

# Frameshift mutation in the *PTCH2* gene can cause nevoid basal cell carcinoma syndrome

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**Abstract** Nevoid basal cell carcinoma syndrome (NBCCS) is an autosomal dominant disorder characterized by developmental defects and tumorigenesis. The gene responsible for NBCCS is *PTCH1*, encoding a receptor for the secreted protein, sonic hedgehog. Recently, a Chinese family with NBCCS carrying a missense mutation in *PTCH2*, a close homolog of *PTCH1*, was reported. However, the pathological significance of missense mutations should be discussed cautiously. Here, we report a 13-year-old girl diagnosed with NBCCS based on multiple keratocystic odontogenic tumors and rib anomalies carrying a frameshift mutation in the *PTCH2* gene (c.1172\_1173delCT). Considering the deleterious nature of the frameshift mutation, our study further confirmed a causative role for the *PTCH2* mutation in NBCCS. The absence of typical phenotypes in this case such as palmar/plantar pits, macrocephaly, falx calcification, hypertelorism and coarse face, together with previously reported cases, suggested that individuals with NBCCS carrying a *PTCH2* mutation may have a milder phenotype than those with a *PTCH1* mutation.

**Keywords** Nevoid basal cell carcinoma syndrome · *PTCH1* · *PTCH2* · Frameshift mutation

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## Introduction

Nevoid basal cell carcinoma syndrome (NBCCS) (OMIM 109400), also known as Gorlin syndrome, is an autosomal dominant disorder characterized by developmental defects including bifid ribs, palmar or plantar pits, and tumorigenesis such as the development of basal cell carcinoma, medulloblastoma, or keratocystic odontogenic tumor (KCOT) (formerly known as odontogenic keratocysts) [1]. It is transmitted with complete penetrance and variable expressivity. The gene responsible for NBCCS is the human homologue of the *Drosophila patched* gene, *PTCH1* [2, 3]. The human *PTCH1* gene contains 23 coding exons spanning approximately 70 kb and encodes a protein of 1,447 amino-acid residues containing 12 transmembrane-spanning domains and two large extracellular loops [2]. The *PTCH1* protein is the ligand-binding component of the sonic hedgehog (Shh) receptor complex. In the absence of Shh binding, *PTCH1* is thought to hold smoothed (SMO), a 7-pass transmembrane protein, in an inactive state and thus inhibit signaling to downstream genes. Upon the binding of Shh, the inhibition of SMO is released and signaling is transduced leading to the activation of target genes by the Gli family of transcription factors [4]. Therefore, aberrant activation of the Shh signaling cascade due to the haploinsufficiency of *PTCH1* is believed to cause NBCCS.

In vertebrates, there exists a close homolog of *PTCH1* named *PTCH2*. The human *PTCH2* gene contains 22 coding exons spanning approximately 15 kb and encodes a protein of 1,203 amino-acid residues [5]. Recently, in six affected members of a Chinese Han family with NBCCS, Fan et al. identified a heterozygous germline missense mutation in the *PTCH2* gene [6]. Here we report a case with NBCCS carrying a frameshift mutation due to a 2-bp

deletion in *PTCH2*. To our knowledge, this is the first report of NBCCS caused by a frameshift mutation and the second report of a germline mutation in the *PTCH2* gene.

### Clinical report

A 13-year-old Japanese girl was referred to our hospital with jaw cysts and a rib abnormality. She was born weighing 2,570 g, with a height of 48 cm and a head circumference of 32 cm. Her two siblings as well as her parents exhibited no similar features. No consanguineous marriage was noticed in her pedigree. At 10 years of age, she had multiple KCOTs (Fig. 1a) and underwent a surgical operation to remove them. At 12 years of age, she exhibited proteinuria, and was diagnosed as having chronic glomerular nephritis. Then, she was referred to our hospital for further investigation. At examination, she was 150.1 cm tall (mean) and weighed 57.8 kg (+1.8SD). Her head circumference was 54.6 cm (mean). She had normal intelligence without neurological deficit. She exhibited no structural abnormalities of face, oral cavity, or limbs. However, chest roentgenogram revealed a left bifid rib without any other bone abnormalities (Fig. 1b). She did not exhibit palmar or plantar pits, falx calcification, medulloblastomas, or basal cell carcinomas at that time. Since she exhibited KCOTs and a rib anomaly fulfilling the diagnostic criteria made by Kimonis et al. (two major criteria), we diagnosed her as having NBCCS.

