Infantile macrocephaly and multiple subcutaneous lipomas diagnosed with PTEN hamartoma tumor syndrome: A case report

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Abstract. A heterozygous loss-of-function mutation of the PTEN gene, one of the tumor suppressor genes, causes a wide variety of disorders, ranging from macrocephaly/autism syndrome to PTEN hamartoma tumor syndrome, including Cowden disease that causes thyroid and breast cancer mainly in the adolescence and young adult generation. An 8-month-old male infant with simple macrocephaly developed a café-au-lait spot and two subcutaneous tumors at the age of 1 year. One of the tumors developed rapidly was resected at the age of 1 year and 9 months and identified as benign lipoma. From the age of 2 years, the patient often threw a tantrum. At the age of 2 years and 9 months, a pathogenic germline mutation was identified in the PTEN gene (NM_000314.7), c.195C>A, p.Y65* in the form of a heterozygous germline variant. Developmental delay was noted but no tumors were found in the thyroid gland and breasts. Immunohistochemistry for PTEN in the resected lipoma demonstrated that the PTEN expression pattern was similar to that in a subcutaneous adipose tissue from a normal subject, suggesting that two-hit was not likely involved in the rapid growth of this lipoma. At the age of 5 years, the patient was diagnosed with autism spectrum disorders with moderate developmental delay. A long-term follow-up is underway to examine developmental changes in psychomotor disorders and possible tumor formation.

Introduction

The phosphatase and tensin homolog (PTEN) gene is one of the tumor suppressor genes (1) located in chromosome 10q23.31, encoding 403 amino acids of 47166 Da protein. Loss of heterozygosity and deletion of this gene was first reported in glioblastomas, and later in several malignant tumors (2). PTEN germline mutations cause a wide variety of phenotypic diseases, such as macrocephaly/autism syndrome (OMIM #605309) usually noticed in infants and PTEN hamartoma tumor syndrome (PHTS, OMIM #601728). PHTS includes Cowden syndrome (CS, OMIM#158350) and Bannayan-Riley-Ruvalcaba syndrome (BRRS, OMIM#153480) (3).

Macrocephaly/autism syndrome is an autosomal dominant disorder characterized by increased head circumference, abnormal facial features, and delayed psychomotor development resulting in autistic behavior or mental retardation (4). Varga et al (5) reported that PTEN mutations were detected in 5 of 60 (8.3%) patients with autism spectrum disorder (ASD) and 6 of 49 (12.2%) patients with developmental delay and macrocephaly without ASD.

CS is a multiple hamartoma syndrome with a high risk for benign and malignant tumors of the thyroid, breast, and endometrium in young adults and adults. Arteriovenous malformation, multiple lipomas, and other soft-tissue tumors are also reported (3,6). Affected individuals usually develop macrocephaly, trichilemmomas, and papillomatous papules by late 20s. On the other hand, BRRS is a congenital disorder characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, and pigmented macules of the glans penis (3). For PHTS patients, 2019.2 NCCN guideline (7) recommends that tumor follow-up involves annual physical examination and thyroid ultrasound, with colonoscopy every 5 years beginning at age 35 or earlier based on family colon cancer history and kidney ultrasound every 1-2 years starting at age 40.

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We found a de novo PTEN germline mutation in a male infant with macrocephaly and lipomas by using NGS analysis. A rapidly growing lipoma was resected and examined for PTEN by immunostaining, since there have been few reports on PTEN inactivation, two hits or one hit, in tumors in PHTS patients.

**Case report**

**Patient.** Male infant was born after 37 weeks gestation with 4.078 g (+2.7 SD) in weight, 52 cm (+1.4 SD) in height and 36 cm (+1.9 SD) in head circumference.

Family history: No physical abnormalities are apparent with the father, 37 years old, the mother, 33 years old and a sister, 3 years old.

Pregnancy history: Pregnancy progressed uneventfully. The delivery was through the vagina after induction.

Postnatal progress: No special findings in one- and four-month postnatal examinations. The infant showed roll-over at 6 months old, neck stabilization at 7 months old, and independent gait at 1 year and 5 months. He exhibited obsession and temper tantrum frequently after 2 years of age. Macrocephaly was pointed out when he was taken to a hospital for treatment of bronchitis at the age of 8 months. His height was then 70 cm (+2.8 SD), weight 8,845 g (+0.2 SD) and head circumference 48.5 cm (+2.8 SD). Brain MRI showed no abnormal signals in cerebral parenchyma (data not shown), indicating that his macrocephaly was a simple one. To elucidate the cause of macrocephaly, genetic testing was performed at the age of 1 year and 9 months under the informed consents of the parents.

