ELECTRONIC LETTER

PTEN hamartoma tumour syndrome: variability of an entity

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Cowden syndrome (CS; OMIM 158350) is an autosomal dominant disorder with age related penetrance characterised by mucocutaneous lesions, macrocephaly and an increased risk of cancer, especially of the breast, thyroid and endometrium.[1 2] 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 five times higher after *PTEN* was identified (estimated incidence before PTEN identification 1:1 000 000,[5] and after >1:200 000.[6 7]). Bannayan-Riley-Ruvalcaba syndrome (BRRS; OMIM 153480) is allelic to CS and is characterised by the triad of macrocephaly, lipomas, and pigmented macules of the glans penis.[8] Proteus syndrome (PS; OMIM 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.[8] Proteus-like syndrome (PLS) is another closely related disorder, where individuals are characterised by the presence of macrocephaly, lipomas and overgrowth not meeting the criteria for CS, BRRS, or PS.[8] 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 PLS.[9 10]

Here, we present a family (a mother and 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 the 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 thirdborn 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 2620 g (97th centile) and occipitofrontal circumference (OFC) was 38 cm (5 cm >98th centile). APGAR scores were 4 and 5 after 1 and 5 min, respectively. Respiratory insufficiency urged immediate artificial ventilation. Physical examination showed his skull to be severely asymmetric, bulging to the left, and there were 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 computed tomography (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 weight 510 g; normal for

Key points

- 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 of 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 that 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. Hemimegalencephaly as part of Jadassohn naevus sebaceous syndrome can be added as further manifestations of germline *PTEN* mutations.

gestational age 217 g (SD 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 neurones, lacking the normal arrangement in six layers. The child was diagnosed as having Jadassohn naevus sebaceous (OMIM 163200).[8] 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 min, respectively. His weight was 4720 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

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Abbreviations: BRRS, Bannayan-Riley-Ruvalcaba syndrome; CS, Cowden syndrome; CT, computed tomography; EEG, electroencephalograph; HME, hemimegalencephaly; MRI, magnetic resonance imaging; OFC, occipitofrontal circumference; PHTS, PTEN hamartoma tumour syndrome; PLS, Proteus-like syndrome

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 macrocephaly and a bicoloured 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 two 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 magnetic resonance imaging (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 4750 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 examination at 9 years, he had an OFC of 60 cm (3.5 cm >98th centile) with a prominent forehead, but otherwise no minor anomalies, neurological, or skin abnormalities (fig 2B). EEG studies and brain MRI scanning gave normal results.

Case 4

The mother of the three 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 had always had a large head circumference. Her cognitive development had been normal, and she followed normal schooling. At the age of 35 years, she was found to be macrocephalic (OFC 63.2 cm; 4 cm>98th centile). Her skin had pigmentation abnormalities (fig 2C), and she had large breasts. Sonography showed multiple mammary cysts. A complete examination failed to show any other signs or symptoms of CS.

Her family history showed that she had two brothers, both with unusually large heads, even as children. They were not available for further investigations. History revealed that the patient's mother had had a normal head size, and no specific pigmentation abnormalities. The patient's 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. The father, who was a smoker, developed fatal lung carcinoma at the age of 68 years, with secondary brain metastasis. 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 three affected sons all showed the same germline PTEN mutation, IVS5+1delG (case 1: liver tissue; all others: lymphocytes). As the hemimegalencephalic part of
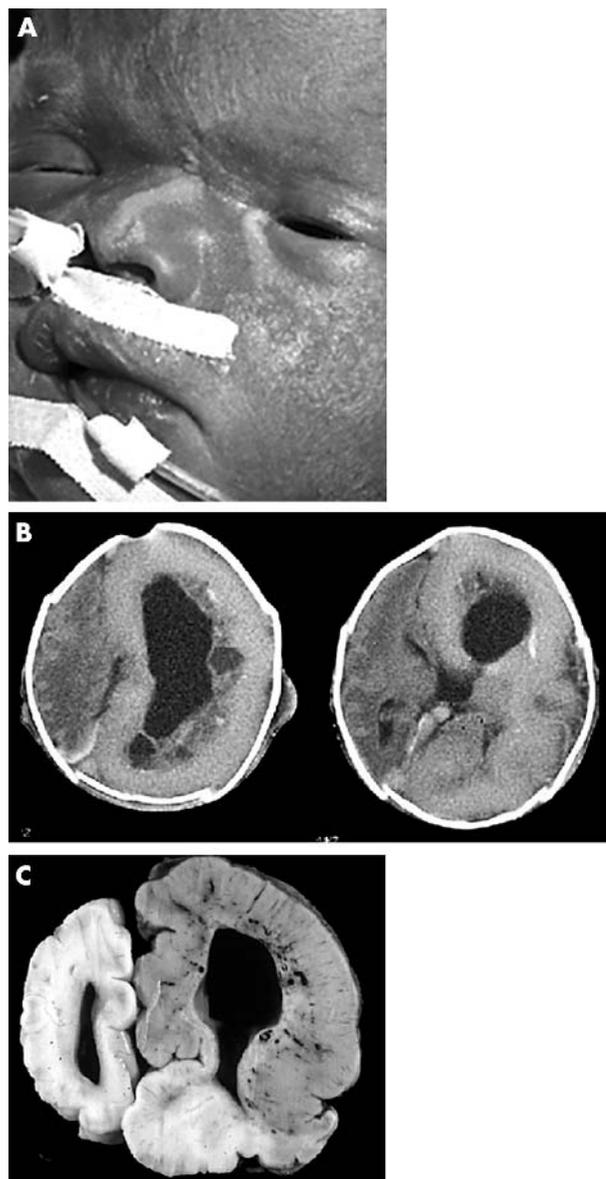

Figure 1 Case 1. (A) Linear naevi on the nose and below the left eye, ipsilateral to the hemimegalencephaly. (B) CT scan of the brain, showing hemimegalencephaly, irregular lateral ventricular dilatation and periventricular calcifications adjacent to the dilated ventricle. This picture was published earlier.[27] (C) Autopsy showed left sided unilateral megalencephaly, periventricular cysts and an extremely thick cortex, the left side showing pachygyria.

