Genetic Heterogeneity in Familial Juvenile Polyposis

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Abstract

Juvenile polyposis syndrome (JPS) is an autosomal dominant syndrome characterized by multiple gastrointestinal hamartomatous polyps in the absence of the extraintestinal features that are classic for other hamartomatous polyposis syndromes, such as Bannayan-Riley-Ruvalcaba syndrome (BRRS) and Cowden disease (CD). About 50% of BRRS and >80% of CD demonstrate germ-line mutations in the tumor suppressor and dual phosphatase, PTEN. Germ-line mutation of PTEN as a cause for JPS in a child is controversial because extraintestinal manifestations that would exclude JPS could appear after adolescence, altering the clinical diagnosis. Here, we investigated a family in which the 55-year-old father, who lacks thyroid or skin findings characteristic of CD, demonstrated a germ-line mutation in PTEN that was passed to identical twin daughters, who both manifested JPS. The mutation was a deletion of five bases beginning seven bases from the start of exon 4 of PTEN, which caused aberrant transcripts by reverse transcription-PCR that were absent from a normal individual. Thus, mutations in PTEN are associated with JPS in addition to CD and some BRRS families, although the incidence of PTEN germ-line mutations in JPS might be more rare than that reported for SMAD4, a gene found to be mutated in approximately one-half of the JPS families investigated.

Introduction

JPS (MIM 174900) is a rare condition characterized by the occurrence of multiple hamartomatous polyps in the gastrointestinal tract. The definition for juvenile polyposis has undergone much modification with delineation of the syndrome based on the presence of the number of polyps and the location of the polyps. The most common variant of the syndrome are juvenile polyps found only in the colon (1). The earliest classification distinguishing solitary juvenile polyps from juvenile polyposis required the presence of: (a) more than 10 colonic juvenile polyps; (b) juvenile polyps throughout the gastrointestinal tract; and (c) any number of juvenile polyps in an individual with a family history of juvenile polyposis (2). Recent authors proposed a working definition requiring only five colonic polyps, and some have suggested screening patients with as few as three juvenile polyps (3, 4).

Juvenile polyposis may occur as a component of BRRS (MIM 175200), which is characterized by macrocephaly, pigmented spotting of the genitalia, delayed development in childhood, and lipid storage myopathy in some cases (5). Hamartomatous polyposis is also seen as a component of CD (MIM 158350), which consists of multiple orocutaneous hamartomas, facial trichilemmomas, fibrocystic disease, and cancer of the breast, nontoxic goiter, thyroid cancer, and hamartomatous polyposis (6). CD carries an age-related penetrance of 90% by 20 years of age and only a 10% penetrance below age 15 (7). Because of this age-related penetrance for CD, a diagnosis of JPS might only be made if extraintestinal features of CD are absent in a patient beyond the adolescent years.

The genetic classification of the hamartomatous syndromes has been investigated in recent years with CD and BRRS mapped primarily to chromosome 10q22–24, the locus for PTEN (7–9), a tumor suppressor gene with dual specificity phosphatase activity (10, 11). Subsequently, germ-line mutations in PTEN were found in some families with CD and BRRS (12, 13). JPS has been shown to map to the SMAD4 gene at chromosome 18q21.1, and SMAD4 is mutated in ~50% of familial cases of JPS (14, 15). This region encodes a critical intracellular mediator in the transforming growth factor β signaling pathway and is thought to play an important role in the development of gastrointestinal tumors (15).

Additionally, studies of large kindreds excluded the linkage of JPS to PTEN (16). However, one report described a deletion at chromosome 10q22 in a JPS patient and another group found three JPS cases harboring germ-line PTEN mutations (17, 18). In the first report, the patient was 10 months old at presentation and demonstrated dysmorphic features, a cardiac lesion, abnormal extremities, and developmental delay as well as hamartomatous polyposis (17). The multiple congenital abnormalities raised the possibility that this patient may have BRRS rather than JPS, but these features are not diagnostic for BRRS. In the second report, one case was a 72-year-old patient with a history of a thyroid nodule, raising the suspicion that the patient had CD (18, 19). The other two cases were children, ages 14 and 3 (18). The young age of the children raises the possibility that they might develop CD at a later age that would change their diagnosis from JPS to CD. Thus, the association of germ-line PTEN mutations as a cause for JPS is not yet clearly substantiated.

In this report, we identified a germ-line mutation of PTEN in a family in which identical twin 6-year-old girls were diagnosed with JPS. Their 55-year-old father lacked manifestations of CD or BRRS and had the same mutation in PTEN.

