

DATA REPORT

A novel *PTCH1* mutation in a patient with Gorlin syndromeNana Okamoto^{1,5}, Takuya Naruto^{2,5}, Tomohiro Kohmoto³, Takahide Komori¹ and Issei Imoto⁴

Gorlin syndrome is an autosomal dominant disorder characterized by a wide range of developmental abnormalities and a predisposition to various tumors, and it is linked to the alteration of several causative genes, including *PTCH1*. We performed targeted resequencing using a next-generation sequencer to analyze genes associated with known clinical phenotypes in an 11-year-old male with sporadic jaw keratocysts. A novel duplication mutation (c.426dup) in *PTCH1*, resulting in a truncated protein, was identified.

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Basal cell nevus syndrome (BCNS; MIM #109400) or Gorlin syndrome,¹ also known as nevoid basal cell carcinoma syndrome (NBCCS) or Gorlin–Goltz syndrome, is a rare autosomal dominant disorder with almost 100% penetrance and variable expressivity. Gorlin syndrome is characterized by a wide range of developmental abnormalities and a predisposition to neoplasms. Mutations in the *PTCH1* gene (MIM #601309) are the main molecular defects associated with Gorlin syndrome,^{2,3} although mutations in other genes involved in the hedgehog pathway, such as *PTCH2* (MIM #603673) and *SUFU* (MIM #607035),^{4,5} have also been reported in patients with this syndrome. Here, we report a novel *PTCH1* gene duplication mutation (c.426dup) that was detected in a patient with a history of multiple keratocystic odontogenic tumors (KCOTs, Figure 1a) of the jaw, which is the most consistent and common manifestation of Gorlin syndrome.⁶

An 11-year-old Chinese male with no family history of consanguinity presented to our clinic with plantar pits (Figure 1b), mild macrocephaly, a coarse face and melanotic macules. His psychomotor development was appropriate for his age, and the results of neurological and ophthalmological examinations were normal. Imaging tests (magnetic resonance imaging and orthopantomography) revealed multiple odontogenic cysts (Figure 1a). A radiograph of the skull revealed bilamellar calcifications of the falx cerebri and bulging of the sella turcica (Figure 1c). Histopathological examination of the odontogenic cysts confirmed the diagnosis of KCOTs. Over 2 years of follow-up, new black pit lesions appeared on his right cheek. However, neither basal cell carcinoma nor bifid, fused or markedly splayed ribs were observed.

On the basis of the presence of three major criteria for clinical diagnosis,⁷ the patient was diagnosed with Gorlin syndrome. The molecular diagnosis was performed using genomic DNA extracted from the patient's whole blood after obtaining informed consent. The study was approved by the ethical committees of Kobe University and The University of Tokushima. Because various alterations, including mutations and copy number alterations (CNAs)⁸ of three genes with numerous exons (*PTCH1*, *PTCH2* and *SUFU*) can be responsible for Gorlin syndrome, we first used a Miseq bench-top sequencer (Illumina, San Diego, CA, USA) to

perform next-generation sequencing with a TruSight One Sequencing Panel (Illumina) for the simultaneous targeted sequencing of the exon regions of 4,813 clinically relevant genes. The sequencing reads were aligned to the human reference genome (hg19) using Burrows–Wheeler aligner (BWA, version 0.7.8; <http://bio-bwa.sourceforge.net/>).⁹ The alignments were converted from a sequence alignment map (SAM) format to a sorted and indexed binary alignment map files (SAMtools version 0.1.19; <http://samtools.sourceforge.net/>). Duplicate reads were removed using Picard (<http://picard.sourceforge.net/>). Local realignment around the indels, base quality score recalibration and UnifiedGenotyper calls were performed using Genome Analysis Toolkit software (GATK, version 3.1-1; <http://www.broadinstitute.org/gatk/>).¹⁰ All sequence variants were annotated using Annovar.¹¹ To identify single-nucleotide variations, we excluded sequence variants with minor allele frequency >0.05 from the 1000 Genomes Project databases (<http://www.1000genomes.org/>), NHLBI GO Exome Sequencing Project (ESP6500, <http://evs.gs.washington.edu/EVS/>) and the Human Genetic Variation Database (HGVD, <http://www.genome.med.kyoto-u.ac.jp/SnpDB/>). To complement the single-nucleotide variations and indel analyses, a CNA detection algorithm was applied to the alignment map files to identify large CNAs as follows: (1) binary alignment map files were converted into files covering target regions using GATK, (2) log coverage ratios and Z scores were calculated between the case and other samples and (3) regions with an abnormal copy number were detected using circular binary segmentation with DNACopy (R/Bioconductor; <http://bioconductor.org/>).¹² These analyses identified one heterozygous frameshift mutation NM_000264.3 (*PTCH1*_v001):c.426dup in exon 3 of the *PTCH1* gene, which caused a frameshift at codon 143, resulting in the introduction of a stop codon at position 155 (NM_000264.3 (*PTCH1*_i001):p.(Thr143Tyrfs*12)). This resulted in the loss of most of the coding region, which resulted in a loss of protein function; thus, this mutation is the disease-causing alteration in our patient. No other alterations, including CNAs, were observed around the three genes. The alteration was then confirmed using PCR-based direct Sanger sequencing with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) on a 3130

