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Postzygotic mosaicism in basal cell naevus syndrome

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Summary

Basal cell naevus syndrome (BCNS) is an autosomal dominant disorder most commonly caused by a germline mutation in the *Drosophila* homologue of patched-1 gene (*PTCH1*). Here we describe a patient with clinical signs of BCNS, caused by postzygotic mosaicism of a *PTCH1* mutation. We performed restriction fragment length polymorphism analysis and Droplet Digital polymerase chain reaction to determine the degree of mosaicism in different tissues of this patient. Our case shows that a relatively low-grade mosaicism can lead to clinical signs reminiscent of those caused by a germline mutation. This finding has important implications for genetic counselling and therefore is pivotal to recognize for dermatologists, as well as for clinical geneticists and clinical laboratory geneticists.

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What's already known about this topic?

- Basal cell naevus syndrome (BCNS) is generally caused by a germline mutation in the patched-1 gene (*PTCH1*).
- Genetic mosaicism in BCNS has been described.

What does this study add?

- A low-grade postzygotic mosaicism of a *PTCH1* mutation can cause a clinical presentation fulfilling the diagnostic criteria of BCNS.
- It is important to determine the degree of mosaicism as accurately as possible with a quantitative technique, for example Droplet Digital polymerase chain reaction, to provide adequate genetic counselling.

Basal cell naevus syndrome (BCNS, MIM #109400), also known as Gorlin syndrome, is an autosomal dominant disorder characterized by multiple basal cell carcinomas (BCCs), maxillary keratocysts and cerebral calcifications.[1] The incidence of BCNS is estimated at 1 in 50 000–256 000.[2] Diagnostic criteria for BCNS were first established by Evans *et al.*,[3] then modified by Kimonis *et al.*[4] and revised by Bree and Shah in 2011.[5] Diagnosis is based on two major criteria, one major criterion and two minor criteria, or one major criterion and genetic confirmation (Table 1). The cause of BCNS is generally a germline mutation in the *Drosophila* homologue of patched-1 gene (*PTCH1*), which encodes the PTCH1 protein.[6,7]

Postzygotic mosaicism has been described in BCNS.[8,9] In postzygotic mosaicism a mutation occurs somewhere during embryogenesis, affecting only cells from the mutant progenitor, resulting in mixed healthy and affected cell populations. Depending on the mutation load and the tissues involved in the mosaicism, an individual with postzygotic mosaicism may develop clinical signs to a certain degree or none at all.[10] Type 1 and type 2 mosaicism as a cause of segmental distribution of clinical symptoms in BCNS have been described.[9,11] Germline cells can be also affected by genetic mosaicism, referred to as gonadal mosaicism. In theory, when the mutational load in gonadal cells is lower than the regular 50% in patients with classic germline BCNS, the risk of BCNS in the patient's offspring is probably $< 50\%$.

Case report

A 22-year-old woman was referred to our outpatient clinic with a BCC in the medial canthus of the right eye (Fig. 1a). During total-body skin examination we detected two

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additional BCCs below her right eye and palmar pits on both hands (Fig. 1b). Orthopantomography was negative for odontogenic keratocysts, and a magnetic resonance imaging scan of the head showed no cerebral calcifications. Based on the presence of two major clinical criteria, BCNS was diagnosed.

Sanger sequencing analysis of the protein-coding exons and flanking introns of *PTCH1* (primer sequences available on request) in DNA extracted from blood (Wizard Genomic DNA Purification Kit; Promega Co., Madison, WI, U.S.A.) detected a barely observable mutation in exon 13, c.1810G>T (NCBI RefSeq: NM_000264.3). Although Sanger sequencing is not a quantitative method, the mutation seemed to have a remarkably lower level than one would expect in the case of a germline mutation (Fig. 2a). Based on this finding we hypothesized that a postzygotic mutation in *PTCH1*, resulting in genetic mosaicism, was responsible for the clinical presentation of our patient. The mutation results in the alteration of a glutamic acid, resulting in a stop at position 604 (p.Glu604*) of the PTCH1 protein. This premature termination codon likely results in a nonfunctional shorter protein. This mutation has not been detected in databases (dbSNP, ExAC) or described in the literature before, although a similar mutation (p.Arg602*) has been described in a family with BCNS.[12]

