Cancer predisposition in children: genetics, phenotypes & screening
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PTEN hamartoma tumor syndrome and Gorham-Stout phenomenon

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Abstract

PTEN hamartoma tumor syndrome (PHTS) is a group of syndromes caused by mutations in PTEN. Gorham-Stout phenomenon (GSP) is a rare condition characterized by proliferation of vascular structures in bones, resulting in progressive osteolysis. Here we present a one-year-old boy with PHTS and GSP. The lesion that later proved to be GSP was evident from the age of four months, and became symptomatic at the age of one year. Eventually, he developed a fatal chylothorax. Mutation analysis revealed a germline heterozygous mutation c.517 C>T (p.Arg173Cys) in exon 6 of PTEN. Analysis of the lymphatic malformation tissue revealed no loss of heterozygosity (LOH) nor a second, somatic PTEN mutation of the remaining wild type allele. The germline p.Arg173Cys mutation was also present in the mother and the propositus’ younger sister and brother. Further molecular work-up showed a heterozygous variant c.2180C>T (p.Ala727Val) FLT4 in the lymphatic malformation tissue, which was also present in the germline of mother and two sibs. Gorham-Stout phenomenon has not been reported before in a patient with a PTEN mutation. Up to this date, this mutation is the only genetic defect possibly involved in the etiology of Gorham-Stout phenomenon which is plausible given the known function of PTEN in angiogenic signalling.
Introduction

The PTEN hamartoma tumor syndrome (PHTS) is a collective term for clinical entities caused by a germline mutation in PTEN and leading to formation of benign and malignant neoplasms. Macrocephaly is common. PHTS includes PTEN mutation-positive subsets of Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), and an entity that resembles Proteus syndrome (PS) but is still different and has been designated with different terms. All PTEN hamartoma syndromes overlap clinically, but can usually be distinguished from one another. Germline mutations in the tumor suppressor gene PTEN (MIM 601728, phosphatase and tensin homolog at 10q23.31) have been found in 80% of individuals seen by specialist tertiary care centers, in 25% from the community who meet the full diagnostic criteria for classic Cowden syndrome, and in 60% of individuals who meet the clinical diagnosis of BRRS.

PTEN encodes a dual phosphatase protein that antagonizes the PI3K/Akt/mTOR pathway and negatively regulates the MAPK pathway. PTEN mutations lead to unregulated cellular proliferation which gives rise to the formation of malignant and benign tumors, especially hamartomas. Because of the increased cancer risks, recognition of PHTS is important.

Gorham-Stout phenomenon, also referred to as “Gorham massive osteolysis”, vanishing bone disease or disappearing bone disease, was first defined by Gorham and Stout. They originally referred to the condition as a “syndrome”. As the cause is likely to be heterogeneous, it is more appropriate to tag it as a sign rather than an entity in itself, and thus we prefer to use the term Gorham-Stout phenomenon (GSP). It refers to progressive idiopathic massive osteolysis and lymphatic malformations (LM) with disappearance of bone. Histologic study shows prominent proliferation of lymphatic and blood vessels, which has been described as “hemangiomatosis” (venous malformation, VM) or “lymphangiomatosis” (lymphatic malformation, LM). The vascular malformed tissue growing rampant invades the bone and its surrounding soft tissue without respect for anatomical boundaries. The condition affects men and women equally, occurs mostly in young individuals, and affects most often the skull, shoulder and pelvic girdle but may affect any part of the body. The course and outcome of the disease is diverse, clinical manifestations depend on the affected site. 17% of the patients have been reported to have chylothorax as a complication of chest involvement, often resulting in respiratory failure and death. The underlying causes of GSP are unknown and no genetic defects have been associated with the disease.

Here we present a boy with GSP and molecularly proven PHTS, and discuss the possible relationship between the two findings.

Clinical report

The patient was born to a mother of Caucasian ancestry and a father of Caucasian/Chinese/Indonesian ancestry after an uneventful pregnancy of 38 weeks. His weight at birth was
3810 g (75th-97.7th centile); length was 52 cm (50th centile) and head circumference 36 cm (50th centile). At birth, a skin lesion was noticed under the right axilla, which initially looked like an open blister surrounded by erythematous skin. At four months, the lesion started growing and developed into a subcutaneous tumor.

