PTCH mutations and deletions in patients with typical nevoid basal cell carcinoma syndrome and in patients with a suspected genetic predisposition to basal cell carcinoma: a French study

N Soufir, B Gerard, M Portela, A Brice, M Liboutet, P Saiag, V Descamps, D Kerob, P Wolkenstein, I Gorin, C Lebbe, N Dupin, B Crickx, N Basset-Seguin and B Grandchamp

The patched (PTCH) mutation rate in nevoid basal cell carcinoma syndrome (NBCCS) reported in various studies ranges from 40 to 80%. However, few studies have investigated the role of PTCH in clinical conditions suggesting an inherited predisposition to basal cell carcinoma (BCC), although it has been suggested that PTCH polymorphisms could predispose to multiple BCC (MBCC). In this study, we therefore performed an exhaustive analysis of PTCH (mutations detection and deletion analysis) in 17 patients with the full complement of criteria for NBCCS (14 sporadic and three familial cases), and in 48 patients suspected of having a genetic predisposition to BCC (MBCC and/or age at diagnosis ≤40 years and/or familial BCC). Eleven new germline alterations of the PTCH gene were characterised in 12 out of 17 patients harbouring the full complement of criteria for the syndrome (70%). These were frameshift mutations in five patients, nonsense mutations in five patients, a small inframe deletion in one patient, and a large germline deletion in another patient. Only one missense mutation (G774R) was found, and this was in a patient affected with MBCC, but without any other NBCCS criterion. We therefore suggest that patients harbouring the full complement of NBCCS criteria should as a priority be screened for PTCH mutations by sequencing, followed by a deletion analysis if no mutation is detected. In other clinical situations that suggest genetic predisposition to BCC, germline mutations of PTCH are not common.

Keywords: PATCHED; NBCCS; multiple basal cell carcinoma; deletion

Nevoid basal cell carcinoma syndrome (NBCCS, or Gorlin’s syndrome) is an autosomal dominant syndrome predisposing to basal cell carcinomas (BCCs) and numerous developmental abnormalities (Gorlin, 2004). The prevalence is estimated at one per 57 000 (Evans et al., 1991); approximately 0.4% of all cases of BCC and 2% of BCC patients under 45 years of age are affected by NBCCS (Farndon et al., 1992).

NBCCS has been linked to germline mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) (Hahn et al., 1996; Johnson et al., 1996), and the rate of neomutation is high (Shanley et al., 1994). The PTCH mutation frequency in NBCCS patients reported varies considerably in the different studies, ranging from 40 to 80% (Kimonis et al., 2004; Marsh et al., 2005).

The PTCH gene consists of 23 exons, and encodes a 1447-amino-acid integral membrane protein, with 12 transmembrane regions, two extracellular loops, and a putative sterol-sensing domain. Most PTCH germline mutations are predicted to lead to premature truncation of the Ptc1 protein, and assumed to represent null PTCH alleles (Wicking and Bale, 1997), suggesting that many aspects of the phenotype apart from BCC result from haploinsufficiency. Tumours in NBCCS patients are likely to arise when the remaining PTCH allele is inactivated, which would be consistent with PTCH acting as a tumour suppressor gene (Gailani et al., 1992).

In addition, deletions of interstitial chromosome 9q have been identified in some NBCCS patients (Olivieri et al., 2003; Haniffa et al., 2004; Boonen et al., 2005).

One problem that arises is the possibility of a misdiagnosis of NBCCS, because of the complex phenotype of this syndrome. Various clinical and radiological criteria have been used to diagnose NBCCS; these are categorised as major and minor criteria. Nevoid basal cell carcinoma syndrome is considered to be certain when at least two of the four major criteria are present (multiple BCC (MBCGs), palmar and plantar epidermal pits, jaw keratocysts, and cerebral calcification) (Shanley et al., 1994).

Patients may also display many other clinical features that are classified as minor criteria (Table 1) (Lo Muzio et al., 1999).
In addition to NBCCS, recent publications suggest that allelic variation of *PTCH* could also influence susceptibility to BCC (Strange et al, 2004a, b; Asplund et al, 2005). In particular, some *PTCH* haplotypes, including polymorphisms in exon 23 (c.3944C), intron 15 (G 2560 + 9), or exon 12 (c.1686C), seem to have a potentially protective effect against BCC (Strange et al, 2004a, b).

