Novel *PTCH1* Mutation Causes Gorlin-Goltz Syndrome

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Objective: *To analyse the aetiology and pathogenesis of Gorlin-Goltz syndrome (GS; also known as nevoid basal cell carcinoma syndrome [NBCCS] or basal cell nevus syndrome [BCNS]) in a Chinese family.*
Methods: *Whole-exome sequencing (WES) was performed on genomic DNA samples from the subjects in a family, followed by the investigation of pathogenesis via bioinformatic approaches and conformational analysis.*
Results: *A novel heterozygous non-frameshift deletion patched 1 (PTCH1) [NM_000264: c.3512_3526del (p.1171_1176del)] was identified by WES and further validated by Sanger sequencing. Bioinformatic and conformational analysis showed that the mutation caused altered PTCH1 protein structure, which may be related to functional abnormalities.*
Conclusion: *This study expands the mutation spectrum of PTCH1 in GS and facilitates the early diagnosis and screening of GS. PTCH1 [c.3512_3526del (p.1171_1176del)] may cause structural abnormalities and functional disabilities, leading to GS in families.*
Keywords: *Gorlin-Goltz syndrome, mutation, nevoid basal cell carcinoma syndrome, PTCH1, whole-exome sequencing*
Chin J Dent Res 2024;27(1):83–88; doi: 10.3290/j.cjdr.b5128601

Gorlin-Goltz syndrome (GS, OMIM 109400), also known as nevoid basal cell carcinoma syndrome (NBCCS), is an ecto-mesodermic polydysplasia that affects multiple organs.[1] It is characterised by multiple basal cell carcinomas (BCCs), multiple odontogenic keratocysts (OKCs), calcification of the falx cerebri, vertebral and rib anomalies, and palmar and/or plantar pits.[2] Other findings, such as other skeletal abnormalities and cleft lip with or without cleft palate, may be noted.[3]

GS has been reported to be related with mutations in patched 1 (*PTCH1*), suppressor of fused (*SUFU*) and *PTCH2*.[4] Among them, *PTCH1*, which is located on chromosome 9q22.3, is the major pathogenic gene involved in GS. It consists of 24 exons encoding PTCH1 protein with 1447 amino acids. PTCH1 is a 12-pass transmembrane protein that negatively regulates the Hedgehog (HH) signalling pathway.[5] In the unliganded state, PTCH1 maintains Smoothened (SMO) in an unphosphorylated state, contributing to its endocytosis and degradation. Upon binding of HH ligands, the repression of PTCH1 on SMO is relieved, leaving SMO hyperphosphorylated, capable of activating glioma-associated oncogene homologue 1 transcription factors from SUFU (encoded by *SUFU*) inhibition to translocate into the nucleus and stimulate the targeted gene expression.[6,7] The HH signalling pathway is fundamental to proliferation and differentiation during embryonic patterning and development and homeostasis. Dysregulation of this pathway leads to a wide variety of developmental deficiencies, including holoprosencephaly, brachydactyly, non-syndromic colobomatous microphthalmia and solitary median maxillary central incisor syndrome.[8-12] It has also been

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This work was funded by the National Natural Science Foundation of China (Grant no. U22A20313 and 81970904).

involved in tumours, including BCCs, medulloblastoma, rhabdomyosarcoma, glioblastoma and breast, ovarian, prostate, colon, stomach, pancreas and lung cancers, making it a potential target for therapy.[13,14]

To date, over 600 *PTCH1* mutations have been identified in patients with GS, most of which are nonsense, small indels and missense, according to the Human Gene Mutation Database (https://www.hgmd.cf.ac.uk/ac/index.php).

In the present study, a novel heterozygous nonframeshift deletion *PTCH1* [NM_000264: c.3512_3526del (p.1171_1176del)] was analysed in a 13-year-old proband and his mother with GS. The clinicopathologic characteristics of the patients and the pathogenic mechanism of the mutant were further explored.

Materials and methods

Pedigree analysis and clinical diagnosis

This study included a 13-year-old Chinese boy who presented to the Department of Oral and Maxillofacial-Head and Neck Oncology, School and Hospital of Stomatology at Wuhan University. The diagnosis of GS was based on the most frequently used criteria proposed by Kimonis et al.[15] The proband's 38-year-old mother had a history of surgery for OKCs and was also enrolled in the study. The study was approved by the ethics committee of the School and Hospital of Stomatology, Wuhan University (2017-09). Peripheral blood samples and clinical data were collected after obtaining informed consent.

