Desmoid fibromatosis is a rare, locally aggressive fibroblastic/myofibroblastic tumor that occasionally involves children. We examined a series of pediatric desmoids for CTNNB1 mutations, seen in sporadic tumors, and APC germline mutations, associated with familial adenomatous polyposis (FAP). Forty-four desmoids in pediatric patients were identified in the pathology files of 2 large referral centers (1995–2009). Clinical charts were reviewed for history of FAP. Germline APC gene mutations were determined on blood samples from patients presenting with FAP. Immunohistochemistry for beta-catenin was performed. CTNNB1 genotyping was done by Sanger sequencing on formalin-fixed paraffin-embedded tissue. CTNNB1 mutations were observed in 29 of 44 (66%) desmoids, with 3 mutations identified: T41A (64%), S45F (29%), and S45P (7%). Germline APC mutations were present in 7 (16%) desmoid patients. Eight (18%) patients had desmoids that were wild type for CTNNB1 and had no known clinical signs or family history suspicious for FAP at the time of testing or with extended follow up (n = 6). Beta-catenin nuclear labeling was observed in 38 of 41 (92%) tested cases, 34 (89%) of which showed mutations in either CTNNB1 (n = 29) or APC (n = 5). Nuclear localization of beta-catenin was seen in the majority of pediatric desmoids and was most often associated with somatic mutations in CTNNB1. However, a significant proportion of pediatric patients harbored germline mutations in APC. Given the implications, genetic counseling is recommended for children diagnosed with desmoid tumors lacking CTNNB1 mutations because this population is enriched for FAP patients.

Key words: APC, beta-catenin, CTNNB1, desmoid, pediatric, Wnt

INTRODUCTION

Desmoid fibromatosis is a rare mesenchymal neoplasm with myofibroblastic/fibroblastic differentiation that affects young to middle-age adults and occasionally children. It can involve various sites, including the extremities, head and neck, trunk, and intra-abdominal locations, and classically arises from the deep aponeurosis. Histologically, these tumors are characterized by long sweeping bundles of bland spindle cells and collagen fibers [1,2]. Surgical excision has been the main treatment modality for desmoids. Despite their bland histologic appearance, these tumors often behave in a locally aggressive manner with frequent recurrences [1–3].

Desmoid fibromatosis most commonly occurs sporadically but can rarely be part of a familial cancer syndrome called “familial adenomatous polyposis” (FAP), which is associated with germline mutations of the APC tumor suppressor gene. Affected members of these families have up to a 15% risk of developing desmoid fibromatosis [4–7]. In both situations, activation of the canonical Wnt/beta-catenin signaling pathway has been identified to play a key role [8–14]. Beta-catenin is encoded by the CTNNB1 gene and acts in both cellular adhesion and transcription. The cytosolic levels of beta-catenin are controlled by the APC complex, which
phosphorylates key sites on the protein marking it for proteosomal destruction [3,12,15]. In sporadic cases, mutations occur in CTNNB1, with the most common types being point mutations involving phosphorylation sites encoded by exon 3 [3,11,12,16–18]. In FAP, germline mutations occur in the APC tumor suppressor gene in which 1 defective copy is inherited, leaving only 1 functional copy and the subsequent loss of heterozygosity at the wild-type allele believed to initiate tumorigenesis. Patients with FAP develop numerous colonic polyps and progress to multiple cancers, most characteristically colon carcinomas, as a result of the loss of the remaining functional copy of APC [8,10,11,18,19]. In both sporadic and FAP-associated desmoids, nonphosphorylated beta-catenin accumulates in the cytoplasm and translocates to the nucleus, activating the transcription of genes that promote proliferation and increased cell survival [3].

Immunohistochemical studies can be used to detect nuclear accumulation of beta-catenin and therefore serve as a useful marker for an activated Wnt pathway [9,16,20–24]. Large studies have shown that beta-catenin nuclear labeling is present in up to 80%–100% of desmoid fibromatosis and is absent in most other fibrous tumors that mimic desmoids [16,20–24]. Consequently, immunohistochemical study can be a useful tool in the diagnosis of desmoid fibromatosis.

Several studies have demonstrated the utility of CTNNB1 and APC mutation testing in the diagnosis and management of patients with desmoid fibromatosis. Large series on sporadic desmoids have revealed a high rate of mutation (up to 87%) in codons 41 and 45 of exon 3 of the CTNNB1 gene [3,11,16,17,24–28]. In patients with FAP, APC gene mutations have also been associated with the development of desmoids [4–6,18,29–31]. Mutational studies in the pediatric patient population may have critical clinical significance, because the presentation of desmoid tumors can sometimes be the initial presenting sign of FAP [32–34]. Therefore, we investigated a series of pediatric desmoid tumors to identify the rate of underlying CTNNB1 mutations and their relationship with previously established germline APC mutations.

