

# Deletion Analysis of the Adenomatous Polyposis Coli and *PTCH* Gene Loci in Patients with Sporadic and Nevoid Basal Cell Carcinoma Syndrome-Associated Medulloblastoma

Alexander O. Vortmeyer, M.D.<sup>1</sup>  
 Theodora Stavrou, M.D.<sup>2</sup>  
 Dena Selby, M.D.<sup>3</sup>  
 Guang Li, M.D.<sup>1</sup>  
 Robert J. Weil, M.D.<sup>1</sup>  
 Won-Sang Park, M.D.<sup>1</sup>  
 Young-Wan Moon, M.D.<sup>1</sup>  
 Roma Chandra, M.D.<sup>3</sup>  
 Alisa M. Goldstein, Ph.D.<sup>4</sup>  
 Zhengping Zhuang, M.D., Ph.D.<sup>1</sup>

<sup>1</sup> Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland.

<sup>2</sup> Department of Hematology-Oncology, Children's National Medical Center, Washington, DC.

<sup>3</sup> Department of Pathology, Children's National Medical Center, Washington, DC.

<sup>4</sup> Genetic Epidemiology Branch, National Cancer Institute, Bethesda, Maryland.

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Address for reprints: Alexander O. Vortmeyer, M.D., Laboratory of Pathology, National Institutes of Health, Building 10, Room 2A33, 9000 Rockville Pike, Bethesda, MD 20892.

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**BACKGROUND.** Medulloblastomas can occur sporadically or may be associated with hereditary tumor syndromes including familial adenomatous polyposis (FAP) and nevoid basal cell carcinoma syndrome (NBCCS).

**METHODS.** The authors performed a retrospective analysis for allelic deletion of the adenomatous polyposis coli (*APC*) and *PTCH* gene loci using paraffin embedded medulloblastoma specimens from patients who were admitted to Children's National Medical Center in Washington, DC, between 1982 and 1997. Thirty-five cases from which tumor and normal tissue could be procured were analyzed. Two of the analyzed cases had a positive family and personal history for NBCCS; in both cases the histology of the medulloblastoma revealed a desmoplastic phenotype. Thirty-three cases were not known to be associated with hereditary disease; 2 of those cases revealed desmoplastic and 31 cases revealed nondesmoplastic "classic" medulloblastoma histology.

**RESULTS.** Although medulloblastoma tumorigenesis has been associated strongly with FAP associated with *APC* germline mutation, none of the 22 informative sporadic cases revealed loss of heterozygosity of the *APC* gene locus. *PTCH* gene deletion was detected in the tumors of both patients with NBCCS. In contrast, only 1 of 33 sporadic medulloblastomas revealed *PTCH* gene deletion. The sporadic case with *PTCH* gene deletion did not demonstrate the desmoplastic phenotype.

**CONCLUSIONS.** In conjunction with previous studies, the data from the current study confirm that allelic deletion occurs in NBCCS-associated medulloblastomas, consistent with the role of *PTCH* as a tumor suppressor gene. However, in sporadic medulloblastomas, allelic deletion of *PTCH* is an infrequent event. Morphologic examination in conjunction with genetic analysis of *PTCH* gene deletion in medulloblastoma tissue may prove to be a quick and efficient test with which to screen for NBCCS in patients with medulloblastomas. Although medulloblastoma is a component of Turcot syndrome with demonstrated *APC* mutations, *APC* gene deletions appear to be absent or very uncommon in patients with sporadic and NBCCS-associated medulloblastomas. *Cancer* 1999;85:2662-7.

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**KEYWORDS:** medulloblastoma, nevoid basal cell carcinoma syndrome, adenomatous polyposis coli, *PTCH*, allelic deletion.

