Aggressive Phenotypic and Genotypic Features in Pediatric and NF2-Associated Meningiomas: A Clinicopathologic Study of 53 Cases

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Abstract. Pediatric and NF2-associated meningiomas are uncommon and poorly characterized in comparison to sporadic adult cases. In order to elucidate their molecular features, we analyzed MIB-1, progesterone receptor (PR), NF2, merlin, DAL-1, DAL-1 protein, and chromosomal arms 1p and 14q in 53 meningiomas from 40 pediatric/NF2 patients using immunohistochemistry and dual-color fluorescence in situ hybridization (FISH). Fourteen pediatric (42%) patients, including 5 previously undiagnosed patients, had NF2. The remaining 19 (58%) did not qualify. All 7 of the adult patients had NF2. Meningioma grading revealed 21 benign (40%), 26 atypical (49%), and 6 anaplastic (11%) examples. Other aggressive findings included high mitotic index (32%), high MIB-1 LI (37%), aggressive variant histology (e.g. papillary, clear cell) (25%), brain invasion (17%), recurrence (39%), and patient death (17%). FISH analysis demonstrated deletions of NF2 in 82%, DAL-1 in 82%, 1p in 60%, and 14q in 66%. NF2-associated meningiomas did not differ from sporadic pediatric tumors except for a higher frequency of merlin loss in the former (p = 0.02) and a higher frequency of brain invasion in the latter (p = 0.007). Thus, although pediatric and NF2-associated meningiomas share the common molecular alterations of their adult, sporadic counterparts, a higher fraction are genotypically and phenotypically aggressive. Given the high frequency of undiagnosed NF2 in the pediatric cases, a careful search for other features of this disease is warranted in any child presenting with a meningioma.

Key Words: Childhood; Chromosome 1; Chromosome 14; DAL-1; Malignant tumor; Meningioma; NF2.

INTRODUCTION

Pediatric and/or NF2-associated meningiomas are distinctly uncommon in comparison to their sporadic counterparts in adults. As a result, few large series have been reported (1–19) and detailed genetic characterization of their tumors is lacking. Pediatric meningiomas are thought to represent <2% of all meningiomas and <3% of childhood brain tumors (1–7, 9–11, 13, 16, 18, 19). Many authors report a high incidence of aggressive histology and biologic behavior (3, 5, 7, 11, 19). In contrast, others have suggested that when hemangiopericytomas and other dural-based sarcomas are excluded, the prognosis remains excellent, despite a relatively high incidence of worrisome features, including brisk mitotic activity and brain invasion (4, 9). Anaplastic (grade III) meningiomas with sarcomatoid features pose a further complicating factor, since they may be difficult to distinguish from primary sarcomas on purely morphologic grounds; one such tumor was included in our series. Other reportedly unique features of pediatric meningiomas include a higher frequency of intraventricular examples, cystic changes, lack of dural attachment, and a lack of the female predilection so characteristic of adult tumors (1–7, 9–11, 13, 16, 18, 19).

As with pediatric meningiomas, NF2-associated tumors comprise only a small fraction of all meningiomas. It is estimated that only about 1% of meningioma patients have NF2, and most of these patients have multiple tumors (8, 15). Within the NF2 patient population however, meningiomas are second in frequency only to vestibular schwannomas. According to Evans et al, meningiomas are seen in 53% of all NF2 patients and in 83% with the early onset, more severe phenotype (“Wishart variant”) (8). Furthermore, NF2 patients presenting during childhood are much more likely to present with meningiomas than are older NF2 patients (8, 14). The NF2 association with pediatric meningiomas is evident in other series, the disorder being seen in 7%–41% of patients (1–3, 9, 11, 13, 16, 19). Also reported is a link between NF2 and meningiomas with high proliferation indices and other aggressive morphologic features (12, 13). Others suggest that NF2-associated meningiomas are no more aggressive histologically than sporadic examples, though relatively few cases had been reported (20). Given the high frequency of NF2 in our pediatric meningioma population, the young age at presentation of patients with NF2-associated meningiomas, and the fact that other features of NF2 may not always yet be evident in pediatric patients, we combined our pediatric and NF2-associated meningiomas in the current clinicopathologic and genetic study.
Our findings a) confirm the high frequency of NF2 in pediatric meningioma patients, b) indicate that meningioma may be the first manifestation of the syndrome, and c) suggest that both pediatric and NF2-associated meningiomas exhibit an aggressive histologic, genetic, and clinical phenotype.