METHODS
Patient and tumor tissues
Following institutional review board approval, 44 formalin-fixed, paraffin-embedded desmoid tumor specimens from 44 patients (age ≤19 years) were retrieved from the pathology archives of The University of Texas M. D. Anderson Cancer Center (UTMDACC) and Texas Children’s Hospital from 1988 to 2009. Dermal scars were used as controls. All specimens were screened by experienced soft-tissue pathologists (A.J.L. and W.L.W.), and only those confirmed to be desmoid tumors adequate for molecular analysis were included. Clinical information including demographics, site, and history of FAP or other hereditary cancer syndromes were obtained from patient medical records. APC gene mutations were previously determined prospectively on blood samples from patients presenting with clinical evidence of FAP under a routine clinical treatment algorithm. Briefly, molecular testing for germline mutations in APC was performed using gene sequencing and/or multiplex ligation-dependent probe amplification (MLPA) techniques. For gene sequencing, all exons were amplified with exon-specific primers and directly sequenced using dye terminator chemistry (Applied Biosystems, Carlsbad, CA, USA). Large heterozygous deletions or duplications were detected using the P043-C1 MLPA probe-mix for APC (MRC-Holland, Amsterdam, The Netherlands), which detects copy number alterations in all 18 exons of the APC gene. The site was categorized as superficial trunk, deep trunk/mesentery, extremity, or head and neck.

Immunohistochemistry
Beta-catenin immunohistochemistry study was performed using the polymeric biotin-free horseradish peroxide method on a Microsystems Bond Max stainer (Leica Microsystems, Buffalo Grove, IL, USA). Four micrometer-thick sections were prepared from formalin-fixed paraffin-embedded tissue blocks and dried in a 60°C oven for 20 minutes. Slides were pretreated with enzyme-induced epitope retrieval for 2 minutes followed by incubation with antibodies against beta-catenin (clone 14, dilution 1:500; BD Biosciences, San Jose, CA, USA) in an automated Bond Max stainer. An anti-mouse secondary antibody and the refine polymer detection kit (Leica Microsystems) were used for immunostaining, with 3,3'-diaminobenzidine used as a chromagen. Positive and negative controls were run in parallel. Labeling intensity was graded as none, weak, moderate, or strong, as previously described [16].

CTNNB1 genotyping
CTNNB1 genotyping was performed as previously reported [16]. Briefly, DNA was extracted from 2 20 μm-thick formalin-fixed, paraffin-embedded tissue rolls cut from blocks with at least 75% tumor using the QiAmp DNA mini kit DNA isolation kit (Qiagen Valencia, CA, USA). The CTNNB1 gene was amplified using primers (BCAT-DES-F: 5-AGTCACTGGCGACAAACAGTC-3 and BCAT-DES-R: 5-TCTTCTCCAGGATTGCCTT-3) and with thermocycling conditions to amplify exon 3 of CTNNB1 (phosphorylation domain, codons 30 to 48) with appropriate controls. Polymerase chain reaction products were detected by gel electrophoresis in 2% agarose. Direct sequencing of the polymerase chain reaction products used the above primers (forward and reverse). Sequencing was performed on an ABI Prism 3100-Avant genetic analyzer (Applied Biosystems, Foster City, CA, USA). Both forward and reverse strands were analyzed by the NCBI Blast Alignment Tool (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi) to identify mutations.
RESULTS

The cohort consisted of 21 males and 23 females (Table 1). The age range was 1 to 19, with a median of 15 years. Sites involved included extremity (n = 16; 36%), deep trunk (n = 12; 27%), superficial trunk (n = 11; 25%), and head and neck (n = 5; 11%). Beta-catenin nuclear staining was observed in 38 of 41 (92%) tested cases, with the majority showing strong nuclear labeling (n = 26), while 9 cases had moderate labeling and 3 cases had weak and no labeling each (Fig. 1). Exon 3 of the CTNNB1 gene was found to be mutated in 29 of 44 (66%) tested desmoids (Table 1). The 3 mutations previously described in the adult desmoids series were identified: ACC to GCC at codon 41 resulting in a coding substitution of threonine to alanine (T41A), TCT to TTT at codon 45 resulting in a coding substitution of serine by phenylalanine (S45F), and TCT to CCT at codon 45 resulting in a coding substitution of serine by proline (S45P). The overall mutation frequency of this pediatric desmoid series was T41A (n = 18; 26%), S45F (n = 9; 20%), and S45P (n = 2; 4%). APC mutations were present in 7 (16%) desmoids. Eight patients were wild type for CTNNB1, but material for APC testing was not available.