The human *PTCH* gene, a homologue of the *Drosophila* segment polarity gene patched, is a regulator of embryonic development and a putative tumor suppressor gene. Inactivation of the recently discovered *PTCH* gene<sup>1,2</sup> has been found in patients with the nevoid basal cell carcinoma syndrome (NBCCS), in which patients are pre-

disposed to developmental abnormalities and a variety of neoplasms including basal cell carcinoma, ovarian fibroma, and medulloblastomas.<sup>3,4</sup> There is increasing evidence that patients with NBCCS carry a germline mutation of the *PTCH* gene, and NBCCS-associated basal cell carcinoma and medulloblastoma develop after inactivation of the second *PTCH* allele.<sup>5</sup>

The situation in sporadic medulloblastomas is less well understood. Although *PTCH* gene mutations and deletions have been detected in small but significant subsets of tumors,<sup>6-10</sup> both copies of the *PTCH* gene appear to be structurally intact in the majority of medulloblastoma cases. In addition, there is increasing evidence that NBCCS-associated medulloblastomas are different from classic medulloblastomas. First, NBCCS-associated medulloblastomas appear to present earlier and to be less lethal than medulloblastomas not associated with the syndrome.<sup>11</sup> Second, NBCCS-associated medulloblastomas have been observed to reveal a so-called "desmoplastic" phenotype, characterized by reticulin free islands of lower cellularity that are surrounded by reticulin-rich, highly cellular tumor tissue.<sup>12</sup> In this study, we performed histopathologic classification and deletion analysis of 35 medulloblastomas. Two of the tumors had developed in unrelated patients with a positive family history of NBCCS.

Turcot syndrome, which is characterized clinically by the concurrence of a primary brain tumor and multiple colorectal adenomas, may occur secondary to mutations of the adenomatous polyposis coli (*APC*) gene or mutations of a mismatch repair gene.<sup>13</sup> Different types of brain tumors observed include medulloblastoma, glioma, lymphoma, meningioma, pituitary adenoma, and craniopharyngioma.<sup>13-15</sup> Medulloblastomas predominantly occurred in patients belonging to familial adenomatous polyposis (FAP) families associated with *APC* germline mutations, suggesting that *APC* gene mutation may predispose to medulloblastoma.<sup>13</sup> However, no loss of heterozygosity (LOH) at the *APC* locus or somatic *APC* gene mutations were found in sporadic medulloblastomas.<sup>16,17</sup> Because to our knowledge only a limited amount of data are available so far,<sup>16,17</sup> and because our material included well documented cases of different histologic subtypes and hereditary background, we investigated whether *APC* gene alterations may be observed in at least a subset of those cases.

## MATERIALS AND METHODS

Tumors from 77 cases of medulloblastoma that were diagnosed at Children's National Medical Center, Washington, DC, between 1982 and 1997 were collected and the histopathology was reviewed. Informed

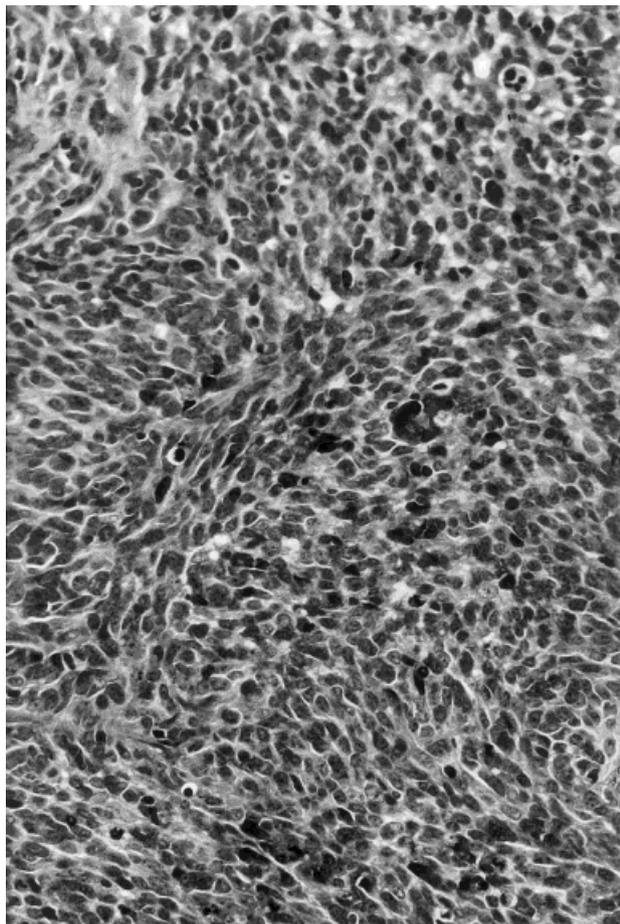
consent was obtained from subjects, parents, and/or guardians under an institutional review board-approved protocol. In a first screening process, only those cases that showed the presence of tumor tissue and at least small amounts of adjacent normal brain tissue for analysis of constitutive heterozygosity were selected (n = 35).

