A Novel Mutation of PTCHD3 Identified in a Chinese Family with Basal Cell Nevus Syndrome-associated Odontogenic Keratocysts

Si Yi ZHOU[1,#], Zi Yu ZHU[1,#], Ke Jie LU[1], Jian Lu KONG[1], Tian Yi GU[1], Lixuen SIOW[1], Ying QIAN[1], Chao Ying ZHANG[1], Si Yu WANG[1], Takseng HONG[1], Jia Xing GONG[1], Hui Yong ZHU[2], Meng Fei YU[1], Hui Ming WANG[1]

Objective: *To elucidate the genetic aetiology of basal cell nevus syndrome (BCNS) within a Chinese cohort featuring an affected family.*

Methods: *The patient's and their parents' peripheral venous blood was collected for high-throughput exon sequencing. Tools such as Mutation Taster2 and SIFT were used to predict mutation harmfulness and obtain the most suspected pathogenic mutation. GeneMANIA was employed to construct the protein interaction network between the gene and Hedgehog pathway. SWISS-MODEL was used to simulate the bioinformatics structure changes of the mutant gene and HDOCK was utilised to simulate molecular docking. Finally, the function of this gene mutation was verified by q-PCR.*

Results: *A novel pathogenic mutation of PTCHD3 was identified. CCDC57 in the Hedgehog pathway was found to interact with PTCHD3 through GeneMANIA. SWISS-MODEL predicted that PTCHD3 mutation would lead to changes in the 3D structure of the protein. HDOCK showed that the binding between PTCHD3 and CCDC57 was significantly reduced. The mutation of PTCHD3 may result in decreased binding ability of PTCHD3 to CCDC57, activate the Hedgehog pathway and lead to the occurrence of the disease. The diagnosis and treatment pathway for clinical discovery of OKC was provided.*

Conclusion: *A novel pathogenic mutation of PTCHD3 was identified in Chinese BCNS patients. PTCHD3 may play a role in various biological processes mediated by the Hedgehog signalling pathway and is expected to be a new potential therapeutic target.*

Keywords: *basal cell nevus syndrome, Hedgehog pathway, odontogenic keratocysts, PTCHD3*

Chin J Dent Res 2026;29(1):61–70; doi: 10.3290/j.cjdr.b6953776

Basal cell nevus syndrome (BCNS, OMIM #109400), also known as naevoid basal cell carcinoma syndrome or Gorlin syndrome, is a rare autosomal dominant multisystem disorder initially delineated by Gorlin and Goltz in 1960.[1] It is characterised by developmental anomalies and a predisposition to various neoplasms, most notably basal cell carcinomas (BCCs). The condition affects both sexes with an approximately equal distribution (1:1.3) across all racial and ethnic groups,[2] though a higher prevalence has been reported among individu-

1 Stomatology Hospital, School of Stomatology, Zhejiang University School of Medicine, Zhejiang Provincial Clinical Research Center for Oral Diseases, Zhejiang Key Laboratory of Oral Biomedical, Hangzhou, P.R. China

2 Department of Oral and Maxillofacial Surgery, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, P.R. China

# These authors contributed equally to this work.

Corresponding authors: Dr Jia Xing GONG and Dr Meng Fei YU, The Affiliated Hospital of Stomatology, School of Stomatology, Zhejiang University School of Medicine, and Key Laboratory of Oral Biomedical Research of Zhejiang Province, Hangzhou 310000, P.R. China. Tel: 86-571-56367276. Emails: gongjiaxing@zju.edu.cn; yumengfei@zju.edu.cn

Dr Hui Yong ZHU, Department of Oral and Maxillofacial Surgery, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310000, P.R. China. Tel: 86-571-87236114. Email: zhuhuiyong@zju.edu.cn

This study was supported by the Young Elite Scientist Support Programme by the China Science Association (CSA) (2023PYRC001), the Zhejiang Xinmiao Talents Programme (2023R401213) and the Scientific Research Foundation of Zhejiang University (XY2023015).

als of White European descent, with an incidence of 1/30,000 in the UK[3] and 1/235,000 in the Japanese population.[4] However, due to phenotypic heterogeneity and potential underdiagnosis, especially in individuals presenting with subtle or atypical manifestations, the true prevalence is likely underestimated.[5,6] The syndrome typically presents with a spectrum of craniofacial and skeletal anomalies, including keratocystic odontogenic tumours (KCOTs), falx cerebri calcification, macrocephaly, hypertelorism, hydrocephalus and cleft lip/palate.[4] Over the past six decades, diagnostic criteria have evolved significantly.[1,7-9] The currently accepted clinical diagnostic criteria require either the presence of two major criteria or one major and two minor criteria.[10] The major criteria include multiple (> 2) BCCs or a single BCC occurring before the age of 20, histologically confirmed odontogenic keratocysts of the jaw, palmar or plantar pitting, bilamellar calcification of the falx cerebri, rib anomalies and familial history of BCNS. Minor criteria include medulloblastoma, macrocephaly, congenital malformations (e.g., frontal bossing, cleft lip/palate, hypertelorism), skeletal anomalies (such as Sprengel deformity, pectus deformity, syndactyly), radiological abnormalities (e.g., bridging of the sella turcica, vertebral anomalies) and ovarian and cardiac fibromas.

BCNS is an autosomal dominant inherited disorder primarily linked to 9q22.3-q31 and is most commonly caused by heterozygous mutations in PTC, the human counterpart of the Drosophila patched gene.[9] Under physiological conditions, PTCH1 acts as a negative regulator of the Hedgehog (Hh) signalling pathway by suppressing the activity of Smoothened (SMO), thereby maintaining proper control of cell proliferation and differentiation. Inactivation or mutation of PTCH1 results in constitutive activation of SMO, leading to dysregulated Hh signalling and the development of congenital malformations and neoplasms.[11] The molecular pathogenesis of BCNS is classically explained by the two-hit hypothesis: affected individuals inherit a germline mutation in a tumour suppressor gene (e.g., PTCH1) and a somatic "second hit", often induced by environmental factors such as ultraviolet radiation or ionising radiation, triggers disease manifestation.[12] Approximately 85% of BCNS cases are attributed to heterozygous germline mutations in PTCH1 located in chromosome 9q22.3.[2,12] Recently, mutations in the SUFU gene on chromosome 10q13 and PTCH2 on chromosome 1p[5,14] have been identified in Gorlin syndrome patients, with SUFU mutations being associated with an increased medulloblastoma risk but a lower risk of BCC development compared to PTCH1 mutations.[13,15]

Moreover, genetic variability has been observed across ethnic populations. For example, in Han Chinese families, mutations in PTCH2 have been reported. Li et al[16] identified a pathogenic locus on chromosome 1p32.3 in a Han Chinese family, implicating PTCH2 as the probable disease-causing gene in that pedigree. Although PTCHD3 is a member of the patched gene family and encodes proteins containing Patched and sterol-sensing domains, possibly participating in Hedgehog signalling,[17] it has not been previously associated with BCNS. Notably, Ghahramani Seno et al[18] demonstrated that individuals harbouring homozygous PTCHD3 deletions, or a compound genotype of deletion and single nucleotide mutation, did not exhibit overt abnormal phenotypes, leading to the assumption that PTCHD3 may be non-essential in humans.

