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CASE REPORT

Germline *PTCH1*: c.361_362insAlu alteration identified by comprehensive exome and RNA sequencing in a patient with Gorlin syndrome

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Abstract

Gorlin syndrome can be caused by pathogenic/likely pathogenic (P/LP) variants in the tumor suppressor gene *PTCH1* (9q22.1-q31), which encodes the receptor for the sonic hedgehog (SHH) ligand. We present a 12-month-old boy clinically diagnosed with Gorlin syndrome who was found to have significantly delayed development, palmar pitting, palmar and plantar keratosis, short hands, frontal bossing, coarse face, hypertelorism, a bifid rib, misaligned and missing teeth, and SHH-activated medulloblastoma. Genetic testing, including a pediatric cancer panel and genome sequencing with peripheral blood, failed to identify any P/LP variants in *PTCH1*. Paired tumor/normal exome sequencing was performed, which identified a germline NM_000264.5 (*PTCH1*): c.361_362ins? alteration through manual review of sequencing reads. Clinical RNA sequencing further demonstrated an Alu insertion at this region (*PTCH1*: c.361_362insAlu), providing molecular confirmation of Gorlin syndrome. This finding exemplifies a unique mechanism for *PTCH1* disruption in the germline and highlights the importance of comprehensive analysis, including manual review of DNA sequencing reads and the utility of RNA analysis to detect variant types which may not be identified by routine genetic screening techniques.

KEYWORDS

Alu insertion, Gorlin syndrome, *PTCH1*, RNA sequencing, SINE

1 | INTRODUCTION

Recent advancements in genetic testing technology, including next-generation sequencing (NGS), have significantly enhanced the precision of germline genetic testing (Ow et al., 2019; Sorscher et al., 2017). Furthermore, the growing use of multigene panel testing and exome sequencing (ES) has expanded our ability to identify pathogenic/likely pathogenic (P/LP) variants in germline cancer predisposition genes based on DNA analysis (Dangoni et al., 2024). However, despite the advancements in DNA sequencing, there remains a limitation that noncoding DNAs are excluded from the analysis and this exclusion reduces the diagnostic yield of such sequencing approaches (Paolillo et al., 2016).

RNA sequencing has ushered in a new era for diagnosing and identifying genetic diseases (Ergin et al., 2022). However, adoption of this technology is still slow in the clinical setting due to challenges

associated with clinical validation (Byron et al., 2016). Nonetheless, this powerful technique enables more precise analysis of genes that have been previously challenging to investigate and comprehend thoroughly (Ergin et al., 2022). By delving into transcriptomes across different species, RNA sequencing provides valuable insights into rare diseases and enhances our understanding of various eukaryotic organisms (Baumhoer et al., 2024; Ergin et al., 2022; Hong et al., 2020). Moreover, recent advancements in computational analysis and RNA sequencing using both blood and tissue biopsy specimens empower clinicians to pinpoint the genetic origins and causes of genetic disorders (Ergin et al., 2022; Hong et al., 2020). This comprehensive approach not only aids in detecting genes resistant to existing therapies but also contributes to refining treatment methods for greater effectiveness and fewer side effects (Aney et al., 2024; Ergin et al., 2022; Hong et al., 2020).

Gorlin syndrome, also known as nevoid basal cell carcinoma syndrome, is caused by a germline alteration in *PTCH1* (OMIM: 601309) or, less commonly, *SUFU* (OMIM: 607035) (Fujii & Miyashita, 2014). Gorlin syndrome is a rare genetic disorder that has a variable prevalence between 1/30,827 and 1/235,800 (Fujii & Miyashita, 2014). There are specific clinical diagnostic criteria used to identify individuals with Gorlin syndrome (Jawa et al., 2009; Shetty et al., 2023). In addition to its association with basal cell carcinoma, Gorlin syndrome is characterized by various tumors, including keratocystic odontogenic tumors, cardiac fibroma, meningioma, and sonic hedgehog (SHH)-activated medulloblastoma (Fernandez et al., 2022; Fujii & Miyashita, 2014; Jawa et al., 2009; Shetty et al., 2023). Beyond these neoplastic features, individuals with Gorlin syndrome may exhibit additional clinical characteristics such as pits or small indentations in the palms and soles, bifid, fused or markedly splayed ribs, misaligned and missing teeth, and calcification of the falx cerebri (Fernandez et al., 2022; Fujii & Miyashita, 2014; Jawa et al., 2009; Shetty et al., 2023). The facial features commonly seen in Gorlin syndrome include macrocephaly, frontal bossing, coarse face, hypertelorism, cleft lip or palate, strabismus, microphthalmia, cataracts, milia cysts, micrognathia, and midface hypoplasia (Fernandez et al., 2022; Fujii & Miyashita, 2014; Jawa et al., 2009; Shetty et al., 2023).

