with the following changes: annealing temperature for exon 2 was 54°C, for exon 6 56°C, for exon 8 54°C, and for exon 13 57°C. Sequencing reactions containing 40 ng of the PCR product with 3.2 pmol of the sequencing primer in a volume of 12 µl were performed using ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Foster City, CA) or ABI Big Dye Terminator Kit (Perkin-Elmer, Foster City, CA) according to the manufacturer's instructions. Sequencing reactions were electrophoresed either on 6% Long Ranger gels, containing 8 mol/l urea, or 5% Long Ranger gels, containing 6 mol/l urea, and analysed on an Applied Biosystems model 373A or 377 automated DNA sequencers, respectively.

Exons 1 and 4 were amplified as described above and analysed using SSCP from 84 and 212 cancer free controls, respectively. After PCR, 5 µl of each sample was mixed with 5 µl of denaturing loading buffer (95% formamide, 20 mmol/l EDTA, 0.05% bromphenol blue, 0.05% xylene cyanole FF), denaturated for five minutes at 94°C and loaded into a 0.4 mm × 30 cm × 45 cm gel. Electrophoresis was performed for exon 1 using gels containing 0.5 × MDE solution (AT Biochem, Malvern, PA) and 0.6 × TBE buffer and were run at 4 W for 20 hours. Exon 4 was analysed using 1 × MDE solution and 2.5 mol/l urea at 6 W for 14.5 hours. The gels were silver stained according to standard procedures.

We detected one potential missense mutation in the coding *E-cadherin* gene sequence (table 1, fig 1, No 1). A C to G change occurred in codon 172 in exon 4 resulting in substitution of proline by arginine (P172R) (fig 2A). This family contains seven gastric cancer cases in three different generations. Three of the affected subjects had gastric cancer under 50 years of age (33, 39, and 40 years). One of them also had ductal breast cancer. In addition, one patient with both bladder and ovarian cancer and another with prostate cancer and basalioma were found in this family. To investigate the segregation of this missense type change in the family, we screened two additional family members with gastric cancer (fig 1). DNA from paraffin embedded tissues was isolated according to standard procedures and mutation analysis was performed as described above. However, neither of them carried the P172R change. One of the patients studied was the mother of the mutation carrier. The father of this patient died at the age of 94 years and was cancer free. This change was also absent in 212 control samples from cancer free subjects, as screened by SSCP analysis (fig 2B). The change appears to be a rare polymorphism.

Four additional polymorphisms of the *E-cadherin* gene were found in this series of gastric cancer patients. A C to T silent change in codon 692 (from alanine to alanine) occurred in eight of 13 (61.5%) gastric cancer patients. A C to T change in codon 751, resulting in aspartate substitution by asparagine, was detected in three of 13 (23%) patients. These two polymorphisms have been previously reported.[10 11] A C to G change was found before the start codon (−71 bp) in the non-coding region in one of 13 (7.7%) gastric cancer patients and in two of 51 (3.9%) cancer free controls. A T to C change at position +6 in intron 1 occurred in five of 13 (38%) gastric cancer patients and in 18 of 51 (35%) cancer free controls.

So far, altogether 14 truncating *E-cadherin* germline mutations have been detected in gastric cancer patients.[8] A few putative missense germline mutations have been reported but their functional significance has not been tested.[1 6 7] In the sporadic type of cancer there seems to be a cluster of mutations between exons 7 and 9 whereas germline mutations are more evenly distributed.[8 11] A novel missense type change, P172R, found in this study is located in exon 4 which encodes a large extracellular domain with Ca^{2+} binding motifs (exons 4-13).[10] Based on the segregation of the mutation in affected cases in this particular family, it seems that this change is not a pathogenic mutation. It seems to be a very rare polymorphism because none of the 212 cancer free controls carries this change. This finding is interesting because altogether seven gastric cancer cases were found in this family. Caldas *et al*[8] have suggested that *E-cadherin* should account for 25% of the families fulfilling the established criteria for HDGC. However, PCR based screening methods used in this study do not allow detection of all mutation types, for example, large deletions.

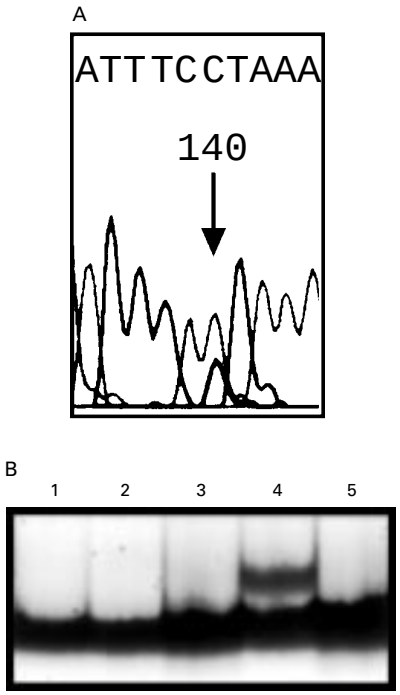

Figure 2 (A) Direct sequencing shows a heterozygous C→G change (P172R, see arrow). (B) SSCP analysis of the P172R change. A positive control (lane 4) was included in all SSCP runs.

Our results support the notion that germline mutations in the *E-cadherin* gene are responsible for only a subset of gastric cancer patients with a family history of the disease. In our study, no mutations were found in 13 gastric cancer probands. Five of the families studied fulfil the criteria for HDGC and one for FIGC. Our data suggest that for the purpose of efficient *E-cadherin* mutation detection, there may be a need for more stringent criteria for HDGC, such as requiring three affected subjects as is common in research on familial breast and colon cancer. However, our data set is limited. Loose inclusion criteria should encourage collection of gastric cancer families. This is important, because further work is necessary to identify predisposing gene(s) for a subset of HDGC families, as well as families segregating intestinal gastric cancer.