Of 38 desmoids with beta-catenin nuclear staining (n = 41 total), 89% showed mutations in either CTNNB1 (n = 29 of 29 total cases) or APC (n = 5 of 7 total cases) (Table 1). Correlation with beta-catenin immunohistochemical studies revealed that the majority of tumors with strong nuclear labeling carried a T41A mutation (n = 15) and that this was the predominant labeling pattern for this mutation type, with the remaining 2 T41A cases exhibiting moderate labeling intensity. Both tumors with S45P mutations had strong nuclear labeling. Tumors with S45F exhibited predominantly moderate nuclear labeling (n = 5, 56%), with some cases demonstrating strong (n = 3) and weak (n = 1) nuclear labeling pattern. Tumors with APC mutations had varied nuclear labeling intensity. The 3 cases that were negative for beta-catenin labeling were wild type for CTNNB1. One patient carried a germline APC mutation; the remaining desmoid associated with germline APC mutation was not available for staining.

Review of the clinical parameters revealed a female predominance in patients with tumors with wild-type genotype and with S45F mutations (n = 6 of 8 wild type; n = 6 of 9 S45F) and a male predominance in those with APC mutations (n = 5 of 7). Those with T41A and S45P had similar sex distribution (n = 8 of 10 T41A; n = 1 of 2 S45P) (Table 1). A greater proportion of desmoids with S45F mutations occurred in the extremities (n = 8 of 9). The majority of patients diagnosed with desmoid tumors were older than 10 years irrespective of mutation type, with patients with T41A, S45P, and wild-type tumors being slightly younger (median ages 11.5, 10.5, and 13 years, respectively) than those with S45F and APC mutations (age 16 and 15 years, respectively).

Table 1. Summary of clinical correlates and beta-catenin immunohistochemical labeling patterns by mutation type

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>41A</th>
<th>45F</th>
<th>45P</th>
<th>APC</th>
<th>CTNNB1 WT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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</tr>
<tr>
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<td>52</td>
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</tr>
<tr>
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<td>48</td>
<td>10</td>
<td>56</td>
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<td>33</td>
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<tr>
<td>Median age (range)</td>
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<td></td>
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<tr>
<td>&lt;10 years</td>
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<td>32</td>
<td>7</td>
<td>39</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>&gt;10 years</td>
<td>30</td>
<td>68</td>
<td>11</td>
<td>61</td>
<td>7</td>
<td>88</td>
</tr>
<tr>
<td>Tumor site</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Superficial trunk</td>
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<tr>
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<td>36</td>
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<td>39</td>
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<tr>
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<td>33</td>
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<tr>
<td>Head and neck</td>
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<td>2</td>
<td>11</td>
<td>1</td>
<td>11</td>
</tr>
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<td>17</td>
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<td>9</td>
<td></td>
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<td>7</td>
<td>0</td>
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</tr>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>11</td>
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<td>63</td>
<td>15</td>
<td>88</td>
<td>3</td>
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</tbody>
</table>

CTNNB1 WT = wildtype for CTNNB1.
Distribution of mutational status tabulated with patient clinical and tumor data.
Total row gives percentage of each tumor type in the population.

CTNNB1 AND APC TESTING IN PEDIATRIC DESMOIDS 363
No CTNNB1 mutations were seen in the desmoid cases associated with germline APC mutation \((n = 7)\). The 8 remaining cases lacked CTNNB1 mutation; APC mutation analysis was not available, because it was not performed due to lack of clinical suspicion. Six of these 8 cases had more extended follow up and none had significant clinical signs or family history of FAP.

DISCUSSION

Similar to their counterparts occurring in adults, the vast majority of pediatric desmoids are associated with inappropriate activation of the Wnt/beta-catenin signaling pathway. Immunohistochemical detection of the nuclear accumulation of beta-catenin in routine surgical pathology specimens has been previously shown to be a useful marker for desmoids and an activated Wnt pathway. In our study, nuclear staining for beta-catenin was detected in almost all cases (90%). Large series on adult desmoids have reported similar high rates of beta-catenin labeling, with some as high as 100%, depending on the antibodies and antigen retrieval techniques used [16,20,21,23]. A lower rate of labeling was seen in 1 large study of pediatric desmoids, in which only 21 of 50 (42%) deep fibromatoses were found to show nuclear beta-catenin labeling [22]. However, a more recent study revealed findings similar to ours, with 30 of 32 (94%) pediatric desmoids having beta-catenin labeling [24]. The differences between studies are not entirely clear, but differences in immunohistochemical sensitivity and detection techniques are a possible explanation. Overall, our findings confirm the diagnostic utility of beta-catenin immunohistochemical study to support the diagnosis of desmoid fibromatosis and the role of Wnt/beta-catenin signaling pathway activation in pediatric desmoids.