In this case, we present a 12-month-old boy who met the clinical diagnostic criteria for Gorlin syndrome. Germline testing was pursued, including a pediatric cancer panel and genome sequencing (GS) which failed to identify a P/LP variant in *PTCH1* or *SUFU*. Comprehensive molecular testing, including paired tumor/normal ES, was performed on the Molecular Characterization Initiative through the Children's Oncology Group which identified a germline insertion of unclear origin (NM_000264.5 [*PTCH1*]: c.361_362ins?), which likely disrupted *PTCH1* within exon 2. Subsequent clinical RNA sequencing of *PTCH1* revealed this finding to be an LP variant resulting from an Alu insertion denoted as NM_000264.5 (*PTCH1*): c.361_362insAlu.

2 | CASE DESCRIPTION

The patient was initially diagnosed with SHH-activated medulloblastoma at 12 months of age after brain magnetic resonance imaging (MRI) was performed due to persistent gross motor delays. Additional syndromic manifestations, including a bifid rib and facial dysmorphic features, prompted a diagnostic workup for Gorlin syndrome.

The patient was delivered via normal spontaneous vaginal delivery at 39 weeks of gestational age with Apgar scores of 8 at 1 min and 8 at 5 min after birth. His body weight was 3.39 kg (54th percentile), length was 52.1 cm (88th percentile), and head circumference was 34.5 cm (51st percentile). An undescended right testicle was noted during the newborn examination. He was referred to audiology for a failed hearing screening on the left side. The patient was found to have a small patent foramen ovale but otherwise normal cardiac anatomy on echocardiogram performed at 17 days of age due to persistent stridor. At 5 months of age, he underwent right inguinal orchiopexy and inguinal hernia repair.

Developmentally, he followed to the midline at 1.5 months, held his hands together at the midline at 4 months, reached out for objects, fed himself at 6 months, and rolled over at 8 months. At 12 months, he could not sit up alone or crawl. He attempted to hold his head upright but had difficulty maintaining head control, mainly secondary to truncal hypotonia. At 6.5 months, he began vocalizing, and by the age of 12 months, he was able to babble, although he had not yet spoken any words. He smiled at 2 months and laughed at 3 months. At 12 months, he could look at people around him and engage with his mother.

Due to significant developmental delays, his primary care physician (PCP) recommended physical therapy evaluation around 6 months of age, resulting in concerns for cerebral palsy. Neurology evaluated him around 9 months of age and recommended follow-up for hypotonia and consideration of a brain MRI if his deficits persisted despite physical therapy. At approximately 8 months old, the mother observed a growing mass on the left side of the chest wall, which had initially been noticed around 2–3 months of age. She promptly informed the PCP about this growing mass. Chest x-ray did not visualize a definite left rib anomaly. However, a chest ultrasound revealed an expansile mass-like lesion at the costal cartilaginous junction concerning chondroma or chondrosarcoma. Pediatric surgery was involved, and a chest MRI was requested, which revealed enlargement and widening of the unossified cartilage in the left seventh rib, indicating a bifid rib. A brain MRI was obtained in conjunction with the chest MRI, which demonstrated a mass of the left cerebellar hemisphere. The patient underwent suboccipital craniotomy with gross total resection of the mass, and the pathology findings were consistent with SHH-activated medulloblastoma. There was no evidence of metastasis on staging workup. He was subsequently admitted for chemotherapy, during which period he underwent genetic evaluation.

The genetic physical examination was notable for broad face, frontal bossing, hypertelorism, epicanthal folds, wide and depressed nasal bridge, and upturned nasal tip. His head appeared large, but his head circumference did not meet the criteria for macrocephaly. Hypotonia was mainly appreciated in his trunk and neck, affecting his motor development. He had several pits and keratosis on his palms and soles. At around 12 months, he did not have any teeth, but by 14 months, it was observed that he had both misaligned and missing

teeth. During the ophthalmologic evaluation, the patient exhibited normal optic nerve function, and both eyes demonstrated conjugated horizontal movement. However, when at rest, there was evidence of dysconjugate gaze. Audiology evaluation revealed normal hearing in the right ear and conductive hearing loss in the left ear with left middle ear dysfunction of unclear etiology. He was clinically diagnosed with Gorlin syndrome based on the criteria of palmar pitting, palmar and plantar keratosis, frontal bossing, a bifid rib, hypertelorism, and SHH-activated medulloblastoma. His family history was notable for schizophrenia, bipolar disorder, attention deficit hyperactivity disorder, autism spectrum disorder, and learning disability, but there was no family history of cancer.