At 12 months he was referred because of progressive dyspnoea. The patient had reached the developmental milestones within the normal range and clinically appeared to follow a normal cognitive development. Clinical exam showed macrocephaly (head circumference 50.5 cm, > 97.7th centile) and a vascular tumor covering part of the right axilla and flank (Figure 1 A).

The chest radiograph showed absence of VIIIth and IXth rib on the right side and extensive pleural fluid. A chest MRI confirmed an extensive tumor resembling a lymphatic malformation, displacing most of the right lung and infiltrating the surrounding tissues (Figure 1C). Ultrasound study of liver and spleen showed venous malformations, and limb radiographs showed osteolytic lesions of humerus, femur and tibia, most pronounced in the

![Figure 1: Clinical pictures A/B: Clinical presentation  A: At age 12 months, note erythematous skin and hemangioma-like appearance of the tumor. B: At age 20 months, note growth of lymphangioma, dark purple discoloration.  C/D: Radiological examinations  C: Coronal STIR (TR4000ms, TE 70.9ms, SL.Th 3mm, FLA 90° showing soft tissue involvement of the right chest wall, right chest cavity and left chest wall. Note splenic involvement (arrow); S= stomach. D: Post-mortem chest radiograph at 24 months, showing marked progression of osteolysis. Note fracture of rib VI, thinned cortex of rib VII, absence of rib VIII en IX and involvement of rib XI and XII. Note bilateral involvement of the proximal humerus and collars of the thoracic vertebrae 8 to 12.  E/F/G: Postmortem examination.  E. Large number of small and medium-sized blood vessels in inner thoracic wall. F. Mid-thoracic vertebrae with destructed vertebral bodies. G. Collagen IV staining of a section through a vertebra showing numerous thin walled and dilated lymphatic vessels (brown outline) that have replaced the pre-existing bone trabecula (B).]
distal femoral metaphyses. The possibility of Maffucci syndrome was raised, but clinically and radiologically the hands were not affected. Biopsy of the intra-thoracic lesion was estimated to have an unacceptable risk for complications, therefore only a chest wall biopsy was taken. This confirmed the clinical suspicion of lymphatic malformation. The dyspnoea was managed by pleural drainage and oxygen supplementation but his general condition deteriorated. Intravenous interferon-alpha 2b (5,000,000 units/ m² per day) stabilized the thoracic lymphatic malformation for 5 months, but was subsequently discontinued as prolonged interferon-alpha therapy has a risk of persisting bone marrow suppression. After 3 months, the lymphatic malformation started expanding again and eventually covered most of his thorax (Figure 1 B). The patient died of respiratory insufficiency at age 2 years.

Pathology examination

Postmortem macroscopic examination showed extensive lymphatic malformation in the thorax, covering most of the right lung and infiltrating the chest wall (Figure 1 E) and vertebral column (Figure 1 F). Most of the ribs in the right hemi-thorax and part of the right clavicle and scapula had disappeared. Lymphatic malformations were found in liver and spleen and in the vertebrae lymphatic vessels were found that had replaced the pre-existing bone trabecula (Figure 1 G).

