Germline and germline mosaic PTEN mutations associated with a Proteus-like syndrome of hemihypertrophy, lower limb asymmetry, arteriovenous malformations and lipomatosis

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INTRODUCTION

Germline PTEN mutations cause Cowden syndrome (CS) and Bannayan–Riley–Ruvalcaba syndrome (BRR), two harmatoma-tumour syndromes, and somatic PTEN alterations have been shown to participate, to a greater or lesser extent, in a wide variety of sporadic neoplasia. PTEN is a tumour suppressor and dual-specificity phosphatase which affects apoptosis via its lipid phosphatase activity in the phosphoinositol-3-kinase and AKT pathway as well as inhibiting cell spreading via the focal adhesion kinase pathway. CS and BRR share some features, such as hamartomas and lipomatosis. To determine whether other syndromes characterized by overgrowth and lipomas are part of the PTEN syndrome spectrum, we ascertained six individuals with overgrowth and lipomas who did not meet the diagnostic criteria for CS or BRR. Five had Proteus syndrome and one, a Proteus-like syndrome. When germline DNA and DNA from at least one involved tissue per case were examined for PTEN mutations, only the Proteus-like patient was found to harbour a germline R335X mutation. Interestingly, a lipomatous mass, an epidermoid naevus and arteriovenous malformation tissue, all of which were sampled from physically distinct sites, were all found to carry a second hit R130X mutation on the allele opposite the germline R335X. Both mutations have been described in CS and BRR. We postulate that the second hit, R130X, occurred early in embryonic development and may even represent germline mosaicism. Thus, PTEN may be involved in Proteus-like syndrome with its implications for cancer development in the future.
any hamartoma and overgrowth, but who did not meet the diagnostic criteria of CS or BRR (4,5), and subjected them to PTEN mutation analysis.

RESULTS

No germline or somatic PTEN mutations were found in the five cases of PS or their correspondingly affected tissues. Interestingly, the individual with the undefined PS-like syndrome was found to have a germline PTEN R335X (exon 8) mutation (Fig. 1). This boy was born with marked hypertrophy of the right lower extremity in girth and length, pink verrucoid epidermoid naevi in whirls and plaques on the right side of his body, and macrocephaly. At age 2.5 years, he was noted to have lipomas. The hemihypertrophy worsened over time such that he presented at the age of 6 years with massive arteriovenous malformations involving the muscles and bones of the entire right lower extremity, pelvis, lower abdomen and buttock as well as diffuse verrucoid epidermoid naevi over his chest, hands, legs and face. The arteriovenous malformations resulted in severe arteriovenous shunting such that it caused the patient to develop progressive heart failure. He appeared malnourished and chronically ill with diminished subcutaneous fat in the lower face, trunk and uninvolved extremities. He had difficulty walking and needed assistance. Laboratory studies showed an iron-refractory normocytic anaemia and elevated thyroid stimulating hormone, denoting hypothyroidism. Plain radiographs showed osteolysis of the femoral head with dislocation, and lucencies of the right ilium and abnormal trabecular pattern of the tibia and fibula, all of which are ascribed to the arteriovenous shunting in the affected limb. Magnetic resonance imaging demonstrated extensive arteriovenous malformations of the right buttock and throughout the right lower extremity. At age 7.5 years, after a series of embolizations, a right hip articulation and amputation were performed. Histopathologic examination confirmed arteriovenous malformations in all tissues, including muscle, nerves and bone. Adipose tissue was present in abundance but did not appear hyperplastic. One year after the amputation, his previously normal-appearing left lower limb started developing intramuscular arteriovenous malformations. Of note, he has no trichilemmomas, papillomatous papules, speckled penis or connective tissue naevi. The patient is doing well intellectually in the third grade of school. No other features of CS, BRR or PS were noted. No family history could be elicited. Blood samples were re-collected from the patient and both parents. Sequencing confirmed that the affected boy carried a germline heterozygous 1003C→T base transversion that results in R335X whereas his parents were wild-type. Non-paternity was excluded by analysis of 10 sets of highly polymorphic microsatellite markers on chromosomes 4, 10 and 14 (data not shown).

Genomic DNA was extracted from an epidermoid naevus, a lipomatous mass and arteriovenous malformation tissue surrounded by muscle, resected from this patient from three physically non-contiguous sites, and subjected to PTEN mutation analysis as well as loss of heterozygosity (LOH) analysis in the 10q23/PTEN region. Mutation analysis revealed ‘somatic’ R130X (exon 5) mutation in the naevus, the lipomatous mass and the arteriovenous malformation tissue (Fig. 1). Genotyping revealed no LOH of 10q23 markers (data not shown). To determine whether the germline R335X and somatic R130X are on the same allele or on opposite alleles, RT–PCR was performed using combinations of a forward primer specific for the presence of R130X (E5F-mut) or a universal forward primer (E4/5F) and a universal reverse primer which lies 3′ of codon 335 (E8/9R) (Fig. 2a and d). Sequence analysis of the amplicon generated with cDNA harbouring both germline R335X and somatic R130X and primers E5F-mut and E8/9R revealed wild-type sequence at codon 335 (Fig. 2b), thus denoting that R335X and R130X are indeed on opposite alleles. As a control, sequence analysis in the exon 8 region was performed on an amplicon generated using the same template and primers E4/5F and E8/9R, revealing the R335X (Fig. 2c).