Given the young age at diagnosis and syndromic clinical features, a germline pediatric cancer panel (Prevention Genetics, WI) and GS (Prevention Genetics, WI) were performed, which were negative. For tumor characterization, solid tumor fusion analysis, paired tumor/normal ES, and methylation array were sent as part of the Molecular Characterization Initiative through the Children's Oncology Group. No fusions were observed by Archer FusionPlex. DNA-based methylation array classified the tumor as SHH-activated medulloblastoma. Paired tumor/normal ES revealed a somatic in-frame 6-base pair duplication in exon 18 of *PTCH1* (NM_000264.5 [*PTCH1*]: c.3125_3130dupTGTGCG, p.Val1042_Cys1043dup) (Figure 1a). Although the functional impact of this in-frame duplication has not been established, this variant has been previously described in an odontogenic keratocyst (Gu et al., 2006), a tumor commonly observed in the setting of Gorlin syndrome and associated with *PTCH1* variation (Qu et al., 2019). While uncommon, in-frame insertions and deletions are described in SHH medulloblastoma (Northcott et al., 2017; Skowron et al., 2021), including a similar duplication (c.3130_3131insTGTGCG: p. Ala1044delinsValCysAla) within exon 18 (Skowron et al., 2021). Given this evidence, there is support that this variant may be deleterious. A second hit in *PTCH1* was not appreciated in either the germline or somatic sequence variants or copy number. In the absence of a *PTCH1* second hit consistent with the mechanism of tumorigenesis, a manual review of the sequencing reads was performed, which revealed a significant drop in coverage within exon 2 of *PTCH1* in the germline peripheral blood sample at nearly a heterozygous allele frequency (Figure 1b). This finding was observed at a similar allele frequency in the tumor sample. Soft-clipped bases which did not align with the reference genome were observed at the region of *PTCH1* disruption. This soft-clipped sequence did not align within the human reference genome (GRCh38) using Ensemble BLASTN or University of California Santa Cruz blat tools, nor did this sequence generate a stop codon. Therefore, this germline variant was reported as NM_000264.5 (*PTCH1*): c.361_362ins? p.? with a recommendation for additional testing to further clarify the origin of this inserted sequence and confirm that it results in loss-of-function of *PTCH1*. Clinical RNA sequencing of *PTCH1* was performed (Ambry, CA), which revealed the presence of an Alu insertion within exon 2 of *PTCH1* (NM_000264.5 [*PTCH1*]: c.361_362insAlu). This finding is predicted to result in loss-of-function of *PTCH1* and is causal for the clinical phenotype of this patient. Additionally, whole chromosome gains of 2, 4, 7, 15, and 21, a gain of terminal 12p, and a loss of distal 13q were observed in the tumor (Figure 1c). The germline copy number analysis was negative.

3 | DISCUSSION

RNA sequencing complements DNA-based methods, offering a functional context for identified variants that may impact RNA splicing (Ergin et al., 2022; Hong et al., 2020). Adding RNA sequencing to germline genetic testing can enhance accuracy and clinical sensitivity compared to DNA sequencing alone (Bouras et al., 2023; Horton et al., 2024; van Schouwenburg et al., 2015). Recently, Horton et al. (2024) found that concurrent DNA and RNA sequencing in a cohort of 43,524 individuals undergoing hereditary cancer testing had a significant impact on variant classifications. Specifically, RNA sequencing influenced the classification of variants in 1.3% of participants. Among those affected, 97 individuals received clinically relevant upgrades in variant classification, and 70 individuals had their variants reclassified from variants of uncertain significance to P/LP variants. Additionally, 27 individuals harbored a novel deep intronic P/LP variant, which was detectable only through RNA sequencing. The authors concluded that RNA sequencing plays a crucial role in refining the classification of genetic variants associated with hereditary cancer risk (Horton et al., 2024). This combined approach enhances the likelihood of detecting relevant P/LP variants and offers greater precision for individuals who are undergoing genetic assessment to evaluate their susceptibility to cancer predisposition syndromes (Aney et al., 2024; Horton et al., 2024).