MATERIALS AND METHODS

Patients/Tumors

Both pediatric (≤ 18 yr of age) and/or NF2-associated meningiomas were retrieved from the surgical pathology files of Washington University School of Medicine, St. Louis, Mo. (1981–2000), Mayo Clinic, Rochester, Minn. (1972–2000), and the University of Texas Southwestern Medical Center, Dallas, Tex. (1985–2000). All available slides were reviewed and classified by one of the authors (AP) according to 2000 World Health Organization (WHO) criteria (21). In accordance with the revised WHO scheme (21), all tumors were graded using previously published criteria (22, 23). Atypical meningiomas (grade II) featured ≥4 mitoses/10 high-power fields (HPF) and/or at least 3 of the following 5 variables: sheeting, small cells, macronucleoli, hypercellularity, and necrosis. The finding of brain invasion was considered equivalent to grade II, even for otherwise histologically benign meningiomas. This is in accordance with current grading recommendations and is based on the fact that such cases have demonstrated similar rates of recurrence as atypical meningiomas diagnosed by the other criteria discussed above (21, 23). Anaplastic meningiomas (grade III) were defined by ≥20 mitoses/10 HPF and/or loss of meningothelial differentiation at the light microscopic level (i.e. histology resembling sarcoma, carcinoma, or melanoma). Meningiomas wherein an aggressive variant morphology (papillary, rhabdoid clear cell, or chordoid) was partly (<50%) expressed were graded using the criteria outlined above. When these morphologies were the dominant (≥50%) feature, the recommended WHO grade was assigned: i.e. grade II for clear cell or chordoid meningioma and grade III for papillary or rhabdoid tumors (21). Patient followup was acquired through clinical chart review.

Immunohistochemistry and FISH

Based on its H&E appearance, a representative paraffin block of each tumor was selected and 5- to 6-μm-thick sections were cut for immunohistochemistry and dual-color fluorescence in situ hybridization (FISH) analysis as previously described (26, 27). Automated immunohistochemistry was performed with a Dako Autostainer® (Carpinteria, CA). Noncommercial affinity-purified rabbit polyclonal antibodies against Merlin (WA30) and DAL-1 (3A1) were each applied at a 1:500 dilution (26). Antigen retrieval was achieved using 4% H2O2 in 0.01 N HCl for 30 min at 37°C. Commercially available monoclonal Ki-67 (MIB-1; Dako, 1:80 dilution) and progesterone receptor (PR) antibodies (PR88; BioGenex, San Ramon, CA; 1:500 dilution) were also applied after utilizing microwave antigen retrieval for 8 min in EDTA buffer (pH 8.0, 1.0 mM). Tumors were considered positive when >1% of neoplastic cells displayed nuclear staining for PR or cytoplasmic staining for merlin or DAL-1. MIB-1 proliferative indices were expressed as percent staining and based on manual counts of 1,000 nuclei in regions of greatest staining. A high MIB-1 labeling index was defined as ≥4.2% (28).

For FISH analyses, sections were deparaffinized, steamed in 10-mM citrate buffer, pH 6.0, and pepsin digested. Bacterial artificial chromosome (BAC) DNA probes targeting chromosomal regions 22q12.2 (NF2 region; CIT987SK-A-390C5, Research Genetics; genome database #712269, http://www.gdb.org), 1p32 (RPCI-11–260123, Research Genetics, Huntsville, AL) (29), 1p36 (p73 gene region, gift of Dr. Robert Jenkins, Mayo Clinic), 14q13 (CIT987SK-A-299G2, Research Genetics; gdb #713290), and 14q32 (CIT987SK-A-899E8, Research Genetics; gdb #1382620) were prepared and labeled with rhodamine or fluorescein according to manufacturer’s instructions. A fluorescein-labeled P1 clone localizing to the DAL-1 region on 18p11.3 was also used, as previously published (30). Paired NF2/DAL-1, 1p32/1p36, or 14q13/14q32 probes were diluted (1:50) in DenHyb buffer (Inisits, Albuquerque, NM), applied to each slide, and co-denatured with the target DNA at 90°C for 13 min. Hybridization was carried out via overnight incubation at 37°C in a humidified oven. The following day, the slides were washed with 50% formamide in 1× SSC, followed by a second wash with 2× SSC, and a third wash with PBS, each wash for 5 min. Nuclei were counterstained with DAPI and fluorescent signals were enumerated under an Olympus BX60 fluorescent microscope with appropriate filters (Olympus, Melville, NY). For each hybridization, 100 to 200 nonoverlapping nuclei were assessed for numbers of green and red signals. Slides were scanned for regional variability (e.g. clonal heterogeneity) and were considered abnormal regardless of whether the alteration appeared focal or diffuse. Cutoffs for 1p and 14q deletions were based on prior studies of non-neoplastic brains, with requirements of 1 signal per nucleus in at least 40%–46% tumoral nuclei, depending on the probe being used (27). The cutoffs for NF2 and DAL-1 deletions were 46% and 45%, respectively (mean plus 3 standard deviations for non-neoplastic control nuclei with 1 signal). Hybridizations were considered noninformative if the FISH signals were either lacking or too weak to interpret.

