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Original Article

Novel mutation of the *PATCHED* gene in a patient with basal cell naevus syndrome and Tetralogy of Fallot

基底細胞痣綜合症合併法洛氏四聯症患者之 *PATCHED* 基因上的新突變

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Basal cell naevus syndrome (BCNS) is an autosomal dominant disease characterised by the presence of basal cell carcinomas, odontogenic keratocysts, palmoplantar pits and calcification in the falx cerebri, and caused by mutational inactivation of the *PATCHED* (*PTCH*) gene. We identified a Chinese family with the proband and her mother having features characteristics of BCNS. Interestingly, the proband also had Tetralogy of Fallot and cerebral atrophy, which had not been described in BCNS. To investigate the molecular basis of BCNS in this family, we have performed a mutational analysis of the *PTCH* gene by denaturing high-performance liquid chromatography. A one-base pair frameshift deletion, 1480delT, leading to truncated patched protein was identified in the proband and her mother. This result is consistent with previous report that mutational inactivation of the *PTCH* gene is the cause of BCNS in Chinese.

基底細胞痣綜合症是一種常染色體顯性疾病，表現包括有基底細胞癌、牙源性囊腫、掌跖點凹及大腦鐮鈣化。其病因是*PTCH*基因突變性失活。我們發現一個華人家庭，其中的先証者及她的母親均有痣樣基底細胞癌的特微。特別的發現是，先証者還併發有法洛氏四聯症及大腦萎縮這兩種未被發現的病症。我們用高效變性液相色譜法來對這個家庭進行突變分析。我們在先証者及她的母親均發現一個鹼基對的架構轉移性缺失(1480delT)，導致截段性片狀蛋白。這項研究與以前的報告吻合，即華人的痣樣基底細胞癌的病因是由於 *PTCH* 基因有突變性失活所引起。

Keywords: Basal cell naevus syndrome, denaturing HPLC, genodermatosis

關鍵詞：基底細胞痣綜合症，高效變性液相色譜法，遺傳性皮膚病

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Introduction

Basal-cell carcinoma (BCC) is the most common tumour in humans,[1] and the incidence continues to increase.[2] Insights into the origin of BCCs come from the identification of mutations of the patched gene *(PTCH)* in patients with basal cell naevus syndrome (BCNS), an autosomal dominant genetic disease with multiple BCCs and developmental abnormalities.[3,4] *PTCH* is the human homologue of the *Drosophila* segment

polarity gene, patched, which encodes a putative 12-transmembrane receptor protein for the secreted molecule, Sonic hedgehog (Shh). An important clue to the understanding of Ptch function comes from study of its interactions with another membrane protein, smoothened (Smo). The free Ptch acts sub-stoichiometrically to suppress Smo activity and is critical in specifying the level of pathway activity. Ptch protein is homologous to bacterial proton-driven transmembrane molecular transporters; the function of Ptch is impaired by alterations of residues that are conserved in and required for function of these bacterial transporters. These results suggest that the Ptch functions normally as a transmembrane molecular transporter, which acts indirectly to inhibit Smo activity, possibly through changes in distribution or concentration of a small molecule.[5] *PTCH* was shown to be a tumour suppressor gene,[3] and *SMO* has been shown to be a proto-oncogene in sporadic BCCs[6] and desmosplastic medulloblastomas.[7] Mutational inactivation of *PTCH* results in the failure of Ptch to inhibit Smo, leading to constitutive activity of the Shh signaling pathway. Thus, *PTCH* acts as a tumour suppressor gene in BCCs, according to the Knudson two-hit model.[8]

The human *PTCH* gene spans approximately 34 kb DNA on chromosome 9q22[3] and contains 23 exons.[9] The cDNA encodes a protein of 1447 amino acids. Extensive allelic heterogeneity has been demonstrated in this gene, and 60 germline mutations have been identified in the *PTCH* gene from analyzing BCNS patients.[10] Most of these mutations were found in individual families.

In our previous investigations of the molecular basis of BCCs,[6,7] we identified three BCNS families. Three mutations, two one-base pair frameshift insertions and one 8-base pair insertion affecting a donor splice site, were identified, and all lead to truncated patched protein. Recently, we identified a fourth Hong Kong Chinese family with the proband and mother having features characteristics of BCNS. To investigate the molecular basis of BCNS in this family, we performed a mutational analysis of the *PTCH* gene by denaturing high-performance liquid chromatography (DHPLC) followed by direct DNA sequencing.