In this case, paired ES revealed a somatic 6-bp in-frame insertion in *PTCH1*. Given the typical mechanism of tumorigenesis for this gene is a two-hit model, germline and somatic variants and copy number alterations were closely reviewed but failed to yield a second alteration in this gene using standard variant calling tools. Therefore, a manual review of sequencing reads was performed, and a germline insertion of unclear etiology within exon 2 of *PTCH1* was identified. Notably, GS was performed by an outside commercial laboratory, which failed to identify the Alu insertion in *PTCH1*. This may be due to limitations on filtering and manual data review or the low depth of coverage (38×) relative to the ES data, which had ~200× and 350× depth across this region of *PTCH1* in the germline and somatic tissues, respectively. RNA sequencing was performed by an outside commercial laboratory which further clarified the germline *PTCH1* insertion as an Alu insertion (NM_000264.5 [*PTCH1*]: c.361_362insAlu), which was interpreted as LP and likely disrupts *PTCH1*. This variant results from the insertion of an Alu element between nucleotides 361 and 362 in exon 2 of *PTCH1*. Overall, this case highlights the need for careful review of sequencing data when a single gene etiology is suspected and utilization of RNA sequencing to assist in understanding functional impact.

Alu elements belong to a class of non-long terminal repeat retrotransposons, known as short interspersed nuclear elements, which are highly represented in the human genome (Lander et al., 2001). Alu