Genomic DNA extraction and whole-exome sequencing (WES)

Genomic DNA was extracted from the peripheral venous blood of the subjects using the improved salting-out method. DNA samples that passed the quality detection analyses were analysed using WES at Genesky Biotechnologies in Shanghai, China. The exons were targeted from the genomic DNA with the SureSelectXT Human All Exon Kit (Agilent, Santa Clara, CA, USA), and then the Illumina HiSeq X-TEN platform (Illumina, San Diego, CA, USA) was used for sequencing. The readings were aligned with the hg38 human genome assembly using a Burrows-Wheeler aligner. Polymerase chain reaction (PCR) duplicates were removed, and the quality of alignments was evaluated in terms of mean coverage depth, effective base, effective reads and 90×-120× coverage ratio using Picard software. The Genome Analysis Toolkit was used to analyse indels and single-nucleotide variants.[16] ANNOVAR was employed for functional annotation with the KEGG pathway, OMIM, Gene Ontology, Mutation Taster, PolyPhen-2, SIFT and the Exome Aggregation Consortium browsers. The detailed and comprehensive variant analysis was performed in accordance with the workflow previously described.[17-20] Candidate variants were amplified by PCR and confirmed by Sanger sequencing. The PCR primers were designed as follows: forward: 5′-TGAATGTGAACTGCGGTTGG-3′ and reverse: 5′-CTCAAAGCTCAAAGCACGGT-3′. PCR was performed at 95°C for 3 minutes (one cycle), 33 cycles at 95°C for 15 seconds, 55°C for 15 seconds and 72°C for 45 seconds, followed by a final extension at 72°C for 5 minutes. DNA from the proband's father and healthy individuals were used as controls. PCR products were sequenced by the forward and reverse primers at Tsingke Biotechnology (Wuhan, China).

Conservation and pathogenicity analysis

The Ptch1 sequences from HUMAN to ZEBRAFISH were downloaded from ENSEMBL. Multiple-species alignment analysis was performed using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Pathogenicity prediction of variants was conducted using the prediction tools mentioned above, in accordance with the 2015 American College of Medical Genetics and Genomics (ACMG) guidelines.

Structural bioinformatic analysis of PTCH1 p.1171_1176del

A diagram schematically displaying the distribution of mutations was generated using Domain Graph (version 2.0). 3D analysis of structural changes in the PTCH1 mutant was performed using SWISS-MODEL (https://swissmodel.expasy.org/) and viewed on the basis of PyMOL 2.1.0.

Results

Clinical findings and mutation screening

The proband was born of a nonconsanguineous marriage (Fig 1a). There was no significant medical history of abnormal birth weight or premature birth. His diagnosis of GS was established based on OKCs of the jaw proven by histology (Figs 1b and c), palmar and plantar pits (Figs 1d and e), a first-degree relative with GS syndrome (three major criteria), microform cleft lip (Fig 1f) and left hand preaxial polydactyl (post-operation, Fig 1g,