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The spectrum and evolution of phenotypic findings in *PTEN* mutation positive cases of Bannayan-Riley-Ruvalcaba syndrome

EDITOR—Bannayan-Riley-Ruvalcaba syndrome (BRRS) is an autosomal dominant condition which includes the features of macrocephaly, hyperpigmented penile macules, and hamartomatous tumours, including lipomas, haemangiomas, and gastrointestinal polyps.[1–4] In 1996, it was recognised that BRRS shared features with Cowden syndrome, another autosomal dominant condition with multiple hamartomas.[5] Cowden syndrome is characterised by trichilemmomas (small, benign hair follicle tumours), oral papillomas, intestinal polyps, and increased frequency of breast and thyroid cancers in affected subjects.[6] Germline mutations in the *PTEN* gene (phosphatase and tensin homologue deleted on chromosome 10), a gene associated with somatic deletion in a number of cancer cell lines and some primary tumours, were identified in families with Cowden disease the following year.[7–10] At the same time, mutations in the *PTEN* gene were identified in several BRRS families,[11] providing evidence that these conditions are allelic. Identical mutations have been identified in some families with Cowden syndrome and in others with BRRS.[12] In addition, families whose members have overlapping features of both conditions have been identified.[13 14] However, publications on BRRS provide little clinical information on the natural history and progression of this condition. Here we review our experience following 10 subjects in three families with BRRS and *PTEN* mutations. The criteria for ascertainment were at least one affected person in a family with at least two of the three features of macrocephaly, hamartomas (including at least one lipoma, haemangioma, or intestinal polyp), and penile macules in males. Affected subjects were found to have germline *PTEN* mutations by DNA analysis.[12 13 15] The pedigrees of the families are presented in fig 1 and their clinical features are summarised in table 1.

Family 1 is of Native American descent and has been followed for six years (fig 1A). The father and four of his five children are affected. The proband, III.2, was born at 37 weeks' gestation after an uneventful pregnancy with birth length and OFC between the 90th and 97th centiles. He has been a healthy child but had markedly delayed cognitive development. He sat at 8 months, walked at 16 months, and had only one word at 4 years of age. He has exhibited autistic behaviour consisting of arm flapping, head banging, and repetitive and self-stimulatory mannerisms. Hyperpigmented macules of the penile shaft were first detected at the age of 7 years 3 months. They had not been present at 4½ years during a previous evaluation when the diagnosis of BRRS had been considered based on the presence of macrocephaly, developmental delay, and a lipoma on the back. Laboratory evaluations included fragile X testing and karyotype analysis, both of which were normal. At his most recent evaluation, at the age of 8 years 10 months, he had height and weight measurements on the 95th centile. His OFC was 58.8 cm, on the 98th centile for an adult male. He had obvious mental retardation, with very few words and markedly autistic behaviour. His palpebral fissures were downward slanting.

The proband's older sister, III.1 in family 1, was 5 years 9 months old at the time this family was first seen. She was born at ~37 weeks' gestation after an uncomplicated pregnancy with birth weight 4000 g (>97th centile) and length 50.8 cm (97th centile). She had an isolated, small, left groin lipoma at the initial evaluation. She sat at 7 months, walked at 15 months, and exhibited delayed speech although to a much lesser degree than her brother. At her most recent evaluation at the age of 9 years 10 months, her height was between the 75th and 90th centiles, her weight was on the 75th centile, and her OFC was 57 cm, just less than the 98th centile for an adult female. She had downward slanting palpebral fissures, joint hypermobility, and a high arched palate.

III.3 in family 1 was first evaluated at 3 years. Her birth history was unavailable. She walked at 18 months and used a few single words at 3 years. When last evaluated, at the age of 7 years 1 month, she had height on the 95th centile, weight greater than the 95th centile, and head circumference of 58 cm, greater than the 98th centile for an adult female. She exhibited mild mental retardation, most notably speech delay, and had joint hypermobility and a high arched palate, but did not have any cutaneous manifestations of BRRS.

III.4 in family 1 (fig 2A) was born at 37 weeks' gestation after an uncomplicated pregnancy with a birth weight of 3490 g. Other birth parameters were unavailable. He walked at 21 months and had three to four words at his first genetic evaluation at 22 months of age. At his next evaluation, aged 4 years 3 months, he had two small hyperpigmented macules involving the penis. He developed seizures related to hypoglycaemia at the age of 12 months, and had an extensive metabolic workup that was not informative. Normal laboratory studies have included electrolytes, thyroid function tests, insulin, growth hormone, cortisol,

lactate, carnitine (urine and plasma), urine organic acids, and plasma amino acids. He has a history of ketonuria associated with hypoglycaemia. A muscle biopsy was normal, without evidence of lipid myopathy, although the muscle carnitine levels were somewhat low and he is being treated with oral carnitine. A cranial MRI performed at 3 years 8 months and repeated two years later showed normal ventricles and patchy increased T2 signal in the deep and subcortical white matter of both occipital lobes with prominent perivascular spaces. His EEG was abnormal, with diffuse slowing and epileptiform discharges over both occipital lobes. When last evaluated at the age of 7 years 2 months, he had height and weight both above the 95th centile and an OFC of 58.2 cm (98th centile for an adult male). He had four visible macules on the shaft of the penis. His back was hirsute. He continued to exhibit global developmental delay and had joint hypermobility and a high arched palate.