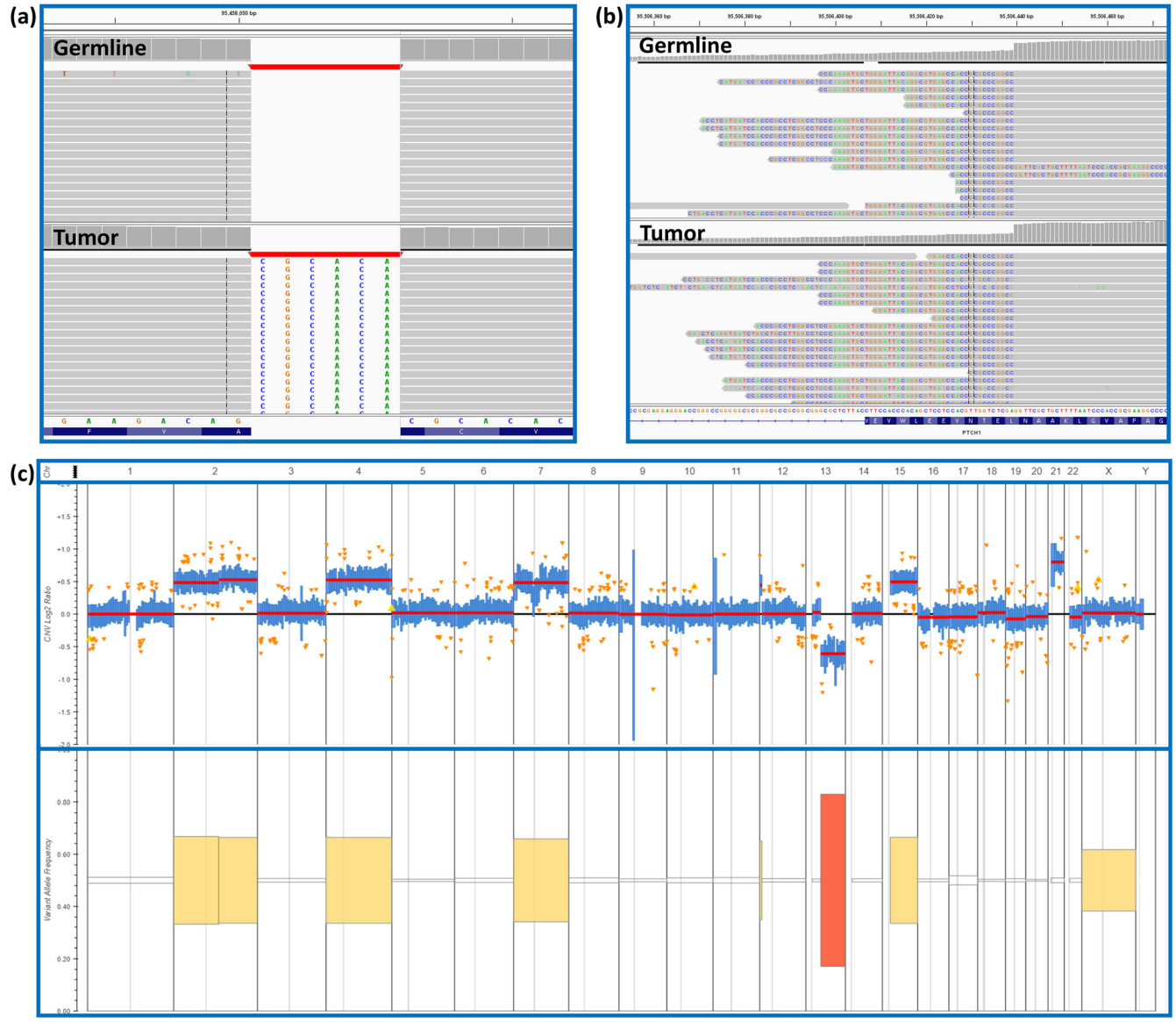

FIGURE 1 (a) Paired tumor/normal exome sequencing (ES) revealed a 6-bp in-frame duplication in exon 18 of *PTCH1* (NM_000264.5 p. Val1042_Cys1043dup). This variant was only identified in the tumor sample and is thus somatic in nature. (b) Paired tumor/normal ES revealed a drop in coverage within exon 2 of *PTCH1*. Soft-clipped reads which do not align to the human reference genome (GRCh38) are visualized. This event was identified in both the germline peripheral blood and tumor sample at a similar variant allele frequency. (c) Somatic copy number analysis. Tumor copy number relative to a panel of normal in log 2 scale is displayed on the top plot. Blue bins represent log 2 values based on sequence depth in 100-bp windows. The yellow and orange triangles represent focal exon and gene level calls, while the red lines indicate segmented copy number calls. The bottom plot displays tumor variant allele frequency for variants that are heterozygous in the normal. The spreading of the bars indicates loss of heterozygosity. The *x*-axis denotes the chromosome number.

elements are ~300 bp in length mobile elements which are found within noncoding regions of the genome, such as intronic sequence, 3′ untranslated sequence, and intergenic regions, and may influence gene and transcript function through altered gene expression and splicing (Deininger, 2011). Although infrequent, mobile Alu element insertions have been shown to contribute to germline disease by either disrupting the coding sequence or inducing aberrant splicing (Belancio et al., 2010; Deininger, 2011; van der Klift et al., 2012). Notably, germline Alu insertions, similar to what is described in this individual, have been described in multiple genes associated with cancer predisposition syndrome (Qian et al., 2017). However, neither germline nor somatic Alu insertions in *PTCH1* have been reported to our knowledge.

With the vast majority of novel information on genes associated with cancer predisposition syndromes, studies are moving from panel testing to more comprehensive testing, including ES, GS, and RNA sequencing, to clarify the genetic etiology of cancer predisposition syndromes (Davidson et al., 2023; Ergin et al., 2022; Esteban-Jurado et al., 2015; Hutchcraft et al., 2024). Enhanced accuracy in genetic testing can significantly impact medical management (Davidson

et al., 2023). It can inform surveillance for at-risk patients, aiding in the early detection of cancer. Additionally, targeted testing facilitates the identification of family members who may be at risk of developing cancer (Davidson et al., 2023; Horton et al., 2024). However, the current cost effectiveness of thorough genetic testing must be considered and, for the time being, may necessitate a narrower testing strategy, including a step-by-step approach (Davidson et al., 2023).

In summary, the inclusion of comprehensive NGS testing, including DNA and RNA sequencing, can lead to better diagnostic outcomes and more precise identification of cancer susceptibility in clinically suspected but traditionally undetected cases of Gorlin syndrome. This case highlights a unique mechanism of inactivation of *PTCH1* and the importance of a thorough analysis of sequencing data when the clinical presentation is consistent with a single genetic etiology.

AUTHOR CONTRIBUTIONS

Aaron Y. Mochizuki: Clinical management; initial assessment of the case; drafting and revision of the manuscript. **Chinmayee B. Nagaraj:** Initial assessment of the case; genetic testing; interpretation of genetic testing; manuscript revision. **Douglas Depoorter and Kathleen M. Schieffer:** Analysis of the exome sequencing; identification of the possible pathogenic variant in *PTCH1*; manuscript revision. **Sun Young Kim:** Conception; interpretation of genetic testing; literature review; drafting and revision of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

INFORMED CONSENT

The patient's family consented to the publication of this case report and signed the informed consent form.

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