### Methods

#### DNA extraction and PCR-sequencing analysis

All experiments described below were approved by the ethics committee at Kitasato University. DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA blood midi kit (QIAGEN). The complete coding region of the *PTCH1*, *PTCH2*, suppressor of fused (*SUFU*) and *SMO* genes, including all splice junctions, was amplified from

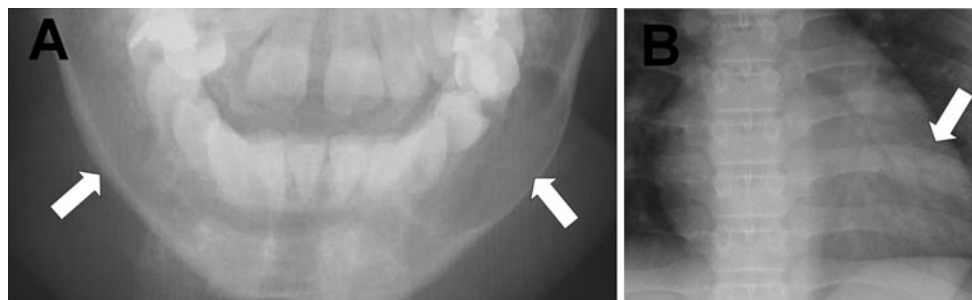
constitutional DNA as described previously [7]. Primers used for amplifying *PTCH1*, *PTCH2* and *SUFU* exons were described previously [5, 7, 8]. Those used for amplifying *SMO* are listed in supporting information Table 1. Amplified products were gel-purified using a QIAEX II gel extraction kit (QIAGEN) and cycle sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in both directions. The sequence was analyzed on a 3130 Genetic Analyzer (Applied Biosystems).

#### Comparison of clinical manifestations

Details of a nationwide survey of NBCCS performed in Japan have been described previously [9]. The survey covered 157 NBCCS patients whose clinical details were available. Major and minor criteria for NBCCS proposed by Kimonis et al. [10] were evaluated in these patients and compared with those observed in patients carrying a *PTCH2* mutation reported previously including the present case [6].

### Results

No mutation in *PTCH1* or the related genes *SUFU* and *SMO* was detected in the peripheral blood from this patient. Although deletion of the entire *PTCH1* gene is a common event in point mutation-negative cases of NBCCS as we reported previously [11], no such deletion was observed using either a ligation-dependent probe amplification method or high-resolution array-based comparative genomic hybridization technology [11–13]. We then sequenced all exons of the *PTCH2* gene, since *PTCH2* is a close homolog of *PTCH1* and is also a suppressor component of the Shh pathway. As a result, a heterozygous 2-base-pair deletion, c.1172\_1173delCT, was detected in exon 9 of *PTCH2* (Fig. 2). This mutation caused a frameshift and created a premature termination codon (PTC) at the site of the deletion resulting in a truncated form of the PTCH2 protein, p.S391X.



**Fig. 1** Roentgenograms of the patient. **a** Pantomography shows bilateral keratocystic odontogenic tumors (Arrows). **b** Chest roentgenogram reveals a bifid anomaly of the left sixth rib (Arrow)

**Table 1** Comparison of the characteristic phenotype of NBCCS in three groups

Patients	Number of patients	Mean age	Mean number of major criteria	Mean number of minor criteria
Nationwide survey	157	33.1	2.4*	1.3**
With <i>PTCH2</i> mutation	7	34.1	1.57	0.14

\* $P < 0.05$  (vs *PTCH2* mutation), \*\*  $P < 0.01$  (vs *PTCH2* mutation)

In order to characterize the phenotype of individuals carrying a *PTCH2* mutation, we next evaluated the number of positive criteria for NBCCS proposed by Kimonis et al. [10] in two groups; first, NBCCS patients collected by a nationwide survey described previously [9], second, reported individuals carrying a *PTCH2* mutation including our case. In spite of the comparable mean ages in two groups (33.1 vs 34.1 years old), positivities of most of the criteria were lower in the *PTCH2* mutation-positive group than in the other, indicating that *PTCH2* mutations cause a milder phenotype than the classical NBCCS (Table 1). In fact, only 4 out of 7 individuals in the *PTCH2* mutation-positive group diagnosed as having NBCCS according to this diagnostic criteria.

