

Case Report

A novel INDEL mutation in the *PTCH1* gene in a Chinese family with Gorlin syndrome

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Abstract: Gorlin syndrome is a rare autosomal dominant disorder, and 50% of the cases are due to the mutation of *PTCH1*, the major receptor of the hedgehog signaling pathway. Here we report a new Gorlin syndrome family found in Xinzhou, China. A further sequence analysis found a novel *PTCH1* INDEL mutation, NM_001083602.2: c.1516_1524delinsTGAGCTGGAGCTCCG (p. Ala506*), leading an N Terminal truncated protein. This truncated *PTCH1* was considered as non-functional version as it loses almost all functional domains, including the 4-12 transmembrane domains and the intracellular and extracellular domains accordingly. Although the effect of the N-terminal truncated *PTCH1* is not clear, Gorlin syndrome in these cases is due to haploinsufficiency. Our report enriches the Gorlin syndrome database and will help to unveil the molecular basis of this condition.

Keywords: Gorlin syndrome, *PTCH1*, novel mutation, INDEL mutation

Introduction

Gorlin syndrome, also called “Basal cell nevus syndrome, BCNS MIM #109400” or “Nevoid basal cell carcinoma, nevoid BCC”, is a rare autosomal dominant disorder with almost 100% penetrance, affecting an estimated 1 in 31,000 people [1, 2]. It is mainly characterized by a wide range of developmental abnormalities and a predisposition to various tumors, including basal cell carcinoma (BCC), medulloblastoma, ovaroma, cardiac fibroma, and keratocystic odontogenic tumor [3]. Besides the high risk of these tumors, some developmental abnormalities are also seen in Gorlin syndrome patients, such as a small depression in the skin of the palms of the hand and the soles of the feet, an unusual large head size with a prominent forehead, as well as skeletal abnormalities involving the skull, spine, and ribs. Mutations in *PTCH1* (*patched homolog 1*, located on chromosome 9q22) account for 50% of Gorlin syndrome cases [4]. Since it was first reported in 1996, more than 900 mutation variants of *PTCH1* have been reported according to 1000 genome (<http://browser.1000genomes.org/>) records, and of these, 635 mutations were pathogenic, most of which were missense, nonsense, and frameshift caused by

either small SNP or deletions. Only 10 INDEL mutations of *PTCH1* are recorded in 1000 genome. The mutations of the other two genes, *PTCH2* (*patched homolog 2*, located on chromosome 1p34) and *SUFU* (*suppressor of fused*, located on chromosome 10q24) were also reported to have a strong association with some Gorlin syndrome cases [5, 6]. In addition, a small deletion in Chromosome 9, the 9q22.3 microdeletion, not only affects *PTCH1* but is also reported to present features of Gorlin syndrome [7]. The *PTCH1*, *PTCH2*, and *SUFU* genes are all suppressors of the hedgehog (Hh) signaling pathway, controlling cell differentiation, proliferation and migration etc. [8]. Here, we report a novel *PTCH1* gene INDEL mutation, NM_001083602.2: c.1516_1524delinsTGAGCTGGAGCTCCG (p. Ala506*), detected in 3 patients from one family with Gorlin syndrome. Our report enriches the Gorlin syndrome database and sheds new light on the molecular basis of this condition.

Subjects and methods

Ethical considerations

All procedures were reviewed and approved by the Ethical Review Board of the Second Hospital

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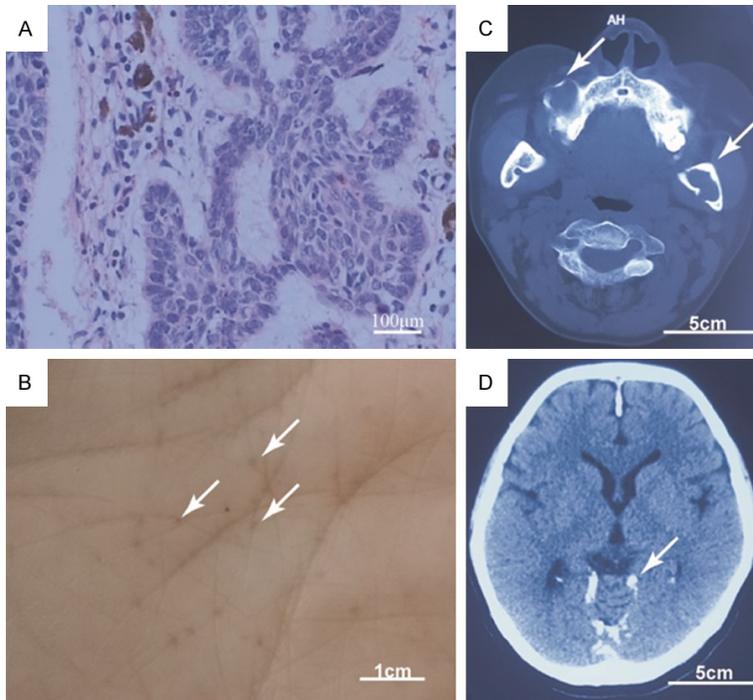