The goal of this study was to search for *PTCH* germline abnormalities both in patients harbouring all the criteria for NBCCS and in those clinically suspected of having a genetic predisposition towards BCC (MBCC and/or BCC while under 40 years of age and/or familial BCC).

**PATIENTS AND METHODS**

**Selection of patients**

This study was performed from 2003 to January 2005. Patients were enrolled at the Saint Louis (60%), Ambroise Paré (25%), Bichat-Claude Bernard (5%), Tarnier (5%), and Henri Mondor (5%) hospitals, all of which are located in or near the city of Paris (France). Sixty-five patients were prospectively enrolled in the study. Ten percent of whom were newly diagnosed cases. Two different categories of patients were studied:

1. Patients affected by the typical NBCCS (17 index cases: three familial and 14 sporadic) who displayed at least two of the major criteria (MBCC, palmo-plantar pits, cerebral calcifications, odontogenic keratocysts) or one major criterion plus at least two minor criteria as defined by Shanley et al (1994). In addition, seven additional NBCCS patients from the three enrolled families were also studied.

2. Patients strongly suspected of having a genetic predisposition towards BCC (48 cases), characterised by either (i) MBCCs (35 cases), defined as the presence of at least two BCCs in the same patient confirmed by pathology reports, and/or (ii) BCC in patients under 40 years of age (28 cases) and/or (iii) familial BCC (10 cases), defined as the presence of at least two BCC cases in first- or second-degree relatives (all cases confirmed by pathology reports). For familial BCC cases, only the proband was enrolled. To exclude the presence of NBCCS features in this ‘BCC-predisposed’ non-NBCCS group, a careful clinical examination was realised to search for BCC, pits, palmo-plantar pits, skin tags, ocular, and limb abnormalities. In addition, dental and cranial X-rays were also performed in order to verify the absence of odontogenic keratocysts and intracranial calcifications.

Written informed consent, agreeing to peripheral blood sampling and genetic analysis, was obtained from each patient enrolled in the study. Genomic DNA was isolated from peripheral blood leucocytes of all the participants by routine methods (Miller et al, 1988).

**PTCH**

The 23 exons of the *PTCH* coding sequence were amplified using 23 primer pairs (Table 2). PCR conditions included 35 denaturing cycles at 95 °C for 30 s, annealing at 60 °C for 30 s, elongation at 72 °C for 45 s for exons 1 and 4; and 35 denaturing cycles at 96 °C for 30 s, annealing at 63 °C for 30 s, elongation at 72 °C for 1 min for exons 5–23. Sequence analysis was performed on an ABI-Prism 3100 automated DNA sequencer using 10 ng PCR purified products and Big-Dye Terminator Cycle Sequencing kits (Perkin Elmer, Courtaboeuf, France), according to the manufacturer's instructions. The functionality of the nonsynonymous variant was predicted using the Polyphen and SIFT informatics program (http://tux.embl-heidelberg.de/ramensky/; http://blocks.fhcrc.org/sift/SIFT_seq_submit2.html).

**PTCH deletion analysis**

Real-time quantitative PCR Real-time quantitative PCR was performed using SYBR Green I dye as a fluorescent signal. This dye binds specifically to the minor groove of double-stranded DNA, making it possible to detect PCR product formation (Ginzinger, 2002).

In order to examine both ends of *PTCH*, two targets were initially chosen on *PTCH* exons 1 and 23, and then, to extend the analysis, two other *PTCH* targets on exons 4 and 15, respectively, were also examined (Table 2). Two single-copy sequences were used as reference sequences: MYH9, mapping at 22q13.1, and Rb, mapping at 13q. Five microlitres of DNA was added to the PCR reaction mixture containing 1 × SYBR Green buffer (Applied Biosystems, Courtaboeuf, France), 300 mM forward and reverse primers, 5 mM MgCl2 (3 mM for 8q11 SST), 200 µM dNTP, and 0.6 U of AmpliTaq Gold (Applied Biosystems) in a final volume of 25 µl.

