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ORIGINAL ARTICLE

MUSCLE HEMANGIOMATOSIS PRESENTING AS A SEVERE FEATURE IN A PATIENT WITH THE PTEN MUTATION: EXPANDING THE PHENOTYPE OF VASCULAR MALFORMATIONS IN BANNAYAN-RILEY-RUVALCABA SYNDROME

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ABSTRACT

Bannayan-Riley-Ruvalcaba syndrome (BRRS) is a rare autosomal, dominantly-inherited, hamartoma syndrome with distinct phenotypic features. Mutations in the *PTEN* gene have been identified in PTEN hamartoma tumor syndromes. Our aim was to determine the correlation of phenotype-genotype relationships in a BRRS case. We have evaluated a *PTEN* mutation in a patient with vascular anomalies and the phenotypic findings of BRRS. We described an 8-year-old girl with the clinical features of BRRS, specifically with vascular anomalies. The mutation in the *PTEN* gene was identified by DNA sequencing. In our patient, we defined a *de novo* nonsense R335X (c.1003 C>T) mutation in exon 8, which results in a premature termination codon. Due to vascular anomalies and hemangioma, the patient's left leg was amputated 1 year after the hemangioma diagnosis. Bannayan - Riley - Ruvalcaba syndrome patients with macrocephaly and vascular anomalies should be considered for *PTEN* mutation analysis and special medical care.

Keywords: Bannayan - Riley - Ruvalcaba Syndrome (BRRS); Hemangioma; Macrocephaly; *PTEN* gene; Vascular anomalies.

INTRODUCTION

Bannayan-Riley-Ruvalcaba Syndrome (BRRS; Online Mendelian Inheritance in Man data base, OMIM 153480) is a rare autosomal, dominantly-inherited, congenital disorder characterized by macrocephaly, lipomatosis, hemangiomatosis, hamartomatous growths, central nervous system abnormalities, genital lentiginosis, intestinal polyposis, vascular malformations, and, less commonly, mental retardation [1-3]. Common facial presentations in BRRS patients include frontal bossing, down-slanting palpebral fissures, strabismus hypertelorism, a depressed nasal bridge, long philtrum, epicanthus inversus, a thin upper lip, a broad mouth and relative micrognathia [4,5]. Hypothonia, delayed psychomotor development, and seizures are also observed in at least half of BRRS patients [2]. Additionally, ocular abnormalities, lipid storage myopathy, and skeletal system abnormalities such as pectus excavatum, joint hyperextensibility and scoliosis are common manifestations of the syndrome [5]. Bannayan-Riley-Ruvalcaba syndrome and Cowden syndrome (CS; MIM 158350) are two hamartoma syndromes that sometimes show clini-

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cal overlap. Thus, at least a subset of BRRS and CS may be allelic. Approximately 60% of patients with BRRS have an identifiable germline mutation in *PTEN* (OMIM 601728), which is a tumor suppressor gene located on chromosome 10 (10q23) [6,7]. The *PTEN* gene has nine exons that encode a protein of 403 amino acids, and is also known as *MMAC1* (mutated in multiple advanced cancers) or *TEP1* (TGFβ-regulated and epithelial cell-enriched phosphatase) [8]. It has been suggested that syndromes characterized by the presence of germline *PTEN* mutations might be referred to as PTEN hamartoma tumor syndromes (PHTS).

In the present study, we have performed *PTEN* mutation analysis in a patient with BRRS. We found a *de novo* nonsense R335X (c.1003C>T) mutation that is rarely reported in the literature for BRRS patients. Furthermore, we attempted to discuss the clinical features of BRRS which should prompt clinicians to test for a PTEN mutation.