Molecular studies

Propositus meets the pediatric criteria for PTEN testing recently developed by Tan et al., in terms of macrocephaly and vascular manifestations 1. PTEN sequence analysis of the patient’s lymphocytic DNA showed a germline heterozygous mutation c.517 C>T (p.Arg173Cys) in exon 6 of PTEN (RefSeq NM_000314.4). Father was normal and mother had the c.517 C>T (p. Arg173Cys) PTEN mutation. Further molecular studies included TSC1 mutation analysis, which did not identify a mutation, deletion or insertion. An unusual polymorphism was found in TSC2 (c.649-26G>T, RefSeq NM_000548.3). However, using splice site prediction software (Alamut, version 2.0), this sequence change was considered as having no effect and thus, was considered non-pathogenic. No loss of heterozygosity or somatic mutation for the remaining wild type PTEN allele was found in DNA from abnormal tissue. Further studies in involved tissue included RASA1, FOXC2, SOX18, and AKT1, all without showing abnormalities. A heterozygous variant c.2180C>T, p.Ala727Val) in FLT4 (RefSeq NM_002020.3, also known as VEGFR3) was found in DNA from lymphatic malformation tissue. This variant was not found in the germline DNA of 100 Dutch controls, nor has it been described as a pathological variant of FLT4. The variant was also found in the germline of mother and two sibs, indicating that the mutation must have been inherited and, thus,
been present in healthy tissue and the germline of the propositus as well. Because of limited stocks of germline DNA of the propositus, we did not want to use this for studying FLT4 as results can be reliably predicted.

**Family history**

Father’s head circumference was 57 cm (25th-50th centile) and mother had macrocephaly (head circumference 60 cm, > 97.7th centile). Mother was known to have fibrocystic disease of the breasts, and had had adenomas of the thyroid and subcutaneous hamartomas of the skin. Mother did not have any sibs, her parents were found not to harbour the mutation. Mother has been under medical supervision according to standard surveillance protocols.

The parents subsequently had 5 further children, 2 of whom were found to harbour the PTEN mutation: both had macrocephaly without vascular malformations, at age 7 years and 7 months, respectively.

**Discussion**

This is the first report of a proven germline mutation (i.e. PTEN, c.517 C>T (p. Arg173Cys)) in a patient with Gorham-Stout phenomenon (GSP). Many causes of GSP have been suggested, summarized in Table 1.

Vascular malformations are known to occur in PHTS and GSP. In an entity that resembles Proteus syndrome but is still different and has been designated with different terms, capillary (CM), venous (VM) or lymphatic malformations (LM) have been reported. Zhou et al. described a case with features resembling Proteus syndrome but still different from PS with a germline R335X mutation who had a second hit (R130X mutation) on the other allele in a lipoma, an epidermoid naevus and an arteriovenous malformation (AVM) 8. This patient did not have lymphatic malformations. Brasseur et al. reported a prenatal case

<table>
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<tr>
<td>Osteolysis through angiomatosis</td>
<td>Gorham and Stout 1955</td>
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<td>Resorption of bone through activation of silent hamartoma</td>
<td>Knoch et al., 1963 (Referred to by Möller et al., 1999)</td>
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<td>Tilling et al., 1968</td>
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<td>Angiomatosis leads to enhanced activity of local hydrolytic enzymes, leading to osteolysis</td>
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<td>Osteolysis through endothelial dysplasia of blood / lymphatic vessels</td>
<td>Young et al., 1983</td>
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**Table 1:** Suggested mechanisms as causes for Gorham Stout phenomenon
with supposed Proteus syndrome and a thoraco-brachial “cystic lymphangioma” (LM) \(^9\). However, both the atypical (prenatal) presentation and lack of phenotypic data on this patient are insufficient to mark this case as a Proteus syndrome patient. Our patient does not meet the diagnostic criteria for PS \(^{10}\) either. In Bannayan-Riley-Ruvalcaba syndrome (BRRS), capillary malformations (CM) are commonly found and patients with BRRS and lymphatic malformations (LM) have been reported \(^{11}\). In half of these cases, the lymphatic malformations were seen in combination with lipomas. There are similarities to our case, but the size and skin discoloration in our proband have not been reported in BRRS. In Cowden syndrome, vascular anomalies are infrequently reported but no prospective, systematical study has been performed. An individual with a “cystic cavernous lymphangioma” (LM) of the mesentery was reported \(^{12}\), and we have personal knowledge of three unrelated patients with PHTS and venous malformations: one child with a single VM in the neck; a second patient with a large lesion in the neck extending to the mediastinum (pathologic study showed lymphatico-venous malformation and lipoma) and several scalp VM’s; and a third patient with several small LVM on one of her fingers and under her feet. In all three patients, the venous malformations remained localized and did not show progressive and invasive growth.