In our pediatric series, the majority of patients (69%) harbored a mutation of the CTNNB1 gene. This is similar to what has been reported for large series of sporadic adult desmoids, in which 73% and even as high as 87% carried a CTNNB1 mutation [3,11,16,17,24–28]. Some of these studies included only sporadic cases and excluded FAP patients. In our series, 29 of 37 sporadic (non-FAP) cases (78%) carried CTNNB1 mutations, similar to recent large adult series [16,17,28]. We saw a similar distribution of mutation types as in previous reports, with the most common type being T41A. S45P was found to be uncommon in pediatric cases, as in other adult desmoid studies. Previous studies have shown that patients with desmoids that are CTNNB1 wild type tend to be female and those with T41F tend to be male; those with S45F tend to occur in the extremities [16]. In our study, a female predominance was also noted not only in patients with wild-type genotype for CTNNB1 but also in patients with tumors harboring a S45F mutation. Occurrence in the extremities was over-represented in our patients with S45F. No male predominance was seen in our patients with T41A, whereas a male predominance was seen in patients with APC mutations. These differences maybe attributed to vagaries of a relatively small sample size.
One large study also suggested that the S45F mutation might be associated with increased local recurrence than other local types, although a 2nd large study did not completely confirm this finding [16,28]. In a series of 138 adults with sporadic desmoids, patients with S45F mutations had a significantly poorer 5-year recurrence-free survival than those with T41A mutations and wild type for CTNNB1 (23% vs 57% vs 65%, respectively) [16]. In that study, desmoids with S45F mutations also exhibited weak to moderate labeling of beta-catenin, and not surprisingly a poorer recurrence-free survival was found in desmoids with weaker nuclear labeling than those with strong nuclear labeling patterns [16]. A similar finding was seen in a series of 32 pediatric desmoids in which patients with recurrent tumor were more frequently found to have S45F than T41A [24]. Our series lacks the power to detect a statistically significant difference between mutation types. A higher percentage of patients with S45F recurred over patients with T41A (33% vs 17%) in our series. We lacked sufficient patients to determine how S45P mutated tumors behaved relative to the other groups. The concept of different mutation types conferring a different phenotype is intriguing and suggests the possibility that mutant beta-catenin variants may affect and interact with the Wnt pathway differently in children than in adults. Patients with mutants associated with a more aggressive phenotype may warrant additional treatment. Further studies are needed and are in progress.

Mutations involving APC are also known to contribute to the occurrence of desmoids. However, unlike desmoids with CTNNB1 mutations that are sporadic, desmoids with APC mutations can be germline and associated with FAP. Sporadic desmoids with APC mutations have been rarely reported [35]. All of our APC mutated cases were discovered from germline DNA testing and thus are FAP related. Besides developing numerous colonic polyps, these patients are at increased risk for developing multiple cancers, including colon cancer, at a young age and are at increased risk for developing desmoids, including as a late complication of surgery. Other associated conditions include multiple follicular cysts, fibromas, osteomas, retinopathies, and dental anomalies with variable penetrance, depending on the APC mutation [29,36,37]. In adults, it is likely that patients with hereditary APC mutations will already have presented clinically with FAP findings. However, children may occasionally present with desmoid tumors as the initial recognized or presenting sign of FAP [32–34]. In our series, APC mutations were present in 7 (16%) of pediatric desmoids. This high frequency demonstrates the importance of correctly identifying and classifying desmoids on the basis of their underlying genetic etiology in children, although the relatively high percentage of