The proband's father, II.5 in family 1, has had learning difficulties and macrocephaly (fig 2B). He was reportedly a large infant. He has multiple hyperpigmented macules on his penis. At the age of 28, he had a thyroidectomy for goitre. Pathological examination showed adenomatous nodular hyperplasia without evidence of carcinoma. At the last evaluation aged 29 years, his height was on the 50th centile, weight was just greater than the 95th centile, and OFC was 64.2 cm, much greater than the 98th centile. He was hirsute and had a high arched palate.

III.5, the youngest child in family 1, is normocephalic, without hamartomas or penile macules. When last evaluated at the age of 3 years 9 months, his height and weight were between the 75th and 90th centiles and his OFC was 52 cm (80th centile). He had mild joint hypermobility and a normal palate. He has had normal development. In this family, there has not been any documented breast or thyroid cancer, nor gastrointestinal polyps, although formal endoscopy has not been performed. The father's sibs and parents were unavailable for evaluation.

Family 2 is of Dutch and other European extraction (fig 1B). The proband, III.1, has been followed for three years. He was initially evaluated for macrocephaly, speech delay, and a family history of Cowden syndrome. Born at term, his birth weight was on the 90th centile and birth length was between the 10th and 50th centiles. Because of a large head and a birthmark along the spine, he had a head CT scan which showed megalencephaly without hydrocephalus, and an MRI of the lumbar spine was normal. When evaluated at the age of 10 months, he had normal development, macrocephaly, and a lipomatous vascular malformation in the lumbar spine region. When evaluated at 3 years 2 months, he had a broad forehead, a fleshy vascular mal-

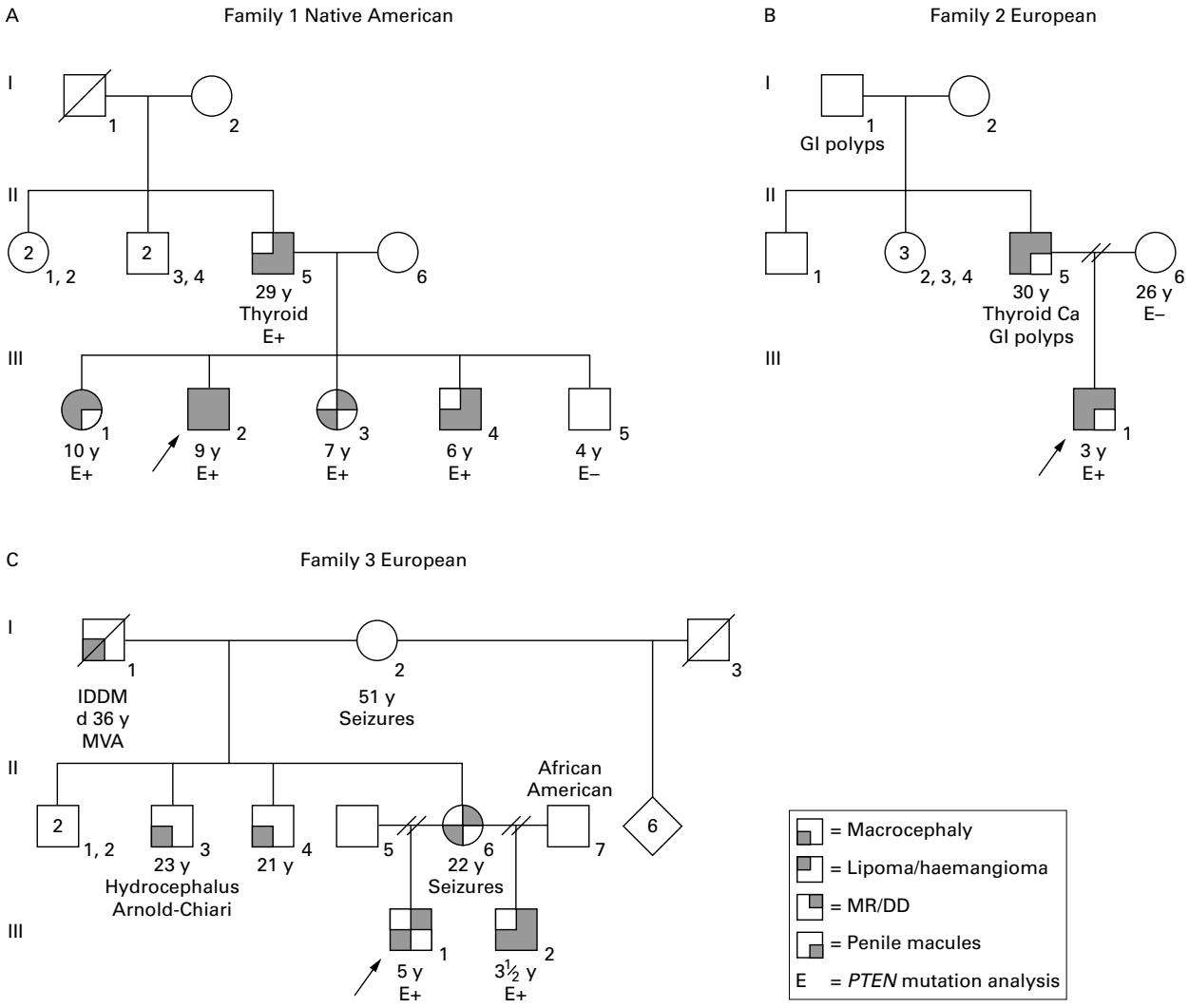

Figure 1 Pedigree of (A) family 1, (B) family 2, and (C) family 3. MR/DD indicates mental retardation/developmental delay. MVA = motor vehicle accident. IDDM = insulin dependent diabetes mellitus.