Among the 35 cases included in the study were 14 females and 21 males. Age at diagnosis ranged from 2 months to 18 years (median, 5 years). During follow-up for medulloblastoma, two patients (Cases 5 and 35) were found to have several features consistent with NBCCS. The patients both had jaw cysts and palmar and plantar pits; one patient also had rib anomalies (bifid ribs) and calcification of the falx cerebri. The patients both were diagnosed with medulloblastoma at age 2 years and subsequently developed multiple basal cell carcinomas in the radiation field 3 and 6 years, respectively, after radiation therapy.

For the 33 remaining patients there was no evidence of NBCCS based on review of medical records (n = 27), review of chest X-rays and computed tomography scans of the brain (n = 22), and clinical evaluation (n = 7). At last follow-up, 21 of the 35 patients still were alive.

Histopathologically, the medulloblastomas were categorized into those with classic features ("undifferentiated" medulloblastoma) and those with "desmoplastic" differentiation. Immunohistochemical or ultrastructural differentiation was not considered in the classification of the samples examined. The "undifferentiated" medulloblastomas were comprised of solid sheets of tumor cells with minimal stroma. Tumors showed monotonous cells with a high nuclear:cytoplasmic ratio, oval-to round nuclei with hyperchromatic chromatin, and scanty cytoplasm (Fig. 1). Few tumors showed Homer Wright rosettes. The desmoplastic variant showed similar features, as well as interspersed paler nodules containing cells with more abundant cytoplasm and an associated delicate fibrillar matrix (Fig. 2).<sup>12,18</sup> There was increased internodular reticulin, as confirmed by reticulin staining that was performed on the four cases classified as desmoplastic medulloblastoma. Undifferentiated medulloblastomas infiltrating into meninges were not included in this group. The histopathology was evaluated blindly, (i.e., without knowledge of the patient's NBCCS status).

Tumor cells were procured and analyzed according to a standard tissue microdissection protocol.<sup>19</sup> Tumor and normal control tissue from adjacent brain tissue were procured separately from each case. After digestion with proteinase K (0.5 mg/mL), the samples were amplified using polymorphic markers flanking



**FIGURE 1.** The “undifferentiated” medulloblastoma was comprised of sheets of undifferentiated cells with a high nuclear:cytoplasmic ratio and hyperchromatic chromatin (H & E,  $\times 312.5$ ).

the *PTCH* gene (D9S252, D9S287, D9S303, and D9S15) and the *APC* gene (APCII, and D5S346 at 5q21-22). The markers for the *PTCH* gene locus are located between 9q21 and the telomeric end of chromosome 9 in the following order: D9S15 (9q21), D9S252, D9S303 (9q21-22), and D9S287 (9q22). D9S15 is located proximal to the *PTCH* gene; the relative position of the other three markers to the *PTCH* gene is not known.

Each polymerase chain reaction (PCR) sample contained 1.5  $\mu\text{L}$  of template DNA as described earlier; 50 pmol of each primer; 20 nmol each of dATP, dCTP, dGTP, and dTTP; 15 mM of  $\text{MgCl}_2$ ; 0.1 U Taq DNA polymerase; 0.05  $\mu\text{L}$  [ $^{32}\text{P}$ ]dCTP (6000 Ci/mmol); and 1  $\mu\text{L}$  of 10 $\times$  buffer in a total volume of 10 mL. PCR was performed with 35 cycles: denaturing at 94  $^\circ\text{C}$  for 40 seconds, annealing at 55  $^\circ\text{C}$  for 1 minute, and extending at 72  $^\circ\text{C}$  for 1 minute. The final extension was continued for 10 minutes.

Labeled amplified DNA was mixed with an equal volume of formamide-loading dye (95% formamide,



**FIGURE 2.** The desmoplastic medulloblastoma showed pale islands in which tumor cells were loosely aggregated (H & E,  $\times 125$ ).

20 mM ethylene diamine tetraacetic acid, 0.05% bromophenol blue, and 0.05% xylene cyanol).

