GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Case Report

Multiple nevoid basal cell carcinoma syndrome associated with congenital orbital teratoma, caused by a *PTCH1* frameshift mutation

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Genet. Mol. Res. 13 (3): 5654-5663 (2014)
Received January 15, 2014
Accepted May 26, 2014
Published July 25, 2014
DOI http://dx.doi.org/10.4238/2014.July.25.21

ABSTRACT. Gorlin-Goltz syndrome, or nevoid basal cell carcinoma

syndrome (NBCCS), is a rare autosomal dominant disorder caused by mutations in the *PTCH1* gene and shows a high level of penetrance and variable expressivity. The syndrome is characterized by developmental abnormalities or neoplasms and is diagnosed with 2 major criteria, or with 1 major and 2 minor criteria. Here, we report a new clinical manifestation associated with this syndrome in a boy affected by NBCCS who had congenital orbital teratoma at birth. Later, at the age of 15 years, he presented with 4 major and 4 minor criteria of NBCCS, including multiple basal cell carcinoma and 2 odontogenic keratocysts of the jaw, both confirmed by histology, more than 5 palmar pits, calcification of the cerebral falx, extensive meningeal calcifications, macrocephaly, hypertelorism, frontal bosses, and kyphoscoliosis. *PTCH1* mutation analysis revealed the heterozygous germline mutation c.290dupA. This mutation generated a frameshift within exon 2 and an early premature stop codon (p.Asn97LysfsX43), predicting a truncated protein with complete loss of function. Identification of this mutation is useful for genetic counseling. Although the clinical symptoms are well-known, our case contributes to the understanding of phenotypic variability in NBCCS, highlighting that *PTCH1* mutations cannot be used for predicting disease burden and reinforces the need of a multidisciplinary team in the diagnosis, treatment, and follow-up of NBCCS patients.

Key words: Basal cell carcinoma; Gorlin-Goltz syndrome; Orbital congenital teratoma; *PTCH1* gene

INTRODUCTION

Nevoid basal cell carcinoma syndrome (NBCCS, MIM No. 109400), also known as Gorlin-Goltz syndrome, is a rare autosomal dominant inherited disorder with complete penetrance and variable expression. The estimated prevalence varies from 1 in 57,000-256,000, with a male-to-female ratio of 1:1 (Lo Muzio, 2008). The condition is characterized by a wide range of developmental abnormalities and predisposition to neoplasms. Clinical diagnosis relies on 2 specific major criteria or on 1 major criteria and 2 minor criteria (Table 1). The main clinical criteria include multiple basal cell carcinomas (BCC), odontogenic keratocysts (OKCs) of the jaws, palmar or plantar pits, skeletal abnormalities, intracranial ectopic calcifications, and facial dysmorphism (Lo Muzio, 2008).

NBCCS is caused by germline mutations in the human homolog of the *Drosophila* patched gene, *PTCH1* (MIM No *601309), which is located on chromosome 9q22.32 (Hahn et al., 1996; Johnson et al., 1996). The human *PTCH1* gene consists of 23 exons and encodes a glycoprotein of 1447 amino acids containing 12 transmembrane-spanning domains and 2 large extracellular loops. *PTCH1* is a particularly interesting gene because it acts both as a development gene and as a tumor suppressor gene. During embryogenesis, *PTCH1* serves as a receptor for the secreted sonic hedgehog protein and is important for proper proliferation,

differentiation, and patterning in nearly every tissue and organ. Gene analysis in NBCCS patients identified more than 230 mutations (including deletion, insertion, splice site alteration, nonsense, and missense mutations), over 80% of which result in a frameshift or premature truncation of the encoded protein; the remaining mutations lead to production of an abnormal receptor (Boutet et al., 2003; Lo Muzio, 2008). All *PTCH1* mutations have been predicted to cause alterations in the hedgehog pathway during development.