Table 1 Clinical features of BRRS patients

	Family 1	*Family 2*	*Family 3*	*Total (%)*
Male:female ratio	3:2	2:0	2:1	7:3
Primary selection criteria for BRRS				
Macrocephaly	5/5	2/2	3/3	10/10 (100)
Penile macules*	3/3 males	0/1 males	1/2 males	4/6 (67)
Hamartomata*	2/5	2/2	0/2	4/9 (44)
Lipomas	2	1	0	3
Haemangiomas	0	1	0	1
Known features of BRRS/Cowden				
MR/DD	5/5	2/2	3/3	10/10 (100)
Motor delay*	4	1	2	7
Speech delay*	4	1	1	6
Thyroid abnormalities*	1/5	1/2	0/2	2/9 (22)
GI polyps*	0/5	1/2	0/2	1/9 (11)
Facial papules*	0/5	1/2	0/2	1/9 (11)
Other findings				
High arched palate*	5/5	1/1	2/2	8/8 (100)
Overgrowth	5/5	1/2	2/2	8/9 (89)
Prenatal*†	2	1	2	5
Postnatal	3	1	0	4
Joint hypermobility*	5/5	0/1	2/2	7/8 (88)
Hypotonia*	2/5	1/1	2/2	5/8 (63)
Frontal bossing*	0/5	1/1	2/2	3/8 (38)
Hypoglycaemic episodes*	1/5	0/1	2/3	3/9 (33)
Seizures	1/5	0/2	2/3	3/10 (30)
Downsl palp fissures*	2/5	0/1	0/2	2/8 (25)
Broad thumbs/big toes*	0/5	0/1	2/2	2/8 (25)
Café au lait macules*	1/5	1/1	1/2	3/8 (38)

MR/DD = mental retardation/developmental delay.
Downsl palp fissures = downward slanting palpebral fissures.
*An affected parent(s) from families 1, 2, and/or 3 was unavailable for assessment of these features and is not included in the total.
†Birth weight or length greater than 95th centile; birth parameters not available for several members of family 1.

formation measuring ~3 × 4 cm, located above the gluteal cleft (fig 3), and several small café au lait macules. He had delayed motor development, and had a vocabulary of only five to ten words. There were no hyperpigmented penile macules. Normal laboratory studies included thyroid profile, growth hormone levels, and karyotype. A cranial MRI at 3 years 9 months showed a small left frontal venous malformation and evidence of mild prominence of the perivascular spaces. At 3 years 10 months, his height and weight were above the 95th centiles, with an OFC of 59 cm (>98th centile for an adult male). There were no penile macules.

II.5 in family 2, the proband's father, has had macrocephaly, multiple lipomas, and learning problems. His birth weight was between the 10th and 50th centiles and the delivery was complicated owing to large head size. At the age of 11 years 11 months, he was documented to have an OFC of 60.2 cm (>98th centile for an adult male), with weight on the 75th and height on the 90th centiles. He had undergone several surgical resections of subcutaneous lipomas. A head CT scan was normal. He exhibited delayed motor and cognitive skills with intelligence in the borderline range (IQ 77-79). He developed a goitre in his teenage years and underwent thyroidectomy at the age of 26 for papillary carcinoma. He was later found to have benign polyposis during evaluation for gastrointestinal complaints. Based on the presence of facial skin papules, he was tentatively given the diagnosis of Cowden syndrome, although the biopsy was not confirmatory for trichilemmomas. We have no information regarding penile macules because he was not examined for this finding and is unavailable for evaluation.

Other contributory history for family 2 is a report of gastrointestinal polyps in I.1, the proband's paternal grandfather. He did not have thyroid or learning problems.

Family 3 is of predominantly European extraction and has been followed in the genetics clinic for three years (fig 1C). The proband, III.1 (fig 4A, B), was born at 36 weeks' gestation by caesarean section for fetal macrocephaly with birth weight 3630 g (97th centile). At the age of 4 months, his OFC measured 45 cm (>98th centile) with weight and height on the 25th centile. He had a generalised seizure

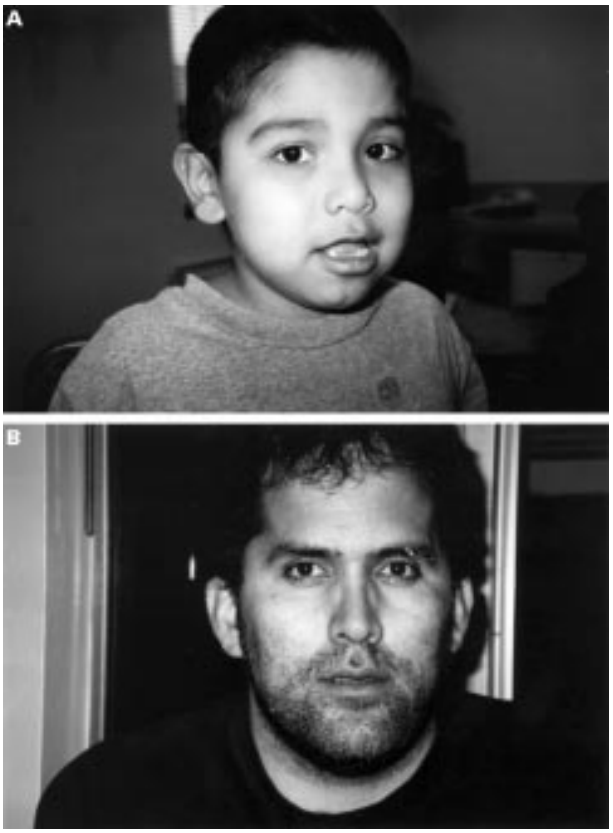

Figure 2 Features of BRRS in family 1. (A) Macrocephaly but otherwise normal cranial configuration of III.4. (B) The father (II.5) also has macrocephaly.