MATERIALS AND METHODS

Clinical Report. The proband was an 8-year-old female, referred to the Neurogenetic Division, University of São Paulo-Clinics Hospital, Ribeirao Preto, São Paulo, Brazil, because of recurrent facial palsy. She was the second child of healthy and non onsanguineous parents. Further family history was unremarkable. She was born by vaginal delivery at term; her birth weight, length, and occipitofrontal circumference (OFC) were not recorded at that time. Her growth parameters were normal during childhood. At 2 years of age, she developed recurrent attacks of facial palsy with full recovery days after the episode; there were no other similar cases in her family. Her medical history included one episode of seizures at 3 years of age, but she did not use anticonvulsants. At 7 years of age, she developed gait problems because of pain in her left leg and was evaluated by an orthopedic surgeon who diagnosed hemangiomas. The hemangioma was excised. After histopathological examination, the lesion was diagnosed as an angiolipoma. However, she had another hemangioma in the same leg several months later. At that time her parents noticed that her leg became thinner. New hemangiomas appeared in her right leg, but the parents decided not to operate until they had a definite diagnosis. Her teachers noticed learning disabilities in school. At the age of 8, her weight and height were in the 50th percentile and her OFC was greater than the 97th percentile for her age. Her parents' OFC were in the normal range. When she was 10, her left leg was amputated because of the hemangioma. On phenotypic inspection, a mild facial dysmorphism consisting of a high forehead, a triangular face, pointed chin, down-slanting palpebral fissures, and a high-arched palate were observed. Several verrucae vulgares on her arms and hands and small café-au-lait spots on her arms and trunk were identified.

Neurological examination identified a mildly asymmetric face, normal eye movements, brisk osteotendinous reflexes, and no signs of cerebellar ataxia. Interestingly, the patient had facial palsy. The weakness in her left leg included a moderate degree of muscle atrophy and aqueous retraction. Her biochemical and serological tests (including thyroid function and karyotype) were all normal. Electroencephalogram (EEG) monitoring recorded as normal. Electromyography (EMG) revealed the presence of myopathic potential units, but nerve conduction velocities were normal. Abdominal ultrasound monitoring recorded as normal. Magnetic resonance imaging (MRI) of the brain showed a small cavitation on the atrium of left lateral ventricle compatible with increased perivascular space. An MRI of the lower limbs showed normal findings in left leg; her right leg displayed the presence of arteriovenous malformation of the right buttock thorough the right lower limbs and severe muscle atrophy. Because of the combination of macrocephaly, angiomyolipomas, and myopathy, we hypothesized that she might have Bannayan-Zonana syndrome, and performed a thyroid ultrasound and colonoscopy. The thyroid ultrasound showed no abnormal findings; colonoscopy showed a few polyps in the sigmoid colon. The results of biopsy on the polyps were compatible with hamartomatous type lesions. In our patient, the existence of hemangiomas, severe vascular malformation leading to amputation of an extremity, macrocephaly, dermatologic and neurologic findings, and facial dysmorphisms in childhood supported a diagnosis of BRRS and an evaluation of *PTEN* mutation analysis. After we obtained written informed consent from her parents (Ethical Committee for Medical Research document

Table 1. The *PTEN* primers [forward (F) and reverse (R)] used in this study.

Primer	Sequence (5'>3')	Tm (°C)	Product Size (bp)
PTEN exon 1ALTF	ATT TCC ATC CTG CAG AAG AAG	58.40	
PTEN exon1R	ATC CGT CTA CTC CCA CGT TCT	60.00	231
PTEN exon2F	CAT TGA CCA CCT TTT ATT ACT CCA	69.33	
PTEN exon2R	CTT TTC TAA ATG AAA ACA CAA CAT GAA	59.88	290
PTEN exon3F	CCA TAG AAG GGG TAT TTG TTG G	59.60	
PTEN exon3R	AAC TCT ACC TCA CTC TAA CAA GCA GA	59.25	301
PTEN exon4F	TGT CAC ATT ATA AAG ATT CAG GCA AT	60.15	
PTEN exon4R	TCT CAC TCG ATA ATC TGG ATG ACT	59.26	246
PTEN exon5F	CCT GTT AAG TTT GTA TGC AAC ATT TC	60.21	
PTEN exon5R	TCT CAG ATC CAG GAA GAG GAA A	60.32	385
PTEN exon6F	GGC TAC GAC CCA GTT ACC ATA	59.00	
PTEN exon6R	GCT TCA GAA ATA TAG TCT CCT GCA T	59.38	365
PTEN exon7F	TGA GAT CAA GAT TGC AGA TAC AGA	59.00	
PTEN exon7R	ACC AAT GCC AGA GTA AGC AAA	59.76	438
PTEN exon8F	AAT AGT CTT TGT GTT TAC CTT TAT TCA G	57.05	
PTEN exon8R	TCA AGC AAG TTC TTC ATC AGC	58.24	488
PTEN exon9F	AAG ATC ATG TTT GTT ACA GTG CTT	57.10	
PTEN exon9R	CTG GTA ATC TGA CAC AAT GTC CT	58.12	434

number 12219/2004), we carried out a direct DNA sequencing of the whole *PTEN* gene exons.

