CLINICAL REPORT

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PTEN Hamartoma Tumor Syndromes in Childhood: Description of Two Cases and a Proposal for Follow-Up Protocol

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PTEN hamartoma tumor syndromes (PHTS) are a spectrum of hamartomatous overgrowth syndromes associated with germline mutations in the tumor suppressor *PTEN* gene located on 10q23.3. It is widely accepted that two of these disorders, Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome, are allelic conditions. Because *PTEN* mutations are not identifiable in every case of the PHTS phenotype, the inability to detect a mutation within the *PTEN* gene does not invalidate the clinical diagnosis of Cowden syndrome, or Bannayan–Riley–Ruvalcaba syndrome, in patients who meet diagnostic criteria for these disorders. *PTEN* mutations are associated with an increased risk for developing breast, thyroid, endometrial, and sometimes renal cancers. Thus, cancer surveillance is the cornerstone of PHTS patient management. Although a consensus cancer surveillance protocol has not been formally instituted, all *PTEN* mutation carriers should adopt the cancer surveillance strategies proposed for patients with Cowden syndrome. In addition, because gastrointestinal and vascular complications can be more severe in Bannayan–Riley–Ruvalcaba syndrome than in Cowden syndrome, patients with Bannayan–Riley–Ruvalcaba syndrome should be monitored from this point of view too. In this study, we report on two cases with Bannayan–Riley–Ruvalcaba phenotype that showed two different *PTEN* mutations. We also propose practice recommendations for management of PHTS patients.

Key words: PTEN Hamartoma tumor syndromes; *PTEN* gene; Cowden syndrome; Bannayan-Riley-Ruvalcaba syndrome

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INTRODUCTION

PTEN hamartoma tumor syndromes (PHTS) are a collection of rare clinical syndromes inherited in an autosomal dominant manner and associated with germ-line mutations of the tumor suppressor gene *PTEN* (OMIM 601728) [Eng, 2000]. The *PTEN* (phosphate, tensin homologue, deleted on chromosome 10) gene encodes a dual-specificity phosphatase that antagonizes the phosphoinositol-3-kinase (PI3K)/Akt pathway, leading to G1 cell-cycle arrest and/or apoptosis and also inhibits cell spreading via the focal adhesion kinase pathway [Sansal and Sellers, 2004]. PHTS encompass Cowden syndrome (CS; OMIM 158350), Bannayan–Riley–Ruvalcaba syndrome (BRRS; OMIM 153480), and Proteus-like syndrome. Of these three entities, CS, occurring in adulthood, and BRRS, a pediatric syndrome, have many overlapping features (macrocephaly, hamartomas, and thyroid abnormalities). Given the clinical similarities between these disorders, the hypothesis of a common genetic pathogenesis has been validated by detection of an identical *PTEN* mutation in different members of the same family [Marsh et al., 1997]. Therefore, the difference between the two conditions is the age of presentation. Both are considered allelic. CS is an autosomal dominant disorder characterized by multiple hamartomatous lesions (trichillemmomas, oral papillomas, intestinal polyps), and by an increased risk of breast, thyroid, and endometrium cancers [Liaw et al., 1997]. Clinical diagnostic criteria for CS are shown in Table I. Both simplex and familial cases of CS have been identified. However, most CS cases are isolated [Marsh

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TABLE I. Operational Criteria for Cowden Syndrome without Family History of Known *PTEN* Mutation Adapted from Tan et al. [2011]

Pathognomonic criteria
Adult Lhermitte–Duclos disease (cerebellar tumors)
Mucocutaneous lesions[a]
Facial trichilemmomas, any number[a] (at least two biopsy-proven trichilemmomas[b])
Acral keratoses
Papillomatous papules
Mucosal lesions
Autism spectrum disorder and macrocephaly[b]
Major criteria
Breast cancer
Epithelial thyroid cancer (follicular and papillary, never medullary)
Macrocephaly (megalocephaly) (i.e., 97th percentile and above)
Endometrial cancer
Mucocutaneous lesions[b]
One biopsy-proven trichilemmoma
Multiple palmoplantar keratoses
Multifocal cutaneous facial papules
Macular pigmentation of glans penis
Multiple gastrointestinal hamartomas or ganglioneuromas[b]
Minor Criteria
Other thyroid lesions (e.g., adenoma, multinodular goiter)
Intellectual disability (i.e., IQ of 75 and below)
Gastrointestinal hamartomas[a] (single gastrointestinal hamartoma or ganglioneuroma[b])
Fibrocystic disease of the breast
Lipomas
Fibromas
Genitourinary tumors (especially renal cell carcinoma)
Genitourinary malformations[a]
Uterine fibroids
Autism spectrum disorder[b]