disorder, with normal EEG and MRI as an infant (except for macrocephaly), episodic ketotic hypoglycaemia, and a history of hypotonia. Motor milestones were moderately delayed with crawling at 17 months and walking at 18 months, and he had language impairment with more severe expressive than receptive delays. When first evaluated in the genetics clinic aged 2 years 4 months, he was noted to have macrocephaly with mild frontal bossing but no dysmorphic facial features. His skin examination was notable for three café au lait macules on his trunk, only one measuring greater than 0.5 cm in diameter, as well as a left preauricular telangiectasia from an involuted capillary haemangioma. No lipomas or penile macules were identified. At his most recent evaluation, aged 5 years 2 months, his height was between the 50th and 75th centiles, weight between the 90th and 95th centiles, and OFC measured 60.2 cm (>98th centile for an adult male). Other findings included a high arched palate, joint hypermobility, and broad thumbs and big toes, but no penile hyperpigmentation. He had speech articulation problems. A karyotype study was normal.

The proband's maternal half brother, III.2 in family 3 (fig 4A, B), whose father is of probable African-American extraction, was born at 37.5 weeks' gestation by repeat caesarean section. Prenatally detected macrocephaly prompted amniocentesis, which showed a normal karyotype. Birth weight was 3940 g (97th centile) and length was 53.5 cm (just greater than the 97th centile). OFC was not available. He had a normal newborn course but was evaluated at 6 months for hypotonia and motor delay. At 7½ months, he was noted to have height and weight on the 50th centile but OFC measuring 51 cm (>98th centile). During genetic evaluation at 10 months, he had macrocephaly, frontal bossing, midface hypoplasia, hypotonia, and joint laxity. Diffuse hyperpigmentation of the penis was

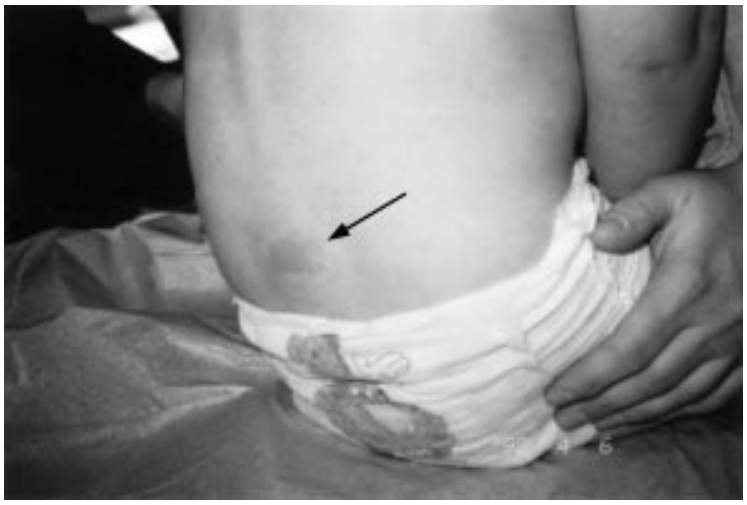

Figure 3 Lipomatous vascular malformation on the back of proband (III.1) in family 2.

noted. At his most recent evaluation, at 3 years 7 months, his growth parameters included height on the 50th-75th centile, weight on the 90th centile, and OFC of 59.8 cm (>98th for adult male). Four discrete hyperpigmented macules were identified on the penile shaft and two on the scrotum. He had mild hypotonia and joint hypermobility, as well as a high arched palate. In spite of mild motor delay, he has had normal language acquisition and has not qualified for special education programmes.

The biological mother of the two boys (II.6 in family 3) has not been available for evaluation and has not been their caregiver owing to concerns of neglect. She is reported to have an OFC of 63 cm (much greater than the 98th centile for an adult female) and a history of childhood seizures, hypoglycaemic episodes, and developmental disabilities. Of her four full brothers, two are reported to have macrocephaly, one with hydrocephalus and an Arnold-Chiari malformation (II.3). The proband's maternal grandfather (I.1) reportedly had macrocephaly and diabetes and died aged 36 in a car accident. There are no reports of breast or thyroid cancer or gastrointestinal polyps in this family, although endoscopy has not been performed.

Molecular analysis was performed on genomic DNA extracted from peripheral blood leucocytes as previously described.[12 15] In family 1, a germline heterozygous nonsense mutation in the *PTEN* gene was identified at codon 130 leading to premature termination of the protein (R130X) within the highly conserved phosphatase domain. The father and four affected children carried the R130X mutation, which was not present in the youngest unaffected child, III.5. In family 2, blood was obtained for *PTEN* mutational analysis on the proband and his unaffected mother, and a mutation was identified in the proband at position 5 in intron 6 (IVS6+5G→T). His mother did not share this allele. In family 3, only the proband and his half brother were available for testing. A mutation in the *PTEN* gene at position 5 in intron 6 (IVS6+5G→A) was detected in one allele from each of the two boys. This specific mutation has not been previously described. The mutations in families 2 and 3 are likely to lead to aberrant RNA splicing and a truncated protein product.