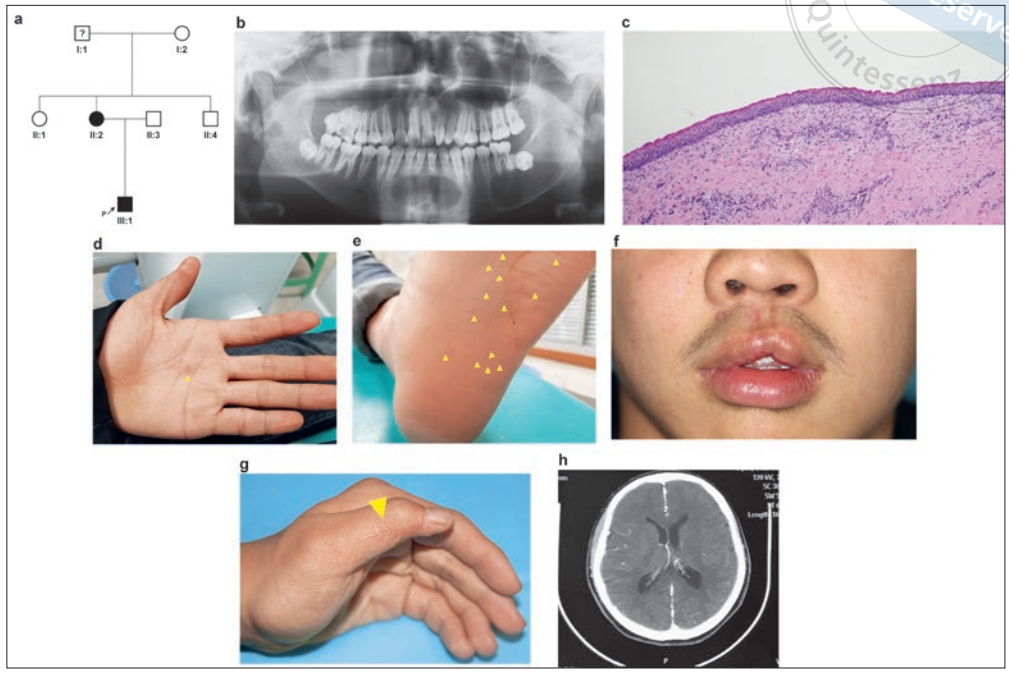

Fig 1 Clinical analyses of patients in the family. Pedigree analysis. Squares, male; circles, female; filled symbols, affected subjects; question mark, unconfirmed diagnosis; symbols with slant lines, the deceased; arrow, proband **(a)**. Panoramic radiograph of the proband **(b)**. Histopathological examination of the lesion revealing typical characteristics of OKCs in the proband **(c)**. Palmar and plantar pits in the proband **(d and e)**. Microform cleft lip in the proband **(f)**. Post-preaxial polydactyl resection of the left hand in the proband **(g)**. Radiograph of the skull showing cerebral falx calcification in the proband's mother **(h)**.

two minor criteria). The proband underwent polydactyl resection at the age of 2 years. The proband's mother had no family history of consanguinity and was diagnosed with GS based on OKCs of the jaw proven by histology, bilamellar calcification of the falx cerebri (Fig 1h) and a first-degree relative with GS syndrome. She underwent surgical excision 6 years previously, and histological examination confirmed OKCs in her right maxilla.

WES identified a heterozygous non-frameshift deletion *PTCH1* [c.3512_3526del (p.1171_1176del)], which was further confirmed by Sanger sequencing (Fig 2a). This mutation was not reported in relevant databases, including1000g, ExAC 03, esp6500, gnomAD_genome, Hrcr1, Kaviar, dbSNP or HUABIAO project (https://www.biosino.org/wepd/). No pathogenic variants were found in other genes related to GS. *PTCH1* (c.3512_3526del) resulted in the deletion of five amino acids (Pro, Val, Leu, Leu and Ser) adjacent to the C-terminal transmembrane region of PTCH1.

Conservation analysis and mutation pathogenecity

Conservative analysis using Clustal Omega showed that amino acids LPVLLS located at the mutant sites were highly conservative among many species (Fig 2b), indicating their important functions in phylogeny. In accordance with the guidelines of the 2015 ACMG, *PTCH1* [c.3512_3526del (p.1171_1176del)] was predicted to be likely pathogenic (PM2 + PM4 + PP1 + PP3 + PP4).

Characteristics of PTCH1 mutant

Structural analysis was performed to investigate the effect of PTCH1 p.1171_1176del on protein function. In comparison with wild-type PTCH1, the mutation p.1171_1176del was located adjacent to a transmembrane helix region, which starts at position 1149 and ends at position 1171, and caused a distinct conformation change. The conformation of the helix at residue 1167-1178 was converted into a loop in mutant p.1171_1176del (Fig 3), which may lead to protein malfunction.

Discussion

GS is a rare autosomal-dominant condition, affecting 1/31,000 to 1/256,000 people in different regions, with no significant difference in morbidity between male and female patients.[2,21,22] It is an ecto-mesodermal dysplasia characterised by a range of clinical manifestations that affect multiple organs. Researchers have reported several diagnostic criteria, and those proposed by Kimonis et al[15] are the most commonly used. According to these, the diagnosis is made on the basis of either two major criteria or one major criterion together with two minor criteria listed below. The major criteria are BCCs, OKCs, palmar or plantar pit, bilamellar calcified falx cerebri, rib abnormalities and a first-degree relative with GS. The minor criteria are macrocephaly, congenital malformation, other skeletal abnormalities,