Samples then were denatured for 5 minutes at 95  $^\circ\text{C}$ , loaded onto a gel comprised of 6% acrylamide (acrylamide:bisacrylamide ratio of 29:1), and electrophoresed at 1800 volts for 2 hours. After electrophoresis, the gels were transferred to 3-mm Whatman paper and dried. Autoradiography was performed with Kodak X-OMAT film (Eastman Kodak, Rochester, NY). Total or near-total loss of one allele was interpreted as LOH. All samples were analyzed in duplicate or triplicate, and the results were evaluated blindly (i.e., without knowledge of the patient’s NBCCS status).

## RESULTS

Histopathologic analysis of the 35 tumors revealed 31 medulloblastomas with “undifferentiated” phenotype (Fig. 1) and 4 medulloblastomas with desmoplastic phenotype (Cases 5, 12, 26, and 35) (Table 1) (Fig. 2). Two of the four desmoplastic medulloblastomas were

**TABLE 1**  
Deletion Analysis of the *APC* and *PTCH* Gene Loci of 35 Medulloblastomas

Case no.	Age at diagnosis (mos)	Heridity	Histopathology	APC gene locus		PTC gene locus			
				D5S346	APCII	D9S15	D9S252	D9S303	D9S287
1	64	sporadic	MB, undiff	na	na	na	na	○	○
2	23	sporadic	MB, undiff	–	–	○	○	○	○
3	13	sporadic	MB, undiff	○	○	○	○	na	na
4	154	sporadic	MB, undiff	○	○	○	○	○	○
<b>5</b>	24	NBCCS	MB, desmoplast BCC	○	○	●	–	–	●
6	135	sporadic	MB, undiff	–	○	–	○	○	○
7	124	sporadic	MB, undiff	na	–	–	○	○	–
8	104	sporadic	MB, undiff	○	○	–	○	○	○
9	135	sporadic	MB, undiff	–	○	○	○	○	na
10	75	sporadic	MB, undiff	–	–	○	○	–	○
11	48	sporadic	MB, undiff	–	–	○	○	○	○
<b>12</b>	2	sporadic	MB, desmoplast	na	na	○	○	○	○
13	83	sporadic	MB, undiff	–	–	–	○	○	○
14	37	sporadic	MB, undiff	na	○	na	○	na	na
15	164	sporadic	MB, undiff	○	na	na	na	–	○
16	224	sporadic	MB, undiff	○	○	○	○	○	○
17	15	sporadic	MB, undiff	○	○	–	○	○	○
18	131	sporadic	MB, undiff	○	○	○	○	○	○
19	45	sporadic	MB, undiff	–	na	na	na	na	○
20	48	sporadic	MB, undiff	○	○	na	○	○	○
21	19	sporadic	MB, undiff	○	○	na	○	○	○
22	4	sporadic	MB, undiff	na	○	–	○	○	○
23	53	sporadic	MB, undiff	○	○	na	○	○	na
24	146	sporadic	MB, undiff	na	na	na	●	–	●
25	113	sporadic	MB, undiff	na	○	na	○	○	○
<b>26</b>	73	sporadic	MB, desmoplast	○	○	na	○	○	○
27	60	sporadic	MB, undiff	○	○	na	○	○	○
28	33	sporadic	MB, undiff	–	–	–	○	○	○
29	16	sporadic	MB, undiff	–	–	–	–	○	–
30	134	sporadic	MB, undiff	–	–	○	na	na	na
31	124	sporadic	MB, undiff	○	○	○	–	○	○
32	37	sporadic	MB, undiff	–	○	○	○	–	○
33	61	sporadic	MB, undiff	○	○	–	–	○	○
34	10	sporadic	MB, undiff	–	–	–	–	○	–
<b>35</b>	31	NBCCS	MB, desmoplast BCC	○	○	●	●	–	●

APC: adenomatous polyposis coli; MB: medulloblastoma; undiff: undifferentiated; na: no amplification product obtained; open circle: no loss of heterozygosity; NBCCS: nevoid basal cell carcinoma syndrome; desmoplast: desmoplastic; BCC: basal cell carcinoma; closed circles: loss of heterozygosity.

Case numbers given in boldface type (5, 12, 26, and 35) refer to cases with desmoplastic morphology.

found in the NBCCS patients (Cases 5 and 35) and two desmoplastic medulloblastomas were detected in the group of sporadic cases (Cases 12 and 26).