[a]Present in this section as defined by ICC criteria only.
[b]Present in this section as defined by NCCN 2010 criteria only.

et al., 1999]. Germ-line *PTEN* mutations have been associated with the majority of CS cases [Liaw et al., 1997; Marsh et al., 1998]. The molecular basis of CS in the remaining cases has yet to be determined. BRRS is a rare congenital syndrome characterized by macrocephaly, multiple hemangiomas, and lipomas (subcutaneous and/or visceral), gastrointestinal hamartomatous polyps, neurologic manifestations (autism or cognitive and motor developmental delay), and hyperpigmented macules on the skin of the shaft and glans penis [Gorlin et al., 1992]. Common features include hypotonia associated with a lipid storage myopathy [DiLiberti et al., 1984] and thyroid abnormalities such as Hashimoto's thyroiditis [Gorlin et al., 1992]. Clinical findings of BRRS are shown in Table II. Most BRRS cases have a family history, and are inherited in an autosomal dominant manner [Zonana et al., 1975]. However, there are sporadic cases that do not belong to classic BRRS pedigrees [Carethers et al., 1998]. Germ-line *PTEN* mutations are reported in over half of the patients with BRRS. In contrast with CS, there are no agreed international criteria for the diagnosis of BRRS. However, after the discovery of the *PTEN* gene, it became apparent that *PTEN* mutation testing might facilitate early diagnosis in childhood [Marsh et al., 1997; Mester et al., 2011]. Recently a prospective multicenter study defined clinical criteria useful to decide which pediatric (<18 years) patients to test for *PTEN* (Table III) [Tan et al., 2011]. However, BRRS is not always associated with germ-line mutations in the *PTEN* gene [Carethers et al., 1998]. Therefore, other molecular mechanisms could have occurred to cause the BRRS cases without germ-line *PTEN* mutations.

MATERIALS AND METHODS

Clinical Report

Patient 1 was a 6 1/2-year-old female, third child of nonconsanguineous healthy parents. After an uncomplicated pregnancy, she was born at 38 weeks gestation by cesarean due to polyhydramnios. Her birth weight was 3,270 g (50th–75th centile), length 51 cm (75th–90th centile), and OFC 36 cm (>97th centile). At 1-month of life, generalized hypotonia was noted, for which physiochynesitherapy was started. Because of development (motor, cognitive, and language) delay, some diagnostic investigations were performed. Neurological examination, electroencephalogram, and cranial Computed Tomography scan did not reveal any brain abnormalities; visual evoked potential (VEP) and auditory brain stem responses to complex sounds (cABRs) were normal too. At 4 years and 10 months of age, she developed a right cervical swelling. Ultrasound study showed a hyperechogenic swelling (4.5 cm × 3.5 cm) in right supraclavicular region. Thorough investigation with Positron emission tomography (PET) failed to reveal any tumor illness with high metabolic activity in right lateral cervical and thoracic regions. Therefore, the patient underwent excision of right cervical-axillary lesion. Findings from histopathologic examination of this lesion were interpreted as a lipoma. As a result of these clinical and anamnestic features the patient was referred to genetic counseling. At first observation, the patient was 6 1/2-years old. Prominent physical findings included macrocephaly (Fig. 1a,b), with OFC of 58 cm (>95th centile), and overweight, with a weight of 33.5 kg (>95th centile). Her height was normal. A severe scoliosis was noted, and bilateral *pes planus* was present too. Examination of patient's parents and siblings didn't reveal physical abnormalities. Because of macrocephaly, psychomotor and cognitive delay, and lipomatosis we decided to study *PTEN* gene.