Here, we describe the case of a boy who presented with multiple basal cell carcinomas associated, for the first time, with congenital orbital teratoma. Because the patient's clinical features prompted suspicion of NBCCS, we performed mutational analysis of the *PTCH1* gene. We identified a germline frameshift mutation, c.290dupA (p.Asn97LysfsX43), present in a heterozygous state. The results of our study reinforce the role of a multidisciplinary team, which is mandatory for the diagnosis, treatment, and follow-up of NBCCS patients.

Table 1. Comparison of diagnostic criteria for nevoid basal cell carcinoma syndrome with the case reported.

Criteria	Patient
Major	
>2 Basal cell carcinomas (BCCs) or 1 nevoid cell carcinoma (age, <20 years)	Multiple (>8 BCCs), first at the age of 15 years
Odontogenic keratocysts (OKCs) of the jaw	2 OKCs, confirmed by histology
>3 Palmar pits	>5 Palmar pits
Bilamellar calcification of cerebral falx	Spotted meningeal calcification
Bifid or fused ribs	No
First degree-affected relative	No
Minor	
Medulloblastoma	No
Macrocephaly	Yes
Congenital malformations	Giant congenital orbital teratoma and hypertelorism
Skeletal malformations	Scoliosis

MATERIAL AND METHODS

A peripheral blood sample was collected from the suspected NBCCS patient after obtaining written informed consent from the legal guardians of the patient. Total genomic DNA was extracted automatically using the MagNA Pure Compact Nucleic Acid Isolation Kit (Roche; Basel, Switzerland). All coding exons of the *PTCH1* gene (GenBank accession No. NM_000264.3), including their splice junctions, were amplified using self-designed primers (Table 2). The polymerase chain reaction was conducted in a total volume of 25 µL containing 200 ng genomic DNA, 400 nM primers, 1X GC-RICH PCR buffer, 1 M GC-RICH resolution (GC-RICH PCR System; Roche), 200 mM of each dNTP, and 2 U GC-RICH PCR enzyme mix (Roche). The reaction included 30 cycles at 95°C for 30 s, 56°C for 30 s, and 72°C for 1 min. Fragments were screened for mutations by direct sequencing using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; Foster City, CA, USA). The sequencing products were separated in an Applied Biosystems® 3500 DX Genetic Analyzer and analyzed using the Sequencing Analysis 5.3.1 software (Applied Biosystems).

Table 2. Primers for the sequencing of all coding exons and intron-exon boundaries of the *PTCH1* gene.