Figure 1. Clinical data of the Gorlin syndrome proband. A. Basal cell carcinoma of the tumor tissue gained from proband's eyelid was confirmed by hematoxylin-eosin staining. B. Multiple palmar pits were found in her left palm as indicated by the arrows. C. Keratocystic odontogenic tumors were found at the maxilla by a CT scan as indicated by the arrows. D. Intracranial calcification spots were detected by a CT scan as indicated by the arrows.

of Shanxi Medical University. Informed consents were obtained from the patients or their family members for all procedures in this study.

Patient evaluation

Gorlin syndrome in the proband was diagnosed according to the criteria described in the literature [3], and confirmed by the *PTCH1* mutation sequence analysis. The patients were examined by an experienced dermatologist, and then they were evaluated radiologically for skeletal deformities and underwent a head CT scan. The basal cell carcinoma from the proband's eyelid was sectioned and stained with H&E for histological examination. A detailed family history was obtained.

Mutation analysis

Peripheral blood samples were collected from the proband and her family members with clinical consent. Total genomic DNA was extracted using a whole blood DNA extraction kit (Tiangen, Beijing, China). The DNA sample of the proband

was sent for whole exome sequencing to Veritas Genetics (Hangzhou, China). The variants were functionally annotated and filtered using our in-house rare disease NGS analysis platform as previously described [9]. Exonic sequence alterations and intronic variants at exon-intron boundaries, with unknown frequencies or minor allele frequencies (MAF) < 1% and not present in the homozygous state in those databases were retained. The mutation of *PTCH1* (OMIM:603673) found by whole exome sequencing was validated by PCR and then Sanger sequencing. The primers used for the mutational exon of *PTCH1* were listed below. Fwd: GTT ATT CTG CCA CGT ATC TGC TC; Rev: TTA AAC AGA GCC TCA AAC ACA GG, Sequencing primer: TCC AGT GCA GCT CTC AGC GCT. The sequence used as reference in this work was NM_0010-

83602.2. The affected transcript and protein denoted using the Human Genome Variation Society (HGVS) nomenclature version 2.0 (Mutalyzer 2.0, <https://mutalyzer.nl/>).

Results

Clinical pathologic characteristics of the proband

A 38-year-old Chinese woman (II-2), the proband, was hospitalized for a left eyelid tumor removal operation. The tumor biopsies were examined by H&E staining, and then diagnosed as basal cell carcinoma (BCC) (**Figure 1A**). Although no severe developmental deformation was seen, multiple palmar pits (**Figure 1B**) and mild palmar fovea, typical characteristics of Gorlin syndrome, were noted. Radiographic examination showed multiple intracranial calcification spots and several keratocystic odontogenic tumors at the maxilla (**Figure 1C** and **1D**). The proband was subjected to the excision of a cyst of the maxilla when she was 26 years old. In addition, her chest X-ray results showed bifid