In identifying our families with BRRS, we focused on subjects possessing at least two of the three features of

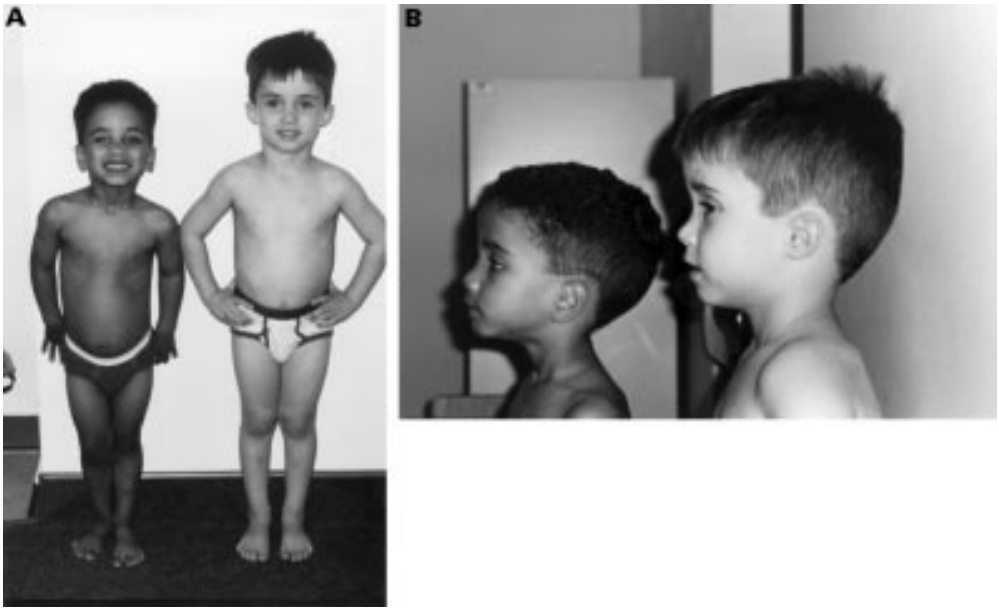

Figure 4 Features of BRRS in family 3. (A) Body habitus of affected boys III.2 (left) and III.1 (right). (B) Macrocephaly in the same two boys.

macrocephaly, hamartomas, and penile macules, criteria which have been used to ascertain BRRS patients for molecular studies.[11 13] It has been suggested that the mere presence of macrocephaly/macrosomia and developmental delay, without hamartomata or penile macules, should warrant further evaluation for *PTEN* mutations.[16] In fact, macrocephaly has been the most consistent finding in BRRS in numerous reviews[17–19] and was present in all of our affected subjects. The macrocephaly consists of megalencephaly without ventricular dilatation and is generally present at birth.[20] We were surprised by the degree of macrocephaly in all affected members of family 1, with head circumference measurements greater than the 98th centile for age, in the absence of facial dysmorphism or other distinguishing cranial features such as frontal bossing. Presumably, the overall large body size masked the disproportionately large head size in these subjects. Families 2 and 3 also exhibited marked macrocephaly, and the affected children had a broad, prominent forehead that has been previously described in BRRS.[21]

The broad spectrum of hamartomata is illustrated by these families. Although family 1 was first brought to medical attention because of familial macrocephaly and developmental delay, the diagnosis of BRRS was not suspected until single lipomas were identified in two of the four affected children. As the children have grown, these lipomas have become less visible. None of the members in family 1 has been evaluated for gastrointestinal polyposis and symptoms such as intestinal bleeding or chronic abdominal pain have not been reported. The father in family 2 had several classical tumours of BRRS, including multiple lipomas evident by his teens and intestinal polyps. His young son had only a vascular malformation along the lumbar spine. In contrast, the affected boys in family 3 have had no obvious hamartomata. These cases illustrate the importance of a thorough skin examination at each evaluation of a child with macrocephaly, although the absence of dermatological abnormalities does not exclude the diagnosis.

Penile macules are perhaps the most distinctive and potentially valuable diagnostic feature of this syndrome. This is shown by family 1, in which penile macules were important in establishing the diagnosis and were not identified in the oldest son (III.2) at the age of 4½ years but were seen at 7 years 3 months. His younger brother had two very small hyperpigmented macules identified at the age of 4½ years and four small penile macules by 7 years 2 months. Only one of the two affected half brothers in family 3 had penile macules, which were first visualised between the ages of 2½ and 3½ years, although generalised penile hyperpigmentation was seen in infancy. He is of mixed racial heritage, with an African-American father, which may increase the likelihood of pigmentation, since his fully white older brother with the identical mutation did not have penile macules. The penile macules detected in all of the children were very small pigmentary changes that might be missed during a cursory examination and were far less obvious than many published photographs.[5 18] We conclude that speckling of the penis is more likely to occur in later childhood, and its absence in infants and toddlers should not exclude consideration of the diagnosis of BRRS.