At the age of one-year, two elastic soft subcutaneous tumors of 1-2 cm in diameter appeared in the abdomen and in the right side of the back. A café-au-lait spot of 2 cm in diameter was also found on the left side of the back. At the age of 1 year and 9 months, one tumor in the abdomen rapidly enlarged to 6x5 cm (Fig. 1A) and was surgically removed. The removed tumor was soft and yellowish and macroscopically diagnosed as a lipoma.

At the age of 2 years and 9 months, the results of genetic testing were reported, and genetic counseling was performed. At this time, his height was 92 cm (+0.2 SD), weight 16 kg (+2.1 SD), and head circumference 55 cm (+3.7 SD). He showed a broad and projected forehead, a flat nasal root and low-set deformed auricles. A café-au-lait spot of 2 cm in size was present on the left back and subcutaneous tumors of 2x2 cm in the right back (Fig. 1B) and in the sole (not shown). Developmental test by Kyoto Scale of Psychological Development 2001 (8) showed 64 scores in total developmental quotient (100 as an average), indicating that he had moderate developmental delay.

At the age of 3 years, follow-up brain MRI showed no abnormality in the cerebral white matter (Fig. 1C and D), except for the hypertrophied corpus callosum (Fig. 1E) and enlargement of the perivascular space (Fig. 1F), of which findings were consistent with simple macrocephaly.

At the age of 5 years, subcutaneous tumors in the back and the sole remained the same in size, and no newly developed tumors and café-au-lait spots were detected. He could not communicate normally and was diagnosed as ASD with moderate developmental delay.

**Chromosomal analysis.** Using peripheral blood, G-banding was performed.

**Whole-exome sequencing and Sanger sequencing.** DNA was extracted from peripheral blood of the patient and the parents and Whole exome sequencing was performed as previously described (9). Regions suspected of containing pathological mutations were amplified by PCR and subjected to Sanger analysis (HGMD® Professional 2016.1)

**Pathologic examination.** The surgical specimen was fixed in formalin and embedded in a paraffin block. Sections cut from the block were stained with hematoxylin-eosin and with an immunoperoxidase method using anti-PTEN antibody (Dako/Agilent Technologies, Santa Clara, CA). Stained sections were examined under a light microscope. As a control, a subcutaneous fat containing skin sample obtained from a one-year-old male infant without CS were used anonymously.

**Chromosomal analysis.** Chromosomal analysis showed a normal karyotype.

**Mutation of the PTEN gene.** In exon 3 of the PTEN (NM_000314.7), c.195C>A, p.Y65? was found as a heterozygous germline variant in the patient. This mutation is considered as pathogenic, since the same mutation has been reported in one young adult female with macrocephaly/autism syndrome and one adult female with CS (10,11) (referred HGMD® Professional 2019.1). The predicting truncated PTEN protein with deletion of most of the C-terminal region is likely unstable leading to haploinsufficiency. This mutation is sporadic, since his parents did not carry the mutation. Based on these results, we diagnosed this patient with PTEN hamartoma tumor syndrome (PHTS). As this mutation was reported in a CS case, we performed ultrasound analysis of the thyroid and visual and palpitation inspection of breasts of the patient at 2 years and 10 months. No abnormalities were found in both tissues. Endoscopic examination of gastrointestinal tract was not performed because of his young age.

**Pathologic findings of the subcutaneous tumor.** The surgical specimen had a lobulated macroscopic appearance and was encapsulated with delicate fibrous veil (Fig. 2A). Histologically, it consisted of mature adipocytes, sparse blood vessels and thin collagen bundles (Fig. 2B), and the diagnosis of lipoma was confirmed. Immunohistochemically, PTEN expression was observed in vessels in the control sample, as was expected (Fig. 2C and D). A few subcutaneous adipocytes were also stained. Similarly, in the lipoma tissue, vessels and a small number of neoplastic adipocytes were PTEN-positive (Fig. 2E and F). No obvious differences in PTEN expression, its distribution pattern and intensity, were detected between the lipoma tissue and the control tissue. Fig. 3 shows the position of the mutation in the PTEN protein (12). Anti-PTEN antibody binds to a C-terminal region (13). It is not possible to bind to the truncated protein due to the nonsense mutation in this patient (Fig. 3).