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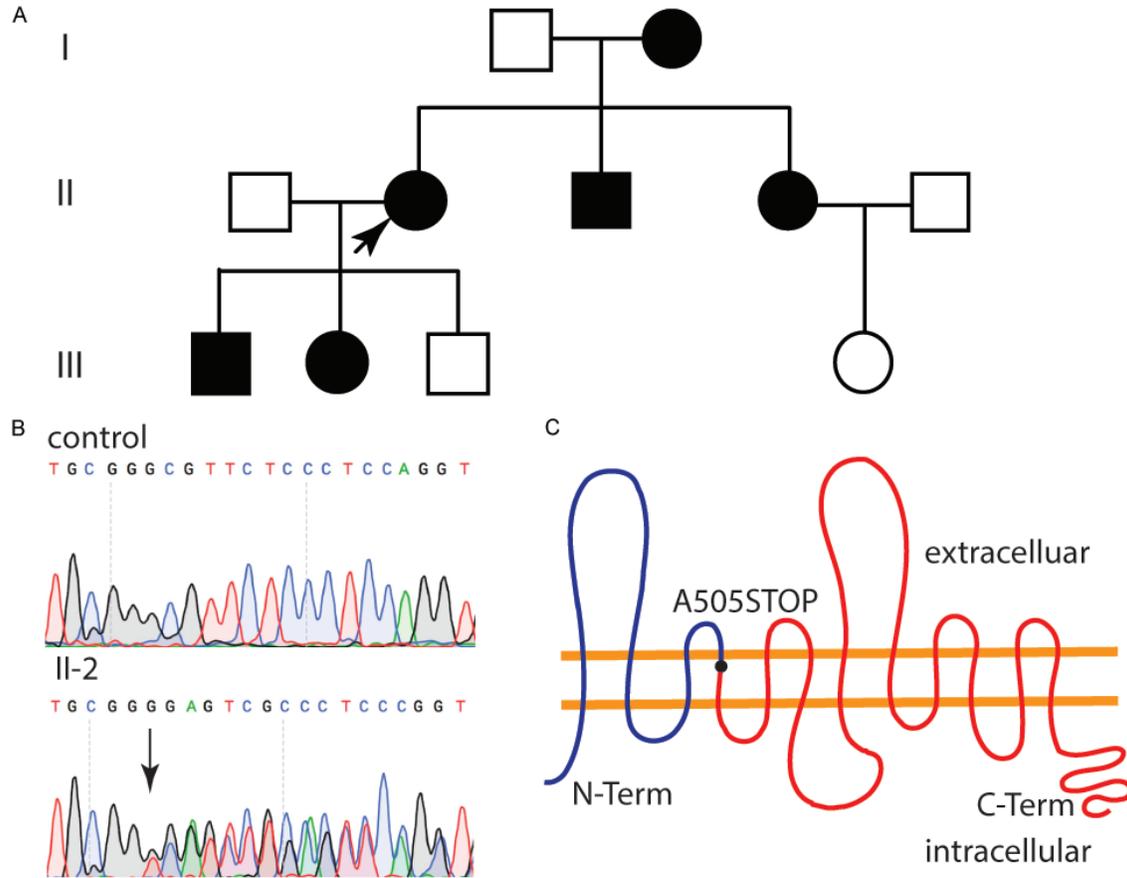


Figure 2. Family pedigree and sequence information of the BCNS family. A. Pedigree of the family in this study. B. Sanger sequencing validation of the proband's *PTCH1*; the arrow indicates the mutation start site. C. Schematic diagram of the *PTCH1* protein with the mutation found in this study; the blue part indicates the remaining N terminal region, and the red part indicates the missing region.

ribs at the upright side. Based on these clinical and radiological findings, the patient was believed to have Gorlin syndrome.

Pedigree characteristics of the proband

A follow up pedigree analysis was carried out with the family members. In total, 5 members with similar symptoms were found, including the proband's mother (I-2), brother (II-3), sister (II-4), and one son (III-1) and daughter (III-2) (**Figure 2A**). However, the symptoms and severity slightly differed among the individuals (**Table 1**). The proband's mother, 62-years-old, showed the most severe symptoms among all the members in our study. Besides the symptoms listed above, she also showed spinal deformities and squinted. In addition, she was subjected to a basal cell carcinoma removal operation on the upper right arm when she was 54 years old. Compared with the proband, her brother,

and two sons all showed multiple palmar pits, palmar fovea, multiple intracranial calcifications and several keratocystic odontogenic tumors at the maxilla, but less severe (**Table 1**). The proband's brother and sister were both subjected to the excision of a cyst of the maxilla. No basal cell carcinoma was found in these four members. According to these clinical and radiological findings, it is suggested that these members have Gorlin syndrome and this family could be a BCNS family.