RESULTS

Patient/Tumor Cohort

The study cohort consisted of 33 pediatric (ages 3–18 yr, median 12 yr) and 7 adult (ages 20–54 yr, median 33 yr) patients. There were 21 males and 19 females, yielding a male to female ratio of 1.1. Nine (27%) of the pediatric patients and all of the adults were known to have NF2 at the time of surgery. An additional 5 pediatric patients originally thought to have sporadic meningiomas only later qualified for the diagnosis of NF2. Therefore, 14 (42%) of the pediatric patients were known to have NF2 by the end of the study and 21 NF2 patients were studied in total. These patients often harbored bilateral vestibular schwannomas and in many instances, parasellar schwannomas and/or meningiomas.
Tissue was available for study from 53 surgically resected meningiomas. Twelve (57%) NF2 patients had radiologic and/or histologic evidence of multiple meningiomas. In contrast, none of the pediatric, non-NF2 patients had multifocal disease (p < 0.001; Fisher exact test). Multiple meningioma specimens from the same patient were examined in 3 cases. Of the meningiomas studied, 43 (81%) were primary and 10 (19%) were recurrent. In 5 cases, both primary and recurrent specimens were examined. Sites of disease are summarized in Table 1. Cerebral convexity and/or parasagittal tumors were most common, with roughly half of all cases presenting at this site. In comparison to sporadic tumors occurring in adults, an intraventricular location was relatively common (13%). Information regarding surgical extent of resection was not readily available for most of our cases.

Pathology and Genetics

A wide morphologic spectrum was encountered in pediatric and NF2-associated tumors (Fig. 1; Table 2). Transitional meningioma was the most common histologic variant, accounting for either the primary or secondary morphology in 51% of cases. No statistically significant differences between NF and non-NF-associated meningiomas were found, though there was a trend for more transitional meningiomas in the former (p = 0.170; Fisher exact test). Aggressive variant morphology (clear cell, chordoid, papillary, rhabdoid) was seen in 13 (25%) cases, composing the primary (>50%) pattern in 2 clear cell (grade II) and 2 papillary (grade III) meningiomas. In the remaining cases, the aggressive morphology was secondary or only focal.
TABLE 2
Histologic Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Pediatric (n = 23)</th>
<th>NF2 (n = 15)</th>
<th>Adult NF2 (n = 15)</th>
<th>Total (n = 53)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transitional</td>
<td>9 (39%)</td>
<td>9 (60%)</td>
<td>9 (60%)</td>
<td>27 (51%)</td>
</tr>
<tr>
<td>Meningothelial</td>
<td>7 (30%)</td>
<td>4 (26%)</td>
<td>2 (13%)</td>
<td>13 (25%)</td>
</tr>
<tr>
<td>Fibroblastic</td>
<td>4 (17%)</td>
<td>0</td>
<td>2 (13%)</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Papillary</td>
<td>3 (13%)</td>
<td>2 (13%)</td>
<td>0</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Rhabdoid</td>
<td>2 (9%)</td>
<td>2 (13%)</td>
<td>1 (7%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Chordoid</td>
<td>0</td>
<td>0</td>
<td>2 (13%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Clear Cell</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (13%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Microcystic</td>
<td>1 (4%)</td>
<td>1 (7%)</td>
<td>0</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Psammomatous</td>
<td>0</td>
<td>1 (7%)</td>
<td>1 (13%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Metaplastic-xanthomatous</td>
<td>0</td>
<td>1 (7%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Lympholasmacyte-rich</td>
<td>0</td>
<td>1 (7%)</td>
<td>0</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Variants add up to >53 cases and >100% because some cases had more than 1 histologic pattern. No statistically significant differences were found between NF2- and non-NF2-associated meningiomas, though there was a trend for more transitional meningiomas in the former (p = 0.170).