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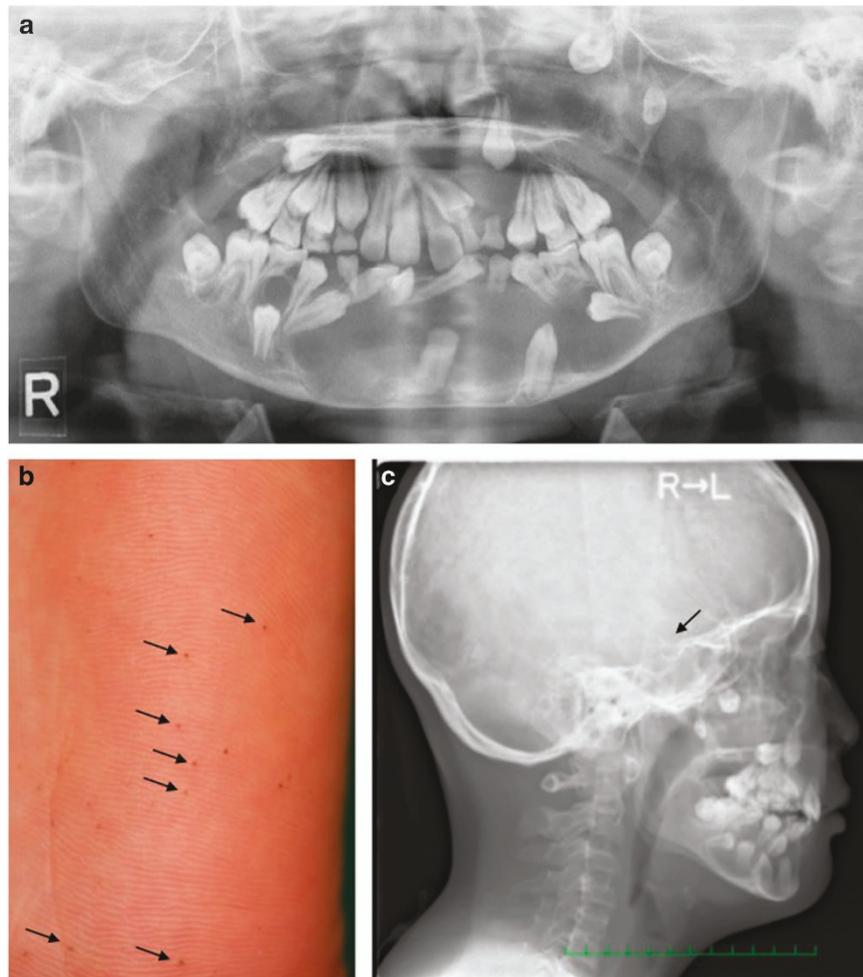


Figure 1. (a) Dental orthopantomography of a patient affected by multiple keratocystic odontogenic tumors (KCOTs). (b) Numerous tiny black pits over the right plantar area (arrows). (c) Radiograph of the skull revealing bulging of the sella turcica (arrow).