In this study, the present authors investigated the genetic aetiology of BCNS in a multigenerational Chinese family using whole-exome sequencing (WES) and the phenotype-driven gene prioritisation tool Phenolyzer. The high-throughput RNA sequencing (RNA-seq) and transcriptome analysis were performed by LC-BIO Biotech (Hangzhou, China). They systematically screened known BCNS-associated genes (PTCH1, SUFU and PTCH2) and identified a novel variant in PTCHD3. The present findings aim to expand the current spectrum of pathogenic mutations involved in BCNS and contribute to a more nuanced understanding of genotype–phenotype correlations in this rare disorder.

Materials and methods

Patient enrolment

A BCNS family was recruited from the First Affiliated Hospital of Zhejiang University School of Medicine. Comprehensive clinical data were collected, including detailed personal and family medical history, maxillofacial and intraoral photographs and radiographic images. Written informed consent was obtained from all participating individuals in accordance with the Declaration of Helsinki. This study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital of Zhejiang University School of Medicine (ethics approval no. 2023-0549-Quick).

DNA extraction

Peripheral blood samples were collected in EDTA-coated tubes, and genomic DNA was extracted using a DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany),

following the manufacturer's instructions. DNA integrity and potential degradation were assessed by 1% agarose electrophoresis. Sample purity was evaluated using a NanoPhotometer Spectrophotometer (IMPLEN, Munich, Germany), and DNA concentration was quantified using a Qubit 3.0 Flurometer (Life Technologies, Carlsbad, CA, USA).

WES

WES was performed in accordance with standard operating protocols. Initially, the AgilentV6 Exon targeted sequence enrichment system was used to capture human exon sequences. Library construction involved hybridisation-based enrichment using the SureSelectXT target enrichment system, followed by real-time PCR amplification of target fragments. Upon completion of library preparation, preliminary quantification was carried out using the Qubit 3.0 fluorometer, and libraries were subsequently diluted to a concentration of 1 ng/ul. Library fragment size was evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) to confirm that insert sizes met quality standards. Quantitative PCR (Q-PCR) was then performed using the Bio-Rad CFX 96 real-time system and the iQ SYBR Green kit (both Bio-Rad, Hercules, CA, USA) to accurately determine library concentration. Libraries with an effective concentration greater than 10 nM were considered qualified for sequencing. Finally, qualified DNA libraries were sequenced on the HiSeq sequencing platform.

Prediction of mutation harmfulness

Harmful mutations were predicted by several tools, such as Mutation Taster2, Sorting Intolerant From Tolerant (SIFT), Polymorphism Phenotyping v2 (PolyPhen2), Combined Annotation-Dependent Depletion (CADD) and Functional Analysis Through Hidden Markov Models (FATHMM). MutationTaster2 includes all publicly available single-nucleotide polymorphisms (SNPs) and indels from the 1000 Genomes Project as well as known disease variants from ClinVar and HGMD Public.[19] It is designed to predict the functional consequences of not only amino acid substitutions but also intronic and synonymous alterations, short insertion and/or deletion (indel) mutations and variants spanning intron-exon borders. SIFT is a program that predicts whether an amino acid substitution affects protein function and can distinguish between functionally neutral and deleterious amino acid changes in mutagenesis studies and on human polymorphisms.[20] The SIFT score ranges from 0 to 1. If the score is less than 0.05, it is deleterious (D), and if it is more than 0.05, it is tolerated (T). PolyPhen2 is based on evolutionary conservatism and the 3D structure of proteins, using a Bayesian classifier to calculate a posterior probability to predict the pathogenicity of mutations and employing existing protein annotation databases to identify the importance of replacement positions for scoring matrix scoring. Polyphen2_HVAR, based on the HumanVar, is commonly used for monogenic inherited disease. Polyphen2_ HDIV, based on the HumanDiv database, is commonly used for complex diseases. D means probably damaging, P means possibly damaging and B means benign. CADD is an integrative annotation built from more than 60 genomic features and can score human single nucleotide variants and short insertion and deletions anywhere in the reference assembly.[21] FATHMM predicts the functional effects of protein missense mutations by combining hidden Markov models (HMMs) with sequence conservation, and its prediction results represent the impact of the mutation on protein sequences. D means deleterious, whereas T means tolerated.

Candidate gene function prediction

GeneMANIA (http://genemania.org/) was used to query genomic, proteomic and gene function data. Hundreds of data sets and hundreds of millions of interactions were collected from GEO, BioGRID, IRefIndex and I2D, as well as organism-specific functional genomics data sets. The present authors also employed GeneMANIA to predict and construct an interaction network between candidate genes and key genes, including protein-protein, protein-DNA interactions, pathways, physiological and biochemical reactions, co-expression and co-localisation. If two gene products were found to interact in protein-protein interaction studies, they were linked with a pink line. Most of these data were collected from primary studies found in a protein interaction database, including BioGRID and PathwayCommons. Two genes were linked with a purple line if their expression levels were similar across conditions in gene expression research. Most of these data came from the Gene Expression Compilation (GEO). An orange line represents predicted functional relationships between two genes, often protein interactions. One major source of predicted data was mapping known functional relationships from another organism through orthology. Two gene products were linked with a blue line if they participated in the same reaction in a pathway. These data were collected from various source databases, such as Reactome and BioCycle, via PathwayCommons.

