Coincident Two Mutations and One Single Nucleotide Polymorphism of the *PTCH1* Gene in a Family with Naevoid Basal Cell Carcinoma Syndrome

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Sir,
Naevoid basal cell carcinoma syndrome (NBCCS, OMIM #109400), also called Gorlin's syndrome, is an autosomal dominant disease that affects about 1 in 60,000 individuals (1, 2). NBCCS is associated with various skeletal and neurocutaneous abnormalities. Major manifestations are multiple basal cell carcinomas (BCCs), odontogenic keratocysts, palmoplantar dyskeratotic pits and intracranial calcification (3). In addition, rib and vertebral malformations, epidermal cysts, macrocephaly, facial anomalies, ovarian fibromas and medulloblastomas are associated with this syndrome.

It was reported that NBCCS results from germline mutations in the human homologue of the *Drosophila* segment polarity gene patched (*PTCH1*) (4, 5). *PTCH1* maps to 9q22.3 and contains 23 exons coding for a transmembrane protein with 12 transmembrane regions, two extracellular loops, and a putative sterol-sensing domain. PTCH1 binds the secreted factor sonic hedgehog (SHH) and represses the signalling activity of the co-receptor smoothened that is required for transmission of the hedgehog (Hh) signal to the nucleus (6–8). Here we report three cases of NBCCS in a family with two mutations and one single nucleotide polymorphism (SNP) in *PTCH1*.

PATIENTS AND METHODS

A 70-year-old woman (case 1) was referred with multiple tumours on the face and neck. Clinical and histological findings identified them as BCC. The patient also showed frontoparietal bossing, hypertelorism and mental retardation. A computed tomography (CT) scan, X-rays of the skull and an orthopanogram examination revealed odontogenic keratocysts and intracranial calcification. Her daughter was a 36-year-old female (case 2) with similar clinical findings, i.e. odontogenic keratocysts, intracranial calcification, frontoparietal bossing, hypertelorism and palmoplantar pits. The daughter of case 2 was a one-year-old female (case 3) exhibiting the clinical findings of frontoparietal bossing, hypertelorism and mental retardation. Based on the clinical diagnostic criteria (9), all three cases were diagnosed as NBCCS. To date, however, cases 2 and 3 had no clinical signs of BCC, medulloblastoma or ovarian fibromas, presumably because of age.

Since case 2 asked us to perform the genetic analysis, *PTCH1* status was investigated. After informed consent was obtained, blood samples were collected and genomic DNAs were extracted. All coding exons of *PTCH1* were examined by single strand confirmation polymorphism assay followed by direct sequencing of exons with intron-exon junctions (4). Exons that showed a variant band pattern were sequenced to confirm the presence of a mutation. Two mutations of the *PTCH1* gene; a deletion of AGAC at nucleotide position 667 within exon 3 and an intervening sequence (IVS)16 -3T>C, and a SNP; IVS10 -8T>C, were detected in all three cases. A deletion of AGAC causes a frameshift and a subsequent stop codon in exon 3, which prematurely truncates the protein (Fig. 1a). In addition, the IVS16 -3T>C could lead to an aberrant splicing and truncation of *PTCH1* (10–12).

To detect the expression level of PTCH1 protein in the skin, an immunohistochemical study was performed using goat polyclonal anti-PTCH1 antibody (G-19; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Enzyme reactions were developed with conventional substrates for diamino-benzidine (Sigma, St Louis, MO, USA) (13). In sporadic BCC from a non-NBCCS patient as a control, moderate staining of the peripheral outer palisading cell layer and focal aggregates within the lesion were observed (Fig. 1b, left). Although it was expected that the staining was weaker in a NBCCS-related BCC (case 1), similar staining pattern and intensity of PTCH1 was observed (Fig. 1b, right).