Exon	Name	Sequence (5'-3')	Amplicon (bp)	Tm (ºC)
1	PTCH1-E1-F	CGCAATGTGGCAATGGAAGG	835	64
	PTCH1-E1-R	GCAGGAGGAAGAAGTTCAGGGC		
2	PTCH1-E2-F	CTTTATGACCGAGCCCCG	505	58
	PTCH1-E2-R	GCCCAAACAATAAACAATCCC		
3	PTCH1-E3-F	GCCCCCCACTTTCGTCACAC	530	64
	PTCH1-E3-R	ACCAGCAGCCTTCTCCCACC		
4-5	PTCH1-E4-5-F	TTGCTGGGTCTCTACTTGGC	528	58
	PTCH1-E4-5-R	TTTCAATGTTTTTATTTCTTGTTCA		
6	PTCH1-E6-F	GCAGATATGCTGGAAAGGAG	439	54
	PTCH1-E6-R	ATAAAGTGAACGATGAATGGAC		
7	PTCH1-E7-F	CTCTGAAACACACAAGCCCT	287	58
	PTCH1-E7-R	GGAGGGAAGTGGCTTTTGA		
8	PTCH1-E8-F	AGCCAGTGAGTTGGGGGAG	336	58
	PTCH1-E8-R	TAAAGCGAATGGAAAGAAATG		
9	PTCH1-E9-F	GCACCCACTGCCACTGATTA	441	58
	PTCH1-E9-R	TAAGAAGCAGGAGCAGTCAT		
10	PTCH1-E10-F	GTGATGGGTGGAGGGAAAC	435	58
	PTCH1-E10-R	GACTTCCTACCCACTTCCCTGA		
11	PTCH1-E11-F	GAGTTGGATTCCTTCTCATTTAC	501	54
	PTCH1-E11-R	GCTTATTTCATTGACTGGCA		
12	PTCH1-E11-F	GTGTTAGGGGGTAAGGCAG	404	56
	PTCH1-E11-R	CAGGCATTTCTATTTCACTTCAT		
13	PTCH1-E13-F	AGAGGAAAGGGAAAGAAAAAT	289	56
	PTCH1-E13-R	GAGTTCTCTCACAGCACCATTC		
14	PTCH1-E14-F	TACACAGTGAAAAATGGCAGA	554	54
	PTCH1-E14-R	CAATCTGATGAACTCCAAAGG		
15	PTCH1-E15-F	GAGAACAACCCCTACAAGATAAAT	743	58
	PTCH1-E15-R	GGAAAGAGCACCAGAAGCCT		
16	PTCH1-E16-F	CGTGTCTTTCCGTCGCACTC	546	62
	PTCH1-E16-R	CTGTCAAGCAGCCTCCACCA		
17	PTCH1-E17-F	GGTGTTCTGATGGGATTTTCG	531	60
	PTCH1-E17-R	ATGTGATAGAGTGCGGGGGT		
18	PTCH1-E18-F	AGACCCCTCACAAAGAATGAC	623	56
	PTCH1-E18-R	GCCCAGACATAAACAAAACTT		
19	PTCH1-E19-F	AGTCAGTCCATTCATTGTTTTG	453	54
	PTCH1-E19-R	ATACCCCTCTCCTTAGCCTC		
20	PTCH1-E20-F	GTCAACACCAAATATGACCCAGTG	956	56
	PTCH1-E20-R	TTTCTAACCCGAGACAATAATG		
21	PTCH1-E21-F	TTGTTCATTTCTGGCGTTGC	273	60
	PTCH1-E21-R	AGTGAAGAGCGGCACAGGA		
22	PTCH1-E22-F	GCCCCTGAAAAATACCGTG	450	58
	PTCH1-E22-R	TCTGCCTGTGTGATGTGCTG		
23	PTCH1-E23-F	GGCTTTTCTTTTGTGGGTGG	957	60
	PTCH1-E23-R	GTGGTGCTGTTTGTGTCCTTG		

RESULTS

The patient was the first child of nonconsanguineous and clinically unaffected parents, born spontaneously after an uncomplicated pregnancy at 40 weeks of gestation. He had severe right proptosis at birth. Computerized tomography showed a right orbital soft tissue mass without intracranial extension. Histopathological examination revealed various mature tissues of all 3 embryonic germinal cell lines, representing a congenital orbital teratoma. This teratoma was successfully managed by multiple surgeries during the patient's first year of life.

At the age of 15 years, the patient was evaluated at the Dermatology Department of Hospital do Divino Espírito Santo de Ponta Delgada, EPE, for multiple skin-colored round papules affecting the face, primarily the nose, forehead, left zygomatic, and left temporal areas.

All of these lesions were excised. Pathological examination revealed that all lesions were BCC (Figure 1). In addition, panoramic radiography performed for orthodontic purposes showed 2 radiolucid images, suggesting multiple OKCs in the inferior jaw (Figure 2). The patient was then examined by a maxillofacial surgeon who removed the tumor using the enucleation technique. Histopathological analysis confirmed OKCs, showing characteristically parakeratinized epithelium (Figure 3). These 2 features, BCC and OKCs, prompted clinical reevaluation, which revealed macrocephaly, ocular hypertelorism, kyphoscoliosis, and multiple palmar pits (more than 5) in both hands (Figure 4). Imaging exams identified calcification of the falx cerebri, as well as spotted meningeal calcifications by head computed tomography (Figure 5). During the subsequent 4 years of follow-up, the patient had 2 or 3 BCCs excised *per* year, and began treatment with topical imiquimod and oral retinoids beyond solar protection.