Protein structure simulation

The SWISS-MODEL web server (https://swissmodel.expasy.org) was used to predict the tertiary structure of the mutant protein based on homology modelling. This approach infers unknown protein structures by aligning the target sequence to known templates derived from experimentally resolved natural protein structures. Template selection and model reliability were primarily assessed based on sequence identity. A sequence identity above 40% indicates a high likelihood that the target and template proteins belong to the same structural family, allowing for reliable homology-based structural prediction. Structural quality was evaluated using Global Model Quality Estimation (GMQE) and Qualitative Model Energy Analysis (QMEAN) scores. GMQE values range from 0 to 1, with values approaching 1 indicating superior model reliability. QMEAN scores range from −4 to 0, where values closer to 0 denote greater congruence between predicted and reference structures.

Molecular docking

To investigate the potential impact of PTCHD3 mutations on protein–protein interactions, molecular docking was performed using the HDOCK server (http://hdock.phys.hust.edu.cn/). This tool simulates biophysical interactions, including electrostatic, hydrogen bonding, hydrophobic, van der Waals and hydrophobic forces, between two molecules.[22] Binding affinity and interaction modes between wild-type or mutant PTCHD3 and its candidate partner protein CCDC57 were calculated and scored, providing insight into structural perturbations caused by the mutation.

RNA isolation and quantitative real-time PCR analysis

Total RNA was extracted using TRIzol reagent (Life Technologies), and cDNA was synthesised using the PrimeScript RT Reagent kit (Takara Bio, Dalian, China) according to the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green Master Mix (Thermo Fisher Scientific, Waltham, MA, USA) in a final volume of 10 µl. Gene expression levels were normalised to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table S1 (provided on request).

Statistical analysis

Statistical comparisons were performed using a Student *t* test in GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA). Data were presented as mean ± standard deviation (SD). $P < 0.05$ was considered statistically significant.

Results

Clinical features of a BCNS family

A Chinese family presenting with BCNS was identified. The proband was a 17-year-old girl who exhibited multiple odontogenic keratocysts (OKCs) in both the maxilla and mandible (Fig 1a). Clinical examination revealed multiple facial pigmented nevi, notably two larger lesions (diameter ~ 0.3 cm) adjacent to the nasal bridge on the left cheek (Fig 1b). Craniofacial anomalies included increased interorbital distance and frontal/parietal bossing (Fig 1c). The proband's father had a documented history of recurrent jaw cysts, while her mother was phenotypically normal, consistent with autosomal dominant inheritance (Fig 1d).

Identification of PTCHD3 mutation

Peripheral blood samples were collected from the proband and her parents for WES. Variant analysis revealed predominantly transition-type single nucleotide variants (Fig S1, provided on request). SNP difference analysis identified 17,279 SNPs shared by the patient and the father, but absent in the mother (Fig 1a), consistent with autosomal dominant (AD) inheritable disease transmission requiring a "second hit" mutation. Moreover, dominant homozygous mutation sites are generally heterozygous in parents, but heterozygous or homozygous mutation sites in diseased individuals, and are different from the genotypes of normal brothers and sisters (normal brothers and sisters are homozygous sites). Based on inheritance models and variant distribution, 14,357 candidate heterozygous SNPs were selected, from which 534 rare exonic or splice-site SNPs were retained after excluding variants present in the 1000 Genomes database and synonymous mutations (Fig 2a and b). GO and KEGG pathway analyses were performed on 534 SNPs, which conformed to dominant genetic characteristics. GO analysis revealed enrichment in cellular processes and protein binding. In the cellular component, the differentially expressed genes were enriched in the membrane and membrane part (Fig S2, provided on request). The KEGG pathway analysis revealed significant enrichment in cancer-related signalling pathways (Fig S2). GO and KEGG pathway analysis results were highly consistent with the classical pathogenic pathway HH-PTCH-

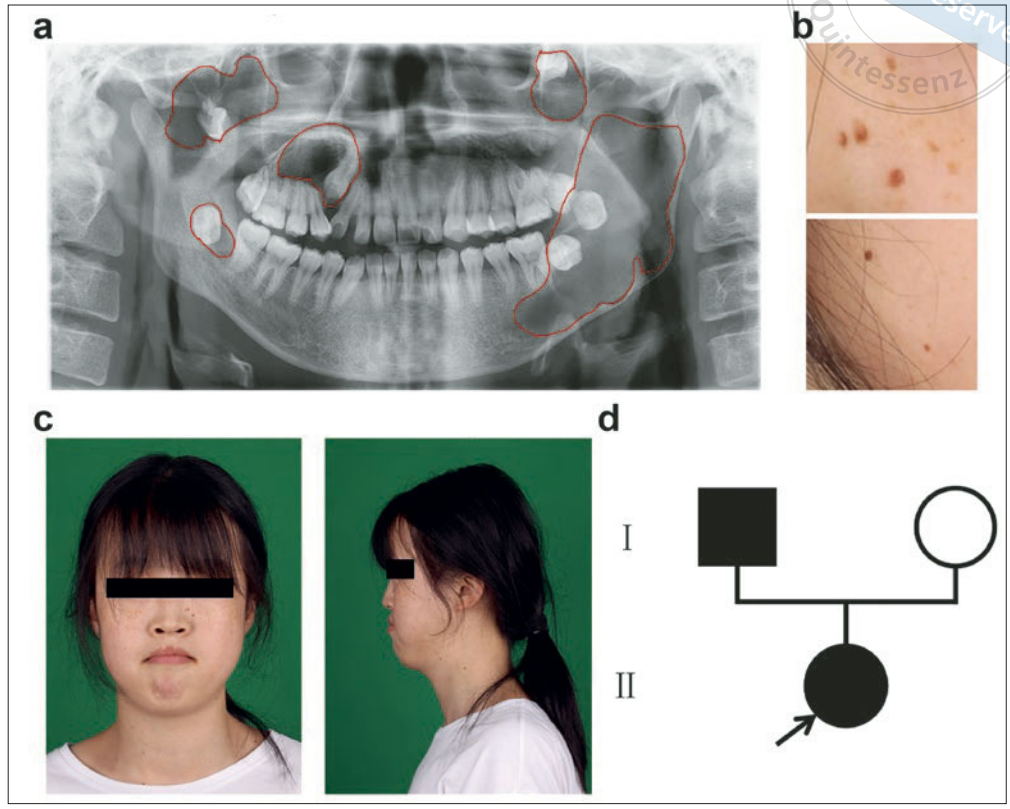
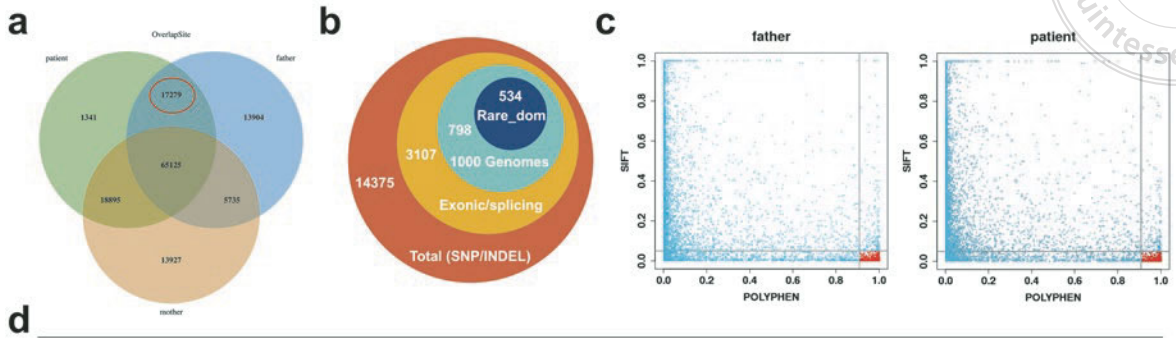