DISCUSSION

Screening for the mutation database for *PTCH1* (http: www.cybergene.se/PTCH, http://www.hgmd.cf.ac.uk/ac/index.php/ HGMD) revealed that the two mutations detected in our patients (667 del AGAC and IVS16 -3T>C) have not been reported. 667 del AGAC within exon 3 of the *PTCH1* gene causes a frameshift and subsequent stop codon in exon 3. Therefore, this mutation truncates the protein made from that copy of the gene and impairs the second extracellular loop (Fig. 1a). IVS10 -8T>C is a SNP (rs2277184). And IVS16 -3T>C potentially leads to an aberrant splicing and truncation of *PTCH1*. More than 20 mutations have been reported to result in aberrant splicing in *PTCH1* and some of them have been proven experimentally (10–12). On the other hand, these two mutations and a SNP were penetrant in the individuals examined here, suggesting that they occurred simultaneously in one allele. However, the frameshift mutation at exon 3 seems not to affect the translation of the following SNP at the intron 10 and mutation at splicing site of intron16-exon17. It is not certain whether these mutations occurred merely by chance or during recombinant repair processes (14).

It was anticipated that the truncating *PTCH1* mutation reduces the predicted amount of PTCH1 protein by 50%, and that a premature termination in an aberrant mRNA possibly initiates the process of nonsense-mediated decay resulting in reduced protein levels. Therefore, immunohistochemistry could be a candidate for screening NBCCS. However, as shown in Fig 1b, BCC of case

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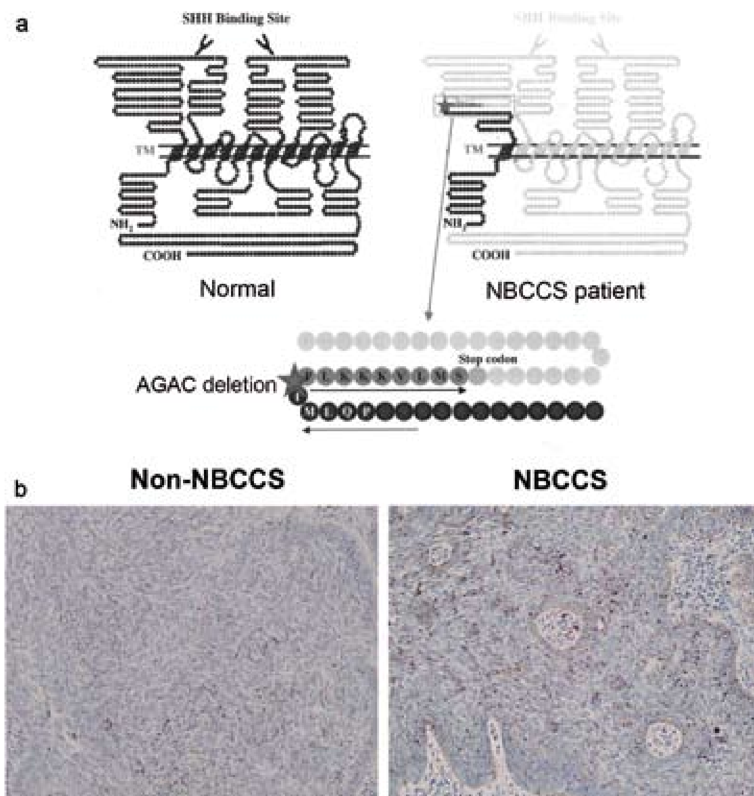

Fig. 1. Deletion of AGAC at exon 3 and immunohistochemical analysis. (a) Predicted effect on the PTCH1 protein. The deletion of AGAC causes a frameshift and a subsequent stop codon, which prematurely truncates the PTCH1 protein made from that copy of the gene. (b) Basal cell carcinoma from sporadic non-NBCCS patient stained with antibody to PTCH1 (*left panel*) showed moderate staining of the peripheral palisaded layer and focal aggregates within the lesion. The similar staining was observed in basal cell carcinoma from a NBCCS patient (case 1) (*right panel*). NBCCS: naevoid basal cell carcinoma syndrome; SHH: sonic hedgehog.

1 and non-NBCCS (no mutation of *PTCH1* had been confirmed) exhibited an immunohistochemically similar pattern and intensity of PTCH1. The commercially available antibody to PTCH1 protein targets the N-terminus of PTCH. Since the truncating point existed at exon 3 in our case, the N-terminus of PTCH1 produced by the mutated allele could be intact, resulting in the similar staining pattern to the sporadic BCC. It is suggested that the intensity of PTCH1 staining alone cannot predict the existence of *PTCH1* mutation. It is important to bear in mind that the loss of staining depends on the mutational sites, the target regions of antibodies used, and the process of nonsense-mediated decay.

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