Mental retardation has been a feature in case reports of BRRS[22] and has been reported in 15-20% of affected subjects in two previous surveys.[17 20] More common are motor and speech delays occurring in childhood in approximately 50% of patients. These delays are reported to improve with age in many cases,[18] and adults are often described as having motor dysfunction with normal IQ.[20] In our families, all affected subjects had some degree of learning impairment. In family 1, the degree of cognitive disability was highly variable, with the father exhibiting the mildest learning problems. All of his affected children were in special education programmes, and the two affected sons had the greatest impairment, with the oldest son at the age of 9 years showing autistic behaviour and minimal expressive language. To our knowledge, autism has not been described previously in this condition. The second family had two affected males with learning problems; the father had borderline intelligence on repeated testing and his son had significant global delay and behavioural problems. The affected children in both of these families appeared to have more severe cognitive impairment than their parents, a phenomenon which has been described in other families with BRRS or Cowden syndrome.[14 23 24] In family 3, both children exhibited motor and speech delay, although the oldest son was more severely affected; their mother was reported to have developmental disabilities as well. The true prevalence of mental retardation in this disorder remains to be established, but for those children suspected of carrying this diagnosis, developmental assessments and appropriate therapeutic interventions are important aspects of care.

Based on the clinical features in these affected subjects, summarised in table 1, we suggest that some "soft" clinical signs may aid in diagnosis when a young child exhibits macrocephaly and learning delay but may not have developed hamartomata or, if male, penile macules. One of these features is a high arched palate, which has been identified in 56-70% of BRRS patients in other surveys[17 20 25] and was identified in all of our patients. Overgrowth, of either prenatal or postnatal onset, is a feature exhibited by almost 90% of our cohort. Approximately 50% of newborns with BRRS have been reported to have large birth weight and length, with subsequent postnatal growth deceleration resulting in normal growth parameters by adulthood.[18 20] As newborns, five of the patients were macrosomic, with either birth weight or length greater than the 95th centile. Three of the affected children in family 1 have exhibited postnatal overgrowth, as has the affected boy in family 2. The father and oldest daughter in family 1 now have normal height and weight, suggesting that the overgrowth resolves by adulthood and perhaps puberty. Since the disturbance in growth velocity appears to be age dependent, it may not be appreciated without following growth curves over time. Other findings with greater than 50% incidence in our cohort include joint hypermobility and hypotonia. Hyperextensibility of joints has been reported in approximately 50% of patients with BRRS,[17 20 25] and hypotonia in approximately 20%.[17 20] These reports also identified downward slanting palpebral fissures in a majority of their patients, a finding which was less common in our cohort. Frontal bossing, hypoglycaemic episodes, seizures, and café au lait macules were all identified in approximately 1/3 of our patients. The presence of these less specific findings may support the diagnosis of BRRS.

It has been suggested that BRRS shows a male preponderance, and that this reflects the overall increased incidence of macrocephaly in males.[19 26] In previous surveys, even before the recognition of penile macules as a clinical feature of the disorder, ~70% of identified patients were male.[17 20] In our three families, seven out of 10 subjects at risk for BRRS were male, with six of these males mutation positive. However, both at risk females in family 1 were also affected, so we do not have adequate numbers to draw conclusions regarding this male preponderance. Since our inclusion of penile macules as a diagnostic criterion may lead to bias in recognising males with this disorder, further studies incorporating mutation analysis are warranted to confirm the observation that more males are affected with BRRS. The opposite sex ratio may exist for

Cowden syndrome, which has been reported to exhibit a female preponderance.[24 27] However, breast cancer is much more prevalent in females in general, and women may be more likely to report facial papules because of cosmetic concerns, so females with Cowden syndrome may be more readily identified than affected males. As a consequence of these sex specific clinical features, the reported ratios in each disorder may merely reflect ascertainment bias.

PTEN mutations have now been identified in up to 80% of patients with Cowden syndrome and in up to 60% of those with BRRS, indicating that they are allelic disorders.[7 12 13 15] In several cases, the same *PTEN* mutation has been identified in families with a diagnosis of either Cowden syndrome or BRRS, and in other cases family members carrying the same *PTEN* mutation have different diagnoses.[13 14 23] There is significant overlap between Cowden syndrome and BRRS, and two of the features found in both conditions are macrocephaly and thyroid abnormalities.[5 6 18] The father in family 1 had thyroidectomy for adenomatous nodular changes although he and his children fit the description of BRRS better than Cowden syndrome. Family 2 also illustrates the overlap in phenotypic features of these conditions. The son had the BRRS findings of macrocephaly, a haemangioma, and developmental delay, while his father had macrocephaly, borderline intelligence, and features more consistent with Cowden syndrome, including GI polyposis, thyroid cancer, and facial papules. In contrast, family 3 has no distinctive features usually associated with Cowden syndrome, although assessment of all at risk subjects has not been performed and the boys are still quite young. Cowden syndrome may be a more likely diagnosis in adolescents or adults because the cardinal features of GI polyposis and thyroid and breast carcinomas are of later onset than the findings of macrocephaly and developmental delay identified in children diagnosed with BRRS. As we accumulate more data on these children with *PTEN* mutations and the diagnosis of BRRS, they may develop features classically associated with Cowden syndrome.

Correlations between genotype and phenotype are beginning to be elucidated for Cowden syndrome and BRRS, with resulting implications for genetic counselling for cancer and related health risks. An association between the presence of a *PTEN* mutation and the development of cancer or breast fibroadenomas has been observed in both BRRS and Cowden syndrome.[13] Thus, affected females in particular may have an increased risk of breast cancer, and we have recommended breast cancer surveillance beginning at puberty for the daughters in family 1. Mutations in the core phosphatase domain are common, and this domain appears to be a crucial region for the function of the tumour suppressor.[12 13] The *PTEN* mutation identified in family 1, R130X, is present in this core motif, and has been described in other families with either Cowden syndrome, BRRS, or features that overlap both of these conditions.[12 13 23] The *PTEN* mutation in family 2 has also been identified as a somatic mutation in a tumour (C Eng, unpublished data) and probably results in abnormal RNA processing with a truncated protein product. Since the father with overlapping BRRS/Cowden syndrome features had GI polyposis as well as thyroid cancer in his mid-20s, we have recommended that the son has an annual manual thyroid examination as well as thyroid function studies, and that he seek medical attention for the development of any breast or neck masses or rectal bleeding. The mutation in family 3 is at the same intronic position as that in family 2, although the symptoms appear to be more subtle in the two half brothers, who are still less than 6 years old. We have recommended that the same evaluations as those for family 2 be offered to these brothers and their other at risk family members.

