Original articles

Phenotypic findings of Cowden syndrome and Bannayan-Zonana syndrome in a family associated with a single germline mutation in PTEN

Julide Tok Çelebi, Hui C Tsou, Fei Fei Chen, Hong Zhang, Xiao Li Ping, Mark G Lebwohl, Jeffrey Kezis, Monica Peacocke

Abstract
Cowden syndrome (CS) and Bannayan-Zonana syndrome (BZS) are two hamartoma syndromes with distinct phenotypic features. Although partial clinical overlap exists between CS and BZS, they are considered to be separate entities. PTEN has been identified as the susceptibility gene for both disorders, suggesting allelism. We have identified a germline mutation, R335X, in PTEN in a family consisting of two female members with the phenotypic findings of CS and two male members with the phenotypic findings of BZS. To our knowledge, this is the first report that shows the presence of separate subjects with CS and with BZS in a single family associated with a single germline PTEN mutation.
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Department of Dermatology, Columbia Presbyterian Hospital, Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York, New York 10032, USA
J T Çelebi
H C Tsou
F F Chen
H Zhang
X L Ping
J Kezis
M Peacocke

Department of Dermatology, The Mount Sinai School of Medicine, The Mount Sinai Medical Center, One Gustave L Levy Place, Box 1130, New York, New York 10029, USA
M G Lebwohl

Correspondence to:
Dr Peacocke.

Cowden syndrome (CS) and Bannayan-Zonana syndrome (BZS) are both autosomal dominantly inherited diseases characterised by hamartomas and distinct phenotypic features. CS, described by Lloyd and Dennis[1] in 1963, also known as multiple hamartoma syndrome, is characterised by skin findings and hamartomas in a variety of tissues including the breast, thyroid, skin, gastrointestinal tract, endometrium, and the brain.[1 2] Many, but not all patients with CS have skin findings, usually developing in the second decade of life. The skin lesions fall into four broad categories including facial papules (trichilemmomas), oral papillomatoses, and acral and palmoplantar keratoses.[3] Identification of multiple trichilemmomas, or benign tumours of the hair infundibulum, are pathognomonic of CS.[4] In addition to the hamartomas and benign tumours, patients with CS have an increased risk of developing breast (25-50%) and thyroid cancers (3-10%).[5 6] Moreover, women with this disease appear to develop breast cancer at a younger age.[7] Central nervous system (CNS) manifestations of CS include macrocephaly (38%), Lhermitte-Duclos disease (LDD) or dysplastic gangliocytoma of the cerebellum, and less commonly mental retardation.[8 9]

Bannayan-Zonana syndrome (BZS), described by Riley and Smith,[10] Bannayan,[11] Zonana *et al*,[12] and Ruvalcaba *et al*,[13] has been recognised under different names, including Bannayan-Zonana syndrome, Ruvalcaba-Myre syndrome, Riley-Smith syndrome, and recently Bannayan-Riley-Ruvalcaba syndrome.[10–17] Macrocephaly is the predominant finding in patients with this disease.[18] Characteristic features other than macrocephaly are CNS abnormalities, lentigines of the penis, and hamartomatous growths, such as subcutaneous and visceral lipomas, intestinal polyposis, and vascular malformations. At least half of the patients with BZS have CNS anomalies including hypotonia, delayed psychomotor development, mild to severe mental retardation, and seizures. Ocular abnormalities, such as prominent Schwalbe lines and corneal nerves, lipid storage myopathy, and a variety of skeletal system abnormalities, including joint hyperextensibility, pectus excavatum, and scoliosis are additional associated manifestations of the syndrome.[19] Unlike CS, an increased risk of malignancy has not been documented in patients with BZS.[20] Although CS and BZS have distinct phenotypic features, the presence of macrocephaly, intestinal polyposis, and lipomas in both diseases suggests a partial clinical overlap. Moreover, the presence of features common in CS and BZS within the same family has been reported in a few kindreds.[21]

Linkage analysis of 12 families with CS have determined the locus for this syndrome to be on chromosome 10q23.[22] Subsequently, a putative tumour suppressor gene, PTEN/MMAC1/TEP1, was identified in this CS critical region[23–25] and germline mutations in the PTEN gene were found in subjects with CS.[26–28] PTEN is a dual specificity phosphatase. While the core phosphatase domain is present at residues 122-134, other areas of significance include three potential tyrosine phosphorylation sites at

residues 233-240, 308-315, and 336. Sequence analysis of PTEN shows homology to tensin, a protein associated with focal adhesions, and to auxillin, a cytoskeletal protein.[24]