Deletion analysis of the *APC* gene locus with primers D5S346 and APCII revealed amplification products in 32 cases for at least 1 of the 2 markers, and 22 of these 32 cases were informative. None of the informative cases revealed LOH of the *APC* gene locus (Table 1).

Deletion analysis of the *PTCH* gene locus with D9S252, D9S287, D9S303, and D9S15 showed all cases to be informative for at least one of the applied mark-

ers. Three cases revealed LOH of the *PTCH* gene locus. Among these three cases were the medulloblastomas of both NBCCS patients (Cases 5 and 35). The third case with *PTCH* LOH was a sporadic medulloblastoma with “undifferentiated” morphology (Case 24). The remainder of the cases did not show *PTCH* LOH.

Paraffin embedded material from two NBCCS-associated basal cell carcinomas also were obtained (Cases 5 and 35) and analyzed. Both cases revealed *PTCH* LOH, similar to the medulloblastoma tissue from the same patients.

## DISCUSSION

Thirty-five medulloblastomas were subclassified histologically according to standard criteria and subsequently analyzed for allelic deletion of the *APC* and *PTCH* gene loci. Because FAP associated with *APC* gene mutation predisposes to medulloblastoma,<sup>13</sup> it has been hypothesized that *APC* gene alterations may be associated with the development of sporadic medulloblastomas. We found no evidence of allelic deletion in the medulloblastomas analyzed in this study. In conjunction with a previous mutation analysis of 41 cases<sup>16</sup> and a previous deletion analysis of 27 sporadic cases,<sup>17</sup> we were able to confirm the absence of *APC* gene alterations in sporadic medulloblastomas.

On histopathologic evaluation, both NBCCS-associated medulloblastomas (Cases 5 and 35) revealed a desmoplastic phenotype. This finding is consistent with the hypothesis that NBCCS is associated preferentially with medulloblastomas of desmoplastic subtype.<sup>12,20</sup> At present, it is not entirely clear whether or to what extent other histologic subtypes can be observed in NBCCS-associated medulloblastomas.

Both desmoplastic, NBCCS-associated medulloblastomas showed LOH of the *PTCH* gene locus, which is consistent with previous studies<sup>12,20</sup> and with the postulated tumor suppressor role of *PTCH*. In addition, we detected *PTCH* LOH in basal cell carcinomas from both patients. However, only one of three NBCCS-associated medulloblastomas showed *PTCH* LOH in an analysis by Cowan et al.<sup>20</sup> and a more detailed study including a larger number of NBCCS-associated tumors and sufficiently fine deletion mapping is needed. In addition, tissue microdissection should be performed in each case to avoid "contamination" of tumor tissue with normal or reactive cells.<sup>19</sup>

Two additional sporadic cases revealed desmoplastic morphology but no evidence of *PTCH* LOH. A recent study indicated that *PTCH* mutations occurred exclusively, and *PTCH* deletions preferentially, in sporadic desmoplastic medulloblastomas.<sup>21</sup> It has been concluded that among sporadic medulloblastomas, the desmoplastic subtype may not only represent a morphologically but also a genetically distinct subgroup. However, Wolter et al. detected *PTCH* mutations in 2 of 15 nondesmoplastic and 2 of 17 desmoplastic sporadic medulloblastomas.<sup>9</sup> Further studies will be needed to clarify to what extent *PTCH* gene alterations play a role in sporadic desmoplastic and nondesmoplastic medulloblastoma tumorigenesis.

By far the largest subset of tumors in our study was comprised of sporadic medulloblastomas with nondesmoplastic, classic morphology. Among those tumors, we were able to detect deletion of the *PTCH*

gene locus in one case. From these findings it can be concluded that allelic deletion of *PTCH* is an infrequent finding in sporadic classic medulloblastomas, and a major role for *PTCH* in sporadic medulloblastoma tumorigenesis appears to be unlikely.

NBCCS-associated medulloblastomas are reported to occur between the ages of 2 and 3 years whereas the mean age at the time of the first basal cell carcinoma presentation among NBCCS patients without medulloblastoma is 20 years.<sup>3</sup> In the majority of NBCCS patients who develop medulloblastoma, medulloblastoma is expected to present as the first NBCCS-related tumor. Therefore, prospective deletion analysis of desmoplastic medulloblastomas may help to identify NBCCS more efficiently among patients presenting with medulloblastoma in the future.

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