In summary, our three families with *PTEN* mutation confirmed BRRS illustrate the phenotypic variability within family members with this condition and the time course for the development of some of the manifestations. Macrocephaly appears to be the most consistent feature in BRRS, but may not be obvious and requires measurement and documentation. The natural history of this disorder suggests that the distinctive finding of penile macules in males may not appear until mid childhood, and that cognitive impairment, in addition to macrocephaly, may be a prominent feature of BRRS in many families. We suggest that postnatal overgrowth during childhood may be common in this condition, and other features such as high arched palate, joint hypermobility, and hypotonia may aid in diagnosis. With the availability of *PTEN* molecular analysis, genotype-phenotype correlations may be feasible. Our cases confirm the observation that the clinical features of BRRS and Cowden syndrome show significant overlap, and suggest that until these conditions are better understood, genetic counselling should include information about the risk of developing thyroid and breast cancers and gastrointestinal polyps for anyone with a documented *PTEN* mutation.

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A frameshift mitochondrial complex I gene mutation in a patient with dystonia and cataracts: is the mutation pathogenic?

EDITOR—Mitochondrial DNA (MtDNA) is highly polymorphic. Each person is estimated to differ from another on average at about 25 base pairs among the 16 569 that comprise the mitochondrial genome.[1] Thus, only a small fraction of mtDNA variants are likely to be of pathogenic significance. Criteria currently used for determining the likelihood that a missense mutation is pathogenic include heteroplasmy (the percentage of mtDNA molecules within a cell or tissue harbouring a mutation), evolutionary conservation of the altered amino acid, a maternal inheritance pattern, absence of the mutation in controls, clinical features commonly linked to known pathogenic mtDNA mutations, and defects in mitochondrial morphologies and enzyme activities.[1] However, these criteria are inadequate for several reasons. Many mitochondrial missense mutations are homoplasmic. Pathogenic mtDNA mutations are typically characterised by incomplete penetrance, even when homoplasmic, presumably reflecting interactions with environmental and genetic factors.[2] As a result, inherited mtDNA mutations may manifest as "sporadic" disorders rather than with the classical maternal inheritance pattern. Biochemical assays may also be inconclusive, as the expression of a defect in mitochondrial function depends on the nuclear background and tissue type in which the mutation is studied.[3 4] As a result, mtDNA mutations identified in rare families or subjects with a putative mitochondrial genetic disorder are often of uncertain pathogenic significance.

Over 100 point mutations have been identified in mitochondrial genes in association with human disease, at least 45 of which are missense mutations in protein encoding genes.[5] However, frameshift mtDNA mutations are exceedingly rare. An acquired frameshift 4 bp deletion mutation was identified in the cytochrome b gene at nucleotide position (np) 14 787-14 790 in a patient with parkinsonism-MELAS overlap syndrome[6] and somatic mutations including frameshift mutations have been found in human cancers.[7 8] In contrast, inherited frameshift mutations in mtDNA have not previously been reported. We now report the identification of an inherited frameshift mutation in a patient with dystonia and maternally inherited cataracts. The normal base pair (T) is replaced by AC at np 3308 (T3308AC) in the mitochondrial gene encoding the ND1 subunit of complex I. Dystonia[9 10] and cataracts[11–13] have each been linked previously to complex I dysfunction and to mtDNA mutations but, for the reasons outlined above, the pathogenicity of the T3308AC mutation remains uncertain.

DNA was isolated by standard proteinase K and SDS digestion followed by phenol and chloroform extractions. DNA was isolated from muscle (III.4), fibroblasts (II.8), or blood (I.1, III.1, IV.1, IV.5, and IV.6). Each of these subjects (except IV.1) underwent neurological and ophthalmological examinations. Clinical and molecular data were unavailable from other family members. Polymerase chain reaction (PCR) amplification of mtDNA and sequencing on an ABI 377 automated sequencer (Perkin-Elmer) were performed as previously described.[14] PCR reactions for restriction digests were performed with primers at np 3207-3223 (upper) and 3414-3401 (lower). Restriction digests were performed with *Msi*I (New England Biolabs) and analysed by ultraviolet illumination of a 2% agarose gel permeated with ethidium bromide. The mutation eliminates the single restriction site for this enzyme. The normal 208 base pair (bp) PCR product is cut into two 104 bp fragments, but in the presence of the T3308AC mutation, a single 208 bp fragment remains. A normal control DNA sample was included in each assay to confirm complete digestion by the enzyme. Other PCR and sequencing primers have been published previously.[14]

Immunoblotting of the ND1 subunit of complex I was performed using lysates of fibroblasts obtained from three affected family members and one control. Samples (10 μg) were loaded and run on a 12% acrylamide minigel, rinsed, transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MD), and incubated in blocker containing primary antibody (1:500), as described previously.[15] Membranes were thoroughly rinsed, then incubated with