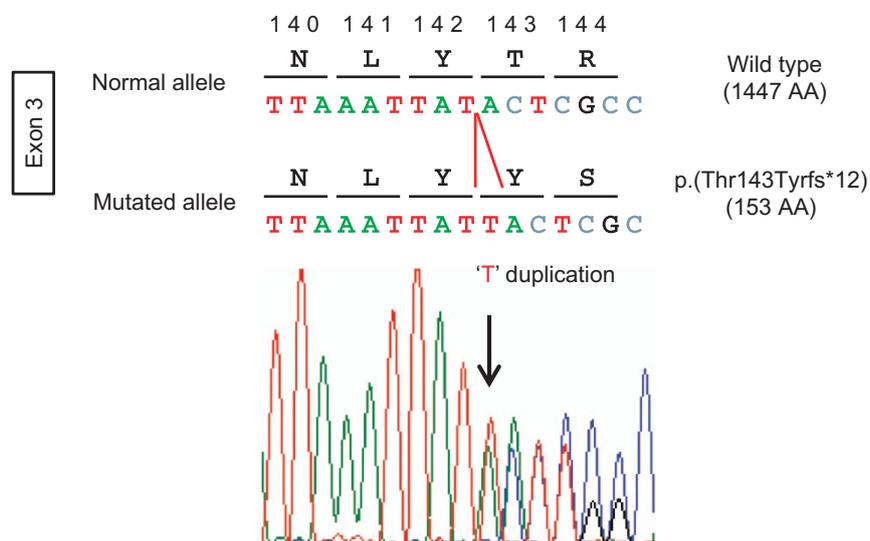


Figure 2. Electropherogram of the *PTCH1* exon 3 sequence showing the heterozygous germline duplication of a thymine (T) in the patient DNA. The DNA and corresponding amino-acid sequences of wild-type and mutant *PTCH1* alleles are also shown. The affected transcript and protein were labeled NM_000264.3 (*PTCH1_v001*):c.426dup and NM_000264.3 (*PTCH1_i001*):p.(Thr143Tyrfs*12), respectively, using the Human Genome Variation Society (HGVS) nomenclature version 2.0 (Mutalyzer 2.0.beta-32, <https://mutalyzer.nl/>). The arrow indicates the duplication point.

Genetic Analyzer (Applied Biosystems) in both directions (Figure 2, primer information is available on request). This mutation has not been previously reported in any patients with Gorlin syndrome (Human Gene Mutation Database professional 2014.2, <http://www.hgmd.org/> and ClinVar, <http://www.ncbi.nlm.nih.gov/clinvar/>) and is not present in the 1000 Genomes, ESP6500 or HGVD databases. Because parental DNA was not available, this mutation could not be confirmed to be *de novo*.

The human *PTCH1* gene contains 23 coding exons that span ~70 kb and encode a protein composed of 1,447 amino acids with 12 transmembrane-spanning domains and two large extracellular loops.³ *PTCH1* is the ligand-binding component of the sonic hedgehog receptor complex. Aberrant activation of the sonic hedgehog signaling cascade owing to haploinsufficiency of *PTCH1* is believed to cause Gorlin syndrome.¹³ As germline mutations of *PTCH1* in patients with Gorlin syndrome were first reported by two groups in 1996,^{2,3} >300 different mutations covering almost all exons, with no hot spots and various CNAs, have been reported. Most of these are frameshift or nonsense mutations that lead to the synthesis of a truncated protein. In a series of 313 *PTCH1* mutations reported in the Human Gene Mutation Database, most of which were detected in cases of Gorlin syndrome, 33.9% were missense or nonsense mutations, 47.6% were small insertions and/or deletions and 8.3% were gross deletions, insertions or duplications. In the current study, we reported a novel germline duplication (insertion) mutation of *PTCH1*, c.426dup, in a Chinese patient with sporadic Gorlin syndrome. This mutation created a premature termination codon in the mutant allele that resulted in the truncation of *PTCH1*. This truncation of *PTCH1* is one of the shortest forms reported to date; this truncation lacks 11 of the 12 transmembrane-spanning domains. Because a premature termination codon leads to mRNA degradation via nonsense-mediated mRNA decay,¹⁴ the haploinsufficiency of *PTCH1* likely had an important etiological role in this case.

A combination of clinical and molecular screening tools could be useful for the identification of Gorlin syndrome in patients who were previously diagnosed with multiple KCOTs of the jaw. These patients could be identified by retrospectively evaluating the pathological records.¹⁵ Although this case presented with several symptoms that fulfilled the criteria for the diagnosis of Gorlin syndrome, identifying the specific pathogenic mutation is useful for genetic counseling. Therefore, a proper diagnosis is essential, particularly during the early stages of life. Gorlin syndrome is primarily caused by mutations in *PTCH1*. However, no hot-spot mutations within the 23 coding exons have been reported in this gene; CNAs are also known to be responsible for Gorlin syndrome. Genetic alterations in *PTCH2* (22 coding exons) and *SUFU* (12 coding exons) have also been identified, although they are rarely observed. Previous studies have revealed that there were no genotype–phenotype correlations between mutations in *PTCH1* or other genes and the major clinical features of Gorlin syndrome.^{16,17} Target resequencing of the possible disease-causing genes and other genes associated with various known clinical phenotypes using next-generation sequencing technology enables the simultaneous evaluation of mutations and CNAs. Therefore, this could form a simple, efficient and economical genetic test for Gorlin syndrome.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.513>.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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