Materials and Methods

Family Pedigree and Clinical Evaluation. Twin A presented to the Pediatric Gastroenterology group at University of California-San Diego with a 2.5-cm prolapsed polyp and >1 year of hematochezia. A complete history revealed normal body growth and development pattern. Physical examination indicated a normal-sized head circumference, lack of cutaneous abnormalities, and a nonpalpable thyroid. Endoscopic evaluation revealed five hamartomatous polyps to the region of the splenic flexure. These polyps were removed and confirmed by histology to be juvenile polyps. Consequently, a diagnosis of...
JPS was established. Because of the presentation of Twin A, Twin B, a monozygotic twin, was also evaluated and noted to have a normal development and growth history as well as a normal physical exam. Twin B was also noted endoscopically to have five hamartomatous polyps distal to the splenic flexure and the diagnosis of JPS was made. Both parents were evaluated with complete histories and physical examinations and endoscopic evaluations. Both parents had a normal developmental history and growth. There was no past history of any malignancies or thyroid abnormalities. Physical exam revealed normal systems and lack of any cutaneous findings along with nonpalpable thyroids. Colonoscopies were performed on both parents and no polyps were noted on either evaluation.

**Polyp Histology.** Endoscopic polypectomies were performed on both twins, and tumor pathology revealed multiple dilated glandular epithelium with mucin-filled cysts and inflammatory infiltrates in the lamina propria. No adenomatous changes were noted, and stromal infiltrates were absent. There were no bands of smooth muscle present. The histological findings were consistent with juvenile polyps.

**PCR Amplification and Sequencing.** DNA was extracted from whole blood lymphocytes from the twin girls as well as from both parents using phenol-chloroform for phase extraction and subsequent ethanol precipitation. All nine exons of PTEN were analyzed by PCR as described previously (20). The resultant PCR products were confirmed for length by electrophoresis on a 1% agarose gel stained with ethidium bromide. PCR products were then filtered through a Microcon YM-30 filter (Millipore Corp, Bedford, MA) and subjected to cycle sequencing using the Big Dye Terminator chemistry supplied by Applied Biosystems (PE Applied Biosystems, Foster City CA). The dye-terminated product was filtered through a Sepharose column (Princeton Separations, Adelpha, NJ), and subjected to analysis in an Applied Biosystems 310 Genetic Analyzer (PE Applied Biosystems). All of the sequences were confirmed in both the forward and reverse direction.

**Cloning of PCR Products.** PCR products were then cloned to separate individual allelic sequences with the TA cloning kit (Invitrogen, Carlsbad, CA). PCR products were amplified and ligated into a pCR2.1 plasmid vector. Clones were screened for insertion sequences by amplification with primers specific for the cloning vector. DNA was extracted from isolated subclones using a Minprep kit (Life Technologies, Inc., Rockville MD) and subjected to sequencing analysis.

**RT-PCR.** Total RNA was extracted from peripheral blood lymphocytes from the father, using triazol reagent (Life Technologies, Inc.) by following the manufacturer’s protocol. Reverse transcription of mRNA was performed using random hexamer primers. Primers were designed to amplify a cDNA template encompassing the contribution from exons 2 to 5 of PTEN. The cDNA oligomers used were 5'-GACTTGAAAGCGATACAGG-3' and 5'-TTCAGTTGAAATTGCTGCAAC-3'. PAGE of the RT-PCR products was performed to assess for aberrant transcripts.

### Results

**Family Presentation.** Fig. 1 shows the immediate family pedigree. Twin A presented with a prolapsed polyp and a long standing history (>1 year) of hematochezia. Endoscopic evaluation to the splenic flexure revealed the presence of five hamartomatous polyps, which were removed by endoscopic polypectomy. Twin B, a monozygotic twin, also had a >1 year history of hematochezia. A complete colonoscopy revealed five hamartomatous polyps, which were subsequently removed endoscopically. The twins’ biological mother was evaluated by colonoscopy and was found not to have any mucosal abnormalities. Neither parent had a history of hematochezia, thyroid cancers or diseases, or other types of malignancies. Colonoscopic
workup of the father also revealed normal colonic mucosa. The father has three adult daughters from a previous marriage who by history have not had hematochezia or malignancies and do not carry the diagnosis of JPS. Blood was not available from these siblings for DNA analysis.

**DNA Analysis.** Genomic DNA sequencing revealed a deletion of five bases proximal to the start of exon 4 of PTEN. This deletion was seen in the PCR-derived product from each twin as well as the father’s genomic DNA, with overlapping signals on DNA sequencing analysis indicating the presence of heterozygous alleles (Fig. 2). The mother’s DNA was wild type by sequencing analysis. We cloned each sequence to separate each of the alleles from the affected family members which confirmed the presence of a wild-type PTEN allele and a mutated PTEN allele (Fig. 2). The mutation occurred 7 bp proximal to the start of exon 4 and comprised a deletion of CTTTT. This region is the splice acceptor site for exon 4, and loss of this critical pyrimidine tract is predicted to alter splicing. This mutation was not seen in at least 20 normal controls in addition to the mother.