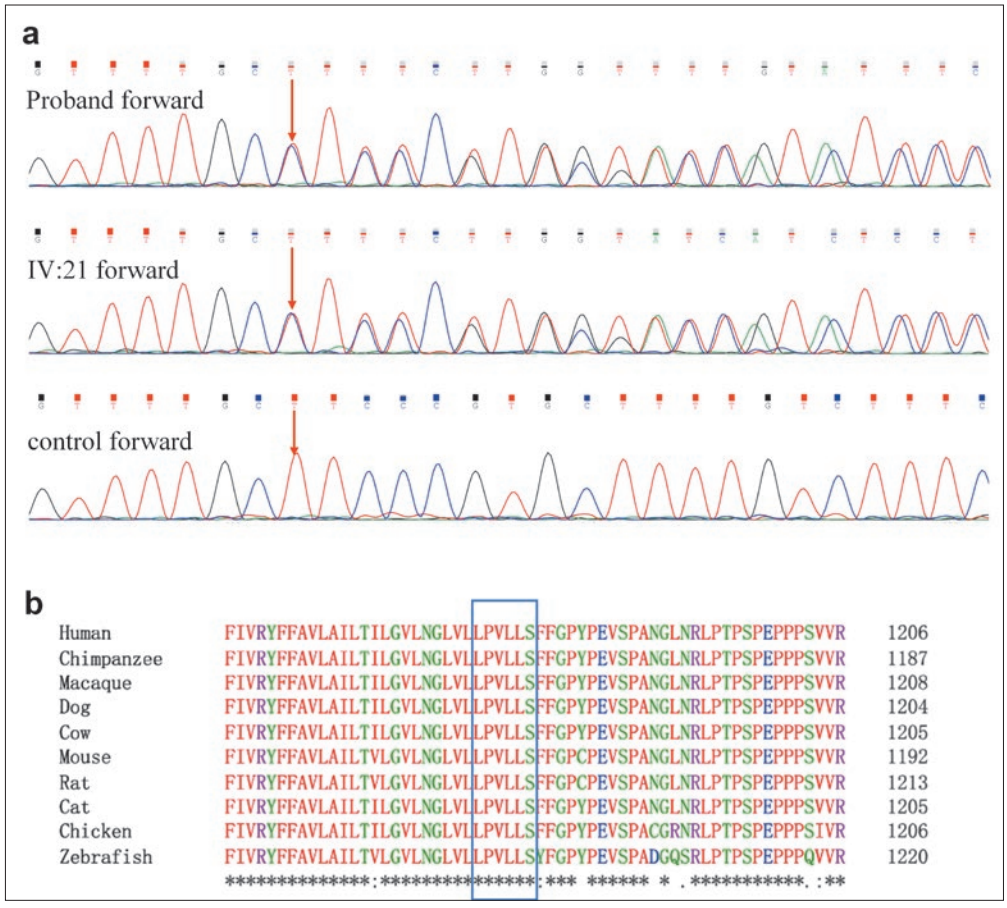

Fig 2 Mutation and conservation analysis. Sanger sequence chromatograms revealing a novel heterozygous non-frameshift deletion *PTCH1* [c.3512_3526del (p.1171_1176del)] in the proband and the mother (IV:21) **(a)**. Conservation analysis indicating p.1171_1176 is well conserved across orthologues **(b)**. Numbers at the end of each line denote the position of the rightmost residue.

radiological abnormalities, ovarian fibroma and medulloblastomas.[2]

In this study, the proband met three major criteria (histologically verified OKCs of the jaw, palmar and plantar pit, and a first-degree relative with GS), and two minor criteria (microform cleft lip and left hand preaxial polydactyl). The proband's mother met three major criteria (histologically verified OKCs of the jaw, bilamellar calcification of the falx cerebri and a first-degree relative with GS). The proband's mother reported another family member with GS.