In postzygotic mosaicism, the percentage of affected cells can vary between different tissues and they may be segmentally distributed. To determine the degree of mosaicism in other tissues, we isolated DNA from urine-derived urothelial cells and buccal mucosa cells of the patient (Wizard Genomic DNA Purification Kit). On these samples we performed restriction fragment length polymorphism (RFLP) analysis with the restriction enzyme *Bfa*I (digesting the mutant allele, Fig. 2b). In addition we performed Droplet Digital polymerase chain reaction (ddPCR) (QX200 ddPCR; Bio-Rad Laboratories, Hercules, CA, U.S.A.) on DNA of the same tissues and BCC tumour cells of this patient (DNA isolation with Maxwell 16 FFPE plus LEV DNA Purification Kit, Maxwell 16 MDX; Promega) to determine the degree of mosaicism (Fig. 2c). ddPCR technology makes use of standard TaqMan probe-based assays (custom Taqman assays, catalogue no. 4331349, ID no. AHN1XWW; Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). The results are summarized in Table 2. Our patient has a low-grade mosaicism of approximately 16%, based on the average of the different degrees of mosaicism determined by ddPCR.

Table 1 Diagnostic criteria for basal cell naevus syndrome (BCNS). Two major criteria, one major criterion and two minor criteria, or one major criterion and genetic confirmation are required for diagnosis

Major criteria	Minor criteria
Multiple BCCs or one BCC in a person younger than 20 years	Bifid, fused or splayed ribs
Odontogenic keratocysts	Other specific skeletal and radiologic abnormalities (i.e. pectus excavatum, scoliosis, hemivertebrae, Sprengel's deformity, syndactyly of digits, bony bridging of the sella turcica, flame-shaped lucencies of phalanges)
Palmar or plantar pits	Macrocephaly
Lamellar calcification of the falx cerebri	Cleft lip or palate
Medulloblastoma in early childhood	Ovarian or cardiac fibroma
First-degree relative with BCNS	Lymphomesenteric cysts
	Ocular anomalies (i.e. congenital cataract, coloboma, glaucoma, hypertelorism)

Discussion

Our findings confirm that a low-grade postzygotic mosaicism of a *PTCH1* mutation can cause a clinical presentation fulfilling the diagnostic criteria for BCNS. Phenotypic variability between patients with BCNS is common, even within families.[13] Such variability also exists between patients with genetic mosaicism. Therefore the phenotype may be difficult to predict, even when the degree of mosaicism is known. Segmental arrangement of symptoms has been described in

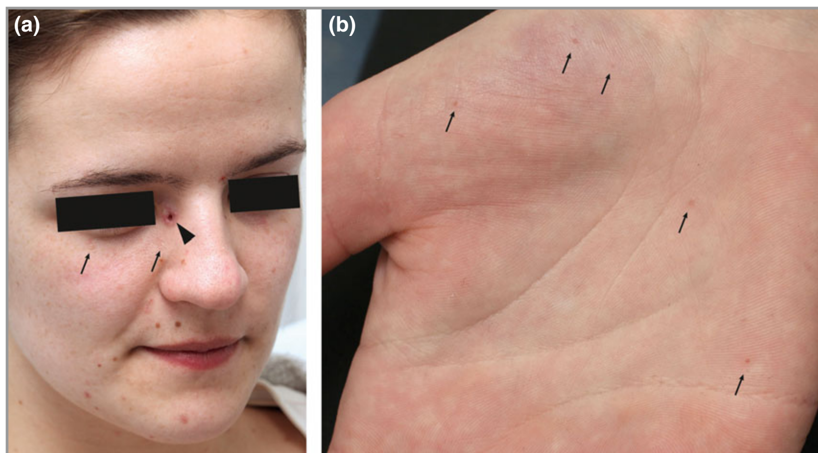

Fig 1. (a) A patient with a basal cell carcinoma (BCC) in the medial canthus of the right eye (arrowhead), and two additional small BCCs (arrows) and (b) multiple palmar pits (arrows).