Fig 1a to d Patient medical history and examination findings. Panoramic radiograph showing multiple OCKs in both the maxilla and mandible, with several embedded teeth. The left mandibular lesion involved almost the entire mandibular ramus, angle and part of the body **(a)**. Multiple pigmented nevi were observed on the facial skin, including two prominent nevi (~ 0.3 cm in diameter) located on the left cheek adjacent to the nasal bridge **(b)**. Facial examination revealed hypertelorism (left panel) and frontal-parietal bone protrusion (right panel) **(c)**. Pedigree chart indicating autosomal dominant inheritance of BCNS in this family. The arrow denotes the proband **(d)**.

SMO-Gli in BCNS. However, no PTCH1, PTCH2 or SUFU mutations were found in this BCNS family. The present authors used the base database and mutation frequency database to screen for low-frequency variants and novel variants. They then employed SIFT and PolyPhen2 HVAR, as well as other tools such as Mutation Taster2 and FATHMM to screen pathogenic and deleterious mutations (Fig 2c), along with phenotype databases (Fig S3, Table S2, provided on request). Finally, the present authors found a PTCHD3 mutation, adenine mutating into cytosine at chr10:27700847, which was most likely pathogenic with the same structural domain as PTCH1 (Fig 2d). They further explored the pathogenic mechanism of PTCHD3 mutation.

Functional analysis and expression profiles

PTCHD3 has Ptc and Sterol-Sensing domains (SSD), indicating that PTCHD3 may play a role in the HH pathway. The present authors employed GeneMANIA to construct the network of PTCHD3 and HH pathway interactive proteins based on physical interactions, co-expression, predicted, co-localisation, pathway, genetic interactions and shared protein domains. They found that CCDC57, which co-expressed with IHH, might interact with PTCHD3 (Fig 3a) and, based on the relationships in the network, guessed that PTCHD3 could competitively bind to the CCDC57 complex. However, once PTCHD3 mutated, its binding force with CCDC57 decreased, activating the HH pathway, release Gli activator (GliA). GliA entered the nucleus to activate the expression of the target gene (Fig S4, provided on request). Using SWISS-MODEL, the PTCHD3 structure was predicted. The mutation caused premature termination and structural disruption (Fig S5, provided on request). Then, HDOCK server was employed to redock and score the bond of PTCHD3 mutations and CDCC57 compared to before. Comparing the binding scores of PTCHD3 and CDCC57 before and after mutation, the present authors found that the binding force between PTCHD3 and CDCC57 after mutation was significantly reduced (Fig 3b). The results suggest that the mutation of PTCHD3 reduced the binding force with CCDC57, which might activate the HH pathway and lead to disease. BCNS-associated OKC myofibroblasts are known to exhibit enhanced proliferative and osteoclastogenic potential compared to sporadic OKCs.[23] Furthermore, BCNS-associated OKCs show more aggressive tissue infiltration and a higher recurrence rate than sporadic OKCs.[24] To assess the osteoclast differentiation-inducing capacity of fibroblasts derived



d

Transcript ID	Mutation	Mutation Taster prediction	FATHMM	SIFT	Polyphen2_ HDIV	Polyphen2_ HVAR	CADD
ENST00000043870	Chr10:27700847 A>C	Diseae_causing (0.970923832)	-1.94, D	0.168, T	0.297, B	0.381, B	1.215

Fig 2a to d Screening of pathogenic gene mutations. Venn plot of SNP distribution between samples. A total of 17,279 SNPs were shared between the patient and her father but not her mother, serving as the initial screening pool **(a)**. Stepwise filtering for candidate pathogenic mutations: from 14,357 dominant mutations, 3,107 exonic/splice region mutations were selected. After excluding SNPs with > 1% frequency in the 1000 Genomes database, 798 remained. Synonymous variants were removed, yielding 534 rare, non-synonymous mutations **(b)**. Pathogenicity prediction of SNPs using SIFT (score ≤ 0.05) and PolyPhen2_ HVAR (score ≥ 0.909). Grey lines indicate harmful thresholds of SIFT or POLYPHEN, and red dots represent SNPs predicted deleterious by both tools. Upper and lower plots represent the patient and her father, respectively **(c)**. PTCHD3 mutated at Chr10:27700847 from A to C leading to a stop-loss variant. This mutation was predicted to be disease-causing by Mutation Taster (score 0.9709) and deleterious by FATHMM (−1.94), but benign by PolyPhen2 and tolerated by SIFT **(d)**.

from PTCHD3-mutated and sporadic OKCs, the present authors measured the mRNA expression of interleukin (IL)-1α, cyclooxygenase (COX)2, RANKL and osteoprotegerin (OPG). While RANKL levels were similar between groups, IL-1α and COX2 transcripts were 1.69-fold and 4.29-fold higher, respectively, in PTCHD3-mutated than sporadic OKC fibroblasts (Fig 4a). The RANKL/OPG ratio was 1.44-fold higher in PTCHD3-mutated than sporadic OKC fibroblasts (Fig 4b). To evaluate proliferation and stemness of fibroblasts derived from PTCHD3-mutated and sporadic OKC, Ki67 and SOX2 expression was quantified. Ki67 and SOX2 transcripts were elevated 3.13-fold and 2.5-fold, respectively, in PTCHD3-mutated compared to sporadic OKC fibroblasts (Fig 4c). MMPs are known to remodel the ECM in OKCs.[25-29] In addition, stromal myofibroblasts (characterised by αSMA and caldesmon) contribute to ECM remodelling.[30] In Fig 4d, it showed MMP-7, MMP-9, ACTA2 and CALD-1 transcript levels were significantly increased in PTCHD3 mutated OKCs when compared to sporadic OKCs ($P < 0.0001$).