**Discussion**

We found a PTEN mutation by NGS analysis in a male infant with macrocephaly. Brain MRI examination showed...
simple macrocephaly consistent with *PTEN* macrocephaly/autism syndrome reported by Vanderver *et al* (14) and Bhargava *et al* (15).

Table I shows 30 patients of <3 years of age with macrocephaly or autism spectrum in whom *PTEN* mutations were detected (4,11,14-20). Clinical manifestations of these patients are presented in Table II. All cases showed developmental delay with 8 cases diagnosed as autism spectrum and 10 hypotonia.

Ten of 30 cases showed frontal bossing with 8 exhibiting café-au-lait spots and skin features, and 5 diagnosed with tumors and hamartomas, such as gastrointestinal polyps and cutaneous lipomas. Our case, an only in infant patient with macrocephaly/autism syndrome reported, suggests that the *PTEN* mutation detected is responsible for the syndrome.

All the 30 patients with macrocephaly in Table I showed no apparent genotype-phenotype correlation nor malignant
Table I. Reported 30 patients diagnosed as having PTEN mutation at <3 years.

| Author, year | Case no. | Age | PTEN mutation | Inheritance | (Refs.) |
|--------------|----------|-----|---------------|-------------|---------|
| Vanderver et al, 2014 | 1 | 0d | partial deletion of exon 6, identified on array-CGH; arr10q23.31 (89,683,610 -89,702,204) | de novo | (14) |
| Vanderver et al, 2014 | 2 | 3m | c.1120_1121dup : p.D375+ | de novo | (14) |
| Vanderver et al, 2014 | 3 | 5m | c.A17T : p.K61 | ND | (14) |
| Tan et al, 2011 and Vanderver et al, 2014 | 4 | 7m | c.253+5G>T | de novo | (11,14) |
| Vanderver et al, 2014 | 5 | 7m | Yes | ND | (14) |
| Tan et al, 2011 and Vanderver et al, 2014 | 6 | 8m | c.T149C : p.I50T | de novo | (11,14) |
| Present study | 7 | 8m | c.A17T : p.K61 | de novo | (14) |
| Varga et al, 2009 | 8 | 9m | p.R173H | Maternal | (5) |
| Varga et al, 2009 | 9 | 9m | c.IVS8-2A>G | Paternal | (5) |
| Vanderver et al, 2014 | 10 | 10m | c.A80G : p.Y27C | de novo | (14) |
| Vanderver et al, 2014, Bhargava et al, 2014, and Rodriguez-Escudero et al, 2011 | 11 | 10m | c.G131A : p.G44D | ND | (14,15,16) |
| Vanderver et al, 2014 and Nelen et al, 1997 | 12 | 10m | c.C388T : p.R130 | ND | (14,17) |
| Vanderver et al, 2014 | 13 | 10m | c.C511G : p.Q171E | Familial | (14) |
| Vanderver et al, 2014 and Eng, 2003 | 14 | 10m | c.C633G : p.C211W | Familial | (14,18) |
| Herman et al, 2007 and Vanderver et al, 2014 | 15 | 10m | c.C1003T : p.R335 | de novo | (4,14) |
| Vanderver et al, 2014 | 16 | 11m | c.A166G : p.K61E | ND | (14) |
| Vanderver et al, 2014 | 17 | 11m | c.G853T : p.G285 | de novo | (14) |
| Vanderver et al, 2014 | 18 | 12m | c. A320G : p.D107G | ND | (14) |
| Vanderver et al, 2014 | 19 | 1y3m | c.C138G : p.Y46 | ND | (14) |
| Herman et al, 2007 and Varga et al, 2009 | 20 | 1y4m | c.520insT | de novo | (4,5) |
| Tan et al, 2011 and Vanderver et al, 2014 | 21 | 1y6m | c.A45T : p.R15S | de novo | (11,14) |
| Hansen-Kiss et al, 2017 | 22 | 1y6m | c.G1004A: p.R335Q | Paternal | (19) |
| Varga et al, 2009 | 23 | 1y8m | p.T202I | de novo | (5) |
| Vanderver et al, 2014 and Eng, 2003 | 24 | 2y | c.T959G : p.L320 | ND | (14,18) |
| Hansen-Kiss et al, 2017 | 25 | 2y | c.607_608delAT : p.L230 | Maternal | (19) |
| Hansen-Kiss et al, 2017 | 26 | 2y | c.A667T : p.K223 | ND | (19) |
| Varga et al, 2009 | 27 | 2y3m | p.G44D | ND | (5) |
| Butler et al, 2005 | 28 | 2y6m | p.F241S | ND | (20) |
| Bhargava et al, 2014 | 29 | 2y7m | No protein | ND | (15) |
| Bhargava et al, 2014 | 30 | 2y8m | No protein | ND | (15) |

ND, not determined; d, days; m, months; y, years.