At the age of 22 years, the genetic basis of the patient's disease was investigated by mutational analysis of the *PTCH1* gene. One heterozygous mutation, c.290dupA, was identified at codon 97 in exon 2 (Figure 6). This dupA mutation generated a frameshift predicted to result in a prematurely truncated protein, p.Asn97LysfxX43, with only 138 amino acids. The protein lacked the 1309 C-terminal amino acids of the full length protein. Moreover, the mutation was likely a *de novo* mutation, as his parents were unaffected.

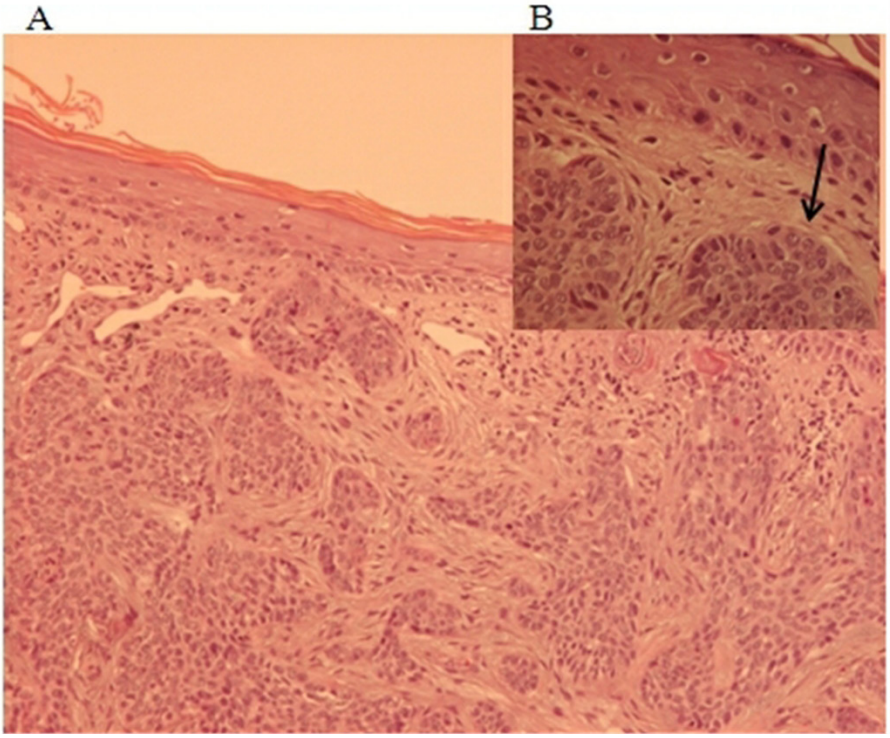

Figure 1. Histopathological findings of solid basal cell carcinoma. (**A**) Hematoxylin and eosin (H&E) stain (100X original magnification) with characteristic palisade arrangement of the nuclei forming a peripheral cell layer. (**B**) H&E stain (400X original magnification) shows the retraction artifact between the tumor and stroma (arrow).

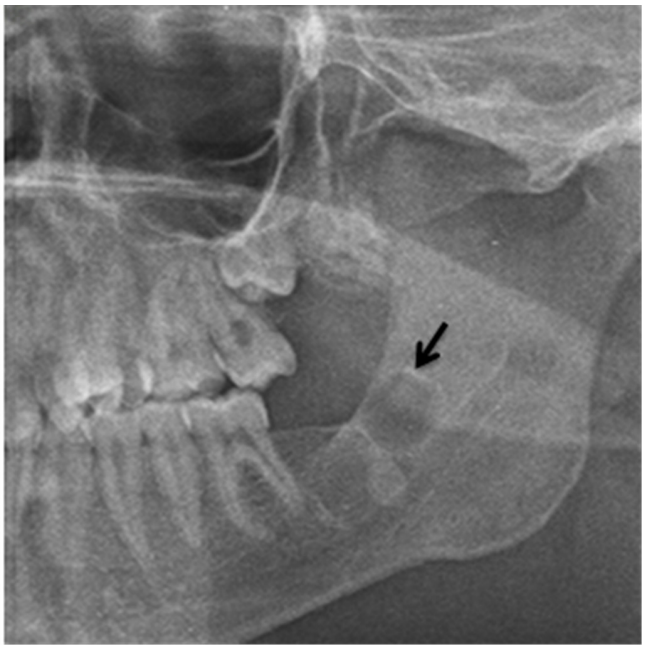

Figure 2. Imaging findings of nevoid basal cell carcinoma syndrome. Orthopantographic examination suggested the presence of odontogenic keratocysts in the mandible (arrow).