DISCUSSION

De novo germline PTEN mutation is associated with a Proteus-like syndrome. Germline PTEN mutation in addition to the ‘second hit’ found in this child’s epidermoid naevus, fatty tissue and arteriovenous malformation tissue provide genetic evidence that PTEN dysfunction is causative of this syndrome. The clinical diagnosis of CS or BRR in this child can be excluded. Overgrowth with asymmetry has never been described in true CS or BRR cases (Table 1). The progressive course of this patient would argue against hemihyperplasia. Although this child has several clinical features consistent with the consensus criteria for PS, such as being sporadic, the presence of lipomatosis and epidermoid naevi, these features are not sufficient to meet the consensus criteria (12) (Table 1). In contrast, the affected tissues appear to be more than a mosaic distribution (a mandatory diagnostic criterion for PS).

Germline R335X has been previously described in a family with CS and cancers (3) and an unrelated family with CS/BRR overlap syndrome (4). In other words, R335X appears to be highly penetrant in both instances. Although this PS-like patient does not have a family history, his young age at onset and severe
been shown to destabilize the predicted secondary structure resulting in haploinsufficiency and to affect phosphatase function (13). Further, the ‘second hit’ mutation R130X truncates the remaining protein within the phosphatase core motif. Both mutations, therefore, affect phosphatase activity as well as truncate within (R335X) or before (R130X) the C-terminal C2 domain, which is important for phospholipid membrane binding (14). The occurrence of the identical somatic mutation in an epidermoid naevus, derived from ectoderm, as well as the lipomatous mass and arteriovenous malformation tissue, derived from mesoderm, is worthy of note. Instead of postulating that each somatic mutation occurred independently three times, one could instead infer that a single R130X somatic mutation occurred relatively early in embryonic development, perhaps immediately preceding gastrulation or during gastrulation. In this regard, we could speculate that this patient has a germline R335X as well as a germline mosaic R130X. Perhaps the germline heterozygous R335X, i.e. a single PTEN hit resulting in PTEN haploinsufficiency, is causative of this patient’s features which are more reminiscent of BRR, e.g. macrocephaly and thyroid disease. The second hit, R130X, on top of the first would result in PTEN null status in the affected tissues. Consequently, progressive and severe neoplastic-like overgrowth is the phenotype in those tissues which completely lack PTEN function. In sum, our observations suggest that this type of Proteus-like syndrome can be associated with pathogenic germline PTEN mutations and is part of PHTS, with its implications for cancer risk and future surveillance.

**MATERIALS AND METHODS**

**Patients**

Over the course of a single year, six unrelated individuals from two tertiary institutional practices were ascertained that met the minimal criteria of having lipomas, any single hamartoma and overgrowth but that did not meet the diagnostic criteria of CS or BRR as set forth by the International Cowden Consortium (4,5). Five of these patients had classic PS diagnosed in accordance with the consensus guidelines (12) and one with a syndrome that resembles PS but does not meet the consensus diagnostic criteria.

**Mutation analysis**

Genomic DNA was extracted from peripheral blood lymphocytes and from at least one affected tissue from each of the six cases using standard techniques, as previously described (15–17). PTEN mutation analysis using PCR-based DGGE and semi-automated sequencing using the ABI-377 or PE-3700 (4) was performed on genomic and somatic DNA from each of the six cases. Genomic and exonic PTEN primers and PCR conditions have been previously published (1,18,19) except for the following exonic primers: E5F-mut, 5′-CTGTAAAGCTGGAAAGGGAT-3′; E8/9R, 5′-TGAAGTACATTACATTCTTCTTATTATG-3′; E4/5F, 5′-CAATTTAATTGCAGAGTTGCA-3′; E8/9R, 5′-TGAAGTACATTCTTCTTATTATG-3′; E8/9R, 5′-TGAAGTACATTCTTCTTATTATG-3′. When PCR was performed with these exonic primers, the annealing temperature was 65°C.

**Genotyping at microsatellite loci**

Genotyping was performed to exclude non-paternity using microsatellite markers D4S392, D4S414, D4S2935, D10S579,

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**Table 1. Features in CS, BRR, PS and the case patient**

<table>
<thead>
<tr>
<th>Feature</th>
<th>CS</th>
<th>BRR</th>
<th>PS</th>
<th>Case patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrocephaly</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mental retardiation</td>
<td>+++</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Thyroid</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Skin trichilemmoma papillomatosis</td>
<td>+++</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Breast tumours</td>
<td>+++</td>
<td>+/–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lipomatosis</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arteriovenous malformation</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Endometrial tumours</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Connective tissue naevi</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Epidermoid naevi</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Hemi hypertrophy</td>
<td>–</td>
<td>+++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Mosaic distribution</td>
<td>–</td>
<td>+++</td>
<td>+/–</td>
<td>–</td>
</tr>
</tbody>
</table>

–, absent in >99% of cases; +, feature present; ++, somewhat prominent feature; ++++, very prominent feature.
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REFERENCES