Patient 2 was a female of 24 months of age. She was first-born to nonconsanguineous healthy parents. She was born at 41.3 weeks gestation from uneventful pregnancy and spontaneous delivery. Her birth weight was 3,730 g (75th–90th centile), length 51 cm (50th–75th centile), and OFC 36.5 cm (>97th centile). By 8 weeks of age, her OFC at 42 cm was >97th centile. Early cognitive and language milestones were reached normally, but she started to walk at 22 months of age. During this period, she was noticed to have multiple thoraco-abdominal swelling (Fig. 2a,b), which revealed lipomas after excision. Thus, she was referred to our genetic evaluation at 24 months of age: she weighed 12.5 kg (25th–50th centile), 87 cm tall (50th centile),

TABLE II. A Broad Spectrum of Clinical Findings can be Associated with Variable Expressivity among BRRS Patients

Features present at birth	Overgrowth of prenatal and/or postnatal onset: OFC and/or weight and length ≥98th centile
	Neonatal hypotonia
Facial dysmorfic features	Frontal bossing, hypertelorism, downward slanting palpebral fissures, depressed nasal bridge, strabismus, epicanthus inversus, small beaked nose, long philtrum, thin upper lip, broad mouth, and relative micrognathia [Hendriks et al., 2003]
Ophthalmologic abnormalities	Pseudopapilloedema
	Prominent Schwalbe lines
	Prominent corneal nerves [Riley and Smith, 1960]
Hamartomatous lesions	Hemangiomas and lipomas (subcutaneous and/or visceral)
	Gastrointestinal polyps
Mucocutaneous abnormalities	Hyper-pigmented penile macules in males
	Cafè au lait spots
	Acanthosis nigricans-like
	Oral, facial and acral warts or verrucous papules Cutis marmorata and telangiectases [Erkek et al., 2005]
Cardiovascular abnormalities	Arteriovenous malformations, shunts, and fistula
	Local overgrowth of small vessels
	Aneurysms of aortic root ad ascending aorta [Tan et al., 2007]
	Unstable angina and atrial septal defect [Halal and Silver, 1989]
Thyroid involvement	Hashimoto's thyroiditis
	Multinodular goiter, adenoma, cancer
Neurological abnormalities	Autism
	Cognitive, speech, and motor development delay
	Incoordination
	Mild mental deficiency
	Seizures (25% of patients) [Gorlin et al., 1992]
	Rare peripheral neuropathy [Erkek et al., 2005]
Skeletal system abnormalities	High palate, scaphocephaly, scoliosis, joint hyperextensibility, pectus excavatum, pes planus [Boccone et al., 2006]
	Punctate cystic changes in acral tubular bones, enostosis of talus, broad thumbs and great toes [Erkek et al., 2005]

and her OFC was 51 cm (>97th centile). Physical examination revealed extreme macrocephaly, epicanthus, and three café-au-lait macules on her trunk, only one measuring greater than 2.5 cm in diameter. A tonsil papilloma was observed too (Fig. 2c). Her parents had not macrocephaly. As result of the phenotype we performed *PTEN* molecular analysis.

TABLE III. Pediatric Clinical Criteria for PTEN Testing* Adapted from Tan et al. [2011]

Clinical features
- Macrocephaly (≥2 SD)
- At least one of the following four additional criteria should be present:
  - Autism or developmental delay
  - Dermatological features, including lipomas, trichillemmomas, oral papillomas, penile freckling
  - Vascular features, such as arteriovenous malformations or hemangiomas
  - Gastrointestinal polyps

*In addition, pediatric-onset thyroid cancer and germ cell tumors (testicular cancer and dysgerminoma) are recognized associations of Cowden Syndrome and should provoke consideration of *PTEN* testing.

RESULTS

PTEN Molecular Analysis

Molecular genetic analysis was performed on genomic DNA extracted from peripheral blood leukocytes. Real-time quantitative multiplex PCR analysis and a sequence analysis of *PTEN* exons (1, 2, 3, 4, 5, 6, 7, 8, and 9) and promoter region were performed [Zhou et al., 2003]. In patient 1 a de novo heterozygous mutation in exon 8 of *PTEN* (c.959T>A) was identified at codon 320 leading to premature termination of the protein (p.Leu320X). Based on literature review, germ-line mutation p.Leu320X was already reported in both variants c.959T>A and c.959T>G in patients with Cowden syndrome [Nelen et al., 1999; Eng, 2003]. In patient 2 *PTEN* mutational analysis showed a de novo heterozygous mutation in exon 7 (c.703G>T) resulting in a premature stop at codon 235 (p.Glu235X). To our knowledge, this mutation has never been reported in BRRS patients.

