S804 (Genome Database). Primers were labeled with the dyes fluorescein (FAM) or rhodamine (Tamra) at the 5-terminus. Samples were analyzed under denaturing condition using an ABI Genetic Analyzer 310 (ABI, Foster City, CA). In informative cases, loss of heterozygosity (LOH) was determined by comparing the peak areas of both alleles in blood and corresponding tumors.

The correlations of age versus genetics were evaluated by the unpaired t-test. The associations of genetics with LOH on 17q and genetics with sex were analyzed by the Fisher exact test.
### TABLE 1
Localization of, and Allelic Losses in, 12 NF1-Associated and 25 Sporadic Pilocytic Astrocytomas

<table>
<thead>
<tr>
<th>Localization</th>
<th>Genetics</th>
<th>Number</th>
<th>LOH/Informative cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optic nerve/tract</td>
<td>NF1</td>
<td>10</td>
<td>10/10 (100%)</td>
</tr>
<tr>
<td></td>
<td>Sporadic</td>
<td>4</td>
<td>0/4</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>NF1</td>
<td>10</td>
<td>1/10 (10%)</td>
</tr>
<tr>
<td></td>
<td>Sporadic</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Spinal</td>
<td>NF1</td>
<td>1</td>
<td>1/1</td>
</tr>
<tr>
<td></td>
<td>Sporadic</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Temporal lobe</td>
<td>NF1</td>
<td>11</td>
<td>0/11</td>
</tr>
<tr>
<td></td>
<td>Sporadic</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### RESULTS

WHO grade I pilocytic astrocytomas from 12 unrelated NF1 patients and 25 sporadically arising pilocytic astrocytomas were examined. Age at resection averaged 13.4 yr in the NF1 patients and 22.7 yr in patients with sporadic tumors. This difference was significant (p = 0.024, unpaired t-test). In the group with sporadically arising tumors, 13 patients were female and 12 were male. Among NF1 patients, 8 were female and 4 were male.

Thirty-seven patients were analyzed for allelic losses on chromosome 17. All 12 NF1-associated tumors and 24 of 25 sporadic tumors were informative for at least 1 marker within the NF1 gene on 17q. All sporadic tumors and 11 of 12 NF1-associated tumors were informative for at least 1 marker on 17p. LOH in the NF1 region was detected in 11 of 12 (92%) informative NF1-associated pilocytic astrocytomas. In contrast, only 1 of 24 (4%) informative sporadic pilocytic astrocytomas exhibited LOH within the NF1 gene. This difference was highly significant (p < 0.0001, Fisher exact test). LOH in the NF1 region was detected in 10 of 10 (100%) informative NF1-associated optic gliomas, but in 0 of 4 informative sporadic optic gliomas. This difference was significant (p = 0.001, Fisher exact test). LOH on the short arm of chromosome 17 was detected in 5 of 11 informative NF1-associated and in none of the 25 informative sporadic pilocytic astrocytomas. All cases with LOH on 17p also exhibited LOH within the NF1 region. Representative data is shown in Figure 1. Patient #187 exhibits LOH in the intragenic NF1 markers IVS38GT53 and IVS27CA28.4. The findings are summarized in Tables 1 and 2. We did not detect microsatellite instability in any of the markers tested on the 37 pilocytic astrocytomas.

### DISCUSSION

The present study provides evidence for involvement of different genetic pathways in the genesis of NF1-associated and sporadic pilocytic astrocytomas. This hypothesis is supported by the highly significant difference of allelic losses in these groups (p < 0.0001). Detection of LOH in the NF1 region in 11 of 12 pilocytic astrocytomas from patients with NF1 indicates that virtually all these tumors have lost NF1 function. Only 1 of 24 pilocytic astrocytomas from patients without NF1 exhibited LOH in the NF1 region. In this patient it cannot be assumed that the present NF1 allele is inactivated by a mutation. These structural differences are supported by several previous studies on NF1 expression in pilocytic astrocytomas. The most recent study (7) focused on NF1-associated pilocytic astrocytomas and revealed that neurofibromin was absent in 8 of these tumors, while it was present in 1 sporadic pilocytic astrocytoma. That study also detected LOH in the NF1-region in 2 of 4 NF1-associated pilocytic astrocytomas. In contrast, studies on sporadic pilocytic astrocytomas reported either a slight upregulation of NF1 (9) or NF1 expression levels comparable to other astrocytomas (10). Our data would support these studies since NF1 mutations with an assumed effect on transcription were predominantly seen in NF1-associated lesions, whereas sporadic pilocytic astrocytomas...
with intact NF1 expression did not exhibit chromosomal losses in the NF1 region. These data support a biological difference between NF1-associated and sporadic pilocytic astrocytomas. However, we cannot exclude bi-allelic small mutations in sporadic pilocytic astrocytomas, although this appears quite unlikely. Another explanation for the discrepancy between histology and genetic findings in NF1-associated and sporadic pilocytic astrocytomas could be that different targets in the same functional cascade are affected, i.e. the NF1 gene in NF1-associated and another upstream or downstream acting gene in sporadic pilocytic astrocytomas.

Allelic loss on 17p was detected in 5 NF1-associated pilocytic astrocytomas; however, it was always in combination with allelic loss in the NF1 region. Therefore we interpret LOH 17p to be consequence of inactivation of the second NF1 allele by loss of the entire chromosome 17 rather than an independent event. In these cases we would not expect LOH17 to sufficiently inactivate TP53 function, which is a tumor-promoting mechanism shown to be involved in the genesis of diffuse fibrillary astrocytic tumors.

The majority of NF1-associated pilocytic astrocytomas are localized to the optic nerve, while sporadic pilocytic astrocytomas do occur more frequently in the cerebellum and the temporal lobes. This could point towards a site-specific pattern of structural genomic lesions. However, LOH in the NF1 region was not detected in the 4 sporadically occurring tumors as opposed to 10 of 10 NF1-associated pilocytic astrocytomas of the optic nerve, chiasm, or optic tract (p = 0.001). Only 1 of 21 sporadic pilocytic astrocytomas outside the optic nerve or optic tract had LOH in that region. These data indicate that LOH in the NF1 region should be attributed to the association with NF1 rather than to the association with the optic nerves and tracts.

NF1-associated optic gliomas seem to have a lower rate of recurrences coupled with a longer latency to tumor regrowth (12, 13). On the other hand, an extensive review on optic gliomas indicated a higher risk for treatment failure (defined as death, tumor progression, or recurrence) in NF1 than in sporadic patients (14). Our set of sporadic optic glioma included only 4 cases and therefore is too small to allow statistics regarding tumor site. However, the localization of 2 of 4 sporadic optic glioma in the optic tract and 0 of 10 NF1-associated optic glioma at that site supports the previous studies (12, 13). It is tempting to speculate that the different genetic findings in NF1-associated and sporadic optic gliomas contribute to the differential biological behaviors.

No consistent gene mutations have been detected in pilocytic astrocytomas. Most emphasis has been put on analysis of TP53, which turned out to be affected in a few pilocytic astrocytomas (15–17). However, one study reported on TP53 mutations in 7 of 20 pilocytic astrocytomas (18), possibly as a result of a more sensitive detection system. PTEN mutation, an alteration frequently seen in high-grade gliomas, has been detected in a single pilocytic astrocytoma (19). Pilocytic astrocytomas in NF1 patients have not been systematically examined for alterations other than NF1.

In conclusion, our data provide strong supporting evidence for the hypothesis that the genetic pathways leading to pilocytic astrocytoma differ between tumors arising sporadically and those arising in association with NF1.

REFERENCES


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