Absence of PTEN/MMAC1 Germ-Line Mutations in Sporadic Bannayan-Riley-Ruvalcaba Syndrome

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Abstract

Bannayan-Riley-Ruvalcaba syndrome (BRRS) is a rare hamartomatous polyposis condition with features of macrocephaly, intestinal juvenile polyposis, developmental delay, lipomas, and pigmentation spots of the male genitalia. An autosomal dominant pattern of inheritance exists in some families, but others appear as sporadic cases. Germ-line mutations in PTEN, a tyrosine phosphatase and putative tumor suppressor gene, have been demonstrated in two families with BRRS, and chromatin loss at the PTEN gene locus on chromosome 10q23 has been demonstrated in two BRRS patients. Germ-line mutations in PTEN have also been described in Cowden disease and in a small number of patients with juvenile polyposis syndrome. In an attempt to assess the nature of PTEN mutations in BRRS, we analyzed three sporadic BRRS patients for chromosome 10q23 deletion or PTEN germ-line mutations. All 3 patients demonstrated no loss of parental alleles at 15 chromosome 10q23 markers that encompassed the region of PTEN. In addition, analysis of mRNA and genomic DNA revealed no nonsense, missense, or insertion/deletion mutations of PTEN. Thus, other mechanisms besides mutation of PTEN must have occurred to cause BRRS in these patients. We speculate that BRRS and juvenile polyposis syndrome may have a heterogeneous etiology to cause their syndromes.

Introduction

BRRS (MIM 153480), also known as Bannayan-Zonana syndrome, Ruvalcaba-Mycr-Smih syndrome, and Riley-Smith syndrome because of its phenotypic variability, is a rare congenital syndrome with features of intestinal hamartomatous polyps, macrocephaly, s.c. and visceral lipomas and hemangiomas, cognitive and motor developmental delays, lipid storage myopathy, Hashimoto’s thyroiditis, and pigmentation spotting of the penis in males (1). Although the prevalence of BRRS syndrome is unknown, patients reported with this syndrome represent sporadic as well as familial occurrences with an autosomal dominant pattern of inheritance (2–16).

Recently, two patients with BRRS were reported with chromosome aberrations yielding loss on chromosome 10q between 10q23.2 and 10q24.1 (17, 18), one as a result of a translocation with chromosome 9 (17). This chromosomal location contains the PTEN (also known as MMAC1 and TEP1) tumor suppressor gene (19–21). In another report, two BRRS families demonstrated germ-line mutations in PTEN (one family demonstrated a missense mutation, and one showed a truncating mutation; Ref. 22). Thus, these two reports suggest that chromatin loss at 10q23 and mutation of PTEN located at chromosome 10q23 can predispose to BRRS. Germ-line PTEN mutations have also been identified in four of five families with CD (MIM 158350; Ref. 23) and in three families with JPS (MIM 174900; Ref. 24). Genetically, germ-line mutations in PTEN make BRRS, CD, and JPS allelic with each other, at least in some families. PTEN encodes a dual-specificity phosphatase with homology to tensin and auxillin and has two potential tyrosine phosphate acceptor domains (19, 20).

Other factors such as modifiers of PTEN or perhaps another gene(s) could also be the causal etiology of BRRS and JPS. To ascertain the nature of PTEN mutations in BRRS, we analyzed mRNA and genomic DNA from three patients with sporadic BRRS. We were surprised to find that no mutations in PTEN occurred, suggesting that another mechanism besides mutations in PTEN must have happened to cause BRRS clinically in these patients.

Materials and Methods

Patients. Patients were referred to a pediatric geneticist because of the suspicion of a genetic syndrome. All were diagnosed with BRRS. Patient 1, a male, presented with macrocephaly, hypotonia, cognitive and developmental delays, cutaneous lipomas, and a 2-cm intestinal metaplastic polyp located in the ascending colon. Patient 2, a male, presented with macrocephaly, multiple intestinal juvenile polyps, pigmentation of the genitalia, cutaneous and visceral lipomas, cutaneous hemangiomas, and hyporeflexia. Patient 3, a male, presented with macrocephaly, multiple rectal juvenile polyps, pigmentation spotting of the penis in males, and hyporeflexia. No other family members of these three unrelated patients were affected with BRRS by family medical history or, if available, by physical examination. Whole blood was obtained from each patient and from the parents of each patient. Buffy coats were exposed to EBV for immortalization of B lymphocytes.

DNA was extracted from peripheral leukocytes by the phenol-chloroform-isoamyl alcohol method (29), and RNA was extracted from immortalized...
lymphocytes using Trizol reagent (Life Technologies, Inc., Gaithersburg, MD) according to the manufacturer's instructions.