DISCUSSION

In this paper we report our experience following two patients with BRRS phenotype and germ-line *PTEN* mutations. Both patients came to our attention for clinical features suggestive of BRRS such as macrocephaly, developmental delay, and subcutaneous

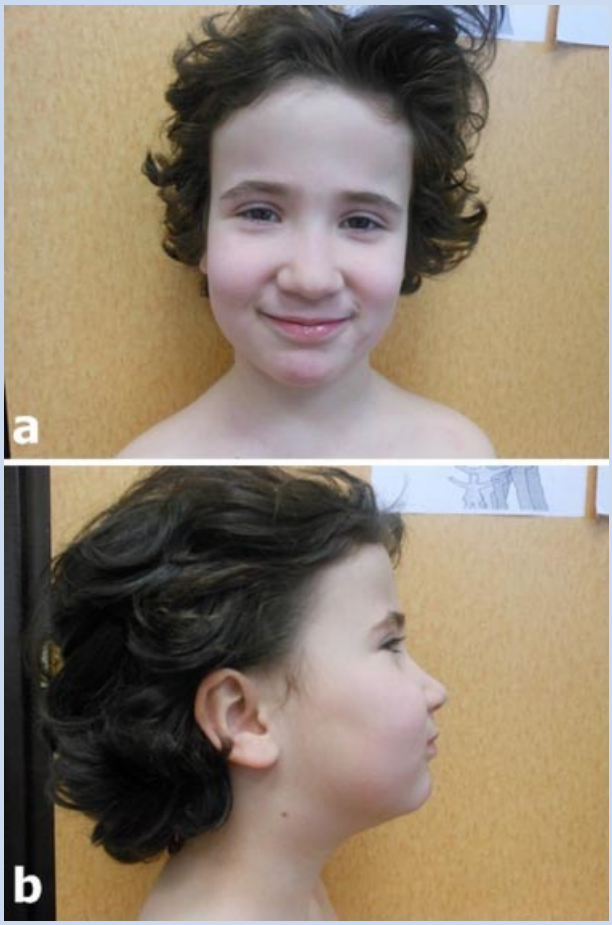

FIG. 1. Patient 1 at age six and half-years. There are no strikingly dysmorphic features; however, macrocephaly was clearly visible both in front (a) and side (b) view.

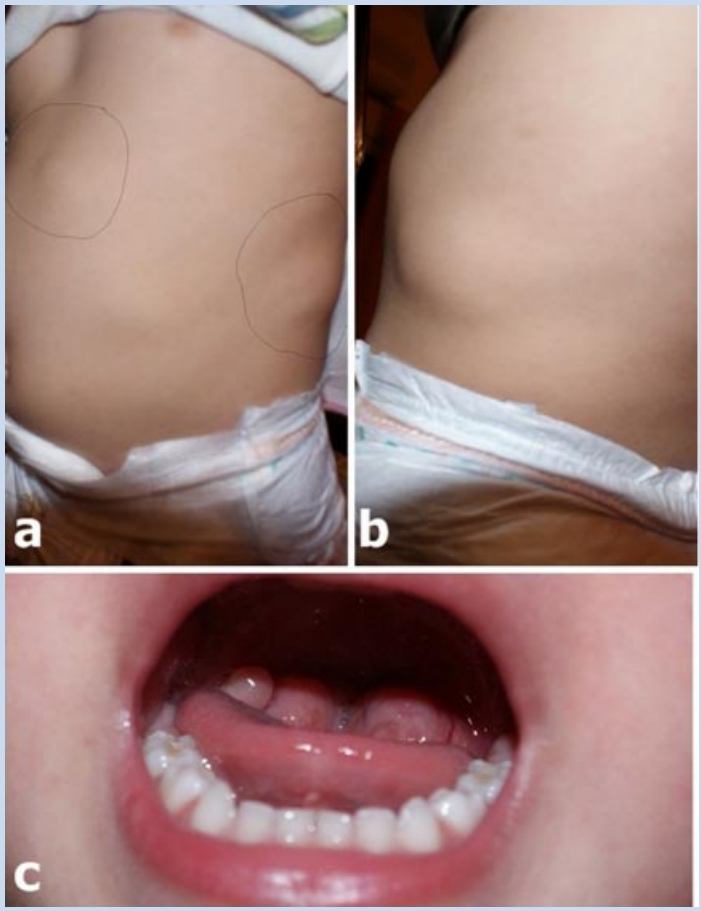

FIG. 2. Patient 2 was first evaluated at age of 24 months for multiple thoraco-abdominal swelling (a,b). Physical examination revealed mild tonsillar hypertrophy and a papilloma on the right side (c).