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**Figure 2. Proposed algorithm in handling pediatric patients with desmoids.** Diagnosis is confirmed by a pathologist, preferably one with expertise, using histology and supplemental beta-catenin immunohistochemistry. Clinical history should be examined for any signs or history of familial adenomatous polyposis (FAP) indicating that the tumor is likely FAP related. All tumors should undergo CTNNB1 exon 3 mutational analysis. If positive for a characteristic CTNNB1 mutation, the tumor should be considered sporadic. If the tumor is negative for common CTNNB1 mutations and the patient is not known to have FAP, the child should be referred to genetic counseling to include work up for FAP and potential testing of APC gene. Patients who are negative for CTNNB1 and APC should be considered to have sporadic wild-type desmoids. Genetic counseling is also recommended to any patient with signs pointing to FAP, because even patients with FAP could conceivably develop a sporadic desmoid. A tumor could also have both an APC (germline) and CTNNB1 (sporadic) mutation, but this has not been previously described.
FAP patients may also be a reflection of referral patterns to our institutions. It is possible that the 8 patients whose tumors were wild type for CTNNB1 but not available for additional testing may harbor a germline APC mutation diagnostic of FAP. However, additional extended follow up for the majority of these patients failed to reveal signs and symptoms of FAP, making FAP very unlikely. These patients could also have somatic (tumor only, not FAP related) mutations in APC, but this possibility was not explored due to the complexity of such testing in formalin-fixed material. Alternatively, other genes in the Wnt pathway may be involved. None of the desmoids in patients with germline APC mutations harbored CTNNB1 mutations, confirming that a lack of such mutations defines a population with increased risk of FAP.

Other fibroblastic/myofibroblastic lesions that are associated with FAP include gardner fibroma and nuchal-type fibromas [38,39]. In contrast to desmoid fibromatosis, these tumors are histologically hypocellular with bland spindle cells admixed with dense thick collagen bundles and without a fascicular architecture. Most occur subcutaneously in the back and neck but can be found at any site. Similar to desmoids, both lesions have been shown to have nuclear accumulation of beta-catenin, indicating the role of Wnt pathway in these lesions [38]. As mentioned above, both can be associated with germline mutations in APC. The examination of gardner fibromas was beyond the scope of this study but are important fibroblastic/myofibroblastic proliferations to recognize in pediatric populations, because they can be the initial presenting sign of FAP similar to desmoid [39].

Given the possible significance of mutation testing, our findings suggest that an algorithm could be applied for suspected desmoid tumors in children (Fig. 2). Clinical algorithms have already been proposed for adults with desmoid and colonic carcinoma and no history of FAP [40]. First, the diagnosis of desmoid should be confirmed by a pathologist, preferably one with appropriate expertise, using histology and with supplemental beta-catenin immunohistochemistry. Because most desmoids will have strong nuclear staining differentiating them from other mimics, this assay can help support the diagnosis, bearing in mind a negative beta-catenin does not entirely exclude desmoid fibromatosis, particularly in needle biopsies (personal observation, W.L.W. and A.J.L.). Upon confirmation of desmoid fibromatosis in a child, the clinical history should be examined for any signs or familial history of FAP. A positive history would likely indicate that the tumor is related to a familial APC mutation. At any rate, all tumors should undergo CTNNB1 exon 3 mutational analysis. The vast majority of sporadic tumors should be positive for 1 of the 3 common mutations in CTNNB1. If the tumor does not have a CTNNB1 mutation and the patient is not known to have FAP, we recommend referring all children for genetic counseling and potential germline testing of the APC gene as per the proposed scheme illustrated in Figure 2. This recommendation is based on our finding that germline APC and somatic CTNNB1 mutations appear to be mutually exclusive. Although sporadic desmoids can lack somatic CTNNB1 mutations (in 15%–20% of adult cases), in our series, this fraction of samples was enriched for patients found to have germline APC mutations and clinical evidence of FAP. A limitation of this study is that not all patients were assessed for germline APC mutations, but these data are not possible to obtain given the expense and difficulty of the analysis and lack of appropriate material (peripheral blood lymphocytes) available for testing in this retrospective study. In some patients, follow up was limited, and it is possible that in some patients the signs of FAP may not have fully developed. Nonetheless, there was no clinical evidence of FAP on follow up in any of the patients with a CTNNB1 mutation.

In summary, we report one of the largest series of pediatric desmoid fibromatosis with the addition of CTNNB1 and APC genotyping. As in their adult counterparts, pediatric desmoids have Wnt pathway alternations leading to increased beta-catenin nuclear accumulation predominantly due to sporadic mutations in CTNNB1 and occasionally germline mutations in APC. Other mechanisms are also likely to exist, such as sporadic APC mutations in neoplastic cells and other alterations leading to Wnt pathway activation and nuclear accumulation of beta-catenin. Our findings suggest that all pediatric patients with desmoid tumors should have genotyping for CTNNB1 performed as a relatively simple screening tool, which in combination with clinical history can help identify a population at higher risk for FAP and potentially avoid more arduous and costly consultations and complex APC gene mutation testing. Given the serious implications, we recommend that pediatric patients with tumors lacking CTNNB1 mutations be referred for genetic consultation, FAP evaluation, and possible confirmatory APC gene mutation testing based on the pretest likelihood of FAP from the clinical/family history and the wishes of the patient (and family).

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REFERENCES