Histologic grading revealed 21 grade I (40%), 26 grade II (49%), and 6 grade III (11%) meningiomas. The stratification of tumor grades was similar in both the pediatric and NF2-associated subsets. The mitotic index ranged from 0 to 109/10 HPF (median 1/10 HPF, mean 5/10 HPF). A high mitotic index (≥4/10 HPF) was seen in 17 (32%) cases. Brain invasion was encountered in 9 (17%) cases overall, with 8 (35%) of the 23 pediatric non-NF2 cases and 1 (3%) of the 30 NF2-associated cases showing this feature (p = 0.007; Fisher exact test). All 3 patients with multiple meningiomas demonstrated a similar or identical morphology and grade for meningiomas derived from the same patient. In 1 patient with 6 separate meningiomas, the primary morphology was transitional for all 6, though both focal chordoid and rhabdoid features were each seen in 1 tumor. In 4 of the 5 cases in which both primary and recurrent meningiomas were available for study, the latter was of the same histologic grade (2 benign, 2 atypical). The fifth case recurred as an anaplastic (grade III) meningioma, appearing 11 yr after the primary, benign (grade I) tumor.

Immunohistochemical and FISH results are summarized in Table 3 and representative cases are illustrated in Figure 2. With 6 probes applied to each of the 53 tumors, FISH signals were interpretable in 304 of the 318 hybridization targets, yielding a success rate of 96%. FISH analyses and immunohistochemistry were concordant for NF2/merlin and DAL-1/DAL-1 status in 37/51 (73%) and 41/51 (80%) interpretable cases, respectively. The most common reason for discordance was gene deletion by FISH in the face of retained protein expression by immunohistochemistry. For the entire cohort, deletions were detected for NF2 in 82%, DAL-1 in 82%, 1p in 60%, and 14q in 66%. Merlin expression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grade I</th>
<th>Grade II</th>
<th>Grade III</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merlin loss</td>
<td>10/21 (48%)</td>
<td>21/26 (81%)</td>
<td>4/6 (67%)</td>
<td>35/53 (66%)</td>
</tr>
<tr>
<td>DAL-1 loss</td>
<td>15/21 (71%)</td>
<td>22/26 (85%)</td>
<td>5/6 (83%)</td>
<td>42/53 (79%)</td>
</tr>
<tr>
<td>PR expression</td>
<td>11/21 (52%)</td>
<td>12/26 (46%)</td>
<td>2/6 (33%)</td>
<td>25/53 (47%)</td>
</tr>
<tr>
<td>Median MIB-1 LI</td>
<td>1.2%</td>
<td>4.1%</td>
<td>7.1%</td>
<td>2.8%</td>
</tr>
<tr>
<td>High MIB-1 LI</td>
<td>3/21 (14%)</td>
<td>11/24 (46%)</td>
<td>4/4 (100%)</td>
<td>18/49 (37%)</td>
</tr>
<tr>
<td>NF2 deletion</td>
<td>14/20 (70%)</td>
<td>23/25 (92%)</td>
<td>5/6 (83%)</td>
<td>42/51 (82%)</td>
</tr>
<tr>
<td>DAL-1 deletion</td>
<td>15/20 (75%)</td>
<td>22/25 (88%)</td>
<td>5/6 (83%)</td>
<td>42/51 (82%)</td>
</tr>
<tr>
<td>1p deletion</td>
<td>11/20 (55%)</td>
<td>14/26 (54%)</td>
<td>6/6 (100%)</td>
<td>31/52 (60%)</td>
</tr>
<tr>
<td>14q deletion</td>
<td>13/19 (68%)</td>
<td>15/25 (60%)</td>
<td>5/6 (83%)</td>
<td>33/50 (66%)</td>
</tr>
<tr>
<td>−NF2/DAL-1</td>
<td>11/20 (55%)</td>
<td>21/25 (84%)</td>
<td>5/6 (83%)</td>
<td>37/51 (73%)</td>
</tr>
<tr>
<td>−1p/14q</td>
<td>8/19 (42%)</td>
<td>11/25 (44%)</td>
<td>5/6 (83%)</td>
<td>24/50 (48%)</td>
</tr>
<tr>
<td>All 4 deleted</td>
<td>5/19 (26%)</td>
<td>10/25 (40%)</td>
<td>5/6 (83%)</td>
<td>20/50 (40%)</td>
</tr>
</tbody>
</table>