**Genomic Deletional Analysis.** Microsatellites and flanking DNA sequences were obtained from the Genethon human genetic linkage map (30). The following microsatellites were assessed: D10S1687; D10S541; D10S215; D10S1765; D10S579; D10S1735; D10S1739; D10S564; D10S1753; D10S583; D10S200; D10S185; D10S571; and D10S1736. In some cases, optimal primer pairs were determined using Primer computer software (version 0.5; Whitehead Institute for Biomedical Research, Cambridge, MA). One oligomer of each primer pair was end-labeled by [γ-32P]ATP in a 5-μL reaction containing 0.0625 μM primer, 10 units of T<sub>v</sub> polynucleotide kinase (Life Technologies, Inc.), and 20 μCi of [γ-32P]ATP (DuPont New England Nuclear, Boston, MA) and incubated at 37°C for 60 min. In general, each PCR reaction was carried out in 4-μL volumes containing 200 ng of genomic DNA template and a final magnesium concentration of 1.875 mM. PCR reactions were carried out in a thermocycler (MJ Research, Watertown, MA) at 94°C for 45 s, 55°C for 45 s, and 72°C for 45 s and cycled up to 40 times for most primer pairs. PCR products (1 μL) were electrophoresed on a 6% polyacrylamide gel containing 7.5 M urea and exposed to X-ray film. The patient's alleles were then compared to each parent's alleles to identify the presence or absence of a contributing allele.

**IVTT Assay.** Templates were generated by PCR from first-strand cDNA using forward primer 5'-GGATCTCTGCTAGAATTCATACGTC-3' and reverse primer 5'-CATGGAATTCTCAGACTTTTGTAATTTGTGTATGC-3'. PCR products were purified using QIAquick PCR columns (Qiagen, Chatsworth, CA), and 100 ng of PCR product were used in a TNT T7 quick coupled transcription/translation reaction (Promega, Madison, WI) with the addition of 0.3 mM magnesium acetate. The resulting products were electrophoresed through a 10% discontinuous SDS polyacrylamide gel. The gel was fixed, dried, and exposed to a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA) for 12 h.

**cDNA Sequencing.** First-strand PTEN cDNA synthesis was accomplished by using each patient's peripheral leukocyte mRNA primed with oligodeoxynucleotide acid and random hexamer oligonucleotides and reverse-transcribed with Superscript RT enzyme (Life Technologies, Inc.). Subsequent PCR amplification of the PTEN coding region was performed by using the primers described for the IVTT assay. Sequencing of the cDNA was performed using four oligonucleotides (two in the sense direction, and two in the antisense direction). Primers used were: FFS, 5'-CTAATGATACGACATCCTTTGTT-3'; F3AS, 5'-ACATGATGTTGCTCATCTCCTTCTTAAGT-3'; and reverse primer 5'-ATTCTGAGGTTATCTTTTTACC-3'; exon 8 (forward), 5'-GATTCAGGCAATGTTTGTTAG-3'; and FF4AS, 5'-TTTAGAAGATATTTCATACCAG-3'; exon 3 (reverse), 5'-TTTAGAAGATATTTCATACCAG-3'; and exon 3 (forward), 5'-ATTCTGAGGTTATCTTTTTACC-3'; and reverse primer 5'-ATTCTGAGGTTATCTTTTTACC-3'; and exon 8 (reverse), 5'-GATTCAGGCAATGTTTGTTAG-3'; and exon 3 (forward), 5'-TTTAGAAGATATTTCATACCAG-3'; and exon 3 (reverse), 5'-TTTAGAAGATATTTCATACCAG-3'; and exon 8 (reverse), 5'-GATTCAGGCAATGTTTGTTAG-3'. Cycle sequencing with these primers was performed using the ABI Prism dye terminator kit (Perkin-Elmer Corp., Foster City, CA), and direct sequencing for PTEN mutations was performed by an Applied Biosystems automated sequencer.