Figure 3. PTEN protein, p.Y65 is the position of the mutation in the PTEN protein. PIP2-binding domain (aa 1-13), a catalytic tensin-type phosphatase domain (aa 14-185), a C2 tensin-type domain, which binds phospholipids (aa 190-350), C-terminal tail, the carboxy-terminal tail of the protein (aa 350-400), and a PDZ-binding domain (aa 401-403) are shown (20). AB 6H2.1: Anti-PTEN antibody binds to C-terminal 100AA (21). Anti-PTEN does not bind to the patients' protein due to truncation of the binding site. PIP2, phosphatidylinositol 4,5-bisphosphate.
tumors that are frequently observed in patients with CS. The risk of developing malignant tumors in later years is not clear, without follow-up data.

Tan et al (21) reported lifetime cancer risks of individuals with PTEN germline mutation listing a variety of cancers (breast, thyroid, endometrial, colorectal, renal cell, tumors). Table II. Clinical characteristics of 30 patients diagnosed as having PTEN mutation at <3 years old.

| Case no. | Age  | Sex | Growth | Neurological findings | Physical features | Others |
|---------|------|-----|--------|-----------------------|------------------|--------|
|         |      |     | MC     | MS                    | Hypotonia        | Nevus and hamartoma/tumor |
| 1       | 0d   | M   | +      | +                     | +^a              | +      | Postaxial polydactyly |
| 2       | 3m   | F   | +      | +                     | +^b              | +      | Pigmented speckled macules of the glans penis |
| 3       | 5m   | M   | +      | +                     | +^c              | +      | Café-au-lait spot, thyroid, nodules testicular hamartomas, rectal and gastric polyps |
| 4       | 7m   | F   | +      | +                     | +^d              | +      | Café-au-lait spot, subcutaneous lipomas |
| 5       | 7m   | M   | +      | +                     | +^e              | +      | Abnormal EEG but no seizures |
| 6       | 8m   | M   | +      | +                     | +^f              | +      | Abdomen and axillary trichilemmomas, subcutaneous lipomas |
| 7       | 8m   | M   | +(2.8 SD) | +                  | +^g              | +      | Mucosal neuroma |
| 8       | 9m   | M   | +(4.4 SD) | +                  | +^h              | +      | Dermalogical features, BRRS |
| 9       | 9m   | M   | +(3.5 SD) | +                  | +^i              | +      | Large café-au-lait spots on chest and abdomen |
| 10      | 10m  | F   | +      | +                     | +^j              | +      | Freckles on the glans penis |
| 11      | 10m  | M   | +      | +                     | +^k              | +      | Left cataract |
| 12      | 10m  | F   | +      | +                     | +^l              | +      | Bilateral hernia |
| 13      | 10m  | F   | +      | +                     | +^m              | +      | Cheek and abdomen |
| 14      | 10m  | M   | +      | +                     | +^n              | +      | Freckles on the glans penis |
| 15      | 10m  | F   | +      | +                     | +^o              | +      | Moles, thyroid nodules, intestinal polyps |
| 16      | 11m  | F   | +      | +                     | +^p              | +      | Bilateral hernia |
| 17      | 11m  | F   | +      | +                     | +^q              | +      | Cheek and abdomen |
| 18      | 1m   | M   | +      | +                     | +^r              | +      | Freckles on the glans penis |
| 19      | 1y3m | F   | +      | +                     | +^s              | +      | Moles, thyroid nodules, intestinal polyps |
| 20      | 1y4m | F   | +(5.8 SD) | +                  | +^t              | +      | Bilateral hernia |
| 21      | 1y6m | M   | +      | +                     | +^u              | +      | Cheek and abdomen |
| 22      | 1y6m | F   | +(5.6 SD) | +                  | +^v              | +      | Freckles on the glans penis |
| 23      | 1y8m | M   | +      | +                     | +^w              | +      | Moles, thyroid nodules, intestinal polyps |
| 24      | 2y   | M   | +      | +                     | +^x              | +      | Bilateral hernia |
| 25      | 2y   | F   | +(6.5 SD) | +                  | +^y              | +      | Cheek and abdomen |
| 26      | 2y   | F   | +(4.7 SD) | +                  | +^z              | +      | Freckles on the glans penis |
| 27      | 2y3m| F   | +(5.0 SD) | +                  | +^aa             | +      | Moles, thyroid nodules, intestinal polyps |
| 28      | 2y6m| M   | +(4.5 SD) | +                  | +^ab             | +      | Bilateral hernia |
| 29      | 2y7m| M   | +      | +                     | +^ac             | +      | Cheek and abdomen |
| 30      | 2y8m| M   | +      | +                     | +^ad             | +      | Freckles on the glans penis |

