ORIGINAL ARTICLE

Patched homologue 1 mutations in four Japanese families with basal cell nevus syndrome

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Aim: To search for patched homologue 1 (*PTCH1*) mutations in four families with basal cell nevus syndrome (BCNS).
Methods: Mutation analysis of *PTCH1* in unrelated Japanese families affected with BCNS was carried out by direct sequencing.
Results: Six novel *PTCH1* mutations, 833G→A in exon 6, 1415C→A and 1451G→T in exon 10, 2798delC in exon 17, 2918–2925dupAGTTCCCT in exon 18 and 3956C→A in exon 23, were identified.
Conclusions: Among the six *PTCH1* mutations, two frameshift mutations (2798delC and 2918–2925dupAGTTCCCT) and one nonsense mutation (833G→A) are predicted to lead to premature termination of *PTCH1* protein translation. Three simultaneous mutations, 1415C→A (A472D) and 1451G→T (G484V) in exon 10, and 3956G→A (R1319H) in exon 23, were found on one allele in only affected members in one family and none of them were found among 90 unrelated healthy Japanese. The three mutations on one chromosome may have resulted from errors in the recombinational repair process and this is the first report on the *PTCH1* mutations due to such a mechanism.

Basal cell nevus syndrome (BCNS, OMIM *109400), known as Gorlin syndrome, is inherited in an autosomal dominant mode. This syndrome is characterised by multiple basal cell nevi, odontogenic keratocysts and skeletal anomalies.[1] At the minimum, its prevalence is estimated to be 1 in 57 000.[2]

BCNS is caused by mutations in the patched homologue 1 (*PTCH1*) gene located at chromosome 9q22.3, the human homologue of the *Drosophila* segment polarity gene patched (*Ptch*).[3 4] *PTCH1* encodes a transmembrane receptor protein for the secreted molecule, sonic hedgehog.[5 6] *PTCH1* gene encompasses about 34 kb and consists of at least 23 exons, encodes 1447 amino acid proteins with a 12-transmembrane domain, two extracellular loops and a putative sterol-sensing domain.[7–9] Bailey *et al*[10] reported that missense mutations could abolish *PTCH1* function, possibly by blocking protein maturation.

It has been reported that basal cell carcinoma occurs in >90% of patients with BCNS by the age of 40 years.[11–13] Identification of mutations is very helpful for genetic counselling and clinical service in the BCNS family, because patients with mutations have a high risk for BCNS. At least 101 *PTCH1* mutations have been reported so far in patients with BCNS.[3–6 14–23]

Here we report the results of a search for *PTCH1* mutations in four unrelated families with BCNS.

MATERIALS AND METHODS

Families

Four unrelated Japanese families, clients of Aichi-Gakuin University, were diagnosed as having BCNS according to the criteria of Shanley *et al*.[12] The four families had a total of 13 affected and 14 unaffected members (fig 1).

Family 1

The proband (fig 1A, III-5) was a boy with cleft lip and palate. His father (II-3) had a cleft lip, multiple basal cell nevi, odontogenic keratocysts, multiple skin pits on the palm and sole; a paternal elder sister (II-2) and her two children (III-1 and III-2) had multiple basal cell nevi and odontogenic keratocysts, whereas the mother (II-4) had no malformations. The paternal grandfather (I-1) had died from skin cancer, according to the father, but the precise diagnosis was not known.

Family 2

The probands (fig 1B, III-1 and III-2) were 10-year-old twins, a girl and a boy with cleft lip and palate. Both the twins and their mother (II-2) had typical BCNS, whereas the father (II-2) was free from malformations on physical examination. The maternal grandparents (I-1 and I-2) were phenotypically normal.

Family 3

The proband (fig 1C, III-2) was an 18-year-old girl with BCNS. Her mother (II-2) also had typical malformations for BCNS, whereas the father (II-1) was phenotypically normal. An elder sister (III-1) of the proband and the maternal grandparents (I-1 and I-2) were all healthy.

Family 4

The proband (fig 1D, III-2) was an 8-year-old girl with odontogenic keratocysts. Her mother (I-4) had malformations typical for BCNS, an elder sister (III-1) had odontogenic keratocysts and the maternal grandfather (I-1) had BCNS. The father (II-3) was phenotypically normal.