Each series of PCR reactions included two negative controls, containing water in place of DNA, and a five-point standard curve. The standard curve was plotted using serial dilutions of normal PBMC in Tris (10 mM) – EDTA (1 mM) buffer, ranging from 10 to 0.02 ng µl−1 (corresponding to 50–0.1 ng of DNA analysed per well). The same dilutions were used for all targets and reference sequences. PCR was performed on the ABI PRISM 7700 Sequence detector system (Applied Biosystems). All analyses were performed in duplicate. The PCR amplification profile was as follows: initial denaturing at 95 °C for 10 min, followed by 40 denaturing cycles at 95 °C for 10 s, and a combined annealing and extension step at 65 °C for 1 min. Detection of the fluorescent product was carried out at the end of the extension period. To confirm amplification specificity, the PCR products from each primer pair were subjected to a melting curve analysis, and subsequent agarose gel electrophoresis. The concentration of each gene was calculated based on the appropriate calibration curve. Relative copy numbers of *PTCH* were then obtained by calculating the ratio of the result obtained for each target to the MYH9 and Rb value. The normalised ratio of each target on MYH9 and Rb was expected to be close to 1, if no deletion had occurred.

**Microsatellite analysis**

Two microsatellite markers were studied: (i) a CGG repeat localised in the 5′UTR, which was genotyped by sequencing *PTCH* exon 1, and (ii) a CA repeat localised in intron 2 (Aboulkassim et al, 2003), which was genotyped by migration of the fluorescent-
labelled PCR product on a 310 Genetic Analyzer (Applied Biosystems).

Multiplex ligation-dependent probe amplification

PTCH deletion was also investigated by multiplex ligation-dependent probe amplification (MLPA), a quantitative, multiplex PCR method, as described previously (Gille et al., 2002). Multiplex ligation-dependent probe amplification was used to determine the relative copy number of each of the 23 PTCH exons, and was performed using an available commercial kit (SALSA MLPA KIT P067 PTCH, mrc-Holland).

RESULTS

Seventeen patients were considered to have NBCCS, on the basis of the presence of two major criteria, or of one major criterion plus two or more minor criteria. Three were nonrelated familial cases, and 14 were sporadic cases. The three families had, respectively, four, three, and two NBCCS patients, all first-degree related.