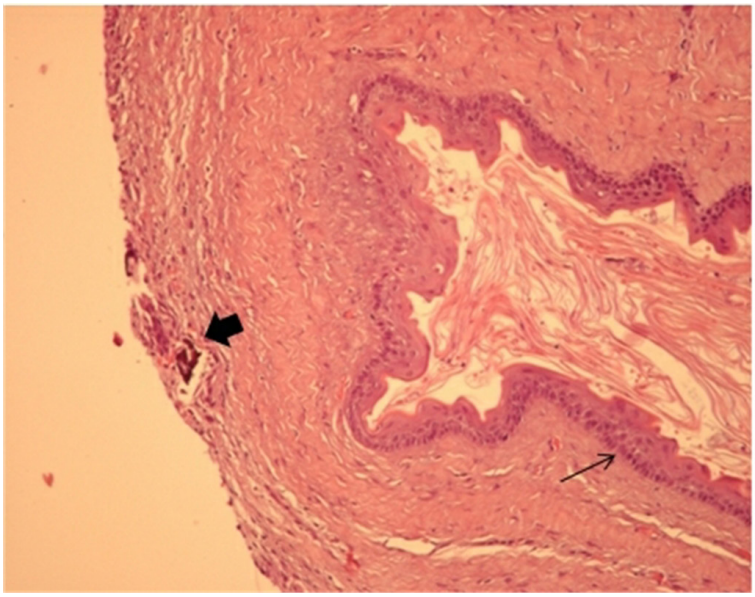

Figure 3. Histopathological features of odontogenic keratocysts (hematoxylin and eosin, 100X original magnification) with dystrophic calcifications (bold arrow). The image shows the lining parakeratinized stratified squamous epithelium with a well-defined basal layer of palisading (thin arrow).

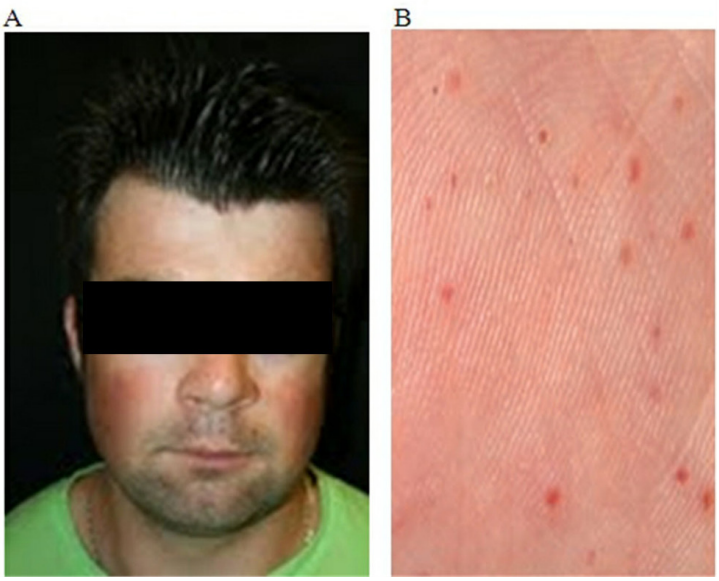

Figure 4. Clinical features of nevoid basal cell carcinoma syndrome. **A.** Facial appearance: macrocephaly, frontal bossing, and hypertelorism. Multiple scars from resection of basal cell carcinomas. Ectropion of the right eye (iatrogenic). **B.** Palmar pits.