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 four 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.[11] The deletion of one of the two canonical splice signals is predicted to result in an aberrant splice, and thus, is almost certainly pathogenic. Owing to the presence of linear verrucous pigmented naevi and hemimegalencephaly, case 1 was first suspected of having Jadassohn naevus sebaceous syndrome,[8] a clinical diagnosis
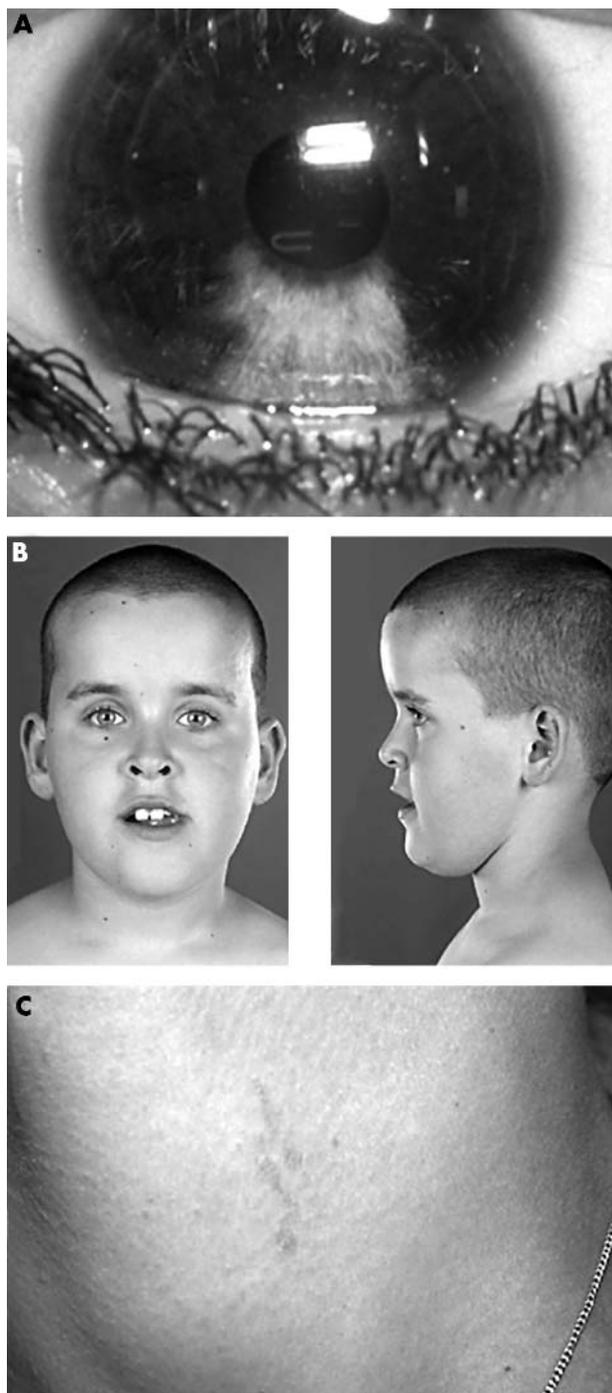
Figure 2 (A) Bicoloured left iris (case 2), not reported previously in a Cowden syndrome/Bannayan-Riley-Ruvalcaba syndrome patient; (B) macrocephaly and prominent forehead (case 3); (C) pigmentation abnormalities of the skin (case 4).

that was disproved only after the birth of the other affected siblings 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 HME in case 1 is unusual and has not been reported previously in a case with CS/BRRS. Flores-Sarnat reviewed HME and proposed a system of classification into three types: isolated HME, syndromic HME and total HME.[12] The present case 1 should be classified as having the syndromic type. HME was found

to have a heterogeneous cause, as several neurocutaneous syndromes presenting with HME have been described. To this, CS/BRRS can be added.

Hamartomas in CS affect derivatives of all three germ layers,[13] but it is not clear why overgrowth in *PTEN* hamartoma tumour 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.[14] Mutation analysis of autopsy material of the brain of case 1 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 130/145 cases with informative tumours,[15] in all four tested tumours from one individual,[16] in 3/11 CS individuals with 20 hamartoma,[17] and in 4/6 skin hamartomas from one CS individual.[18]

The operational diagnostic criteria for CS have been revised in 2000 by the International Cowden Consortium.[1] 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 10 years old. The same principle applies for the increased cancer risk. If the findings of all family members were taken together, then they become closer to the criteria. The family can also be classified clinically as BRRS.[10 14 19] 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.[20] Together with the present observation, this points to a wider indication for the performance of *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.[1 9] 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.[1 21 22] The risk for breast cancer is increased in CS[23–26], and there is also a lowering of the mean age at diagnosis, 10 years (or more) earlier compared with the general population.[23 25] The mother of the present proband is now being screened for cancer according to the guidelines of the NCCN.[2] 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 in which 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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