**Genomic Sequencing.** Genomic DNA was amplified using nine pairs of genomic primers specific for PTEN. Primers used for exon 1 (reverse), exon 2 (forward), and exons 4-9 have been reported previously (23, 31). The other primer sequences used for PCR were as follows: exon 1 (forward), 5'-GAGGATTATTTCTGCTTCTCC-3'; exon 2 (reverse), 5'-GACATATGACTAAATGTAAGAACATACATG-3'; exon 3 (forward), 5'-ATTCTGAGGTTATCTTTTTACC-3'; and exon 3 (reverse), 5'-TTTAGAAGATATTTCATACCAG-3'. PCR was performed in a thermocycler at 94°C for 2 min, 55°C for 1 min, and 70°C for 1 min; followed by 35 cycles at 94°C for 30 s, 55°C for 1 min, and 70°C for 1 min. The length of the expected PCR products was confirmed in a 1% agarose gel. Sequencing primers for exons 6, 7, and 9 (both forward and reverse directions) have been reported previously (23). Other sequencing primers used were as follows: exon 1 (reverse), 5'-ACGTTCTAAGGAGGTGACGACA-3'; exon 2 (forward), 5'-GCTTAAATGTTAACGAGTCTTCTCC-3'; exon 3 (forward), 5'-AAGTCTGCTTAATTCATACGTC-3'; exon 4 (forward), 5'-TCCGCTTCTCTCTCC-3'; exon 5 (forward), 5'-ATTCTGAGGTTATCTTTTTACC-3'; exon 8 (forward), 5'-GATTCAGGCAATGTTTGTTAG-3'; and exon 3 (reverse), 5'-TTTAGAAGATATTTCATACCAG-3'. Cycle sequencing of the PCR products was performed using the ABI Prism dye terminator kit (Perkin-Elmer Corp.), and direct sequencing for PTEN mutations was performed by an Applied Biosystems automated sequencer.

**Results**

We evaluated three unrelated BRRS patients for germ-line PTEN mutations. All three patients were examined by a pediatric geneticist who confirmed the diagnosis of BRRS. To ensure that no chromosomal abnormalities on 10q23 were present, the patients were karyotyped and showed the normal human complement of 22 autosomes and 2 sex chromosomes without any aberration. In addition, we used 15 polymorphic microsatellite markers to amplify each patient's and each patient's parental WBC DNA between D10S1687 and D10S1736 (encompassing the region of PTEN). There was no loss of a parental allele from each patient within this 16.9-cM DNA segment on chromosome 10q.

We initially evaluated each patient for PTEN mutations using an IVTT assay. All three patients demonstrated no truncation of PTEN (Fig. 1). To determine whether missense mutations were present, cDNAs generated from each patient's mRNA were directly sequenced. All three BRRS patients showed only the wild-type sequence for PTEN.

We sequenced all nine exons of PTEN from each patient's genomic DNA to assess any potential mutations missed by mRNA analysis. Only the wild-type PTEN sequence was noted in all three patients. Thus, three clinically identified cases of BRRS demonstrated no germ-line PTEN mutations at the DNA or RNA level.

**Discussion**

Here we show that BRRS is not always associated with germ-line mutations in the PTEN tumor suppressor gene. We searched for missense, nonsense, and deletion/insertion mutations in the mRNA and DNA of these patients. Our approach to mutational analysis would likely eliminate the possibility of whole-gene deletion, based on the microsatellite analysis and the lack of DNA loss compared to parental alleles. However, germ-line microdeletion of PTEN between intact microsatellites could theoretically still be possible. Additionally, mutations in the promoter region of PTEN would not be detected by our approach.

The lack of PTEN mutations in these BRRS patients leads to a number of speculations as to what could be occurring genetically. One possibility is that the PTEN gene product could be inactivated epigenetically, and PTEN gene could be inactivated epigenetically to cause BRRS. Hypermethylation of the promoter region or the gene itself could prevent its transcription without mutation of PTEN. This scenario has not been examined to date.

The intracellular pathway(s) in which PTEN participates is yet unknown, and it is possible that inhibitors of PTEN or its pathway could be present. Alternatively, another gene product, possibly one that regulated PTEN expression, could also be involved in BRRS.
involved in a PTEN intracellular pathway, may be inactivated to cause BRRS. This hypothesis would be similar to that of DNA mismatch repair, in which mutations in several different individual genes that cooperate in DNA mismatch repair can be inactivated to cause one disease, hereditary nonpolyposis colorectal cancer (32). This last scenario allows BRRS to have a heterogenous allelic etiology, one of which may be shared with JPS, because both BRRS and JPS may have intestinal juvenile polyps. Recently, linkage to chromosome 10q22–24 and mutations in PTEN were excluded in several JPS families (31). Thus, we believe that reports of BRRS and JPS demonstrating germ-line PTEN mutations may constitute only a portion of the genetic etiology for their disease. The small number of BRRS patients available (approximately 50 cases reported in the literature) make a determination of the PTEN mutational frequency difficult. Finally, our cases represent sporadic forms of BRRS, and the patients described here do not belong to classic BRRS pedigrees. No other family members of these three patients had signs or symptoms of BRRS, unlike the two pedigrees reported with PTEN germ-line mutations (22). It is possible that the genetic etiologies of sporadic and familial BRRS may be different.

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References

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