PR = progesterone receptor.
All 4 deleted = co-deletion of NF2, DAL-1, 1p, and 14q chromosomes regions.
was lost in 66%, whereas DAL-1 expression was lost in 79%. Most tumors with either 1p or 14q deletion demonstrated simultaneous loss of both of the paired FISH markers, a finding consistent with large deletions or loss of the entire chromosomal arm. However, 7 cases lost 1p32 with intact 1p36, 4 lost 14q13 with intact 14q32, and 1 lost 14q32 with intact 14q12, a pattern suggesting smaller, interstitial deletions.

With a few exceptions, there were no obvious associations between the parameters tested and histologic grade, patient age, or NF2 status (Tables 3 and 4). However, there were significant associations between NF2 status and NF2/merlin losses. Meningiomas from NF2 patients exhibited NF2 deletion and loss of merlin expression in 93% and 80% of cases, respectively. In contrast, non-NF2 meningiomas of pediatric patients showed NF2 deletions and losses of merlin expression in 70% (p = 0.061; Fisher exact test) and 48% (p = 0.020), respectively. The MIB-1 labeling index increased proportionally with tumor grade, although significant overlap was observed among the grades. Median indices and ranges were 1.2% (0.3–8.9%) in benign, 4.1% (1.2–11.2%) in atypical, and 7.1% (4.2–71.1%) in anaplastic meningiomas. A high MIB-1 proliferative labeling index was found in 14% of grade I, 46% of grade II, and 100% of grade III meningiomas. On the other hand, progesterone receptor expression was inversely related to histologic grade, though positive and negative cases were encountered in all grade categories. Co-deletion of all 4 chromosome sites assessed (NF2, DAL-1, 1p, 14q) increased with tumor grade, being encountered in 26% of grade I, 40% of grade II, and 83% of grade III meningiomas.

Among patients with multiple meningiomas, 1 case permitted an analysis of 6 separate tumors; all 6 harbored NF2 and DAL-1 deletions, 4 had combined 1p and 14q deletions, 1 had neither, and 1 had only 1p loss. In another patient, each of 2 separate meningiomas showed co-deletions of NF2, DAL-1, 1p and 14q. In a third patient with 2 separate tumors, 1 was a decalcified psammomatous meningioma, which was noninformative for all FISH assays.

Of the 5 patients in whom both primary and recurrent meningiomas were available for study, the genetic pattern...
of deletions remained the same after recurrence in one. In contrast, DAL-1, 1p, and 14q deletions were observed in the recurrent, but not the primary specimens in 2, 3, and 2 cases, respectively. In each case, however, NF2 deletions were found in both the original and recurrent tumors. Similar findings were seen by immunohistochemistry, with 2 cases losing DAL-1 and 3 cases losing progesterone receptor expression in the recurrences.

Clinical Followup and Associations

Clinical followup was available in 36 (90%) of the 40 patients and ranged from 0.1 to 25.6 yr (mean 7.6, median 7.4) from initial surgery. During this period, 6 (17%) patients died, though the precise cause of death was unknown in most instances. All but 1 of these patients had NF2. They included a 10-yr-old boy with an anaplastic meningioma that had undergone extensive CSF seeding (1-month survival), a 10-yr-old girl with a papillary (grade III) meningioma (10.7-yr survival), an 11-yr-old boy with a suprasellar/third ventricular atypical meningioma (11.4-yr survival), a 44-yr-old man who presented with a benign meningioma, the recurrence of which was anaplastic (11.7-yr survival from presentation, 2 months survival from recurrence), a 10-yr-old boy with a benign spinal meningioma (16.2-yr survival), and a 14-yr-old boy with a benign spinal meningioma (25.6-yr survival).

