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Genetic profiling of basal cell carcinomas detects postzygotic mosaicism in basal cell naevus syndrome*

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Summary

Basal cell naevus syndrome (BCNS) is associated with germline mutations in the PTCH1 gene. Postzygotic mosaicism can also cause BCNS. Here we describe two patients, one with multiple basal cell carcinomas (BCCs) and one with clinical BCNS, who had no PTCH1 mutation in DNA extracted from blood. In both patients, we performed genetic analysis on different BCCs, revealing the presence of a shared PTCH1 mutation in all tumours. Our findings show that in patients with symptoms of BCNS and initial absence of a PTCH1 mutation in blood, genetic profiling of BCCs can detect postzygotic mosaicism.

What’s already known about this topic?

- Basal cell naevus syndrome (BCNS) is associated with germline mutations in the PTCH1 gene, but it can also be caused by low-grade postzygotic mosaicism in PTCH1.

What does this study add?

- In patients suspected of having BCNS or patients with multiple basal cell carcinomas (BCCs) with a special distribution on the body and no mutation detected in blood, it is worthwhile to search for a shared PTCH1 mutation in their BCCs as this can detect postzygotic mosaicism.
- This information is important to ensure proper surveillance programmes, choose the right therapy and provide adequate genetic counselling.
paper we present two patients with symptoms of BCNS who had no PTCH1 mutation detected in blood with Sanger sequencing, and where subsequent genetic profiling of their BCCs led to detection of postzygotic mosaicism.

**Case report**

**Case 1**

A 41-year-old woman was referred to our outpatient clinic because of a history of multiple BCCs exclusively on the right side of her body (Fig. 1). No other symptoms of BCNS were observed. Hundreds of BCCs were treated with excision, Mohs micrographic surgery and photodynamic therapy. The patient was a carrier of a BRCA2 gene mutation and her medical history mentioned left-sided breast cancer, which was treated with mastectomy, radiotherapy and tamoxifen. Sanger sequencing of the coding exons of the PTCH1 gene (BigDye v1.1 sequencing kit, 3730 DNA Analyzer; Applied Biosystems, Foster City, CA, U.S.A.; primer sequences available on request) on DNA extracted from blood (Wizard Genomic DNA Purification Kit; Promega, Madison, WI, U.S.A.) detected no mutation. Because of the unilateral distribution of BCCs we suspected postzygotic mosaicism. Mutation analysis was carried out with single-molecule Molecular Inversion Probes (smMIPs) and next-generation sequencing (NGS) (Illumina NextSeq; Illumina, San Diego, CA, U.S.A.) targeting the coding exons of PTCH1, PTCH2, SUFU and SMO (NCBI RefSeqs NM_000264, NM_003738, NM_016169 and NM_005631) of four different BCCs on the right shoulder and upper arm. This revealed a shared exon 14 PTCH1 mutation in all tumours, designated c.2197_2198del (Table 1 and Fig. S1; see Supporting Information).

In addition, the split halves of the tumours were used for RNA analysis of PTCH1. In two of the BCCs a second somatic mutation was detected in PTCH1 (c.3054G>A in BCC1 and c.1603–1G>A in BCC3). This was also detected in cDNA analysis, the latter affecting splicing (Table 1). In addition, we analysed multiple samples of visibly normal skin on both ends of the fusiform excision specimen of a BCC, fibroblasts grown from one of these ends, subcutaneous healthy tissue underneath the tumour and a biopsy of normal skin on the left, unaffected side of the body (Fig. 2). Mutation analysis on the surrounding skin samples showed variable DNA mutation loads. No mutation was found with smMIP-NGS in the skin biopsy from the left side of the body or in blood. These findings identified type 1 segmental mosaicism of PTCH1 as the cause of her multiple BCCs.

**Case 2**

A 36-year-old woman was referred to our outpatient clinic with a very large basal squamous carcinoma on her forehead, which had been treated with radiotherapy 9 years earlier. On physical examination, a thoracic scoliosis was noticed, and several BCCs, diffusely distributed on the body, together with palmoplantar pits were detected. Based on the clinical criteria we diagnosed her with BCNS. Two mandibular odontogenic cysts were found. A cranial computed tomography scan showed a skin tumour on the forehead with extensive bone destruction and invasion into the dura and cranial part of the superior sagittal sinus. Curative surgery could not be achieved and after deep inferior epigastric perforator flap reconstruction, she was treated with radiation therapy (60–70 Gy). The remaining BCCs were treated with Mohs micrographic surgery, conventional excision and 5-fluorouracil.

Despite her clinical diagnosis of BCNS, Sanger sequencing and smMIP-NGS of the PTCH1 gene on DNA extracted from blood identified no mutation. Mutation analysis was carried out on RNA from two different BCCs on the right shoulder.
and left upper arm (Qiagen RNA isolation kit; Qiagen, Venlo, the Netherlands; iScript cDNA Synthesis Kit; Bio-Rad Laboratories, Hemel Hempstead, U.K.; primer sequences available on request). This showed a shared mutation in exon 15 of PTCH1, designated c.2460C>G, p.(Tyr820*). This mutation was also found with smMIP-NGS on DNA isolated from the split halves of the two BCCs. The results are indicative for type 1 postzygotic mosaicism.