Materials and methods

Subjects

The patient is a 3½-year-old girl. She was born at full term with a birth weight of 3.3 kg. A heart murmur was detected in the early neonatal period and was diagnosed to be Tetralogy of Fallot. Surgery was performed at 2½ years old. She had mild global delay, requiring early educational training. Physical examination showed a body height of 102 cm (75-90th centile), macrocephaly with head circumference of 53.5 cm (>97th centile), hypertelorism, frontal bossing, hirsutism, epicanthic folds, up-turned nose, long philtrum, high-arch palate, numerous pigmented naevi over the body including the palms and soles (Figure 1). There was a small well-circumscribed skin tumour on the right chest wall. Histology showed that the tumour was composed of interconnecting strands of basaloid cells showing peripheral palisading, compatible with trichoepithelioma. CT showed multiple small calcific foci along the dural folds. MRI showed a mild degree of global cerebral atrophy. The proband's mother was 36 years old with no significant past medical history. Physical examination revealed a body height of 165 cm, and head circumference of 56 cm (75-90th percentile), hypertelorism, prominent mandible, multiple pigmented naevi over the neck and shoulders, and multiple skin pits over the palms and soles (Figure 2). Skull X-ray of the mother showed a lucent cystic lesion on the left side of the mandible, calcification over the falx cerebri, bony bridging at sella turcica, bifid spinous process of the second cerebral vertebra. The proband's father has normal appearance. Together, the clinical and radiological features of the proband and her mother are diagnostic of with BCNS.

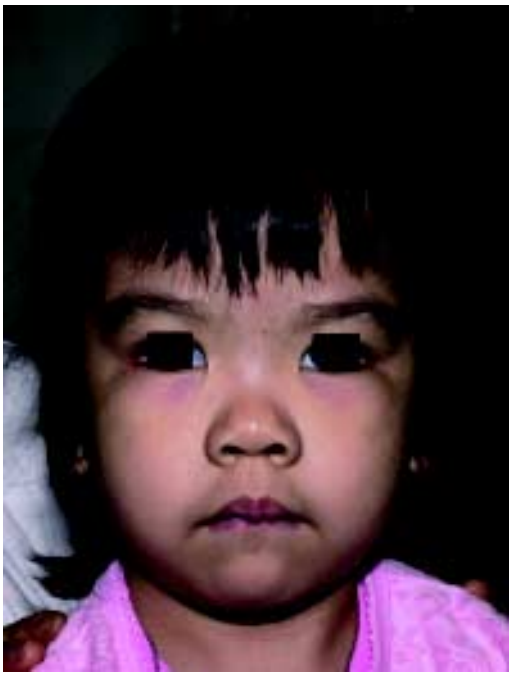

Figure 1. Patient has features of macrocephaly, hypertelorism, epicanthic folds, upturned nose and long philtrum.

DNA extraction and amplification

Genomic DNA of the proband and the mother were extracted from whole blood samples by a QIAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. The study was performed in accordance with the principles of the Declaration of Helsinki. Informed consents were obtained from all subjects. The exons and flanking regions of the *PTCH* gene were PCR amplified as described.[9]

Denaturing high-performance liquid chromatography

Heteroduplex analysis was performed on a WAVE DHPLC instrument (Transgenomic Inc., San Jose, California, USA). The stationary phase consists of 2-μm non-porous alkylated poly (styrene-divinylbenzene) particles packed into a 50 × 4.6-mm ID column (DNASep column, Transgenomic Inc., San Jose, California, USA). Ten microlitres of crude PCR product was loaded onto the column

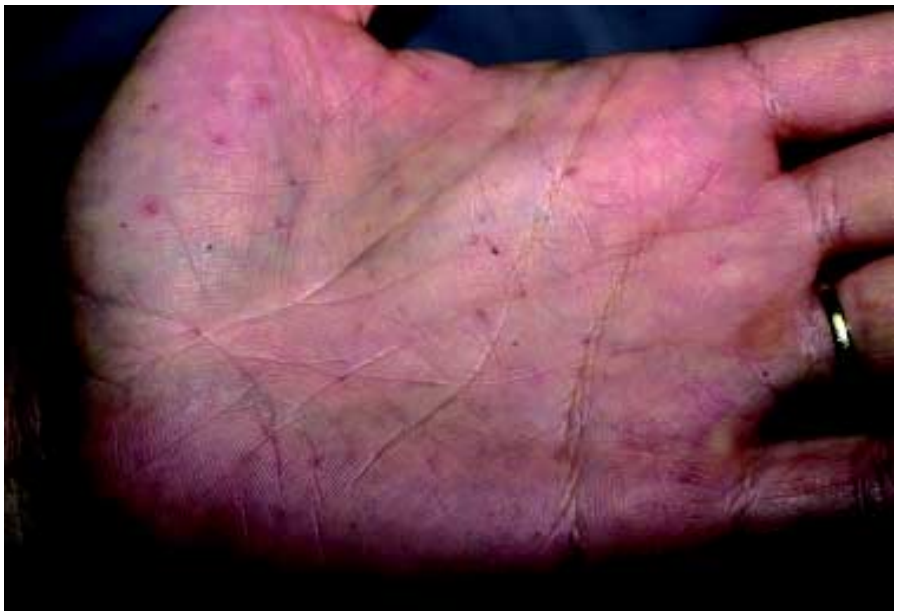

Figure 2. Prominent palmer pits on the left hand of proband's mother.

and was eluted from the column by an acetonitrile gradient in 0.1 mol/L triethylammonium acetate buffer (TEAA), pH 7.0, at a constant flow rate of 0.9 mL/min. The standard buffers are prepared from TEAA buffer concentrate to give A=0.1 mol/L TEAA, B=0.1 mol/L TEAA and 25% acetonitrile. The gradient was created by mixing eluents A and B. The recommended gradient for mutation detection is a slope of 2% increase in buffer B per minute. Eluted DNA fragments were detected with ultraviolet absorption at wavelength 260 nm. The WAVE utility software helps determine the correct temperature for mutation scanning based on the sequence of the wild-type DNA.