The identification of PTEN as the susceptibility gene for BZS followed soon after the identification of germline mutations of PTEN in CS, suggesting allelism.[29] Of interest, a nonsense mutation, R233X, has been previously reported in a family with CS, as well as in an unrelated family with BZS.[20 30] We describe here, for the first time, a family with two female
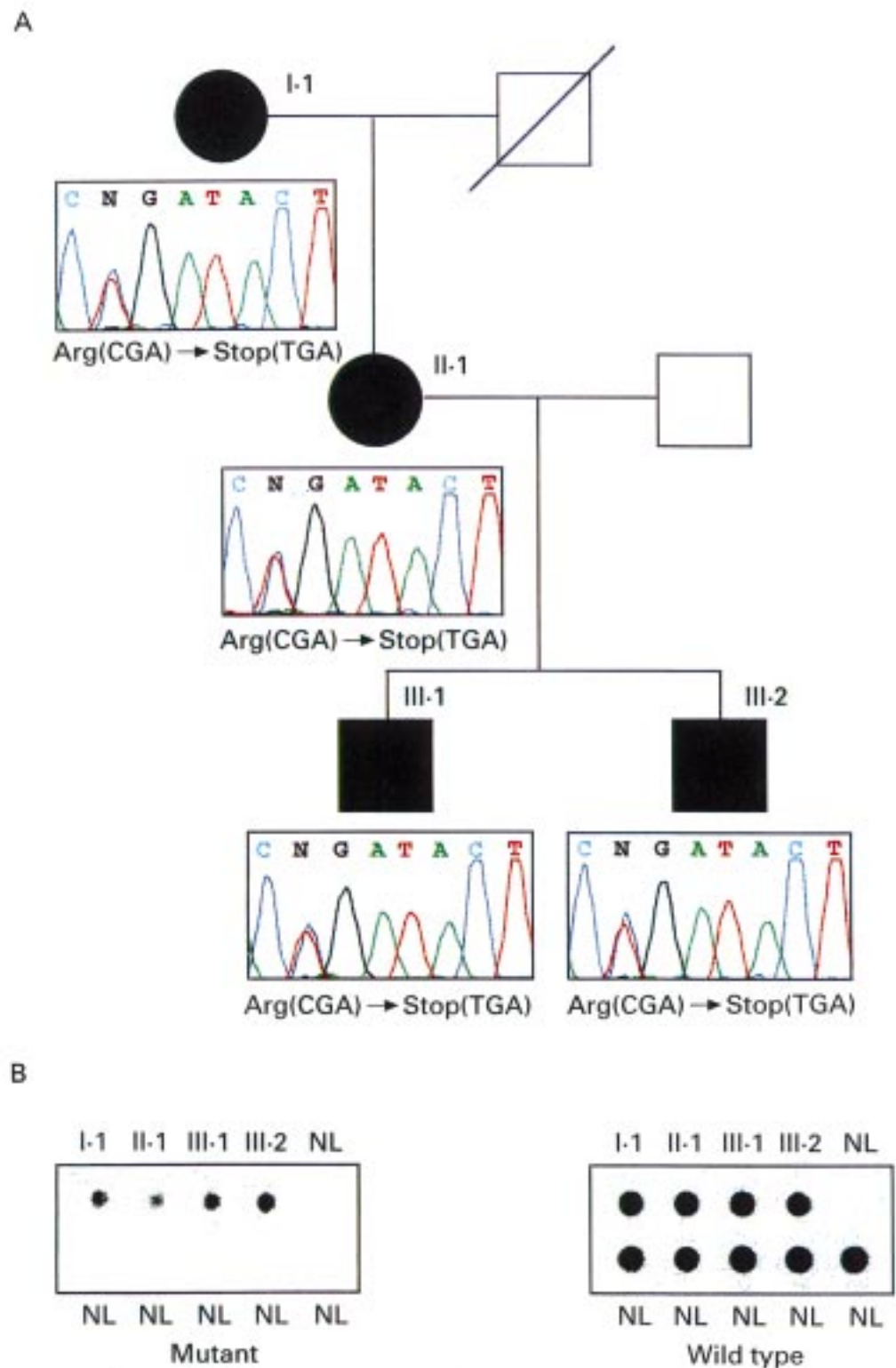