Reinterpretation of the smMIP-NGS data on blood by visual inspection detected the mutation in 1-0% of the sequence reads, a percentage close to the detection limit with the used coverage depth (<1000 ×). One of the tumours was hemizygous for the mutation on cDNA analysis, due to either loss of heterozygosity (chromosomal deletion or allele-specific methylation) of the other allele or nonsense-mediated decay caused by an intragenic PTCH1 mutation (exon deletion or duplication, or splicing). In the other tumour, a second splice-site mutation was identified on DNA, the functional effect of which was also detected with cDNA analysis (r.1603_1607del, Table 1).

**Discussion**

Postzygotic mosaicism as the cause of BCNS can be difficult to detect in DNA from blood with standard Sanger sequencing techniques, as this method’s mutation detection limit is approximately 5%. NGS of a targeted gene panel is more sensitive and has sufficient sequence read depth to detect and quantify the degree of mosaicism reliably. In the patients presented here, a postzygotic mutation could not be demonstrated in blood with Sanger sequencing, but in patient 2 the smMIP-NGS technique did detect the PTCH1 mutation in blood at a very low percentage.

Some forms of segmental mosaicism of dominant mutations can easily be recognized when the presence of the mutation leads to visible skin lesions, such as in mosaic ichthyosis.11 Cutaneous mosaics follow different patterns. Lesions mostly follow the lines of Blaschko, in broad or narrow bands, or appear in a checkerboard pattern.12 In patients with BCNS, skin with only one affected PTCH1 allele has a normal appearance. Only a second hit of the other PTCH1 allele (through loss of heterozygosity or somatic gene mutation) initiates the development of a BCC. The presence of a BCC is a marker for affected skin, genetic mosaicism of a larger cell population in the skin can be demonstrated when different BCCs share the same PTCH1 mutation.

Our first patient clinically had a clear unilateral distribution of BCCs, already indicating genetic mosaicism. The results of the genetic analysis of the different tissue samples suggest a unilateral segmental distribution of the mutated skin following the lines of Blaschko (Figs 1, 2). The mosaicism was probably restricted to the cells of the epidermis, as fibroblasts did not harbour the mutation and subcutaneous tissue underneath the tumour area showed this only to a very low degree. The existence of a blaschkoid pattern in mosaic BCNS is supported by two earlier reports of patients with BCNS by a type 2 segmental PTCH1 mosaicism and type

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**Table 1** DNA mutation loads in different basal cell carcinomas (BCCs) and visibly normal skin samples

<table>
<thead>
<tr>
<th>Case 1</th>
<th>First PTCH1 mutation</th>
<th>Second PTCH1 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTCH1 mutation</td>
<td>smMIP mutation load (%)</td>
</tr>
<tr>
<td>Blood</td>
<td>c.2197_2198del</td>
<td>0</td>
</tr>
<tr>
<td>BCC1</td>
<td>c.2197_2198del</td>
<td>16-3</td>
</tr>
<tr>
<td>BCC2</td>
<td>c.2197_2198del</td>
<td>9-5</td>
</tr>
<tr>
<td>BCC3</td>
<td>c.2197_2198del</td>
<td>14-4</td>
</tr>
<tr>
<td>BCC4</td>
<td>c.2197_2198del</td>
<td>3-4</td>
</tr>
</tbody>
</table>

**Case 2**

- **Blood**
  - c.2460C>G: 1-0

**First PTCH1 mutation**

- BCC1: c.2460C>G: 20-2
- BCC2: c.2460C>G: 15-7

**Second PTCH1 mutation**

- BCC1: c.1603–1_1603delinsAA: 11-2
- BCC2: c.1603–1_1603delinsAA: 11-2

**Hemizygous for the mutation on cDNA analysis, due to either loss of heterozygosity or nonsense-mediated RNA decay; smMIP, single-molecule Molecular Inversion Probe. aZygosity calling is not quantitative but an estimation on the distribution of the Sanger sequencing peaks. bFor splicing effect see Figure S1 in the Supporting Information.**

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1 segmental mosaicism of SMO, showing a comparable pattern.6,7 The second patient had no segmental distribution of symptoms; however, the presence of an identical PTCH1 mutation in two BCCs, a second somatic PTCH1 mutation in one of the tumours and the low percentage of the mutation in blood strongly support our conclusion that PTCH1 mosaicism is the cause of the disease.

Type 1 segmental mosaicism as the cause of BCNS or a partial BCNS phenotype could be more common than previously assumed. It is plausible that these patients do not always fulfil the diagnostic criteria of BCNS and may even present only with multiple BCCs at a young age. A segmental distribution of the BCCs is not always visible. However, it is important to be informed about the
presence of mosaic BCNS as these patients may have other health problems and radiotherapy is contraindicated for the treatment of their BCCs. Also, patients with type 1 segmental genetic mosaicism have a slightly elevated risk of giving birth to a child with a heterozygous PTCH1 mutation, probably depending on the presence and degree of gonadal mosaicism.

In patients with symptoms of BCNS and absence of a mutation in blood, it is worthwhile searching for a shared mutation in the different BCCs with an NGS-based gene panel, to demonstrate genetic mosaicism. This information can be important to ensure proper surveillance programmes, to choose the right therapy and to provide adequate genetic counselling.

References
8 Happle R, Tinschert S. Happle-Tinschert syndrome can be caused by a mosaic SMO mutation and is suggested to be a variant of Curry-Jones syndrome. Br J Dermatol 2016; 175:1108.

Supporting Information
Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Fig S1. Prediction and schematic functional effect of somatic second mutations detected in basal cell carcinomas in case 1 and case 2.