Discussion

BCNS is a rare hereditary multisystem disorder caused by germline mutations, primarily in the PTCH1 gene.[31] The syndrome was first described in 1894 by Jarisch[31] and White,[32], with a more comprehensive delineation provided later by Gorlin and Goltz.[1] The PTCH1 gene encodes receptors for Hedgehog ligands (SHH, DHH and IHH). Ligand binding to the PTCH1 receptor relieves the suppression of the Smoothened (SMO) protein from PTCH1-mediated inhibition, allowing it to translocate to the primary cilia. This translocation facilitates the release of GLI transcription factors (GLI1, GLI2 and GLI3) from SUFU-mediated inhibition, leading to the transcription of downstream target genes involved in cell proliferation.[33] Loss-of-function mutations in PTCH1 result in aberrant activation of the Hedgehog signalling pathway. In this study, the present authors identified a previously unreported pathogenic mutation in the PTCHD3 gene within a Chinese BCNS family. Unlike known mutations in PTCH1, PTCH2 or SUFU, this novel PTCHD3 variant has not been documented in the context of BCNS. Although PTCHD3 is broadly expressed in adult tissues (including the pancreas, placenta, salivary gland, skin, spleen, thymus, thyroid, trachea, bone marrow, brain, colon, heart, kidneys, lungs, lymph nodes, tongue, testicles, ovaries and spinal cord) and foetal sources (encompassing the brain, bladder, kidney, lung, spleen and stomach), and its notably elevated presence

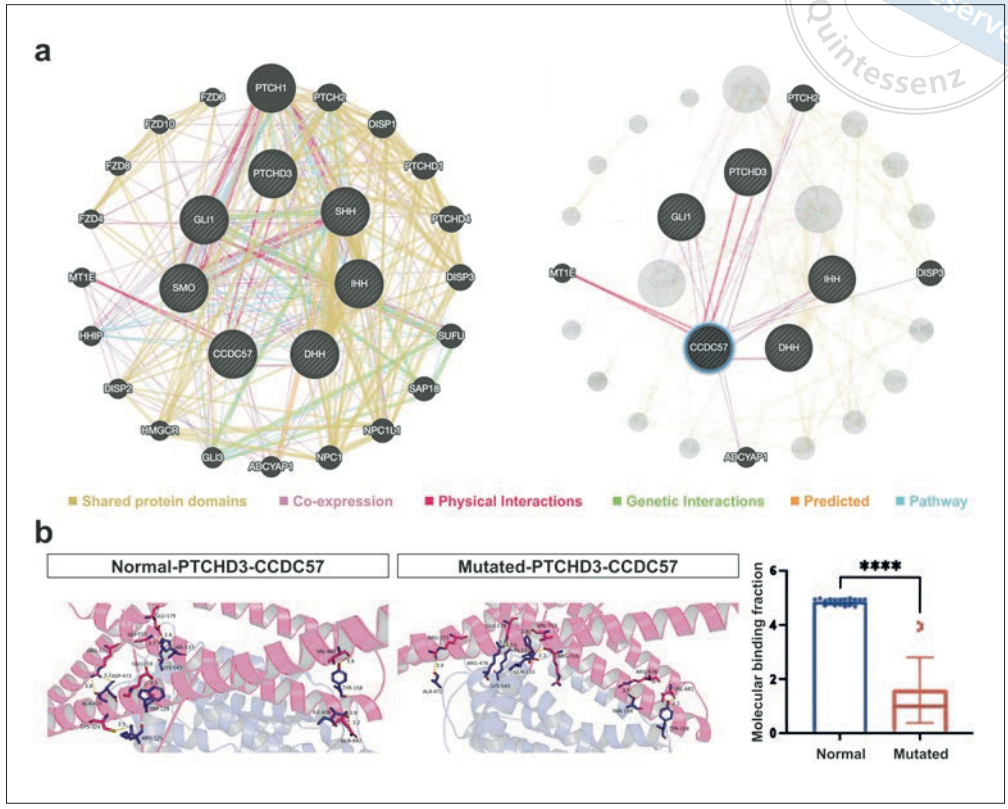


Fig 3a and b Functional prediction of PTCHD3 mutation. GeneMANIA analysis revealed a potential interaction between PTCHD3 and CCDC57, a gene involved in the Hedgehog signalling pathways **(a)**. HDOCK simulations showed that electrostatic, hydrogen bonding, hydrophobic and van der Waals interactions between PTCHD3 and CCDC57 were significantly weakened after mutation (****$P < 0.0001$) **(b)**.

in adult lymph nodes, the testes and the tongue, it has conventionally been classified as non-essential or even a pseudogene.[18] Nevertheless, recent studies have begun to challenge this notion. Notably, in a feature selection study using gene expression data in TCGA, underpinned by robust fuzzy rules,[34] the PTCHD3 gene emerged as a discriminative factor within three of six cancers (PRAD, HNSC and KIRP) encompassed by the analysis (BRCA, PRAD, LIHC, HNSC, KIRP and THCA). This observation suggests a potential significance of PTCHD3 in cancer susceptibility, warranting thorough investigation. The present authors used in silico prediction tools (SIFT, PolyPhen2 HVAR, Mutation Taster2 and FATHMM) to evaluate the pathogenicity of 534 SNPs identified via WES and identified a suspected deleterious mutation in PTCHD3. However, these computational predictions have inherent limitations. Their accuracy is dependent on the quality of existing sequence alignments and protein function annotations, and their ability to predict effects in poorly characterised or novel genes is limited. Moreover, they often reduce complex biological phenomena into binary classifications, such as "deleterious" vs "benign", which may oversimplify the true impact of a given mutation. To better understand the functional relevance of PTCHD3 in the Hedgehog pathway, the present authors propose further experimental validation. Specifically, they will evaluate the activation status of Hedgehog signalling and the expression levels of its downstream targets (GLI1, PTCH1) upon overexpression or knockdown of PTCHD3, using qPCR and RNA-Seq. Co-immunoprecipitation (Co-IP) assays will be employed to determine whether PTCHD3 interacts with key pathway components such as SMO or SUFU.