The diagnosis of GS was further supported genetically by molecular analysis revealing a non-frameshift deletion *PTCH1* (c.3512_3526del) in the proband and his mother. The deletion corresponded to codons 1171-1176 and resulted in p.1171_1176del. PTCH1 p.1171_1176del probably contributes to impaired structure and function. As a ligand binding component in HH signalling pathway, structural abnormalities and dysfunctions in PTCH1 may cause dysregulation of this pathway, leading to the GS in this family. Thus far, over 600 *PTCH1* mutations have been reported in GS. The *PTCH1* mutations identified in GS are mostly nonsense, small indels and missense, followed by splice site mutations and large indels, which are distributed evenly along exons 2-21, with no obvious hotspots.[23] No obvious genotype-phenotype associations have been observed in patients with GS6. Mice heterozygous for *Ptc1* mutant showed developmental abnormalities, including hindlimb defects and medulloblastomas, which recapitulated disease phenotypes seen in patients with GS.[24] In addition to the major causative gene *PTCH1*, *SUFU* and *PTCH2* mutations have been reported in GS. *SUFU* is a negative regulator of the HH pathway, and loss-of-function mutations in *SUFU* were discovered in GS resulting from aberrant regulation of the HH signalling cascade.[25] *PTCH2* consists of 22 exons encoding transmembrane protein PTCH2 with 1203 amino acids, which is highly homologous to the *PTCH1* product. PTCH1 and PTCH2 have closely associated transmembrane modules related to sterol-sensing domains that affect cholesterol modification of HH ligands.[26] Research indicated that *PTCH2* mutations caused inactivation of PTCH2 inhibitory function in the HH pathway.[27] These studies indicated that any perturbations in the HH pathway can cause developmental abnormalities and

neoplastic lesions similar to those caused by PTCH1 mutations. Further research is needed to uncover novel mutant genes in other components of the HH pathway in GS patients with no identified mutation. Accurate genetic diagnosis can facilitate early treatment, prevent further damage to health and improve quality of life in patients with GS.

Conclusion

A novel heterozygous non-frameshift deletion *PTCH1* [c.3512_3526del (p.1171_1176del)] was identified in a family with GS. This study expands the mutation spectrum of *PTCH1* in GS. The mutation caused a distinct conformational change in PTCH1, which may cause protein malfunction and lead to GS in the family.

Acknowledgements

The authors thank all the subjects for their participation in this study.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Dr Hai Tang YUE contributed to the conception, methodology, analysis and draft; Dr Hai Yan CAO contributed to the methodology, analysis and literature; Dr Miao HE contributed to the study design and supervision, manuscript draft and revision.

(Received Aug 21, 2023; accepted Nov 09, 2023)

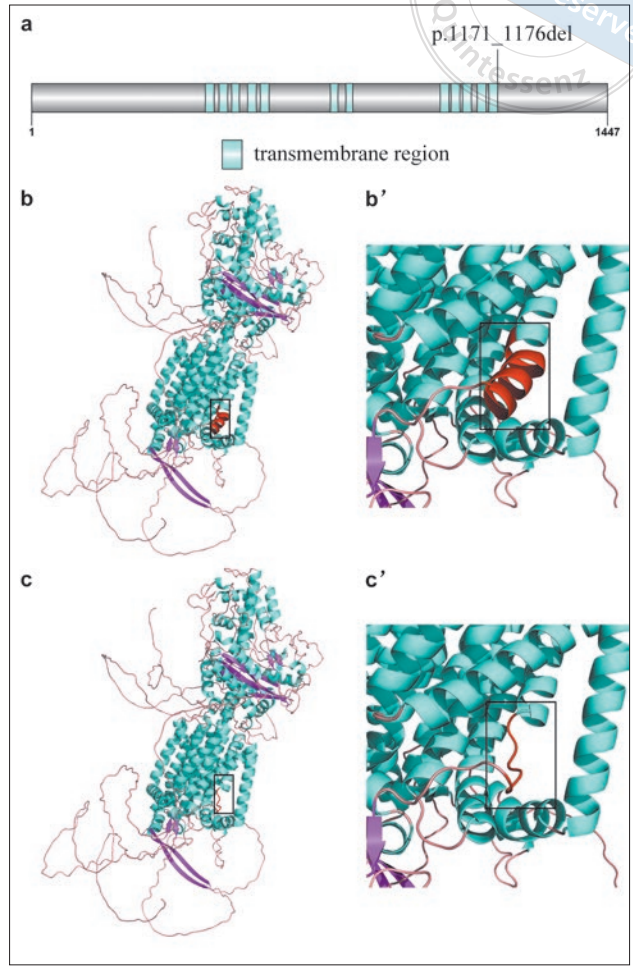

Fig 3 Schematic presentation of the distribution of mutation **(a)** and structural analysis revealing conformational changes in PTCH1 p.1171_1176del **(c and c')** in comparison with wild-type PTCH1 **(b and b')**.

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