Table 2  PTCH PCR primers

| Exon number | Primer name | Primer sequence (5’→3’) |
|-------------|-------------|-------------------------|
| Sequencing primers | | |
| 1 | PTCH Ex 01F | TGG AAG GCG CAG GGT CTG ACT |
| | PTCH Ex 01 R | CTA TCC CAA AGA GGT AGA GGA |
| 2 | PTCH Ex 02F | CTA GGG CCC CTT GAT TGA AC |
| | PTCH Ex 02R | GCT TGG CCT GGC GAA TAT TAT C |
| 3 | PTCH Ex 03F | ACT GCT CAC ACA TCA GCC AGT CTC AT |
| | PTCH Ex 03R | GCA TTT CCC GAA CAC CTT CAT TTA CTA |
| 4–5 | PTCH Ex 04–05F | GCT GGG TCT GTC TCA CTG AAA GAC |
| | PTCH Ex 04–05R | GCC GAC TAT TCA CTC AAA AAA AGC ACA |
| 6 | PTCH Ex 06F | ATT TGT TGT GAT GGC AGA GTC CCA GA |
| | PTCH Ex 06R | GCC TAA TGG GAG GTG TAT GCC AAA TC |
| 7 | PTCH Ex 07F | AAG ATT TGC CAT ACA CCT CCC ATT AGC |
| | PTCH Ex 07R | AAT TCC CCA CAA GGT GCT TTT TTA A |
| 8 | PTCH Ex 08F | GGA AAC ATG TCG TCA CAG AGA AGG AAA |
| | PTCH Ex 08R | TCC CAT CAA GGT CCC AGA ATG GCA |
| 9 | PTCH Ex 09F | CCC TGC CCT GGA ATC AGC TAG AAC |
| | PTCH Ex 09R | GAA GCA GGA GCA GTC ATG AAG TAA |
| 10 | PTCH Ex 10F | TTT GCC GTG TCA CCT TGT ACT C |
| | PTCH Ex 10R | CGG TGA GAA CAC ACA GCA CAC |
| 11 | PTCH Ex 11F | AGG TGC TGG TGG CAG AGT CCT AAC TA |
| | PTCH Ex 11R | GCA GGC AGC GAC ACA TCA TCT GAC AT |
| 12 | PTCH Ex 12F | CTG CCA GTG ATC TGC GCA ACT AGC |
| | PTCH Ex 12R | CAC CCA GTT AAA CAC AGC CTC AAA CAC |
| 13 | PTCH Ex 13F | CAC GGT TTC AAA TGC TTC AAG AGG A |
| | PTCH Ex 13R | CAA ACC CCG TTA CCC ACA TTC CTT |
| 14 | PTCH Ex 14F | CAG GGC ATG AAC CAG GTG ATG TTA T |
| | PTCH Ex 14R | GAA CCA ATC ATC TGC TCA CAG AGT |
| 15 | PTCH Ex 15F | TTG TCC AGG AAG AGT CAG TGG TGC TC |
| | PTCH Ex 15R | GTT GAA GCT GAA CAC GCA AAA GAC C |
| 16 | PTCH Ex 16F | CCC TGC CCT GCT CAG TCT CCT C |
| | PTCH Ex 16R | CTG GCA TGA GTC CAC ACA ATG AGC TG |
| 17 | PTCH Ex 17F | GCC AGT GAT TGC ATC TGC CCT TAA |
| | PTCH Ex 17R | CCA TTA CAT CAC ATC TCT GTC CAG AG |
| 18 | PTCH Ex 18F | CCT CAC AAA GAA TGA TGG TGG |
| | PTCH Ex 18R | CCA GAG GCC CAG ACA TAA ACA AAA CTT |
| 19 | PTCH Ex 19F | AAG GGT CCC ACT TGG AGA AGA AAA TGG |
| | PTCH Ex 19R | TGA ATT AGG CAG TAA AGG CAG TGT CCA |
| 20 | PTCH Ex 20F | TAC GTG AAC ACC AAA TAT GAC CCA GTG |
| | PTCH Ex 20R | TCT GCC TGA GCC GCC CAA GTA GC |
| 21 | PTCH Ex 21F | TGA ATG TGA ACT GCC GTG GGA TAA |
| | PTCH Ex 21R | CCA GTG CAC AGA AGA AAA AAA AGC ACA |
| 22 | PTCH Ex 22F | CCA ATC GCA AAT ACC GTG CTG TTA C |
| | PTCH Ex 22R | ATC TGC GTG GTG GTG GTG CTC |
| 23 | PTCH Ex 23F | GGG TTA ACT GAG TCT GTG TGG AAA C |
| | PTCH Ex 23R | TTG TCC TCC TCT GGT GCC TCT A |

Quantitative PCR primer

PTCHqex1F CCA AAG AGT TAG AGG AGG GAA GAG AAA GT
PTCHqex1R CTA TCT GCC CGC GAG CAC CTA C
PTCHqex4F GCC GGC AGC GAC ACA TCA TCT GAC AT
PTCHqex4R TTT CCA CTG CCT AAT AAA ATG AAA AGC
PTCHqex15F AAG AAA ACA AAC AGC TTC CCA AAA TGG
PTCHqex15R GTT GAA GCT GAA CAC GCA AAA GAC C
PTCHqex23F CAA ACC CCG TTA CCC ACA TTC CTT
PTCHqex23R TCC CAC CCA CAA AAA ACC CTC T

NBCCS = nevoid basal cell carcinoma syndrome; PTCH = patched.
Thirteen patients had two or more major NBCCS criteria (four patients with two major criteria, seven patients with three major criteria, and two patients with all four criteria). Four NBCCS patients had only one major criterion plus two, four or five minor criteria. The frequencies of the major criteria were as follows: MBCC (88%), palmo-plantar pits (78%), odontogenic keratocysts (70%), and cerebral calcifications (57%). The most frequent ‘minor’ criteria were macrocephaly (70%), epidermal cysts (60%), scoliosis (60%), hypertelorism (50%), and strabism (36%). The median age at the first BCC in this NBCCS group was 27 years.

Forty-eight patients suspected of being predisposed to BCC were characterised by either (i) the occurrence of MBCC (35 cases) and/or (ii) the occurrence of BCC before the age of 40 years (28 cases), and/or (iii) the presence of familial BCC (10 cases), defined as the presence of at least two BCC cases in first- or second-degree relatives. The median age at the first BCC in this group was 42 years.