BCNS presents significant therapeutic challenges, primarily due to the multiplicity and recurrent nature of associated lesions. Current treatment paradigms for BCCs in BCNS mirror those employed in sporadic cases, as no syndrome-specific management guidelines exist.[2] Modalities include surgical, nonsurgical and combinatory approaches tailored to lesion burden and anatomical considerations. OKC are among the most common maxillofacial manifestations of BCNS, occuring in 75% to 89% of patients and often serving as the first clinical symptom.[35] Compared with sporadic OKCs, which peak between the second and third decades and are usually solitary, syndrome-associated OKCs tend to appear earlier, are frequently multiple and have a higher recurrence rate following surgical resection.[36] Notably, up to 85% of BCNS-related OKCs involve mutations in PTCH1,[37] whereas SUFU mutations are associated with

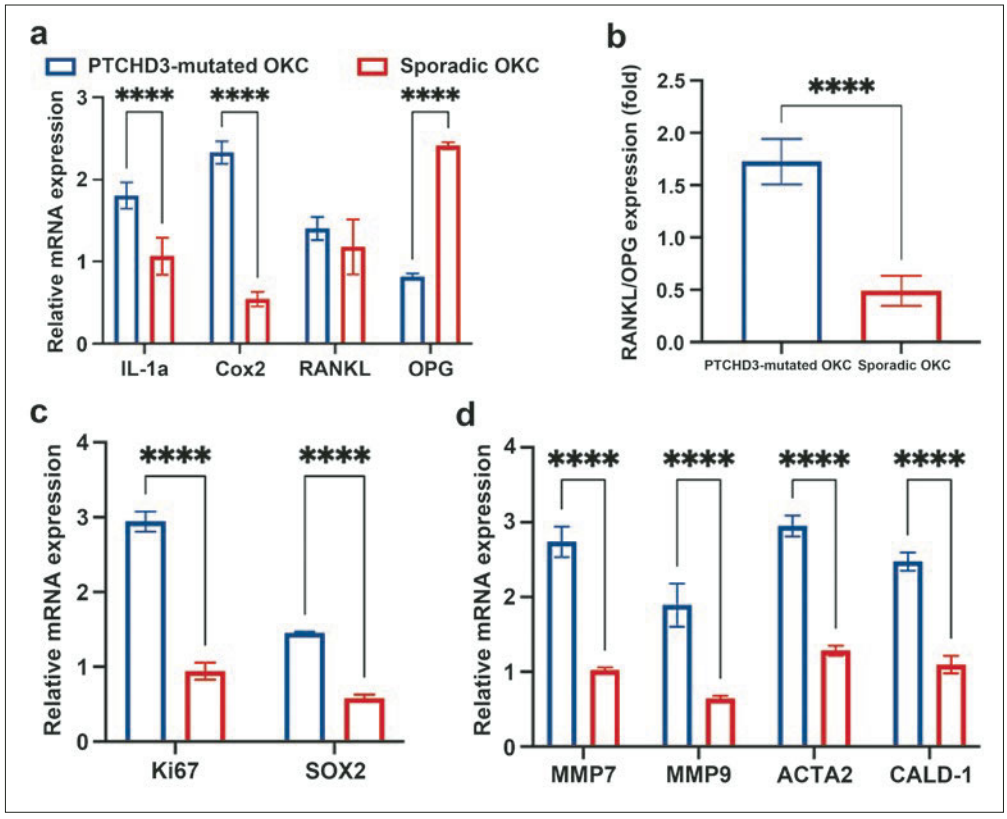


Fig 4a to d Comparison of biological function between PTCHD3-mutated and sporadic OKC fibroblasts. RT-PCR analysis of osteoclast differentiation-related genes in PTCHD3-mutated or sporadic OKC fibroblasts (n = 4, ****P < 0.0001) **(a)**. RANKL/OPG mRNA expression ratio calculated to assess osteoclastogenic potential **(b)**. Relative mRNA expression of Ki67 and SOX2 in PTCHD3-mutated or sporadic OKC fibroblasts determined by RT-PCR (n = 4, ****P < 0.0001) **(c)**. Expression of invasion-related genes assessed via RT-PCR (n = 4, ****P < 0.0001) **(d)**.

a lower risk of OKC development.[35] The present authors' identification of a PTCHD3 mutation in a BCNS patient suggests it may act in a manner similar to PTCH1 and serve as a novel homolog of PTCH1, meriting further investigation. OKCs are mainly treated with enucleation, marsupialisation, decompression or hybrid techniques. Enucleation, though commonly employed, is associated with procedural trauma, incomplete cyst removal, increased risk of adverse outcomes such as pathological fractures, impaired wound healing and high recurrence rates. In cases involving large lesions or those encroaching on critical anatomical structures like the mandibular condyle, marsupialisation and decompression offer advantages including less trauma, fast recovery, reduced bone defects and prevention of postoperative deformities. Optimal care for BCNS patients necessitates a multidisciplinary approach, integrating expertise from maxillofacial surgery, dermatology, genetics and oncology. Recent advances in understanding the molecular pathogenesis of OKCs, particularly the Sonic Hedgehog (SHH) signalling axis, have paved the way for targeted therapies. Vismodegib (GDC-0449), the first FDA-approved SHH pathway inhibitor, received approval in 2012 for the treatment of advanced BCC.[38] A case report has shown partial regression of concomitant OKC lesions in BCNS patients undergoing GDC-0449 therapy.[39] Notably, its efficacy appears to be influenced by specific mutational profiles, such as truncating mutations in PTCH1, while resistance may arise from variants like SMO c.2081C>G (p.P694R).[40] In such scenarios, alternative inhibitors targeting downstream effectors, such as GANT61, which acts at the level of GLI transcription factors, may prove beneficial.