Figure 1   (A) DNA sequence analysis of the PTEN gene in four members from three generations in this family. A C→T transition was evident in all four affected subjects which resulted in a premature stop at codon 335. (B) Analysis of PCR products flanking exon 8 of the PTEN gene by allele specific hybridisation with mutant and wild type oligonucleotides showed the presence of the two alleles in the affected subjects but not in the unaffected population.

Table 1 Summary of family MHSF-066, clinical phenotype and associated mutations

Patient	Age/ sex	Diagnosis	Mutation/ predicted effect	Skin	Thyroid	Breast	CNS	GI	Other
MHSF066-1	31/F	CS	1003 C→T R335X	Trichilemmomas Oral papillomas Acral keratoses	Follicular carcinoma (10) Metastasis (lung) (12)	Ductal carcinoma (21)	Macrocephaly Multiple sclerosis (26)	—	Osteosarcoma (12) Lipoma (12)
MHSF066-2	54/F	CS	1003 C→T R335X	Trichilemmomas Oral papillomas Acral keratoses	Hashimoto's thyroiditis (42)	Ductal carcinoma (48)	Macrocephaly Slurred speech	—	—
MHSF066-3	9/M	BZS	1003 C→T R335X	Lentigines of the penis	—	—	Macrocephaly Developmental delay Dysarthria Hypotonia		
MHSF066-4	6/M	BZS	1003 C→T R335X	Lentigines of the penis	—	—	Macrocephaly Developmental delay Dysarthria Hypotonia		

(): age at diagnosis.

members phenotypically fulfilling the criteria for CS and two male members with the phenotypic findings of BZS associated with a single germline mutation in the PTEN coding sequence. The transition, C to T, at nucleotide 1003 in exon 8 resulted in the arginine being replaced by a stop codon (R335X). While CS and BZS are considered to be allelic disorders, this is the first report showing the existence of a single PTEN mutation in the same germline conferring the phenotypic findings of both syndromes. Moreover, these data support the notion that a family history of breast tumour can be identified in males with BZS.

Materials and methods

FAMILY
The diagnosis of CS was made using the international Cowden's Consortium CS diagnosis criteria. The diagnosis of BZS was made according to accepted clinical criteria. Blood samples were obtained after informed consent from subjects with CS.

DNA EXTRACTION
Genomic DNA was extracted from whole blood using a QIAamp Blood Maxi Kit (Qiagen). DNA concentration was measured by OD_{260} and purity was checked by the ratio of OD_{260}/OD_{280}.

PCR AMPLIFICATION AND MUTATION DETECTION
Genomic DNA was amplified with primers flanking the exons of PTEN/MMAC1 as described.[7] [24] For mutation detection, PCR products were sequenced using an Applied Biosystem 310 automated sequencing system.

ALLELE SPECIFIC HYBRIDISATION
Oligonucleotides (20 base pairs) were synthesised with either the wild type or the mutant nucleotide located in the centre. DNA from subjects with a mutation and normal controls were amplified and blotted onto the nylon membranes. Oligonucleotides were then labelled with $^{32}P\gamma ATP$ and used as probe in the hybridisation reactions. Two identical membranes containing mutant and normal PCR products were hybridised with wild type and mutant oligonucleotides respectively in a buffer (5 × Denhardts, 5 × SSPE, 0.5% SDS) at 48°C for two hours. Blots were then washed in 2 × SSPE, 0.1% SDS at 48°C for 30 minutes. The blots were then air dried and autoradiology performed at –70°C with Kodak XAR film and intensifying screens.