Mutation searches

Mutation analysis of *PTCH1* (NM_000264) by direct sequencing was carried out in the four families. After written informed consent was obtained from the participants of these families, DNA was extracted from their peripheral blood cells by standard methods or from their fingernails by using ISOHAIR (Nippon Gene, Tokyo, Japan). For fingernail DNA, clipped fingernails were cut into small pieces with scissors and DNA was extracted according to the supplied manual of ISOHAIR. However, as DNA from some fingernail samples

Abbreviations: BCNS, basal cell nevus syndrome; PCR, polymerase chain reaction; PTCH1, patched homologue 1

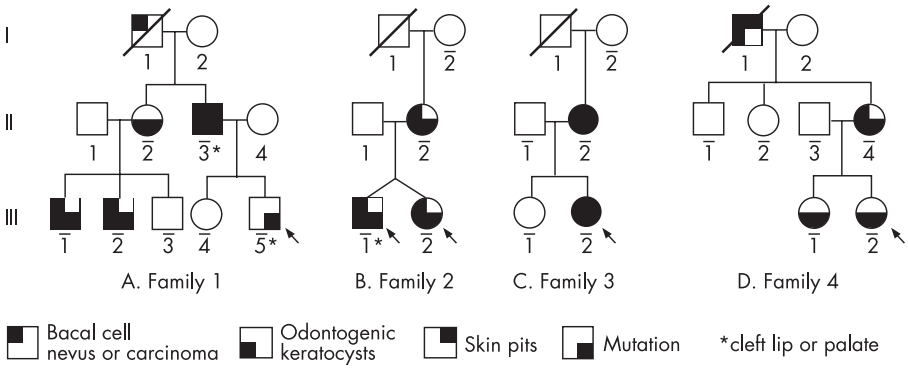

Figure 1 Four families with basal cell nevus syndrome. Arrow indicates proband. People who were analysed are indicated with a horizontal line below their individual symbol.

showed low quality and was a small amount, the method of extracting fingernail DNA was improved. We adopted a frozen-sample crusher SK-100 (Tokken, Kashiwa, Chiba, Japan) to crush the fingernail as finely as possible and extract DNA with ISOHAIR. After the DNA was extracted with ISOHAIR, it was dissolved in extraction buffer (10 mM TRIS–hydrochloric acid, pH 7.5; 100 mM EDTA, pH 8.0; 0.5% sodium dodecyl sulphate), treated with 50 μg/ml proteinase K at 55℃ for 3 h, extracted with phenol or chloroform, and collected with ethanol and sodium acetate. All exons and exon–intron boundaries of *PTCH1* were amplified by polymerase chain reaction (PCR) using our primer pairs designed from its genomic sequence. Amplification of exon 1 was carried out using two sets of pairs because exon 1 is too large as a single fragment for PCR. PCR was carried out in a mixture (10 μl) containing 5 ng genomic DNA, 1 μM each primer, 200 μM deoxynucleotide triphosphates, 0.3 unit TaKaRa ExTaq HS version (Takara, Kyoto, Japan) and 1× PCR buffer supplied by Takara. PCR conditions were as follows: initial incubation at 94℃ for 2 min, followed by 35 cycles of denaturation at 94℃ for 30 s, annealing at 60℃ for 30 s, elongation at 72℃ for 30 s and final elongation at 72℃ for 7 min. PCR products were treated with ExoSAP-IT (Amersham Biosciences, Piscataway, New Jersey, USA) following the instruction manual supplied by the company, and sequenced directly using BigDye Sequencing Kit V.3.1 (Applied Biosystems, Foster City, California, USA). Sequenced samples were purified using SephadexG50 (Amersham Biosciences) and run on an automated sequencer Model 3100 (Applied Biosystems). Sequence electropherograms were aligned and analysed using the Auto Assembler software V.2.1 (Applied Biosystems). We carried out sequencing in both directions and repeated it two times to confirm mutations independently.

RESULTS

All affected members in family 1 had a 1-base deletion at nt 2798 (2798delC) in exon 17. This deletion is predicted to lead to frameshift with 28 amino acids after codon 933, create a premature stop codon at nt 961 (934fsX961) and cause the loss of 487 C-terminal amino acids. Similarly, in the proband of family 2 and his mother, an 8-nucleotide duplication at nt 2925−2926 (2918−2925dupAGTTCCCT) in exon 18 was found. The duplication leads to frameshift with substitution of 15 amino acids after codon 976, introduces a premature stop codon at nt 991 (977fsX991) and causes the loss of 457 C-terminal amino acids. In the proband of family 3 and her mother, a nonsense mutation at nt 833 (833G→A) in exon 6 was found. The mutation leads to premature termination codon (W278X) and loss of the 1170 C-terminal amino acids. None of the mutations were observed among the 90 unrelated healthy Japanese.