^aMild motor only at 2.5 y; ^bdiagnosed at 2 y 8 m; ^cmild motor delay only; ^dmotor speech disorder; ^ediagnosed at 5 y; ^fdiagnosed at 3 y 0 m; ^hdiagnosed at 3 y; ^itranslated at 2 y 1 m; ^jfrontal bossing; ^kenotched ears, right ear larger than left and facial asymmetry; ^lfrontal bossing and hypertelorism; ^mfrontal bossing, depressed nasal bridge, bulbous nose, and smooth philtrum. MC, macrocephaly (head circumference >2 SD); MS, macrosomia; MR, mental retardation/developmental delay; AS, autism spectrum; BRRS, Bannayan-Riley-Ruvalcaba syndrome; d, days; m, months; y, years.
and melanoma) found in a cohort of 368 children and adults aged 0.4-83 years (median age; 39 years). The earliest age of cancer onset reported was 3 years for melanoma. In this paper, no follow-up analysis from childhood to adulthood was reported. Consequently, it is not clear as to how many infant macrocephaly cases with PTEN mutation developed to CS in adulthood. Smokou et al (22) reported that, in the case of a 7-year-old patient with a thyroid cancer, clinical description would allow better formulation of clinical guidelines in children with PHTS. For infant patients with PTEN mutation, pediatricians have tendency to focus attention on macrocephaly, developmental delay or autism. It is therefore important to conduct follow-up assessment for both developmental problem and cancer incidence, and carry out lifelong and total medical management.

On this basis, we conducted thyroid ultrasonography of our patient at the age of 2 years and 10 months, 4 years and 5 years of age and found no abnormality. Regarding gastrointestinal tract hamartomas, we did not perform endoscopic examination, since there have been no reports on the onset in childhood. We examined breasts only by inspection and palpation since the possibility of breast cancer was considered to be lower for male infant patients than female (23).

Lipomas and a café-au-lait spot on the skin were also found in our case. Our immunohistochemical examination revealed that lipoma tissue and the skin tissue obtained from a control subject showed very similar PTEN expression. No obvious differences in PTEN expression were detected among them, suggesting that two-hit in the PTEN gene by loss of heterozygosity was unlikely even in the rapidly growing lipoma. A previous report shows that loss of heterozygosity of markers in endometrial cancer, glioblastoma, and breast cancer (13). In this study, genetic counseling for the patient’s family was performed by a geneticist, who may feel at risk. Taken together, long-term follow-up plans for soft-tissue tumors, thyroid cancer, breast cancer and GI-hamartomas, as well as psychosocial problems are indispensable.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

YY wrote the manuscript. YY, AH and JT acquired the patient data and contributed clinical advice. YI pathologically diagnosed the patient and wrote the manuscript. HU evaluated the images. SM, NM and YK performed genetic analysis. THT performed genetic counseling and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Gene analysis was conducted with approval of Ethical Review Board of Takatsuki General Hospital (IRB no. 2012-8). Histological analysis was conducted after obtaining approval of Ethical Review Board of Takatsuki General Hospital (IRB No. 2017-25).

Patient consent for publication

Informed consent was obtained from a parent of the patient for the publication of the case details and any associated images since the patient was a child.

Competing interests

The authors declare that they have no competing interests.