The present p.Arg173Cys mutation has been described before only once as a germ line mutation, in a study of >3,000 prospectively ascertained PHTS presentations \(^1\). The mutation is also found as somatic mutation in atypical endometrial hyperplasia, endometrial carcinoma, and glioblastoma \(^{13}\). The mother of the present patient is therefore under surveillance according to the currently advised cancer screening guidelines in PHTS with special attention to endometrial cancer screening \(^7\).

The mother and two siblings of the propositus have the \(PTEN\) mutation but do not show any signs of vascular malformations or GSP. We hypothesize that our patient may have had a second (somatic or germline mosaic) mutation. Alternatively, gene-gene and gene-environment interactions may have been involved. Loss of heterozygosity was not detected in affected tissue of the present patient. We considered a digenic pattern of inheritance. The genes causing tuberous sclerosis (\(TSC1/TSC2\)) and \(PTEN\) function in the PI3K-Akt pathway. In addition, \(TSC2\) mutations have been found in lymphangioleiomyomatosis (LAM) \(^{14}\). LAM shares features with GSP and is usually confined to the lung. Mutation analysis and MLPA in lymphatic malformation tissue did not show any pathogenic \(TSC\) aberrations in the propositus however. Lindhurst et al. described in Proteus syndrome somatic activating mutations in \(AKT1\), which also functions in the PI3K-Akt pathway \(^{15}\), but \(AKT1\) mutation analysis in lymphatic malformation tissue failed to show abnormalities. Somatic analysis of genes involved in vasculogenesis, angiogenesis and lymphangiogenesis \(^{16}\) were studied in lymphatic malformation tissue: \(RASA1\) (mutated in capillary malformation-arteriovenous malformation, CM-AVM), \(FOXC2\) (mutated in lymphedema-distichiasis/lymphedema-ptosis/yellow nail) and \(SOX18\) (mutated in hypotrichosis-lymphedema-teleangiectasia syndrome) were all without detectable mutations.
A novel variant of unknown significance in \( \text{FLT4} \) (also known as \( \text{VEGFR3} \)) was found. In silico prediction of pathogenicity yielded contradictory results; SIFT predicts the variant to be deleterious (score 0.00), while POLYPHEN2 predicts it to be benign (scores 0.001 HumDiv en 0.006 HumVar). Pathogenicity of this variant remains uncertain. \( \text{FLT4} \) is involved in lymphatic development and missense mutations in \( \text{FLT4} \) lead to an entity known as primary congenital lymphedema or Milroy disease \(^{17}\), which was not present in the propositus. The somatic variant in \( \text{FLT4} \) was also found in the germline of mother and a sib of the propositus who also harbour the \( \text{PTEN} \) mutation. However, the fact that these individuals do not share the phenotype of the propositus and that one sib who tested negative for the \( \text{PTEN} \) mutation harbours the same \( \text{FLT4} \) variant, does not support the \( \text{FLT4} \) variant alone being responsible for the severity of the phenotype. No direct relationships between \( \text{FLT4} \) and \( \text{PTEN} \) have been described and we have been unable to find proof for digenic inheritance. We cannot exclude the presence of undetected variants or variants in other genes which explain the severity of the phenotype in the propositus, and still favour this digenic or polygenic hypothesis as the most likely explanation. When this patient was evaluated, next generation sequencing analysis techniques were not yet available and because of limited availability of DNA derived from unaffected tissue from the propositus, next generation techniques are no longer possible. Therefore, we chose to specifically look at logical candidates because of their function in vasculogenesis, angiogenesis and lymphangiogenesis in DNA of affected tissue.

In conclusion, we report a \( \text{PTEN} \) mutation in a patient with Gorham-Stout phenomenon. We hypothesize that the \( \text{PTEN} \) mutation was the first of two or more steps in the relentless growth of lymphatic malformation in the propositus. Additional (epi)genetic, either somatic or germline, or environmental factor(s) might have led to the severity of the phenotype in the propositus. Further studies of other genes, especially in the PI3K-Akt pathway and performed in affected tissue may explain this.

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