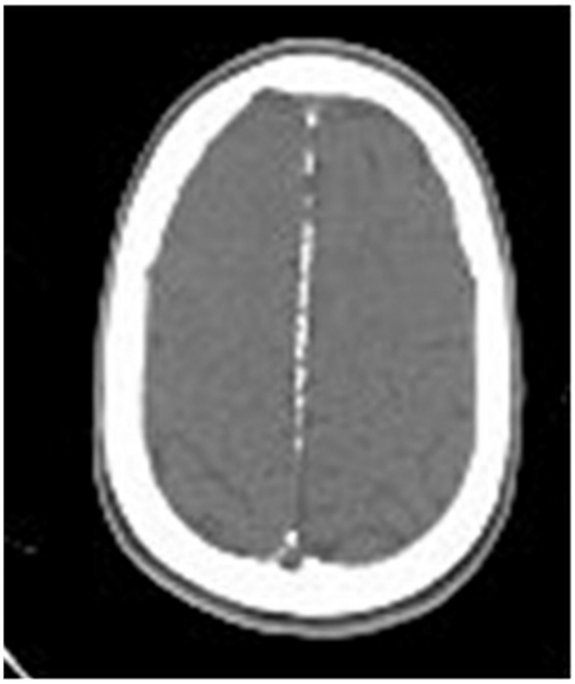

Figure 5. Tomography showing calcification of the cerebral falx.

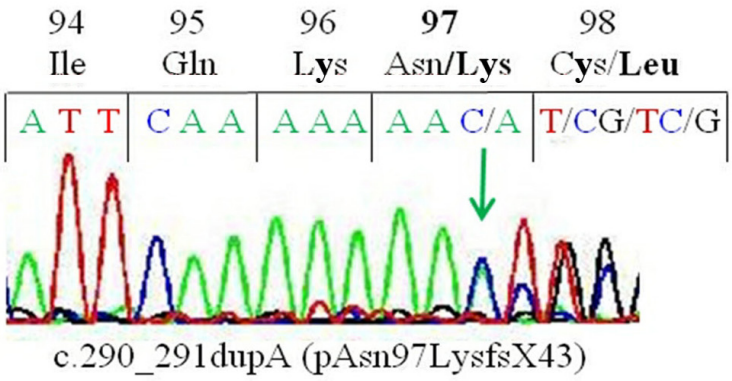

Figure 6. Partial sequence analysis of the *PTCH1* gene in the patient with nevoid basal cell carcinoma syndrome. The electropherogram of genomic DNA revealed a heterozygous frameshift mutation, c.290dupA (p.Asn97LysfsX43), in exon 2 of the *PTCH1* gene.

DISCUSSION

Here we report the case of a boy with various features that resulted in the clinical diagnosis of NBCCS. Multiple BCCs in a teenager should alert physicians to the possibility of a genetic syndrome. This should be followed by careful physical examination that includes the search for other symptoms and signs (Efron et al., 2008).

NBCCS patients have a higher risk of ocular problems, including hypertelorism, exofthalmus, internal strabism, anterior segment dysgenesis with congenital cataracts, vitreoretinal anomalies, coloboma of the iris, choroid and optic nerve microphthalmia, and orbital cysts (Ragge et al., 2005; Lo Muzio, 2008; Yamamoto et al., 2011). Previously, no case of congenital orbital teratoma associated with NBCCS had been described. Our patient had a congenital orbital teratoma during the neonatal period. Neonatal neoplasms are very rare, and their incidence is estimated to be approximately 1 in every 12,500-27,500 live births (Moore et al., 2003). Pediatric germ cell tumors represent only 1-3% of childhood tumors, both in gonadal and extragonadal sites; the latter is typically in midline locations, such as the sacrococcygeal area, retroperitoneum, mediastinum, neck, and intracranial region (Isaacs, 2004), rarely presenting as orbital teratomas. A congenital orbital teratoma is composed of 1 or more embryonic germ layers (ectoderm, mesoderm, and endoderm) and contains tissue that is typically foreign to the anatomic site of origin. Primordial germ cells are originated in the allantois of the yolk sac endoderm around the 4th fetal week, and then they migrate along the dorsal mesentery to the genital ridge, arriving around the 6th fetal week, where they develop into gonads. Arrested migration is thought to account for germ cell deposition at nongonadal sites, whereas aberrant migration deposits cells that develop into germ cell tumors (Rescorla, 2012). Genetic factors have been shown to play a major role in development of tumors during the neonatal period. Neonatal neoplasms are associated with congenital malformations in 15% of cases, there are genetically determined syndromes with an increased risk of malignancy, and environmental factors have a limited influence on oncogenesis during this period (Moore et al., 2003; López et al., 2006).