**PTCH** mutations were identified in 12 out of 17 patients harbouring the full complement of criteria for NBCCS. These were frameshift mutations in five patients, nonsense mutations in five patients, and one in frame deletion in one patient (see Table 3). An identical, nonsense mutation, W129X, was characterised in two unrelated patients. **PTCH** mutations were detected in all three familial cases, and were shown to segregate with the disease in the families, as they were detected in all the seven relatives affected by NBCCS (Table 3).

In addition, a large germline deletion was detected in another typical NBCCS patient. Quantitative PCR analysis showed that three of the four exons examined (4, 15, 23) were deleted, whereas the first exon was not. As the patient was heterozygous for a microsatellite localised in intron 2, this means that the deletion must begin after exon 2 of **PTCH**. These results were confirmed by MLPA, with a 50% reduction in signal intensity from exons 5 to 23, whereas exon 3 was normal. As the MLPA kit does not explore exon 4, both results are concordant and show the presence of a large **PTCH** deletion including exons 4–23. **PTCH** deletions were also looked for in the five remaining NBCCS patients who did not harbour any **PTCH** mutation, but none was found. To summarise, therefore, germline mutations or deletions of **PTCH** were present in 70% of NBCCS patients.

In contrast, in the BCC group without any other criterion for NBCCS, only one missense variant, G774R, was found in a patient affected with MBCC. This patient had five different BCCs, all localised in the head and neck region, the first BCC being diagnosed at the age of 46 years. This variant localised in the putative fourth extracellular domain, and is predicted to be damaging by the SNP prediction programs Polyphen and SIFT (http://tux.embl-heidelberg.de/ramensky; http://blocks.fhcrc.org/sift/SIFT.html). No large deletions of **PTCH** were observed by real-time PCR or MLPA in the remaining patients with a suspected genetic predisposition to BCC.

**DISCUSSION**

In this study, we identified **PTCH** mutations or deletions in 12 out of 17 patients with NBCCS (70%). As far as we know, only one study has been performed in the French population (Boutet et al, 2003). Of the 11 mutations identified in NBCCS patients, 10 resulted in truncation of the **PTCH** protein owing to frameshifts or nonsense mutations. This is consistent with the finding that most (86%) mutations lead to premature termination of the protein (Wicking et al, 1997; Fuji et al, 2003).

Previously, **PTCH** mutations have been found in 40–80% of NBCCS patients (Chidambaram et al, 1996; Wick et al, 1997; Boutet et al, 2003). Although our group is quite small, the exhaustive screening for **PTCH** exons and flanking intronic regions by direct sequencing and deletion analysis may have increased the mutation detection rate.

We identified a large **PTCH** deletion in a patient harbouring the typical signs of NBCCS. In all, five patients that share NBCCS features were previously been reported to carry an interstitial chromosome 9q deletion identified by cytogenetic analysis (Shimkets et al, 1996; Sasaki et al, 2000; Haniffa et al, 2004; Midro et al, 2004). This indicates that large **PTCH** deletions are not a rare mechanism of **PTCH** inactivation, and this possibility should be investigated if no **PTCH** mutation is detected.

Despite the exhaustive analysis, no **PTCH** mutation or large deletion was found in five of the NBCCS patients. This is likely to be due to the existence of mutations outside the regions analysed possibly in introns or regulatory elements. An alternative hypothesis could be the presence of a somatic mosaicism, or the existence of mutations in another gene implicated in the sonic hedgehog pathway, as has been shown to occur in sporadic BCC (Reifenberger et al, 1998; Xie et al, 1998).

In the group of BCC patients without any other NBCCS criterion, only one missense mutation (G774R) was found in a patient with MBCC without any other NBCCS criteria (in particular, this patient had a normal head circumference, no facial or ocular abnormalities, and the chest and cran X-rays did not show any skeletal abnormality or intracranial calcification). Unfortunately, segregation could not be assessed because his parents were deceased. Therefore, the significance of these