Characterization of the PTEN Mutation. We extracted genomic DNA from peripheral blood leukocytes by means of a commercial kit (QIAamp 51104; Qiagen, Hilden, Germany). We then amplified genomic DNA using primers flanking the exons of *PTEN*. Primer sequences and their Tm values are given in Table 1. Reaction conditions were as follows: after initial denaturation at 98°C for 1 min., three steps were repeated for 35 cycles (denaturation for 8 seconds at 98°C, annealing for 8 seconds at 60°C and extension for 15 seconds at 72°C). The reaction was finished with a final extension for 1 min. at 72°C. The polymerase chain reaction (PCR) products were directly sequenced by a sequencing service company, Iontek (Istanbul, Turkey), to detect mutation. We used the Mutation Surveyor software package (v 3.10, SoftGenetics, LLC, State College, PA, USA) for mutation screening.

RESULTS

A nonsense R335X (c.1003C>T) mutation was identified in exon 8 that resulted in a premature termination codon (Figure 1). The mutation was confirmed with the second sequencing reaction and another round of mutation screening procedures. Sequencing analysis of *PTEN* exon 8 in the patient's parents failed to reveal any alteration.

DISCUSSION

The *PTEN* mutation is related to hamartoma syndromes, displaying partial clinical overlap. The determination of germline mutations in PHTS can allow the identification of new genotype-phenotype correlations, particularly in BRRS. However, it was reported that males are more frequently affected than females but our female patient was in the group of lower penetrance group [9]. We have identified a *de novo* mutation, R335X, in the PTEN coding sequence in a child with the phenotypic findings of BRRS. Although this truncating *PTEN* mutation is common in CS, the R335X mutation is rarely reported in BRRS [2]. The R335X mutation at nucleotide 1003 (C>T) in exon 8 results in a truncated protein lacking the C-terminal portion that is needed for phosphatase function [2]. Destabilization of the predicted secondary structure by point mutations on the C-terminal has been shown to result in haploinsufficiency and to affect phosphatase function [10]. Since we did not analyze the DNA of affected tissues, we speculate that a single *PTEN* hit resulting in *PTEN* haploinsufficiency may be causative of the patient's clinical features, such as macrocephaly, verrucae vulgares, café-au-lait spots, seizures and hemangiomas.

Çelebi *et al*. [2] described for the first time a family with two female members fulfilling the criteria for CS and two male members with the phenotypic findings of BRRS associated with

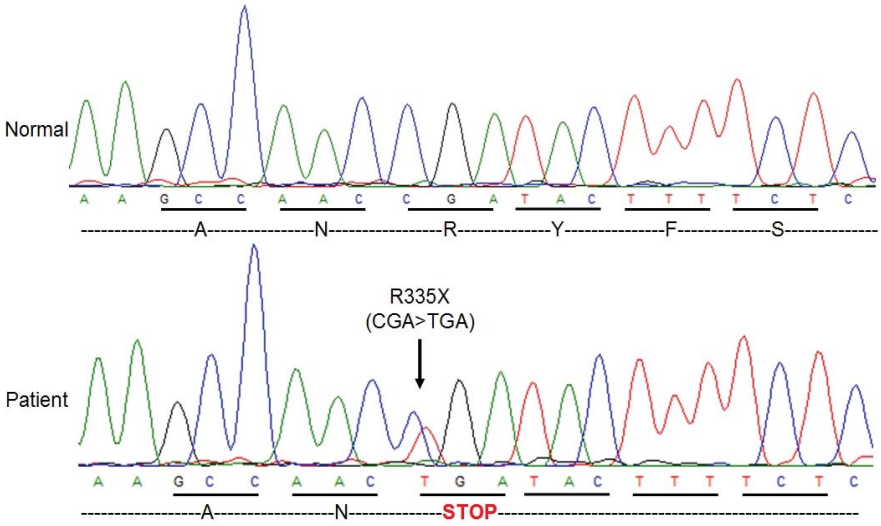

Figure 1. Electropherograms of a normal and patient's DNAs showing the heterozygous R335X mutation (Ensembl Transcript ID: ENST00000371953).