We hypothesize that this rare phenotype likely results from the shortened and nonfunctional truncated PTCH1 protein (p.Asn97LysfxX43), which is expressed from the mutated allele (c.290dupA). To date, more than 80% of reported PTCH1 mutations responsible for NBCCS are nonsense, splice site, insertion, deletion, or duplication mutations that lead to expression of nonfunctional truncated proteins (Boutet et al., 2003; Lo Muzio, 2008), some of which have been confirmed experimentally (Fujii et al., 2003; Pastorino et al., 2005; Pan et al., 2010; Suzuki et al., 2012). These mutations spread along the *PTCH1* gene with no preferential hotspot and can arise by several mechanisms. The c.290dupA mutation may be the result of a slippage of DNA polymerase along the stretch of 7 consecutive adenine nucleotides (wild-type allele position: c.284_c.290) during DNA replication. This error adds 1 adenine nucleotide, c.290_c.291dupA, disrupting the open reading frame of the *PTCH1* mRNA. The mutated allele, which is originated with 8 adenine nucleotides, also escapes the repair process. Because more than 100 *PTCH1* germline mutations associated with NBCCS have been reported and because our patient is the first case in his family (his parents and 1 young sister were clinically unaffected), we propose that the mutation occurred *de novo* in parental germline cells (prezygotic); however, we cannot exclude a mutational event in cells after fertilization (postzygotic) very early in embryogenesis. However, identification of the disease-causing mutation in the reported case provides information that is useful for genetic counseling.

PTCH1 mutations that have been described are primarily located in exons and spread throughout the gene, indicating the absence of a mutational hotspot. Although no founder mutation has been described in the literature, 2 specific mutations in different exons have been reported (Chidambaram et al., 1996; Wicking et al., 1997; Boutet et al., 2003; Ponti et al., 2012). The c.290dupA mutation identified in this Azorean patient is recurrent, as it was also recently described in a Japanese patient with multiple BCCs, jaw OKCs, calcification of the falx cerebri, and a meningioma at the age of 66 years (Kijima et al., 2012). These 2 patients had a different clinical outcome, indicating the involvement of modifier genes that are distinct from the disease locus, as identified for instance in neurofibromatosis type 1 (Bahuau et al., 2001; Ponti et al., 2011), or may have occurred because of different genetic backgrounds (Sunyaev, 2012). In addition, we cannot exclude the effects of epigenetic and environmental factors on the final NBCCS phenotypic outcome, as the same *PTCH1* mutation in monozygotic twins displays different clinical features (Matsuzawa et al., 2006). The case reported here supports the absence of genotype/phenotype correlation in NBCCS. This aspect is clinically relevant, as *PTCH1* mutations do not provide further information regarding prognosis, likely age of onset of BCC, or clinical disease burden.

In summary, the present report describes, for the first time, an NBCCS patient with congenital orbital teratoma caused by a heterozygous frameshift mutation in exon 2 of the *PTCH1* gene. This case increases the understanding of phenotypic variability and highlights that *PTCH1* mutations cannot be used to predict disease burden. In addition, our results reinforce the role of a multidisciplinary team in the diagnosis, treatment, and follow-up of Gorlin-Goltz syndrome patients, as recently proposed by Kiwilsza and Sporniak-Tutak (2012).

ACKNOWLEDGMENTS

The authors gratefully acknowledge the cooperation of the patient, including the permission to publish photographs. They also wish to thank Claudia Castelo Branco, Renato

Pires, and Claudia Nóbrega for providing useful comments on the article.

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