Phenotypic abnormalities in childhood cancer patients; clues for molecular defects?
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Chapter 6

PTEN Hamartoma Tumor Syndrome: variability of an entity

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Abstract

Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), Proteus and Proteus-like syndrome are entities that can show remarkable clinical overlap, and are all shown to be caused by germline PTEN mutations (80% of CS cases, 60% of BRR cases, up to 20% of Proteus syndrome cases, and 50% of Proteus-like cases). We describe four members of a single family with a heterogeneous phenotype, that at present most closely fits BRRS, although further development of symptoms with time may eventually lead to the diagnosis CS. All four cases were shown to harbour the same PTEN mutation (IVS5+1delG). One of the cases was first suspected of having Jadassohn naevus sebaceous syndrome, a diagnosis which was refuted only after the birth of the other family members and PTEN mutation analysis. This patient also had a hemimegalencephaly, not reported before in a case with BRRS or CS. No loss of heterozygosity was found in the megalencephalic part of the brain. The family can best be classified by the molecular cause as having PTEN Hamartoma Tumour Syndrome (PHTS). Hemimegalencephaly as part of Jadassohn naevus sebaceous syndrome can be added as further manifestations of germline PTEN mutations.
Variability in *PTEN* hamartoma tumor syndrome

**Introduction**

Cowden syndrome (CS [MIM 158350]) is an autosomal dominant disorder with age related penetrance characterized by mucocutaneous lesions, macrocephaly and an increased risk of cancer especially those of the breast, thyroid and endometrium. The phenotype in CS has proven to be highly variable, which became especially evident after identification of the susceptibility gene *PTEN*3,4. This is also shown in the change in incidence figures, which were found to be at least 5 times higher after *PTEN* was identified (estimated incidence before *PTEN* identification 1:1,000,000, and afterwards >1:200,0006,7). Bannayan-Riley-Ruvalcaba syndrome (BRRS [MIM 153480]) is allelic to CS and characterised by the triad of macrocephaly, lipomas, and pigmented macules of the glans penis. Proteus syndrome (PS [MIM 176920]) is a disorder characterised by overgrowth of hands and/or feet, asymmetry of limbs, connective tissue and epidermal naevi, vascular and lymphatic malformations, and cranial hyperostosis. Another closely related disorder is Proteus-like syndrome where individuals are characterised by the presence of macrocephaly, lipomas and overgrowth, not meeting the criteria for CS, BRRS, or PS. Germline *PTEN* mutations have been found in 80% of individuals with CS, 60% of individuals with BRRS, up to 20% with PS, and 50% with Proteus-like syndromes.9,10

Here, we present a family (a mother and her three sons) in which phenotype was extremely variable, one member having macrocephaly, normal intelligence, and minimal pigmentation abnormalities; another member with macrocephaly with developmental delay; another with macrocephaly, delay and lipoma; and a last member having hemimegalencephaly (HME), Jadassohn naevus sebaceous, and neonatal demise. All were found to have the same germline mutation in *PTEN*.

**Case reports**

**Case 1**

The proband was the 3rd-born child of non-consanguineous parents. His two older sisters were healthy. Prenatal routine sonography showed a unilateral ventricular dilatation. He was born preterm at 32 2/7 weeks, possibly in part because of the positive discrepancy due to the macrocephaly. Weight at birth was 2,620 g (97th centile) and occipito-frontal circumference (OFC) was 38 cm (5 cm > 98th centile). APGAR scores were 4 and 5 after 1 and 5 minutes, respectively. Respiratory insufficiency urged immediate artificial ventilation. Physical examination showed his skull to be severely asymmetric, bulging to the left, and ipsilateral linear naevi on nose and forehead (Fig. 1a). He developed hemiconvulsions which did not respond to therapy, and caused progressive cardiorespiratory problems. Ultrasonography and CT-scanning of the brain showed ipsilateral hemimegalencephaly, irregular lateral ventricular dilatation and periventricular calcifications adjacent to the dilated ventricle (Fig. 1b). The seizures were uncontrollable and eventually led to an early demise on the third day of life. Autopsy showed left-sided unilateral megalencephaly (total brain
Figure 1

**Figure 1a** Linear naevi on the nose and below the left eye, ipsilateral to the hemimegalencephaly (case 1). This picture was published earlier. 