In total, 14 patients (39%) experienced 1 or more recurrences. In the 8 cases in which the primary tumor was available for review, recurrence was noted 1 to 9 yr (median 2.4) after resection. Of the respective primary tumors, 3 were benign, 4 atypical, and 1 anaplastic (grade III); 2 patients are subsequently free of disease at 1 month and 15.9 yr, 2 are alive with residual disease at 4.2 and 13.1 yr, 2 died at 2 months and 6.4 yr, and 2 were lost to followup. In the 6 cases in which the primary tumor was not available for review, recurrences occurred between 1 and 7 yr (median 5.9) postoperatively. Of these recurrences, 2 were histologically benign (grade I) and 4 were atypical (grade II). After re-resection, and in some cases adjuvant radiation therapy, 4 patients are disease-free from 2.3 to 7.1 yr (median 5.4), 1 is alive with residual disease at 1.1 yr, and 1 recurred after 1.9 yr.

Fifteen patients (42%) have had no recurrences and are currently alive and well without evidence of disease from 0.3 to 14.5 yr (median 4.3) after surgical resection. Histologic grading revealed 9 benign, 5 atypical, and 1 papillary (grade III) meningioma. Three patients (8%) are alive with residual disease 0.9 to 10.5 yr (median 3.5) from surgery.

In total, recurrences and/or death occurred in 18 patients (50%) with followup: 7 of 16 patients (44%) with grade I, 8 of 14 patients (57%) with grade II, and 3 of 4 patients (75%) with grade III meningiomas. Although pediatric non-NF2-associated meningioma patients had a few more recurrences and NF2-associated meningioma patients suffered more deaths, the differences were not statistically significant (Table 4). With respect to chromosome 1p, 1q, or combined 1p/1q deletions, there were also no significant associations with clinical outcome. Admittedly, the number of patients with adequate followup was relatively small. Of the 12 meningioma patients with primary tumors who subsequently recurred...
and/or died, 7 tumors had 1p loss, 6 had 14q loss, and 4 had both. Of the 7 patients with no evidence of disease at least 5 yr postoperatively, 4 tumors had 1p deletions, 5 had 14q deletions, and 3 had combined deletions. Neither the MIB-1 labeling index nor progesterone receptor status was associated with clinical outcome (p > 0.05). Of the cases with followup, 60% with a high MIB-1 index and 42% with a low index experienced recurrences and/or death. For progesterone receptor, 53% with expression and 47% without expression experienced recurrences and/or death.

DISCUSSION

Pediatric/NF2-Associated Versus Sporadic Adult
Meningiomas

Given their overall rarity, it is not surprising that conflicting reports exist regarding attributes that may be unique to pediatric and/or NF2-associated meningiomas (1–7, 9–11, 13, 16, 18, 19). One previously suggested explanation of discrepancies regarding prognosis in pediatric meningiomas is that some investigators have included hemangiopericytomas and dural sarcomas, while others have not (9). Though we agree with exclusion of such cases, we believe that some anaplastic (grade III) meningiomas are sarcomatoid in appearance and are difficult to distinguish from primary sarcomas on the basis of histology alone. Given limited pathologic detail in many of the older series, it is often impossible to discern which reported cases were true sarcomas and which were sarcomatoid forms of anaplastic meningiomas. In the current series, we included 1 example that was purely sarcoma-like (Fig. 1F) and another wherein classic meningothelial morphology was juxtaposed to a sarcomatoid element. The former resembled high-grade fibrosarcoma, but we considered it meningothelial based on the karyotypic findings of monosomy 22 and 1p deletion (data not shown), as well as FISH evidence of co-deletion of NF2, DAL-1, 1p, and 14q. In other words, its molecular genetic signature was highly suggestive of meningioma.