The present authors' recent identification of a pathogenic mutation in PTCHD3 introduces a compelling new dimension to the therapeutic landscape. PTCHD3, a relatively understudied homolog within the Patched family, shares structural similarity with PTCH1 and PTCH2, yet its functional roles remain poorly defined. Emerging evidence implicates PTCHD3 as a potential modulator of the Hedgehog pathway, suggesting it may influence pathway activation in developmental disorders, malignancies and fibrotic diseases. Strategies to therapeutically manipulate PTCHD3 could include the development of small molecules or monoclonal antibodies designed to enhance its inhibitory function or disrupt pathological interactions with key signalling components. Currently available SHH-targeted drugs, such as Vismodegib and GANT61, primarily act on SMO or downstream effectors. Agents that modulate PTCHD3 could complement existing therapeutics by enhancing Hedgehog pathway suppression, particularly

in tumours resistant to SMO inhibitors. A combination approach integrating GLI inhibitors may yield more robust inhibition of the signalling cascade, potentially translating to improved clinical outcomes. To support clinical decision making, the present authors propose a diagnostic and therapeutic algorithm for OKC detection and management (Fig S6, provided on request). Given the importance of early diagnosis and surveillance, genetic testing remains the gold standard for BCNS confirmation. Based on current guidelines and the present findings, the authors recommend initiating annual dental assessments from the age of 2 and taking a panoramic radiograph every 12 to 18 months starting at age 8. If no cysts are detected, the imaging interval may be extended to once every 2 years, and subsequently once every 3 years after age 30.[41] In paediatric patients, particularly those under 10 years old, presenting with multiple OKCs, clinicians should maintain high suspicion of BCNS.[41] For lesions of limited extent, enucleation remains the preferred intervention. Larger or multilocular cysts may benefit from initial marsupialisation or decompression, followed by delayed enucleation to reduce recurrence.[42] Adjunctive measures to minimise recurrence include peripheral ostectomy, mucosal stripping, cryotherapy and application of Carnoy's solution.[43] In cases of recurrence, genetic testing is recommended to assess for PTCH or SMO mutations.[41] If such mutations are identified, targeted SHH pathway inhibitors (e.g., GDC-0449, GANT61) may be considered as adjunctive or primary therapeutic options.[40] Given its emerging role in Hedgehog signalling, PTCHD3 represents a novel and promising, yet underexplored, therapeutic target. Its potential utility, either as a monotherapy target or in combination with SMO/GLI inhibitors, warrants extensive investigation. However, confirmation of its pathogenic role in BCNS will require larger multicentre studies and in-depth molecular characterisation. Elucidating its biological function and developing tailored therapeutic strategies remain crucial next steps. Should conservative therapies fail to control disease progression, segmental or marginal arch resection may be considered as a final-line surgical intervention.[44]

Conclusion

Through WES and pathogenicity analysis, the present authors identified a novel pathogenic mutation in the PTCHD3 gene in a Chinese family with BCNS, which had not been previously reported. Although PTCHD3 was previously considered a non-essential gene in studies on autism spectrum disorder, structural modelling using SWISS-MODEL and molecular docking via HDOCK suggest that PTCHD3 contains a Patched (Ptc) domain. This indicates its potential involvement in biological processes mediated by the Hedgehog signalling pathway. These findings provide new insights into the molecular pathogenesis of BCNS and highlight PTCHD3 as a candidate gene for further investigation.

Conflicts of interest

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

Author contribution

Dr Si Yi ZHOU contributed to the data analysis, investigation and manuscript draft; Dr Zi Yu ZHU contributed to the conceptualisation, investigation, data curation and manuscript draft; Dr Ke Jie LU contributed to the software analysis; Dr Jian Lu KONG contributed to the methodology; Drs Tian Yi GU and Chao Ying ZHANG contributed to the data analysis and validation; Drs Li Xuen SIOW, Si Yu WANG and Takseng HONG contributed to the writing, review and editing; Dr Ying QIAN contributed to the data analysis and study supervision; Drs Jia Xing GONG, Hui Yong ZHU and Meng Fei YU contributed to the study conceptualisation and project administration.

(Received Apr 15, 2025; accepted Aug 28, 2025)

References

1. Gorlin RJ, Goltz RW. Multiple nevoid basal-cell epithelioma, jaw cysts and bifid rib: A syndrome. N Engl J Med 1960;262:908–912.
2. John AM, Schwartz RA. Basal cell naevus syndrome: An update on genetics and treatment. Br J Dermatol 2016;174:68–76.
3. Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: Estimates from a UK family genetic register service. Am J Med Genet A 2010;152A:327–332.
4. Endo M, Fujii K, Sugita K, Saito K, Kohno Y, Miyashita T. Nationwide survey of nevoid basal cell carcinoma syndrome in Japan revealing the low frequency of basal cell carcinoma. Am J Med Genet A 2012;158A:351–357.
5. Onodera S, Nakamura Y, Azuma T. Gorlin syndrome: Recent advances in genetic testing and molecular and cellular biological research. Int J Mol Sci 2020;21:7559.
6. Rehefeldt-Erne S, Nägeli MC, Winterton N, et al. Nevoid basal cell carcinoma syndrome: Report from the Zurich nevoid basal cell carcinoma syndrome cohort. Dermatology 2016;232:285–292.
7. Evans DG, Ladusans EJ, Rimmer S, Burnell LD, Thakker N, Farndon PA. Complications of the naevoid basal cell carcinoma syndrome: Results of a population based study. J Med Genet 1993;30:460–464.