## Discussion

NBCCS is caused by a mutation in the gene *PTCH1*, with rare exceptions in which a *SUFU* mutation has been identified [8, 14, 15]. Recently, a heterozygous missense mutation in the *PTCH2* gene, c.2157G>A (p.R719Q), was identified in 6 affected members of a Chinese Han family with NBCCS [6]. Although the pathological significance of missense mutations should be discussed cautiously, this mutation was demonstrated

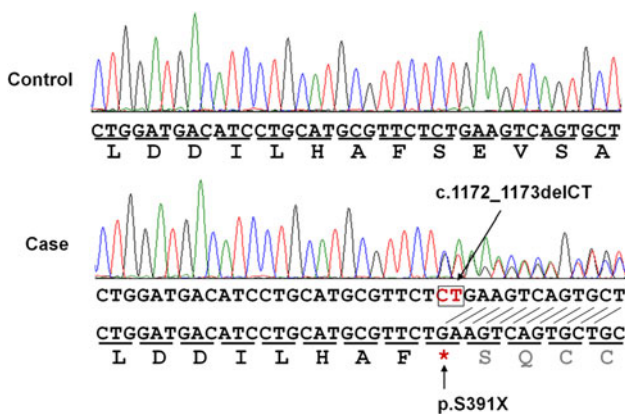
to result in the inactivation of *PTCH2*'s inhibitory activities at least in vitro. In this paper, we reported a second germline mutation of *PTCH2*, c.1172\_1173delCT, found in a Japanese patient with NBCCS. This mutation created a PTC at the site of the deletion in the mutant allele, resulting in the truncation of the *PTCH2* protein. However, since the PTC leads to the degradation of mRNA via a mechanism called nonsense-mediated mRNA decay [16], a haploinsufficiency of *PTCH2* is expected to play an important role in this case.

Interestingly, this mutation is also present in the dbSNP database as rs56126236, submitted by the Center for Genome Medicine, Kyoto University Graduate School of Medicine, Japan. However, details such as frequency are unclear. Therefore, we analyzed 63 healthy Japanese individuals (126 alleles) on this mutation, but found none carrying this deletion. Thus, it is unlikely that this is a rare polymorphism at least in a Japanese population.

However, unfortunately, we were unable to get informed consents from family members and, therefore, could not add data regarding this issue. Nonetheless, considering the deleterious nature of the mutation, we believe that the mutation found in this patient is generated de novo.

Homozygous mutant mice, *Ptch2*<sup>-/-</sup>, developed normally, were viable and fertile, and did not display any obvious defects in hair follicle, limb, neural, or testis development [17]. However, with age, homozygous mutant male mice developed skin lesions consisting of alopecia and epidermal hyperplasia, suggesting a role for *Ptch2* in adult epidermal homeostasis via Shh signaling. In accordance with the milder phenotype of *Ptch2*<sup>-/-</sup> than *Ptch1*<sup>-/-</sup>, which is embryonic lethal [18], it is not surprising that individuals carrying a *PTCH2* mutation also exhibit milder clinical manifestations than those with classical NBCCS. Our patient lacked typical NBCCS phenotypes such as palmar/plantar pits, falx calcification, macrocephaly, hypertelorism and coarse face, the frequencies of which are 60.1, 79.6, 26.5, 68.8, and 27.9 %, respectively, in the Japanese population [9]. In fact, this case does not fulfill the criteria by Evans et al. [19] because a rib anomaly is considered to be a minor criterion. Since the number of cases with a *PTCH2* mutation is still limited, the accumulation of such patients is expected to further clarify their characteristic phenotype.

A genotype–phenotype correlation has not been reported in NBCCS patients [20]. However, mutations in the *SUFU*



**Fig. 2** Electropherograms of the *PTCH2* exon 9 sequence. DNA extracted from the peripheral blood of the patient (Case) as well as a healthy control (Control) was subjected to PCR direct sequencing. The predicted translation is indicated at the bottom of each electropherogram. PTC created by the deletion is indicated by an asterisk

gene were reported to result in a much higher incidence of medulloblastoma than those in *PTCH1* [8, 14, 21]. It is also reported that a large genomic deletion encompassing *PTCH1* leads to NBCCS with atypical clinical manifestations, probably due to a deletion of adjacent gene(s) [12]. Therefore, it should be noted that NBCCS cases caused by a mutation of a gene other than *PTCH1* have phenotypes different from those of classical NBCCS caused by *PTCH1* mutations.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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