To our knowledge, this is the first large series to simultaneously address genotypic, phenotypic, and biologic features of pediatric and NF2-associated meningiomas. It provides evidence of important similarities and differences with the more common sporadic meningiomas of adults. As with meningiomas in general, we encountered a remarkably wide morphologic and clinical spectrum. As stated previously, our pediatric/NF2-associated meningioma population lacked the female predominance noted in sporadic adult meningiomas, had a higher incidence of intraventricular location (13%), and were more often aggressive. Evidence for the latter included high tumor grade (II or III) in 60%, aggressive variant morphology in 25%, brain invasion in 17%, elevated mitotic/proliferative indices in approximately 35%, deletions of the progression-associated markers 1p and 14q in about 60% each, as well as patient death and/or tumoral recurrences in 50% of patients with followup. Therefore, our findings suggest that pediatric and NF2-associated meningiomas are more predisposed towards malignant progression and/or tend to progress at a more rapid rate than their sporadic adult counterparts. Interestingly, the high incidence of brain invasion in our study was restricted to the subset of pediatric non-NF2-associated meningiomas, where it was encountered in 35% of cases. It is possible that tumorogenesis in this patient population involves another gene besides NF2, which facilitates brain invasion.

Although we found an association between clinical behavior and histologic grade, it was not as strong as that we reported for sporadic adult cases (22, 23), in that recurrence/death was relatively common (44%) for patients with grade I tumors. Re-review of these 7 cases revealed no obvious differences from our histologically benign cases associated with favorable clinical behavior (data not shown), though detailed data on extent of tumor resection was lacking. Others have reported that the great majority of recurring, benign pediatric meningiomas have been subtotally resected (1, 7, 9, 18). In terms of aggressive variant morphology, this was most often encountered focally, though 4 cases were primarily papillary or clear cell. This is consistent with the reportedly younger mean ages of patients with these 2 variants (31, 32). In contrast, rhabdoid and chordoid meningiomas have mean ages closer to that of typical meningiomas (33, 34).

As in other recent series (19, 28), we found the MIB-1 labeling index to increase in proportion to histologic grade. However, there was substantial overlap among the grades and many cases with a low index subsequently recurred, thus limiting its potential clinical utility. Unlike our series of sporadic adult meningiomas (26), progesterone receptor expression was only roughly associated with tumor grade and was not at all associated with clinical outcome.

Few studies have focused on the genetic alterations of pediatric and/or NF2-associated meningiomas (17, 35). Our present cases shared with their sporadic adult counterparts the high frequencies of merlin and DAL-1 losses by immunohistochemistry and NF2 and DAL-1 deletions by FISH (26). These 2 techniques were concordant in 73% of cases of NF2/merlin and 80% of cases for DAL-1/DAL-1. These concordance rates are high, considering that one technique measures gene copy numbers and the other detects protein expression. Aside from possible technical difficulties, discordant results could be expected for tumors that delete a genetic region without inactivating the remaining allele. In our series, this pattern of discordance was most common and would be consistent with the higher rates of chromosome 22 loss of heterozygosity (LOH) than of NF2 mutations reported by others (36–41). In such cases, other genes may be targeted for
inactivation or the deletion may represent a random event/genetic epiphenomenon. Alternatively, a gene may be inactivated by mechanisms beyond the detection threshold of FISH, thus leading to loss of expression by immunohistochemistry, despite the lack of a detectable deletion. Examples of the latter would include small deletions, mutations, mitotic recombinations, and hyper-methylation of the promoter region. In any case, since merlin and DAL-1 losses have been commonly found in all meningioma grades, they are currently considered early tumorigenic events (26, 40, 42–47). The precise functions of these related Protein 4.1 tumor suppressors have yet to be elucidated; although merlin has been implicated in the pathogenesis of both meningiomas and schwannomas (48–50). In contrast, DAL-1 is only implicated in meningioma genesis (47, 51). It is of interest that 2 of our pediatric/NF2-associated meningiomas demonstrated DAL-1 gene deletion and loss of protein expression only after a recurrence. In both examples, NF2/merlin losses had been identified in the primary meningioma. Thus, at least in some cases, DAL-1 loss may represent a later event than NF2 inactivation. In both our prior study of sporadic adult meningiomas and in this series, combined losses were particularly common in the high-grade (grade II and III) meningiomas, further suggesting that simultaneous loss of both these Protein 4.1 members may confer additional growth advantages.