8. Bree AF, Shah MR, BCNS Colloquium Group. Consensus statement from the first international colloquium on basal cell nevus syndrome (BCNS). Am J Med Genet A 2011;155A:2091–2097.
9. Kimonis VE, Goldstein AM, Pastakia B, et al. Clinical manifestations in 105 persons with nevoid basal cell carcinoma syndrome. Am J Med Genet 1997;69:299–308.
10. Spiker AM, Troxell T, Ramsey ML. Gorlin Syndrome. In: StatPearls. Treasure Island (FL): StatPearls Publishing; August 8, 2023.
11. Heussler HS, Suri M. Sonic hedgehog. Mol Pathol 2003;56:129–131.
12. Hahn H, Wicking C, Zaphiropoulous PG, et al. Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 1996;85:841–851.
13. Evans DG, Oudit D, Smith MJ, et al. First evidence of genotype-phenotype correlations in Gorlin syndrome. J Med Genet 2017;54:530–536.
14. Fujii K, Ohashi H, Suzuki M, et al. Frameshift mutation in the PTCH2 gene can cause nevoid basal cell carcinoma syndrome. Fam Cancer 2013;12:611–614.
15. Smith MJ, Beetz C, Williams SG, et al. Germline mutations in SUFU cause Gorlin syndrome-associated childhood medulloblastoma and redefine the risk associated with PTCH1 mutations. J Clin Oncol 2014;32:4155–4161.
16. Li JY, Fan ZP, Du J, Shen Y, Wang SL. Localization of the pathogenic gene of nevoid basal cell carcinoma syndrome in a Chinese Han family [in Chinese]. Beijing Kou Qiang Yi Xue 2006;14:1–4.
17. Fan J, Akabane H, Zheng X, et al. Male germ cell-specific expression of a novel Patched-domain containing gene Ptchd3. Biochem Biophys Res Commun 2007;363:757–761.
18. Ghahramani Seno MM, Kwan BY, Lee-Ng KK, et al. Human PTCHD3 nulls: Rare copy number and sequence variants suggest a non-essential gene. BMC Med Genet 2011;12:45.
19. Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: Mutation prediction for the deep-sequencing age. Nat Methods 2014;11:361–362.
20. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31:3812–3814.
21. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: Predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res 2019;47:D886–D894.
22. Yan Y, Tao H, He J, Huang SY. The HDOCK server for integrated protein-protein docking. Nat Protoc 2020;15:1829-–852.
23. Hong YY, Yu FY, Qu JF, Chen F, Li TJ. Fibroblasts regulate variable aggressiveness of syndromeic keratocystic and nonsyndromeic odontogenic tumors. J Dent Res 2014;93:904–910.
24. de Oliveira Ramos G, Costa A, Meurer MI, Vieira DS, Rivero ER. Immunohistochemical analysis of matrix metalloproteinases (1, 2, and 9), Ki-67, and myofibroblasts in keratocystic odontogenic tumors and pericoronal follicles. J Oral Pathol Med 2014;43:282–288.
25. Leonardi R, Matthews JB, Caltabiano R, et al. MMP-13 expression in keratocyst odontogenic tumour associated with NBCCS and sporadic keratocysts. Oral Dis 2010;16:795–800.
26. de Andrade Santos PP, de Aquino AR, Oliveira Barreto A, de Almeida Freitas R, Galvão HC, de Souza LB. Immunohistochemical expression of nuclear factor κB, matrix metalloproteinase 9, and endoglin (CD105) in odontogenic keratocysts, dentigerous cysts, and radicular cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:476–483.
27. Ribeiro AL, Nobre RM, Alves-Junior SM, et al. Matrix metalloproteinases, tissue inhibitors of metalloproteinases, and growth factors regulate the aggressiveness and proliferative activity of keratocystic odontogenic tumors. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114:487–496.
28. Andric M, Jacimovic J, Jakovljevic A, Nikolic N, Milasin J. Gene polymorphisms in odontogenic keratocysts and ameloblastomas: A systematic review. Oral Dis 2022;28:1421–1430.
29. Cavalcante RB, Pereira KM, Nonaka CF, Nogueira RL, de Souza LB. Immunohistochemical expression of MMPs 1, 7, and 26 in syndrome and nonsyndrome odontogenic keratocysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008;106:99–105.
30. Gaitán-Cepeda LA, Quezada-Rivera D, Tenorio-Rocha F, Leyva-Huerta ER. Reclassification of odontogenic keratocyst as tumour. Impact on the odontogenic tumours prevalence. Oral Dis 2010;16:185–187.
31. Jarisch W. Zur lehre von den hautgeschwulsten. Archiv für Dermatologie und Syphilis 1894; 28:163-1655.
32. White JC. Multiple benign cystic ephiteliomata. J Cutan Dis 1894;12:477-81.
33. Foulkes WD, Kamihara J, Evans DGR, et al. Cancer surveillance in Gorlin syndrome and rhabdoid tumor predisposition syndrome. Clin Cancer Res 2017;23:e62–e67.
34. Fan S, Tang J, Tian Q, Wu C. A robust fuzzy rule based integrative feature selection strategy for gene expression data in TCGA. BMC Med Genomics 2019;12(suppl 1):14.
35. Guerrini-Rousseau L, Smith MJ, Kratz CP, et al. Current recommendations for cancer surveillance in Gorlin syndrome: A report from the SIOPE host genome working group (SIOPE HGWG). Fam Cancer 2021;20:317–325.
36. Carlson ER, Oreadi D, McCoy JM. Nevoid basal cell carcinoma syndrome and the keratocystic odontogenic tumor. J Oral Maxillofac Surg 2015;73(12 suppl):S77–S86.
37. Ambele MA, Robinson L, van Heerden MB, Pepper MS, van Heerden WFP. Comparative molecular genetics of odontogenic keratocysts in sporadic and syndromeic patients. Mod Pathol 2023;36:100002.
38. Sekulic A, Migden MR, Oro AE, et al. Efficacy and safety of vismodegib in advanced basal-cell carcinoma. N Engl J Med 2012;366:2171–2179.
39. Goldberg LH, Landau JM, Moody MN, Kazakevich N, Holzer AM, Myers A. Resolution of odontogenic keratocysts of the jaw in basal cell nevus syndrome with GDC-0449. Arch Dermatol 2011;147:839–841.
40. Wang S, Hong Y, Qu J, et al. PTCH/SMO gene mutations in odontogenic keratocysts and drug interventions. J Oral Pathol Med 2023;52:867–876.
41. Fernández LT, Ocampo-Garza SS, Elizondo-Riojas G, Ocampo-Candiani J. Basal cell nevus syndrome: An update on clinical findings. Int J Dermatol 2022;61:1047–1055.
42. de Castro MS, Caixeta CA, de Carli ML, et al. Conservative surgical treatments for nonsyndromeic odontogenic keratocysts: A systematic review and meta-analysis. Clin Oral Investig 2018;22:2089–2101.
43. Janas-Naze A, Zhang W, Szuta M. Modified Carnoy's versus Carnoy's solution in the management of odontogenic keratocysts-A single center experience. J Clin Med 2023;12:1133.
44. Liu B, He Y, Peng X, et al. Chinese expert consensus on the diagnosis and treatment of odontogenic keratocysts [in Chinese]. Zhongguo Kou Qiang He Mian Wai Ke Za Zhi 2022;20:209–218.