References

1. Li J, Yen C, Liaw D, Podsyspanina K, Bose S, Wang SJ, Puc J, Miliareis C, Rodgers L, McCombie R, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275: 1943-1947, 1997.
2. Teng DH, Hu R, Lin H, Davis T, Iliev D, Frye C, Svedlund B, Hansen KL, Vinson VL, Gumper KL, et al: MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. Cancer Res 57: 5221-5225, 1997.
3. Eng C: PTEN hamartoma tumor syndrome. Gene reviews https://www.ncbi.nlm.nih.gov/books/NBK1488/ Accessed 1 March 2017.
4. Herman GE, Butter E, Enrile B, Pastore M, Prior TW and Sommer A: Increasing knowledge of PTEN germline mutations: Two additional patients with autism and macrocephaly. Am J Med Genet A 143A: 589-593, 2007.
5. Varga EA, Pastore M, Prior T, Herman GE and McBride KL: The prevalence of PTEN mutations in a clinical pediatric cohort with autism spectrum disorders, developmental delay, and macrocephaly. Genet Med 11: 111-117, 2009.
6. Kurek KC, Howard E, Tennant LB, Upton J, Alomari A, Burrows PE, Chalache K, Harris DJ, Trenor CC III, Eng C, et al: PTEN hamartoma of soft tissue: A distinctive lesion in PTEN syndromes. Am J Surg Pathol 36: 671-87, 2012.
7. NCCN Clinical Practice Guidelines in OncologyVersion 3.2019 Cowden syndrome/PHTS. NCCN Guidelines®: https://www.nccn.org/professionals/physician_gls/pdf/genetics_screening. pdf. Accessed 8 May 2019.
8. Society for the Kyoto scale of psychological development test. Shinpan K Shiki Hattatsu Kenshahou 2001 Nenban (The Kyoto Scale of Psychological Development Test 2001). Kyoto, Japan: Nakanishiya Shuppan, 2008.
9. Iwama K, Osaka H, Ikeda T, Mitsuhashi S, Miyatake S, Takata A, Miyake N, Ito S, Mizuguchi T and Matsumoto N: A novel SLC9A1 mutation causes cerebellar ataxia. J Hum Genet 63: 1049-1054, 2018.
10. D’Gama AM, Pochareddy S, Li M, Jamuar SS, Reiff RE, Lam AN, Sestan N and Walsh CA: Targeted DNA sequencing from autism spectrum disorder brains implicates multiple genetic mechanisms. Neuron 88: 910-917, 2015.

11. Tan MH, Mester J, Peterson C, Yang Y, Chen JL, Rybicki LA, Milas K, Pederson H, Remzi B, Orloff MS and Eng C: A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands. Am J Hum Genet 88: 42-56, 2011.

12. Bermúdez Brito M, Goulielmaki E and Papakonstanti EA: Focus on PTEN regulation. Front Oncol 5: 166, 2015.

13. Perren A, Weng LP, Boag AH, Ziebold U, Thakore K, Dahia PL, Komminoth P, Lees JA, Mulligan LM, Mutter GL and Eng C: Immunohistochemical evidence of loss of PTEN expression in primary ductal adenocarcinomas of the breast. Am J Pathol 155: 1253-1260, 1999.

14. VanderVeer A, Tonduti D, Kahn I, Schmidt J, Medne L, Vento J, Chapman KA, Lanpher B, Pearl P, Gropman A, et al: Characteristic brain magnetic resonance imaging pattern in patients with macrocephaly and PTEN mutations. Am J Med Genet A 164A: 627-633, 2014.

15. Bhargava R, Au Yong KJ and Leonard N: Bannayan-Riley-Ruvalcaba syndrome: MRI neuroimaging features in a series of 7 patients. Am J Neuroradiol 35: 402-406, 2014.

16. Rodríguez-Escudero J, Oliver MD, Andrés-Pons A, Molina M, Cid VJ and Paludo R: A comprehensive functional analysis of PTEN mutations: Implications in tumor- and autism-related syndromes. Hum Mol Genet 20: 4132-4142, 2011.

17. Nelen MR, van Staveren WC, Peeters EA, Hassel MB, Gorlin RJ, Hamm H, Lindboe CF, Fryns JP, Sijmons RH, Woods DG, et al: Germline mutations in the PTEN/MMAC1 gene in patients with Cowden disease. Hum Mol Genet 6: 1383-1387, 1997.

18. Eng C: PTEN: One gene, many syndromes. Hum Mutat 22: 183-198, 2003.

19. Hansen-Kiss E, Beikampen S, Adler B, Frazier T, Prior T, Erdman S, Eng C and Herman G: A retrospective chart review of the features of PTEN hamartoma tumour syndrome in children. J Med Genet 54: 471-478, 2017.

20. Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, Miles JH, Wang CH, Stratton R, Pilarski R and Eng C: Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. J Med Genet 42: 318-321, 2005.

21. Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS and Eng C: Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res 18: 400-407, 2012.

22. Smpokou P, Fox VL and Tan WH: PTEN hamartoma tumour syndrome: Early tumour development in children. Arch Dis Child 100: 34-37, 2015.

23. Pilarski R: PTEN hamartoma tumor syndrome: A clinical overview. Cancers (Basel) 11: E844, 2019.