**Figure 1b** CT-scan of the brain, showing hemimegalencephaly, irregular lateral ventricular dilatation and periventricular calcifications adjacent to the dilated ventricle (case 1).

**Figure 1c** Autopsy showed left-sided unilateral megalencephaly, periventricular cysts and an extremely thick cortex, the left side showing pachygyria (case 1).

weight 510 g; normal for gestational age 217 +/- 49 g), periventricular cysts and an extremely thick cortex, the left side showing pachygyria (Fig. 1c). Microscopy of this side of the brain showed haphazardly arranged neurons, lacking the normal arrangement in 6 layers. The child was diagnosed as having Jadassohn naevus sebaceous (MIM 163200). The parents were given a low recurrence risk for similar problems in future offspring.

**Case 2**

Case 2 was born at term after an uneventful pregnancy. His large head caused a cephalopelvic discrepancy, making a vacuum extraction necessary. APGAR scores were 3 and 9 after 1 and 5 minutes, respectively. His weight was 4,720 g (> 97th centile) and OFC was 38.5 cm (2 cm > 98th centile). Brain ultrasonography in the neonatal period showed dilated lateral ventricles. His height increased following the 50th centile, his macrocephaly persisted, remaining at > 98th centile. He had a single febrile convulsion at 9 months of age. At 12 months of age, he was first seen because of mild developmental delay and his elder brother’s diagnosis of Jadassohn naevus sebaceous. His cognition was estimated to be normal, but his motor development was delayed. Physical examination showed the
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Figure 2

**Figure 2a** Bi-coloured left iris (case 2), not reported before in a CS/BRRS patient.

**Figure 2b** Macrocephaly and prominent forehead (case 3).

**Figure 2c** Pigmentation abnormalities of the skin (case 4).

Macrocephaly and also a bi-coloured left iris (Fig. 2a). His development remained somewhat delayed thereafter, although he was able to attend regular education. Formal intelligence testing showed his IQ to be at the lower range of normal. Around 30 months of age, he developed 2 lipomata, one on his left lower abdomen, the other in the right paravertebral area. Both were surgically removed. At the age of 36 months, he was found to have absences and atonic seizures. Electroencephalograph (EEG) studies revealed bitemporal focal anomalies, but no spikes. Other electrophysiological studies (ElectroMyoGraphy, ElectroRetinoGraphy, Visual Evoked Potentials, and Brainstem Evoked Potentials) gave normal results, as did sonography of the kidneys. A full metabolic screening of urine, plasma, and spinal fluid did not show any abnormality. The brain MRI scan demonstrated dilated lateral ventricles, widened perivascular spaces but otherwise a normal cortical architecture.

**Case 3**

The third brother presented to us at the age of 9 years because of developmental delay, macrocephaly, and the family history. Pregnancy and delivery were uneventful. Weight at birth was 4,750 g. (> 97th centile), OFC was 37 cm (98th centile). He had a slow psychomotor development, being able to walk at the age of 3.5 years. He entered special schooling. At physical exam at 9 years, he had an OFC of 60 cm (3.5cm > 98th centile), a prominent forehead, but otherwise no minor anomalies, neurologic or skin abnormalities (Fig. 2b). EEG studies and brain MRI scanning gave normal results.
Case 4
The mother of the 3 boys was of Caucasian descent. Pregnancy and delivery were uneventful, no reliable data on body measurements in the neonatal period were available. She stated that she always had a large head circumference. Her cognitive development had been normal, and followed normal schooling. At the age of 35 years, she was found to be macrocephalic (OFC 63.2 cm; 4cm > 98th centile). Her skin had pigmentation abnormalities (Fig. 2c), and she had large breasts. Sonography showed multiple mammary cysts. A complete work-up failed to show any other signs or symptoms of CS. Her family history showed that she had 2 brothers, both with unusually large heads, even as children. They were not available for further investigations. History revealed that her mother had had a normal head size, and no specific pigmentation abnormalities. Her father had had a very large head necessitating him to order custom made hats if needed. He was not known to have had pigmentation anomalies. He developed fatal lung carcinoma at the age of 68 years, with secondary brain metastasis. He was a smoker. No reliable data on other family members were available.