Allelic losses in NF2-associated meningiomas were recently reported by Lamszus et al who, in a study of 30 cases, found LOH for 1p in 40% and 14q in 24% (17). By FISH analysis, we found each of these regions to be deleted in approximately 60% of cases. The higher frequencies in our study likely reflect the fact that in some cases, these deletions are patchy or focal and therefore not detectable in LOH studies. In our prior series of sporadic adult meningiomas (27), we found 1p or 14q deletions in approximately 20%–30% of grade I, 55%–60% of grade II, and 70%–75% of grade III meningiomas. In contrast to that study, there was a weaker association of 1p and 14q deletions with histologic grade in the current series, such that pediatric/NF2-associated meningiomas showed deletions to be frequent even in histologically benign meningiomas. Since these genetic alterations have been previously associated with higher tumor grade, they are generally considered progression-associated or later events (27, 42, 46, 52–57). The finding of these deletions only after recurrence in a few of our patients further supports this hypothesis. In our prior evaluation of sporadic adult meningiomas, 14q deletion was also common in histologically benign meningiomas that subsequently recurred (27). Although 1p and/or 14q deletions were frequent in our histologically benign pediatric/NF2-associated meningiomas, they were not particularly associated with subsequent recurrence. Therefore, the clinical predictive value of these alterations would be limited in this patient population. Other genetic alterations may be more biologically relevant. It is of interest, however, that the combined losses of all 4 genetic sites (NF2, DAL-1, 1p, 14q) had the strongest association with tumor grade, being seen in 26% of grade I, 40% of grade II, and 83% of grade III meningiomas. We suggest that the assessment of multiple markers will have greater predictive value than a search for any single biologic marker alone.

Multiple Tumors, Young Patient Age, and Meningioma Tumor Syndromes

Multiple tumors and/or young age at presentation often suggest a cancer predisposition syndrome. In the case of meningioma, NF2 is the most appropriate consideration, although it only explains a fraction of such cases. In a series of 581 consecutive meningioma patients, Stafford et al reported 17 (3%) with multifocal disease, of which only 4 (24%) were known to have NF2 (58). In the current study, all our patients with histologic or radiologic evidence of multifocal meningiomas had NF2 and 12 (57%) of our NF2 patients had multifocal disease. In a study of 12 non-NF2 patients with multifocal disease, Stangl et al reported that in 6, the same NF2 mutation was found in all meningiomas derived from any 1 patient (59). Four had different mutations and 2 had no mutations. Based on these findings, the authors proposed that many so-called “multiple meningiomas” actually represent dural seeding from a single primary meningioma. In NF2 patients, more of a “field effect” may be involved since all meningotheelial cells already harbor a mutation in 1 of their 2 alleles. Lastly, patients with NF2 mosaicism might also be present with multiple meningiomas, but no additional findings of NF2. Unfortunately, in most of our cases of multifocal disease, tissue was not available from more than 1 tumor. Nonetheless, the 2 tumors of 1 of our patients harbored co-deletions for all 4 chromosomal markers. Yet another NF2 case with 6 separate intracranial meningiomas featured NF2 and DAL-1 deletions in all 6 tumors; 4 of the tumors also had combined 1p and 14q deletions, 1 had only a 1p deletion, and the other had neither. All 6 tumors were primarily transitional meningiomas, but 1 had focal rhabdoid and another had focal chordoid features. It is tempting to think that perhaps this patient started with a single transitional meningioma with NF2 and DAL-1 deletions that then spread, allowing each separate focus to progress and accumulate its own additional genetic alterations. However, the possibility that all 6 tumors developed separately cannot be excluded on the basis of our data.

In summary, our findings and those of others highlight the important relationship between pediatric meningiomas and NF2. This conforms to Knudson’s “two-hit” hypothesis that because only 1 additional hit is required, young patient age at disease onset would be predicted for familial tumors (60). We found that by the end of our followup,

42% of our pediatric patients qualified for the diagnosis of NF2 and that in 5 of these 14 patients (36%), meningioma was the presenting manifestation. Evans et al (14) similarly found that 3 of their 22 children presenting with meningioma later developed classic features of NF2. Given this association, we recommend that any child presenting with a meningioma should be carefully examined for other features of NF2 and closely monitored in a multidisciplinary neurofibromatosis center. For those that never qualify for the NF2 designation, it must be assumed that either these patients developed the necessary somatic mutations early in life, or perhaps germline mutations other than NF2 may be involved. Although DAL-1 would seem a logical candidate, assays for mutational screening of this gene have yet to be fully developed.

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