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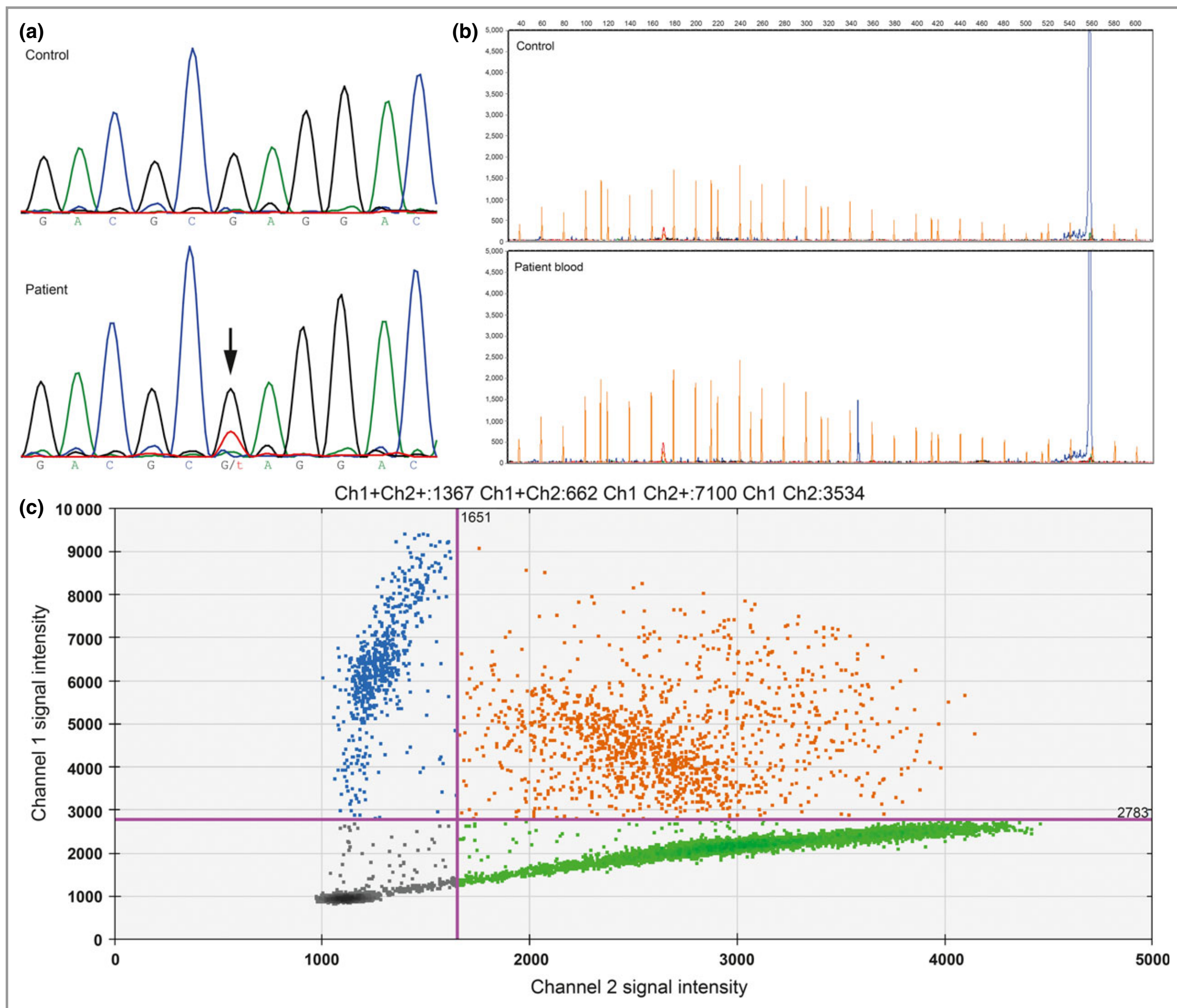

Fig 2. Analyses of genetic mosaicism in *PTCH1* using different techniques. (a) The *PTCH1* sequence trace across the mutation. Top panel: control trace, lower panel: patient trace with c.1810G>T mutation (arrow) from leucocyte-isolated DNA. (b) Restriction fragment length polymorphism analysis with *Bfa*I of wild-type DNA (top) and patient DNA isolated from blood (bottom). Fragment size is depicted horizontally in base pairs, and signal intensity vertically (Genemarker software V2.4.0; SoftGenetics LLC, State College, PA, U.S.A.). The blue peaks represent the 6-carboxyfluorescein (FAM)-labelled polymerase chain reaction products and orange peaks represent fragment-size standards (600 LIZ Size Standard; Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.). The percentage mosaicism was 6·4%, calculated as the mutant peak (348 b.p.) surface area divided by the total peak surface area (348 + 556 b.p.). (c) Representative dot plot picture of the results from Droplet Digital polymerase chain reaction on DNA from the patient's blood. The fluorescence intensity for the 6-FAM TaqMan probe is displayed vertically (channel 1) and the VIC TaqMan probe horizontally (channel 2). The left upper quadrant ($Ch1^{+}$ Ch2) contains positive droplets (blue) for the mutant DNA fragments, the right lower quadrant (Ch1 $Ch2^{+}$) has positive droplets for wild-type DNA fragments (green), the right upper quadrant ($Ch1^{+}$ $Ch2^{+}$) contains positive droplets for one or more wild-type, as well as mutant DNA fragments (orange) and the left lower quadrant (Ch1 Ch2) has empty negative droplets (black). The percentage mosaicism was 13·5%, calculated from positive mutant (blue, orange) droplets divided by the total number of DNA-containing (blue, orange, green) droplets using a Poisson distribution (QuantaSoft software; Bio-Rad Laboratories, Hercules, CA, U.S.A.).