**PTEN Transcript Analysis.** Further analysis of this mutation was performed at the RNA level. Total RNA was extracted from whole blood lymphocytes from the father and reverse transcribed. A cDNA template was designed spanning a region comprising PTEN, exons 2 through 5. The RT-PCR product was amplified and electrophoresed on a 6% denaturing polyacrylamide gel (Fig. 3). Two aberrant migrating transcripts were noted on gel electrophoresis in addition to the expected length product. In our family, DNA sequencing analysis revealed a germ-line mutation in PTEN comprising a loss of a pyrimidine tract in the splice acceptor site for exon 4. Spliceosome formation involves early recognition of a pyrimidine tract at the 5’ splice site. Without this recognition, the components of the spliceosome cannot bind to the appropriate consensus sequences, and splicing of the RNA does not occur correctly. The PTEN mutation found in this family appears to affect RNA splicing as shown by RT-PCR analysis in which two aberrant migrating transcripts are noted. In our family, the diagnosis of JPS in the twin daughters can be firmly established clinically, because they clearly lack extraintestinal manifestations of the other syndromes. However, their age could be argued to be too young to exclude CD.

The 55-year-old father was also found to have a germ-line mutation, identical to his twin daughters. Careful physical examination revealed no skin lesions suggestive of trichilemmomas, a nonpalpable thyroid, and normal thyroid function. Colonoscopic examination also revealed no hamartomatous polyps. Clearly, this patient does not have CD, as would be expected by the age-related penetrance of CD. However, he lacks intestinal hamartomatous polyps that are present in his twin daughters. The penetrance of PTEN mutations manifesting JPS may be less than 100%, or the type of mutation found in this family might cause fewer (<5 polyps) polyps as identified from the twin daughters.

**Discussion**

JPS is a rare condition characterized by the occurrence of multiple juvenile polyps in the gastrointestinal tract in the absence of extraintestinal manifestations that delineate other hamartomatous syndromes. Juvenile polyposis may occur as a component of Bannayan-Riley-Ruvalcaba syndrome and CD. Recently, these syndromes have been associated with germ-line mutations in certain tumor suppressor genes, but there remains some overlap in the manifestations of a mutated phenotype. For instance, PTEN mutations have been found in some but not all BRRS patients, in most CD patients, and in a few JPS patients (12, 13, 18, 20). Other JPS patients were found to have SMAD4 mutations (15). Thus, some of these syndromes may be allelic, or modifying genes that alter phenotypic expressions may be operational.

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The PTEN locus on chromosome 10 has been previously implicated as a germ-line cause for JPS. Jacoby et al. (21) demonstrated loss of germ-line DNA spanning the PTEN locus in a JPS patient. Likewise, Zigman et al. (8) reported deletion of DNA surrounding the PTEN locus in a patient who could only be classified as JPS. Neither of these patients had features that would classify their disease as CD.

Classifying a patient with a germ-line mutation in PTEN has become somewhat problematic. Our 55-year-old father clearly does not have BRRS or CD phenotypically. With a germ-line mutation in PTEN and lack of extraintestinal findings, JPS is the only likely diagnosis for the father, especially because his daughters’ phenotype suggests JPS although the father does not have intestinal polyposis. A description of a mother with CD and a child with BRRS has been reported (22), as well as identical mutations in PTEN present in CD and BRRS (23), which suggests possible genetic overlap in these hamartomatous polyposis syndromes. This creates confusion for screening patients because the malignancy risk is different for the different syndromes. For instance, there is up to a 12-fold increased risk for colon cancer in JPS that is not present in CD (3, 4, 24, 25), and there is an elevated risk for breast and thyroid cancer in CD that is not described in JPS. These elevated cancer risks have not been fully evaluated in BRRS families.

In this family, the diagnosis of JPS is associated with a germ-line mutation in PTEN. There appears to be phenotypic variability in JPS in this family. Whether genetic penetrance plays a role in patients with a germ-line PTEN mutation to cause phenotypic variations remains unclear. Alternatively, the type of mutation of PTEN (e.g., splice variants) may correlate with phenotypic variants of hamartomatous syndromes, as in our family and some cases of JPS (18). Whereas a germ-line mutation in the transforming growth factor β signaler SMAD4 accounts for about one-half of cases of JPS (15), germ-line PTEN mutations may constitute a more rare cause for JPS. There appears to be phenotypic heterogeneity in the manifestation of a germ-line PTEN mutation because it is associated with BRRS, CD, and JPS.

**References**


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