lipomatosis. Macrocephaly with normal ventricular size has been the most consistent finding in BRRS in several reviews [Moretti-Ferreira et al., 1989; Gorlin et al., 1992]. It is noteworthy that macrocephaly is usually present from birth and persists into adulthood [DiLiberti, 1998]. Motor delay or learning difficulties occur in most children diagnosed with BRRS [Gorlin et al., 1992]. However, most specific finding in the PHTS is presence of hamartomatous lesions. In both our patients, the diagnosis of BRRS has not been suspected until lipomas were identified. In Patient 1 we found a de novo heterozygous mutation in exon 8 of *PTEN* (c.959T>A) previously described in CS, confirming that these diseases are allelic, and suggesting that the phenotype may not be predictable on the basis of molecular analysis. It is therefore expected that no clear phenotype–genotype correlation will be made, and that patients with identical mutations may show different phenotype. In Patient 2 a de novo heterozygous mutation in exon 7 of *PTEN* has been identified at codon 703 (c.703G>T). This specific mutation has not been previously described in patients with BRRS, but it is likely to be pathogenic because it inactivates PTEN protein expression. The *PTEN* is a tumor suppressor gene with nine exons, which maps to human chromosome 10q23.31. It is also known as MMAC1 (Mutated in Multiple Advanced Cancers 1) [Steck et al., 1997] and TEP1 (TGF- regulated and Epithelial cell-enriched Phosphatase 1) [Li and Sun, 1997], and it encodes a 403 amino acid protein, which is a dual-specificity phosphatase since it can recognize both protein and phospholipid substrates [Li and Sun, 1997; Myers et al., 1998]. Most PTEN protein functions are to cause G1 cell-cycle arrest and/or apoptosis, and to inhibit cell migration [Sansal and Sellers, 2004]. Overall, decreased PTEN protein expression correlates with unregulated cellular proliferation. Somatic *PTEN* deletions and/or mutations occur with a wide distribution of frequencies in sporadic primary tumors, such as endometrial carcinomas, glioblastoma multiform, prostate cancer, breast cancer, and melanomas [De Vivo et al., 2000]. Germ-line *PTEN* mutations have been identified in patients suffering from CS [Marsh et al., 1998], BRRS [Longy et al., 1998], and Proteus-like syndrome [Zhou et al., 2001]. However, two additional disease phenotypes may also be associated with germ-line mutation or deletion of the *PTEN* gene. Germ-line *PTEN* mutations have been found in 10–20% of patients with autism spectrum disorder (ASD)

TABLE IV. Follow-Up Protocol for PHTS. Modified from Eng [2000] and Tan et al. [2012]

General surveillance
Annual physical examination[a]
Annual dermatologic examination[a]
Formal neurologic and psychological testing (<18 years)
Annual surveillance with fecal occult blood test (FOBT)[b]
Specific surveillance for thyroid cancer
Annual thyroid ultrasound examination[a]
Specific surveillance for breast cancer
Women
Monthly breast self-examination beginning at age 18 years
Annual clinical breast examination beginning at age 25 years[c]
Annual mammography and breast MRI beginning at age 30 years[c]
Men should perform monthly breast self-examination
Specific surveillance for endometrial cancer
Premenopausal women. Annual endometrial sampling beginning at age 30 years[c]
Postmenopausal women. Annual trans-vaginal ultrasound examination with biopsy of suspicious areas
Specific surveillance for other specific cancer
Biannual colonoscopy beginning at age 40 years[c]
Annual urinalysis and biannual renal ultrasound/MRI beginning at age 40 years[c]

[a]Surveillance may be recommend in all patients upon the diagnosis of PHTS, regardless of their age.
[b]FOBT should be considered for the early detection of intestinal hamartomas, which are not believed to increase the risk for colorectal cancer, but may be associated with complications (intussusception, rectal bleeding).
[c]Surveillance may begin 5 or 10 years before the earliest onset of a specific cancer in the family, but not later than the recommended age cutoff point.