---

**Table 3** **PTCH** mutations in NBCCS and MBCC patients

| Patient | Diagnosis | Exon | **PTCH** mutation | Effect on protein | Familial | Segregation |
|---------|-----------|------|-------------------|------------------|----------|-------------|
| B249    | NBCCS     | 4–23 | del               | Truncated        | –        | NA          |
| P270    | NBCCS     | 2    | c.385 G>A         | W129X            | –        | NA          |
| B530    | NBCCS     | 15   | c.2443–2461del18  | p.I815N Del      | +        | Yes, four cases |
| B344    | NBCCS     | 15   | c.2450 T>A        | L818X            | –        | NA          |
| B370    | NBCCS     | 17   | c.2712 C>T        | Q905X            | –        | ND          |
| B395    | NBCCS     | 6    | c.932 delG        | p.A308PomX323    | +        | Yes, two cases |
| B401    | NBCCS     | 18   | c.2962 dup TT     | p.V988LfsX995    | +        | Yes, one case |
| B419    | NBCCS     | 18   | c.3053 G>A        | W1018X           | –        | NA          |
| B420    | NBCCS     | 17   | c.2743 ins CATCATT | p.N915HinsX69X17 | –        | NA          |
| P433    | NBCCS     | 2    | c.260–265delTTTA  | p.F88NdelH6X116  | –        | NA          |
| B484    | NBCCS     | 2    | c.291 inA         | p.N917KfsX139    | –        | NA          |
| B519    | NBCCS     | 2    | c.385 G>A         | W129X            | –        | NA          |
| P345    | MBCC      | 15   | c.2320 G>A        | G774R            | –        | NA          |

**PTCH** mutations are described using the nomenclature system for human gene mutations (den Dunnen and Antonarakis, 2001); MBCC = multiple basal cell carcinoma; NA = not applicable; NBCCS = nevoid basal cell carcinoma syndrome.
amino-acid substitution will not become completely clear until a functional analysis is performed. However, this could be a causative mutation, as (i) it is predicted to be damaging by two bioinformatic programs Polyphen and SIFT and (ii) it was not reported in any previous study or in the NCBI SNP database. On the other hand, we cannot exclude the possibility that this could be a rare polymorphism.

We did not find any other PTCH mutation in this group (P<0.00001), which indicates that when other NBCCS criteria are absent, PTCH mutations are rarely involved in predisposition to BCC. Nevertheless, it remains possible that PTCH polymorphisms located outside the coding sequence or intron-exon junctions could influence BCC susceptibility, as has been suggested by recent publications (Strange et al, 2004a,b; Asplund et al, 2005).

In conclusion, germline abnormalities (mutations and deletions) of PTCH are very predominantly observed in patients with the full criteria for NBCCS. We therefore suggest that patients harbouring the full complement of NBCCS criteria should, as a priority, be screened for PTCH mutations by sequencing, followed by a deletion analysis if no mutation is detected. The finding of a PTCH mutation confirms the clinical diagnosis of NBCCS, therefore validating the clinical and radiological diagnostic criteria of this syndrome. The molecular confirmation of NBCCS diagnosis permits a better clinical monitoring (in particular, dermatological), a choice of the rational therapeutic (e.g. avoiding radiotherapy for treatment of BCCs). Moreover, it makes it possible to carry out a genetic council in the families concerned, and to offer the possibility of antenatal diagnosis if the families wish it.