In all the affected members (the proband, her elder sister and mother) in family 4, three simultaneous nucleotide substitutions leading to amino acid replacements were found—that is, 1415C→A (A472D) and 1451G→T (G484V) in exon 10 and 3956G→A (R1319H) in exon 23. Neither mutation was observed in the father or in unaffected family members, as well as among the 90 unrelated healthy Japanese.

DISCUSSION

The mutation search showed six novel *PTCH1* mutations in the four families (table 1).

Mutations observed in family 4 merit comment. These mutations could be errors in the recombination repair process rather than three independent single-nucleotide substitutions, because they are all located on the same allele. Two (A472D and G484V) of the three are located around the third transmembrane domains. The other (R1319H) is in the last intercellular C-terminal domain of *PTCH1*; the C-terminal domain was recently identified as an important regulatory region essential for proper signalling of sonic hedgehog or *PTCH1*.[24] On the other hand, according to the Prediction of Transmembrane Regions and Orientation programme (http://www.ch.embnet.org/), it is predicted that A472D changes the length of the transmembrane region of *PTCH1*, but G484V never alters the transmembrane topology dramatically. These findings favour 1415C→A or 3956G→A as the causative mutation for BCNS in the family.

Most (around 90%) reported *PTCH1* mutations in BCNS were nonsense, splice site, insertion, deletion or duplication mutations leading to frameshift, and each of them was predicted to generate a truncated protein, although there were a few missense mutations. These mutations were located in most exons and spread all over the *PTCH1* gene,

Table 1 Six mutations in patched homologue 1 (*PTCH1*) found in four families with basal cell nevus syndrome

Exon	Mutation	Nucleotide change	Amino acid change	Family no
6	Nonsense	833G→A	W278X	3
10	Missense	1415C→A	A472D	4
10	Missense	1451G→T	G484V	4
17	Deletion	2798delC	934fsX961	1
18	Duplication	2918–2925dup(AGTTCCCT)	977fsX991	2
23	Missense	3956G→A	R1319H	4

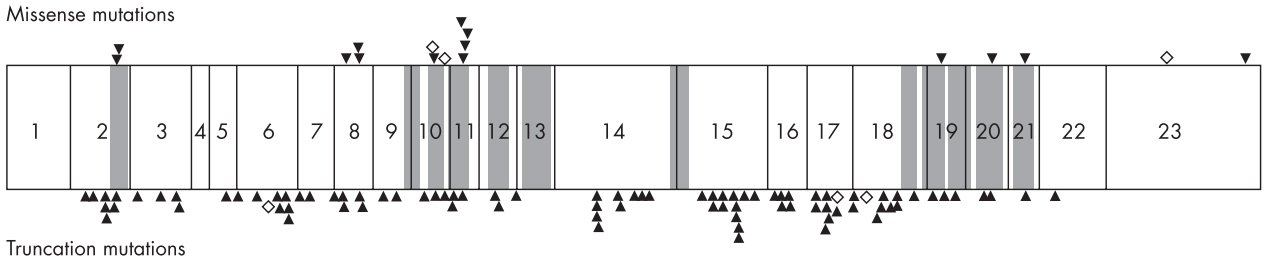

Figure 2 All reported patched homologue 1 (*PTCH1*) mutations causing basal cell nevus syndrome or basal cell carcinoma.[3–6 14–23] Numbers in boxes indicate exons. Missense and nonsense mutations are depicted above and below the gene, respectively. Shaded regions, closed triangles and open diamonds indicate transmembrane domains, previously reported mutations and mutations observed in the present families, respectively.

indicating the absence of a mutational hotspot (fig 2). Three nucleotide changes observed in families 1–3 result in protein truncation corresponding to the previous findings. Although three missense mutations were simultaneously observed on one chromosome in family 4, it remains uncertain which mutation actually affects protein function and is pathogenic. Nevertheless, the identification of mutations is very helpful for genetic counselling and clinical service in the family, because patients with mutations have a high risk for BCNS.

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