and extreme macrocephaly, even without other features of BRRS or CS [Butler et al., 2005]. A germ-line mutation (H61D) has also been reported in a patient with features of VATER association (vertebral, anal, radial, and renal malformations in patients with esophageal atresia or tracheoesophageal fistula), hydrocephalus, and macrocephaly [Reardon et al., 2001]. To date, over 100 germ-line *PTEN* mutations have been found in CS e BRRS [Eng, 2003]. These mutations are distributed along the length of the gene, with the exception of exon 9 (no mutation reported) [Bonneau and Longy, 2000]. Most of the mutations have been found in exon 5, 7, and 8, especially in exon 5, which encodes the N-terminal phosphatase catalytic domain [Marsh et al., 1998].

Although the clinical manifestations of PHTS differ significantly, all four syndromes are characterized by aberrant tissue growth likely related to tumor suppressor role of *PTEN* gene. Genotype–phenotype analyses revealed an association between the presence of *PTEN* mutation in BRRS and the development of benign tumor of various tissues and organs (lipomas, hemangiomas, thyroid adenoma, gastrointestinal hamartomatous polyps) [Hendriks et al., 2003]. However, the most serious consequences of PHTS relate to the increased risk of cancers including breast, thyroid, endometrial, and to a lesser extent, renal. Thus far, an increased risk of malignancy has only been documented in CS [Hendriks et al., 2003]. Cancer risk associated with BRRS is unknown. However, several studies reported the presentation of thyroid nodules and thyroid cancer in young children with PHTS [Ngeow et al., 2011; Smith et al., 2011]. Another report of a malignancy concerned a case of breast cancer in a BRRS patient (the paternal grandmother of the proband died at the age 53 years of breast cancer and endometrial adenocarcinoma) [Longy et al., 1998]. Furthermore, genotype–phenotype analysis within the BRRS group confirmed that breast cancer and fibroadenomas have been found in patients with BRRS, CS, or patients with overlapping features [Marsh et al., 1999]. Therefore, since BRRS and CS are allelic conditions, it may be warranted to follow patients with BRRS and germ-line *PTEN* mutation with the same surveillance protocol. However, no guidelines currently exist from this point of view. Recently a prospective international study proposed new recommendations for management of patients with *PTEN* mutation [Tan et al., 2012]. We advise that all patients with PHTS follow a cancer surveillance strategy and also a specific surveillance for gastrointestinal and vascular complications, as shown in Table IV. In both our patients thyroid ultrasound examination has never shown any tumor illness. The fecal occult-blood testing has been negative too. In Patient 2, the tonsillar mass on the right side became larger and rougher after only 6 months (Fig. 3); hence, tonsillectomy should be performed for histopathologic examination.

In conclusion, our finding suggest that molecular testing for *PTEN* gene mutations in patients with extreme macrocephaly, developmental delay, and hamartomatous lesions, even in the absence of other CS/BRRS related clinical features, should be considered. Clinical management of PHTS patients consists of an early multidisciplinary surveillance program starting from diagnosis.

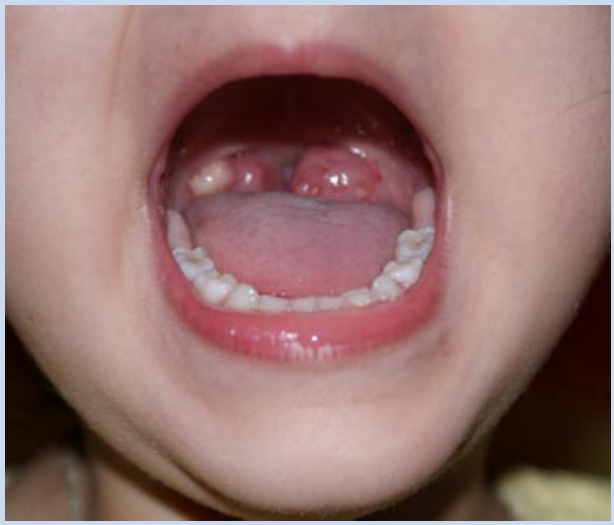

FIG. 3. Patient 2: The tonsil papilloma became larger and rougher after 6 months.

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