PTEN R335X mutation. Marsh *et al*. [11] reported a CS patient with the *PTEN* gene R335X mutation with malignancy. Parisi *et al*. [10] reviewed their experience in three families with BRRS and *PTEN* mutations. Similarly, we encountered common phenotypic features identified in approximately one-third of their patients, who had downward-slanting palpebral fissures, high-arched palates, macrocephaly, frontal bossing, vascular malformations, café-au-lait macules, seizures, and some degree of learning impairment [10]. Fifty-six to 70% of BRRS patients also exhibited high-arched palates [10].

Zhou *et al.* [12] intended to define whether other syndromes of overgrowth and hamartomas are part of PHTS. They also studied six unrelated individuals who had minimal features of lipomas, hamartomas, and overgrowth but did not meet the diagnostic criteria of CS and BRRS; they subjected these patients to *PTEN* mutation analysis. In their study, a germline *PTEN* R335X mutation and a second "hit" germline mosaic R130X mutation, were detected in affected tissues of a boy with clinical features of marked hemihypertrophy, macrocephaly, epidermoid nevi, and lipomas. The patient had been diagnosed with an undefined Proteus-like syndrome. The patient had arteriovenous malformations in all tissues, including muscle, nerve, and bone. The malformations progressively worsened, and after a series of embolizations, a right hip amputation was performed. Since he did not have any gastrointestinal symptoms, this patient did not meet the criteria for a diagnosis of CS or BRRS [12].

Another small series of Proteus syndrome patients was examined for *PTEN* mutations [13-15]. Interestingly, R335X was found in two of five Proteus-like individuals and the same mutation has also been found in CS and BRRS patients [15]. Vascular abnormalities, arteriovenous malformations, vascular hamartomas, or hemangiomas, have been reported in some BRRS and CS patients [16-18]. These vascular anomalies are typically multifactorial intramuscular combinations of fast-flow channels and ectopic fat in patients with *PTEN* mutations [19]. Nevertheless, monitoring for vascular anomalies is not a standard diagnostic procedure for BRRS patients [19]. Erkek *et al*. [1] reported tender and painful arteriovenous hemangiomas that were misdiagnosed as angiolipomas in their patients. In this report, the patient's most striking features were the limitation of vascular hamartomas, myopathy, axonal neuropathy and enostosis to the right lower extremity.

Tan *et al*. [19] identified vascular anomalies in 54% of their patients positively identified as having a *PTEN* mutation, although none of them had

hemangiomas or other pure vascular tumors. They found the R335X mutations in two male patients, one with vascular anomalies and one without. They concluded in this research that macrocephalic patients with fast-flow vascular anomalies or multiple intracranial developmental venous anomalies should be tested for *PTEN* mutation [19]. Although a few cases have been reported with the association of CS, hemangiomas and arteriovenous malformations are reported features of BRRS [20]. Electromyography may reflect the myopathic process [21].

We showed the presence of myopathic potential units in our patient. Bannayan-Riley-Ruvalcaba syndrome is also associated with muscular abnormalities [1]. Our patient had weakness in her left leg with moderate degree of muscle atrophy and aqueous aquileus retraction. Hamartomatous polyposis occurs in 35-45% of BRRS cases, mainly in the colon and rectum [5,8,22]. The results of our patient's colonoscopy showed a few polyps in the sigmoid colon; a biopsy showed that the polyps were compatible with a hamartomatous type. Timely identification of *PTEN* mutations, regardless of clinical presentation, would redefine a diagnosis. Severe arteriovenous malformations/ hemangiomas may be a particular presentation of BRRS patients carrying the R335X mutation and can be a debilitating feature of the disease.

We aimed to evaluate genetic features of BRRS, demonstrating clinical correlations in a demonstrated case. Our study has contributed to the knowledge of vascular anomalies in patients with a *PTEN* mutation. This case report reminds the clinicians to consider a *PTEN* gene mutation testing for diagnosis of patients with vascular anomalies and other clinical features of CS and BRRS.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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