Sequence analysis

PCR products were purified by Microspin S300-HR columns (Amersham Pharmacia, Uppsala, Sweden), and both strands were sequenced using the amplification primers as sequencing primers and BigDyeDeoxy terminator cycle sequencing reagents, according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Products of sequencing reactions were purified by Centri-Sep spin columns (Princeton Separations, Adelphia, NJ). Purified sequencing fragments were separated by capillary electrophoresis and detected via laser-induced fluorescence on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). Sequencing results were compared to the established human *PTCH* sequence (GenBank accession number U59464), and the mutation was designated according to the recommendations by Dunnen and Antonarakis.[11]

Results

The DHPLC chromatogram showed a heteroduplex in exon 10 and the flanking introns of the proband (data not shown). Direct sequencing of the PCR product showed a double heterozygous pattern (Figure 3). Comparison of the double heterozygous pattern with the wild-type sequence indicates a deletion of the first base of codon 494 (TCC) of the *PTCH* gene, i.e., 1480delT. This mutation alters the reading frame of the encoded protein such that a stop codon, TAA, is generated at codon 541, i.e., Leu541Stop. The mutation, 1480delT, causes shifting of the open reading frames and leads to premature termination of *PTCH* protein translation. This mutation was also identified in the mother.

Discussion

We have established that mutational inactivation of the *PTCH* gene causes BCNS in Chinese.[12] Two insertion mutations affecting the coding sequence and one deletion mutation affecting a splicing site have been previously identified. All of these mutations cause shifting of the open reading frames and lead to premature termination of *PTCH*

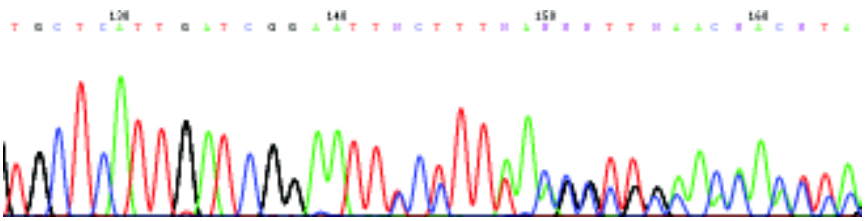

Figure 3. DNA sequence showing the novel *PTCH* mutation, 1480delT. The sequence is shown in the sense direction.

protein translation. This is consistent with an earlier study showing that most germline mutations in BCNS lead to truncated proteins.[13] The mutation in the proband of the first family we studied is a one-nucleotide insertion, 2392insA at codon 797. This mutation alters the reading frame of the encoded protein such that a stop codon, TAA, is generated at codon 797, i.e., Tyr797Stop. The mutation in the proband of the second family is a one-nucleotide insertion at codon 490, i.e., 1468insA. This mutation alters the reading frame of the encoded protein such that a stop codon, TAA, is generated at codon 496, i.e., Asn496Stop. The mutation in the proband of the third family is a deletion, in the 5' splicing site of intron 10, causing a deletion of 8 bases from the first base of intron 10, i.e., IVS10+delGTAAGTGT. The wild-type sequence of the 5' splicing site of intron 10 is 5'-gtaagtgtgtgatcatgctttctg-3'. The mutation retains a GT dinucleotide, i.e., 5'-gtgatcatgctttctg -3', but the sequence of the splicing site will change from IVS10+3 position onwards. Using SpliceView (http://www.itba.mi.cnr.it/webgene/), the mutant splice site was not identified to be a donor splice site while the wild-type splice site has a consensus score of 83. This mutation is predicted to cause aberrant splicing with partial intron 10 retention and premature termination.

Although we have studied only four BCNS families, each of the probands has a different mutation, demonstrating the genetic heterogeneity of BCNS in Chinese, and perhaps the strong selective pressure against these alleles. Interestingly, in this study, the proband also had Tetralogy of Fallot and cerebral atrophy, which had not been reported in BCNS. Chromosome 22q11 microdeletion associated with Tetralogy of Fallot was examined by fluorescence in-situ hybridization and was not detected in this patient. Thus, although these findings may be just coincidental, it is more likely that the spectrum of developmental defects associated with BCNS is wider than previously reported. Lack of common mutations for this condition makes mutation identification more difficult; *PTCH* has 23 exons and mutations have been observed in most of these. Even in this case, however, denaturing high-performance liquid chromatography, as shown here, proves to be both efficient and economical for detection of disease causing mutations.

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