REFERENCES

Aboulkissim TO, LaRue H, Lemieux P, Rousseau F, Fradet Y (2003) Alteration of the PATCHED locus in superficial bladder cancer. Oncogene 22: 2967 – 2971
Asplund A, Gustafsson AC, Wiklund NM, Sela A, Leffeld DJ, Kidd K, Lundeberg J, Brash DE, Ponten F (2005) PTCH codon 1315 polymorphism and risk for nonmelanoma skin cancer. Br J Dermatol 152: 868 – 873
Boonen SE, Stahl D, Kreiborg S, Rosenberg T, Kalscheuer V, Larsen LA, Tommerup N, Brondum-Nielsen K, Tumer Z (2005) Delineation of an interstitial 9q22 deletion in basal cell nevus syndrome. Am J Med Genet A 132: 324 – 328
Boutet N, Bignon YJ, Drouin-Garraud V, Sarda P, Longy M, Lacombe D, Gattamaneni HR, Birch JM (1991) The incidence of patched mutations in French patients with Gorlin syndrome. J Invest Dermatol 121: 478 – 481
Chidambaram A, Goldstein AM, Gailani MR, Gerrard B, Bale SJ, DiGiovanna JJ, Bale AE, Dean M (1996) Mutations in the human homologue of the Drosophila patched gene in Caucasian and African-American nevoid basal cell carcinoma syndrome patients. Cancer Res 56: 4599 – 4601
den Dunnen JT, Antonarakis SE (2001) Nomenclature for the description of human sequence variations. Hum Genet 109: 121 – 124
Evans DG, Farrond PA, Burnell LD, Gattamaneni HR, Birch JM (1991) The incidence of Gorlin syndrome in 173 consecutive cases of medulloblastoma. Br J Cancer 64: 959 – 961
Farrond PA, Del Mastro RG, Evans DGR, Kilpatrick MW (1992) Location of gene for Gorlin syndrome. Lancet 339: 581 – 582
Fujii K, Kohno Y, Sugita K, Nakamura M, Moroi Y, Furue M, Urabe K, Fujii M, Waseda W, Watanabe K, Kirchhoff M, Kalscheuer V, Michalova K, Tommerup N (2004) Interstitial deletion 9q22.32 – q33.2 associated with additional familial translocation t(9;17)(q34.11;p11.2) in a patient with Gorlin–Goltz syndrome and features of Nail–Patella syndrome. Am J Med Genet A 124: 179 – 191
Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 2157
Olivieri C, Maraschio P, Caselli D, Martini C, Beluffi G, Maserati E, Danesino C (2003) Interstitial deletion of chromosome 9, int del(9)(q22.31–q32.1), including the genes causing multiple basal cell nevus syndrome and Robinow/brachydactyly 1 syndrome. Eur J Pediatr 162: 100 – 103
Reifenberger J, Woltzer M, Weber RG, Megahed M, Ruzicka T, Lichter P, Reifenberger G (1998) Missense mutations in SMOH in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 58: 1798 – 1803
Sasaki K, Yoshimoto T, Nakao T, Minagawa K, Takahashi Y, Watanabe Y, Tanabe C (2000) A nevoid basal cell carcinoma syndrome with chromosomal aberration. No To Hattatsu 32: 49 – 55
Shanley S, Ratcliffe J, Hockey A, Haan E, Oley C, Ravine D, Martin N, Wicking C, Chenevix-Trench G (1994) Nevoid basal cell carcinoma syndrome: review of 118 affected individuals. Am J Med Genet 50: 282 – 290
Shinmets K, Gailani MR, Siru VM, Yang-Feng T, Pressman CL, Levant S, Goldstein A, Dean M, Bale AE (1996) Molecular analysis of chromosome 9q deletions in two Gorlin syndrome patients. Am J Hum Genet 58: 417 – 422
Strange RC, El-Gindy N, Ramachandran S, Lovatt T, Fryer AA, Smith AG, Lear JT, Ichii-Jones F, Jones PW, Hoban PR (2004a) PTCH polymorphism is associated with the rate of increase in basal cell carcinoma numbers during follow-up: preliminary data on the influence of an exon 12–exon 23 haplotype. Environ Mol Mutagen 46: 469 – 476
Strange RC, El-Gindy N, Ramachandran S, Lovatt T, Fryer AA, Smith AG, Lear JT, Wong C, Jones PW, Ichii-Jones F, Hoban PR (2004b)
Susceptibility to basal cell carcinoma: associations with PTCH polymorphisms. *Ann Hum Genet* 60: 536–545

Wicking C, Bale AE (1997) Molecular basis of the nevoid basal cell carcinoma syndrome. *Curr Opin Pediatr* 9: 630–635

Wicking C, Shanley S, Smyth I, Gillies S, Negus K, Graham S, Suthers G, Haites N, Edwards M, Wainwright B, Chenevix-Trench G (1997) Most germ-line mutations in the nevoid basal cell carcinoma syndrome lead to a premature termination of the PATCHED protein, and no genotype–phenotype correlations are evident. *Am J Hum Genet* 60: 21–26

Xie J, Murone M, Luoh SM, Ryan A, Gu Q, Zhang C, Bonifas JM, Lam CW, Hynes M, Goddard A, Rosenthal A, Epstein Jr EH, de Sauvage FJ (1998) Activating smoothened mutations in sporadic basal-cell carcinoma. *Nature* 391: 90–92