Molecular studies
Because of the combination of macrocephaly (4/4), pigmentation anomalies (2/4), megalencephaly (2/4), lipoma (1/4), and developmental problems (2/3 in this single family), a leading diagnosis was CS/BRRS, and PTEN mutation analysis was performed, after appropriate informed consent. The mother and her 3 affected sons all showed the same germline PTEN mutation, IVS5+1delG (case 1: liver tissue; all others: lymphocytes). As the hemimegalencephalic part of the brain of case 1 might have arisen due to loss of the remaining wild-type allele (Loss-Of-Heterozygosity; LOH), mutation analysis was performed in a biopsy of the autopsy material. However, no LOH was found.

Discussion
We describe a family in which 4 different members had findings that fitted CS/BRRS, and who were all shown to harbour a germline PTEN mutation. This germline PTEN mutation IVS5+1delG has been reported at least once before in a proband with CS. The deletion of one of the two canonical splice signals is predicted to result in an aberrant splice, and thus, is almost certainly pathogenic. Due to the presence of linear verrucous pigmented naevi and hemimegalencephaly, case 1 was first suspected of having Jadassohn naevus sebaceous syndrome, a clinical diagnosis which was disproved only after the birth of the other affected sibs and the molecular PTEN analysis. We have tested two other affected patients with Jadassohn naevus sebaceous syndrome, but did not find a PTEN mutation in them (Eng and Hennekam, unpublished data). The hemimegalencephaly (HME) in case 1 is unusual and has not been reported in a case with CS/BRRS before. Flores-Sarnat reviewed HME and proposed a classification system in three types: isolated HME, syndromic HME and total HME. The present case 1 should be classified as having the syndromic type. HME was
found to have a heterogeneous cause, as several neurocutaneous syndromes have been described presenting with HME. To this, CS/BRRS can be added.

Hamartomas in CS affect derivatives of all three germ layers, but it is not clear why overgrowth in PTEN Hamartoma Tumor Syndrome (PHTS), for example Proteus syndrome, may be patchily distributed. This may be dependent on the pattern of genetic and epigenetic alterations subsequent to the germline mutation. Mutation analysis of autopsy material of the brain of case one did not show LOH of PTEN. This is in line with the results reported by other groups where LOH was found in different percentages in tumours and hamartomas: LOH was found in 130 out of 145 cases with informative tumours, in all four tested tumours from one individual, in 3 of 11 CS individuals with 20 hamartoma, and in four out of six skin hamartomas from one CS individual.

The operational diagnostic criteria for CS have been revised in 2000 by the International Cowden Consortium. In the present family, no single case fulfilled these criteria. However, the typical skin findings pathognomonic for CS usually develop only in the second decade of life (or later), and three out of four family members were younger than ten years old. The same principle applies for the increased cancer risk. If the findings of all family members were taken together, then criteria may be approached. The family can also be classified clinically as BRRS.

Recently three patients with Benign Familial Macrocephaly (macrocephaly and delayed motor milestones) were reported to harbour a PTEN mutation, one of these patients having a juvenile polyp. Together with the present observation, this points to a more wide indication to perform PTEN mutational analyses. Therefore, with the broadening clinical spectra associated with germline PTEN mutations, it has become more useful to use a molecular-based approach: an individual found to carry a germline PTEN mutation should be classified as having PHTS, irrespective of clinical presentation. Clinicians are urged to manage all PHTS individuals as having CS with respect to cancer risk and surveillance.

The increased risk in CS of developing malignancies mainly involves breast cancer, epithelial thyroid cancer, and endometrial carcinoma. The risk for breast cancer is increased in CS as well as a lowering of the mean age at diagnosis, 10 years (or more) earlier compared with the general population. The mother of the presented proband is now being screened for cancer according to the guidelines of the NCCN. Her affected sons will enter the screening program at 18 years of age.

In conclusion, we have found a germline PTEN mutation in a family, of whom none of the individual affected members fulfilled the international diagnostic criteria for CS. In the presence of a PTEN mutation, the family can be classified as having PHTS, with all implications for further medical management. Hemimegalencephaly as part of Jadassohn naevus sebaceous syndrome may be another phenotypic finding associated with germline PTEN mutation.
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References