*Identification of *PTCH1* mutation of this family*

To determine the caustic gene mutation in this family, a whole-exome sequencing on genomic DNA was performed. A total of 124,667 genetic variants, including 15,036 non-synonymous changes, occurred at the coding sequence or the canonical dinucleotide of the splice site junctions. One heterozygous Indel mutation in

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Table 1. The clinical and sequences data of all the members examined

Family members	Sex	Age	Multiple palmar pits	Palmar fovea	Intracranial calcification	Keratocystic odontogenic tumors	Ribs deformation	Basal cell carcinoma	PTCH1 sequencing validation
I-2	F	38	+	+	+	+	-	+	+
II-2	F	62	+	+	+	+	+	+	+
II-3	M	35	-	+	+	+	+	-	N.A.
II-4	F	30	+	+	+	+	-	-	+
III-1	M	19	-	+	+	+	+	-	+
III-2	F	9	+	+	N.A.	N.A.	N.A.	-	+

(+) indicates “positive” for examination items, while (-) indicates “not found”, N.A. indicates not available, which means the examination was not performed.

exon 12 of *PTCH1* gene has been identified (c.1516_1524delinsTGAGCTGGAGCTCCG) and further validated by Sanger sequencing (**Figure 2B**).

This variant has not been described in any other databases, including dbSNP, OMIM, ESP, Clinvar, 1,000 Genomes, Human Gene Mutation Database, gnomAD and ExAc. Although the mutation did not lead to a reading frame shift, a stop codon TGA was inserted at c.1516, causing an N-truncated *PTCH1* (p. Ala506*). This resulted in the loss of most of the coding region, including 4-12 transmembrane domains, extracellular and intracellular loops accordingly as well as the C-terminal domain (**Figure 2C**), suggesting this truncated form was non-functional protein. Thus, the mutation was believed to be the disease-causing alteration in the proband. No other mutations were observed in the other Gorlin syndrome-related genes, *PTCH2*, and *SUFU*. To further confirm the mutation in this Gorlin syndrome family, we performed a Sanger sequencing validation for I-2, II-3, III-1, III-2 and III-4. Consistently, this variant co-segregates with all affected family members and was not found in healthy family members (**Table 1**).

Discussion

Gorlin syndrome is an autosomal dominant disorder most likely due to a mutation of the *PTCH1* gene [4]. Here we reported a new case of Gorlin syndrome caused by a novel *PTCH1* INDEL mutation in a Chinese family. In general, most patients with Gorlin syndrome develop basal cell carcinoma (BCC), which is one of the crucial standards for diagnosis [10]. In this family, only the proband and her mother developed

BCC, yet no BCC was found on the other individuals, probably due to their ages. There is no doubt that as a person with Gorlin syndrome ages, he or she is likely to have a higher incidence of tumors. Thus, the younger brother, the sister of the proband, and her two affected children have a high likely incidence of BCC development. In addition, because *PTCH1* is not the only Hh pathway receptor, the presence of *PTCH2* could partially compensate for the inhibition function of *PTCH1* in the Hh pathway [11]. Thus, it is reasonable that some Gorlin syndrome patients may not present with BCC.

The human *PTCH1* gene contains 23 coding exons and encodes a 12-transmembrane protein composed of 1381 amino acids according to NM_001083602.2 [12]. It has been shown that *PTCH1* functions as a negative regulator of the hedgehog (Hh) signaling pathway, playing critical roles in both embryogenesis and adulthood metabolism homeostasis [8]. The aberrant activation of the Hh pathway by the haploinsufficiency of *PTCH1* is believed to cause Gorlin syndrome [13]. Since it was first reported in 1996, more than 900 mutation variants have been cataloged according to 1000 genome (<http://browser.1000genomes.org/>) records, within which 635 mutations were found to be pathogenic, including 277 deletions and 336 SNPs mutations. Based on 1000 genome, only 10 INDEL mutations were recorded, suggesting the INDEL is relatively rare. Most of these pathogenic mutations are either frameshift or stop gain mutations, leading to a truncated protein. In this study, we reported the novel INDEL mutation c.1516-1524delinsTGAGCTGGAGCTCCG in a Chinese family. Although the novel INDEL mutation did not result in a frameshift of *PTCH1*, it created a prema-

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ture termination codon in the mutant allele and led to a truncation of *PTCH1*, which lacked the domains starting from transmembrane 4. Functional studies suggested that the first, the third extracellular loops, and the intracellular loop are very important for *PTCH1* function [14]. Thus, the N terminal truncated *PTCH1* was considered as the loss-of-function version. Although it is not clear whether the N truncated *PTCH1* may cause any gain-of-function roles *in vivo*, the haploinsufficiency of *PTCH1* is supposed to be etiological in our cases.

In conclusion, a new Gorlin syndrome family with novel INDEL *PTCH1* mutation was identified in our study. These findings enriched the Gorlin syndrome database and indicate the genetic testing for *PTCH1* gene status could help in the early diagnosis of Gorlin syndrome.

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Disclosure of conflict of interest

None.

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