Results
As shown in the pedigree (fig 1), the MHSF066 family consists of affected family members in three generations, two subjects with CS and two with BZS. MHSF066-01 and her mother, MHSF066-02, both phenotypically fulfil the criteria for CS, by the presence of classical skin lesions and benign and malignant neoplasms. The germline mutations in PTEN and associated phenotypes are listed in table 1. MHSF066-01, who has been reported previously,[31] is a 31 year old white female with a unique presentation of CS. She was diagnosed with a follicular thyroid carcinoma at the age of 10, which later metastasised to the left lung and was treated successfully with surgical resection. At the age of 12, she developed an osteosarcoma of the left proximal tibia and a large lipoma (10 × 15 cm) in the mediastinum. Intraductal breast carcinoma was diagnosed at the age of 21 and multiple sclerosis at the age of 26. In addition to having a complex medical history, she gave birth to two male infants subsequently identified as having BZS. MHSF066-03 and MHSF066-04, currently 9 and 6 years old respectively, were both born with macrocephaly (>95th centile) with normal ventricular size. During their early childhood years, they showed signs of developmental delay, hypotonia, and lentigines of the penis, all characteristic of BZS.

The affected and unaffected members of family MHSF066 were analysed for germline PTEN mutations. Direct sequencing analysis of the PCR product flanking exon 8 showed a transition of C to T at nucleotide 1003, resulting in a change from arginine to a stop codon (R335X). This nonsense mutation was identified in all four affected subjects with CS and BZS in this family.

The mutation was subsequently confirmed by allele specific oligonucleotide (ASO) analysis (fig 1), which showed both the wild type and the mutant allele from each of the affected subject's genomic DNA. By contrast, ASO of 30 alleles from unaffected, unrelated subjects failed to identify the mutant allele.

	Mutation	Phenotype	Reference
1	S170R	BZS	20
2	520del25	BZS	20
3	1390delC	BZS	32
4	Y178X	BZS	32
5	Q214X	BZS	32
6	E256X	BZS	32
7	Y68H	BZS	20
	Y68H	CS	28
8	R233X	BZS	20
	R233X	CS	30
9	R335X	CS, BZS	Present case
	R335X	CS	30
	R335X	CS	30
	R335X	CS	20

Discussion

PTEN has been identified as the susceptibility gene for two hamartoma syndromes, CS and BZS.[20] Although distinct phenotypically, these two disorders display partial clinical overlap.[21] We have identified a germline mutation, R335X, in the PTEN coding sequence, in a family with two members with the classical phenotypic findings of CS and two members with the classical phenotypic findings of BZS. To our knowledge, this is the first report that contains subjects with the CS and the BZS phenotype in a single family resulting from the same PTEN mutation. Since patients with CS usually develop characteristic skin findings in the second decade, it will be crucial to follow the two affected male children with BZS in this family to evaluate whether the features of CS develop. The severe tumour phenotype of proband MHSF 066-01 also deserves comment. In addition to developing malignant tumours at a young age, including osteosarcoma, follicular thyroid carcinoma with metastasis to the lung, and breast carcinoma, she also shows an unknown association of CS, that is, multiple sclerosis.

To date, nine families with BZS have been screened for PTEN mutations, of which five were found to exhibit mutations in this gene.[20 28 30 32] These are summarised in table 2. Of interest, two of the mutations identified in BZS families, R233X and Y68H, were also independently reported in unrelated families with CS.[20 28 30] R335X represents another example of a particular germline PTEN mutation being present in both syndromes.[20 30] Although the mutation reported herein, R335X, has not been previously reported in BZS, it has been observed in three unrelated families with CS. Moreover, we have recently identified R335X in an unrelated female proband with BZS (J Tok *et al*, unpublished data), suggesting that this truncating PTEN mutation is common in both hamartoma syndromes. Additionally, we have tested a frameshift mutation, 915del13, resulting in a premature stop at codon 312 in exon 8, and identified almost a complete loss of the PTEN phosphatase activity (unpublished data). Similar functional changes are predicted to occur with the truncating PTEN mutation R335X.

These data, together with our own, clearly indicate that an identical mutation, found in a single germline, in the PTEN gene can confer susceptibility to two distinct phenotypes, CS and BZS. These observations suggest that increased susceptibility to breast and thyroid cancer may be found in members of BZS families associated with germline PTEN mutations. Moreover, it also suggests that women with CS may give birth to offspring with the findings of BZS as well as CS. While the notion that these two diseases may be within the spectrum of the same primary disorder awaits further study, we cannot exclude the presence of epigenetic factors, such as sex hormones or other modifying genes playing a role in the pathogenesis of the phenotypic findings of both syndromes.

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