mosaic BCNS. In our patient we did not observe this phenomenon, and she also developed a BCC on the left cheek during dermatological follow-up. However, a segmental distribution could become apparent later in life.

Previously it has been suggested that in patients with segmental Darier disease the percentage of affected gonadal cells may be comparable with that of other tissues.[14] A lower level of mosaicism can very well translate into a lower recurrence risk of BCNS for future offspring. Mutational analysis in gonadal cells (oocytes and spermatocytes) is extremely challenging. In our patient we could estimate the degree of gonadal mosaicism to be somewhere between 13% and 17%, based on the

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Table 2 Tissues analysed for degree of genetic mosaicism

Sample	*Bfa*I RFLP	Droplet Digital PCR
Blood	6·4%	13·5%
Urine-derived endothelial cells	14·7%	17·3%
Buccal mucosa	8·7%	17·0%
Tumour	–	36·1%

Percentages of DNA mutation load with *Bfa*I-restriction fragment length polymorphism (RFLP) and Droplet Digital polymerase chain reaction (PCR). Assessment of the tumour sample with RFLP was not feasible because the PCR amplicon size (556 b.p.) was too large for analysis on fragmented formalin-fixed, paraffin-embedded tissue DNA.

percentages found with ddPCR in the different tissues. If the proportion of gonadal cells with *PTCH1* mutation is < 50%, chance of transmission to the offspring will probably be < 50%, and in this patient it may be even as low as 13%. Providing a more precise recurrence risk is beneficial for genetic counselling. Only then can patients make an adequate informed decision about prenatal diagnosis or preimplantation genetic diagnosis.

Different techniques can be used to determine the degree of mosaicism. With standard Sanger sequencing it may be difficult to detect low-grade mosaicism and impossible to quantify the degree of mosaicism. We used quantitative techniques, RFLP and ddPCR, leading to different results. RFLP analysis showed a lower degree of mosaicism than ddPCR. This difference may be due to less accurate calculation of the peak surface area in RFLP and lack of complete digestion control in the *Bfa*I RFLP. ddPCR does not have these limitations, as it makes use of massive partitioning of the sample. This way, thousands of individual data points are produced, rather than a single result, generating a powerful tool for statistical analysis. We therefore think that ddPCR will provide a better measure of the actual degree of mosaicism than RFLP.

As a control we performed ddPCR on the patient's BCC tumour tissue DNA. In theory, the tumour sample has to consist of cells containing the c.1810G>T *PTCH1* mutation along with an acquired second somatic mutation or loss of heterozygosity to inactivate the tumour suppressor function of *PTCH1*. The mutation load in the tumour is therefore expected to be ≥ 50%. However, ddPCR detected the mutation in only 36% of tumour DNA. This is probably due to the presence of a certain percentage of normal tissue, as we did not microdissect the tumour from the tissue. Histologically the percentage tumour in the sample was estimated to be 60–70%. Based on these findings, loss of heterozygosity is probably less likely than a second somatic mutation.

In conclusion, our results show that a low-grade postzygotic mosaicism of a *PTCH1* mutation can cause a clinical picture fulfilling the diagnostic criteria of BCNS. This is an important finding for dermatologists, as well as for clinical geneticists and clinical laboratory geneticists, because it has implications for genetic counselling. Standard techniques are not always suitable to pick up low-grade postzygotic mosaicism, which makes additional quantitative techniques essential. When initial genetic tests in patients with BCNS suggest an underlying genetic mosaicism, we recommend determining the degree of mosaicism as accurately as possible, for example with ddPCR, for adequate genetic counselling.

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