Case study

Multidisciplinary Approach for Mapping Genetic Variants in Naevoid Basal Cell Carcinoma Syndrome. Newly Identified Patched 1 Mutations in Half-Sisters.

ABSTRACT

Objective: Naevoid basal cell carcinoma syndrome is a rare and autosomal dominant inherited condition with variable expressivity. Several genetic mutations have been identified but clear genotype-phenotype correlations have not been demonstrated; therefore, the diagnosis is still based on clinical criteria that may not be present or are not recognized. Numerous medical specialties have a role in the care of these patients, so the number of cases treated in a single centre is usually low, thus making the implementation of standardized diagnostic and treatment protocols difficult.

Materials and Methods A multidisciplinary research team was set up to collect, diagnose and treat naevoid basal cell carcinoma syndrome patients according to the same principles. All patients suspected for this syndrome underwent the same diagnostic steps involving molecular genetic investigation.

Results: One of the first results of this study was the identification of a novel mutation of the patched-1 gene in two maternal half-siblings. Both patients fulfilled the specific criteria for diagnosis of naevoid basal cell carcinoma syndrome. Detailed descriptions of clinical, radiological and genetic findings are given in this paper. Molecular genetic analysis identified a new deletion mutation in exon 13 of the Patched 1 gene in both half-siblings.

Conclusion: Genetic mapping of mutations can facilitate our understanding of the correlation between genetic background and clinical manifestations.

Keywords: naevoid basal cell carcinoma syndrome, odontogenic keratocysts, basal cell carcinoma, genetic polymorphism, mutation

1. INTRODUCTION

Gorlin syndrome, also known as naevoid basal cell carcinoma syndrome (NBCCS), is a rare, hereditary condition that is characterized by a wide range of developmental disorders and a predisposition to different malignancies. It was first reported by Jarisch and White in 1894 and recognized as a syndrome by Gorlin and Goltz in 1960[1-3]. Its incidence is difficult to determine due to its rarity and misdiagnosis. It is estimated to affect an average of 1 in 19.000 to 1 in 256.000 people worldwide, with a male-to-female ratio of 1:1[4, 5]. Its signs and symptoms are extremely variable; the central nervous, ocular, auditory, genitourinary, cardiovascular

and skeletal systems may be affected [6]. NBCCS is most frequently characterized by the development of multiple jaw keratocyst and/or basal cell carcinomas. Keratocysts usually first appear during adolescence and rarely develop after the age of 30 [7]. The diagnosis of NBCCS is usually based on clinical criteria that were suggested by Evans et al. in 1993 and later modified by Kimonis et al. in 2004[8,9]. According to the current principles, the diagnosis can be established if two major criteria (basal cell carcinomas, odontogenic keratocysts, palmar and/or plantar pits, ectopic calcifications of the falx cerebri, bifurcated ribs or a first degree relative with NBCCS) and one minor criterion (spina bifida, macrocephaly, cleft lip and palate, hypertelorism, ovarian fibroma, medulloblastoma) or one major and three minor criteria are present [10]. Approximately, 70%–80% of NBCCS patients have an affected parent and the remaining 20%–30% are due to a de novo pathogenic variant [10,11]. In most cases, mutations of the patched-1 (PTCH1) gene are responsible for the development of NBCCS [12,13]. The PTCH1 is a tumour suppressor gene, a member of the sonic hedgehog pathway (SHH). It encodes a membrane receptor Patched (Ptch), which binds and inhibits another transmembrane protein and activator, smoothened (Smo), thus inactivating SHH pathway signalling. The SHH pathway plays an essential role during embryonic development. Aberrations of this pathway are associated with birth defects or tumorigenesis. Despite investigations, the molecular mechanism of the interplay among Ptch and Smo remains undefined [14]. Mutations in other genes in the hedgehog signalling pathway, such as patched-2 (PTCH2) and suppressor of fused homolog (SUFU) genes, have also been associated with this syndrome. These may be deletions, insertions, splice site alterations, nonsense or missense mutations [15]. Clear genotype-phenotype correlations have not been demonstrated [16]. Multi-layered mutations in genes involved in the hedgehog pathway may explain the wide phenotypic variability of Gorlin–Goltz syndrome [17]. If clinical features are inconclusive, identification of a *PTCH1* or *SUFU* pathogenic variant on molecular genetic testing can establish the diagnosis [10]. Occasional variants in *PTCH2* may not be conclusive [18].

2. AIMS

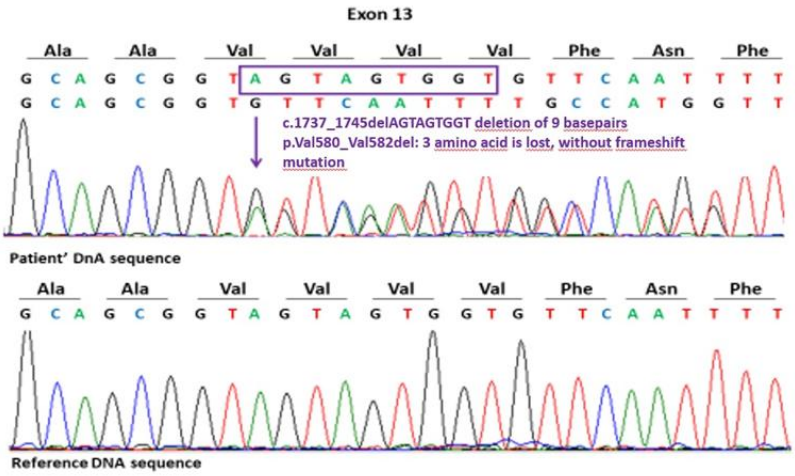
Because of the variable expressivity, a significant part of the NBCCS patients is not recognized as they are scattered amongst different medical specialties. Due to the low incidence and diagnostic difficulties, studies with a large number of patients are hardly available [18]. To understand this complicated disorder better, information on a large number of patients is necessary. The aims of this study were to diagnose and treat NBCCS patients from different medical centres according to the same principles and to collect data for further analysis.

3. MATERIAL AND METHODS

A multidisciplinary team, including oral and maxillofacial surgeons, dermatologists, radiologists, and clinical geneticists worked out a diagnostic protocol for all patients suspected of NBCCS. Panoramic radiography, chest x-ray (chest wall deformity) posterior-anterior skull radiography (falx cerebri), abdominal and pelvic ultrasound are performed in all cases. If a genitourinary or a central nervous system disorder is suspected, magnetic resonance imaging of the region of interest is carried out too.

Routine chromosomal analysis was performed by standard techniques. Genomic DNA was extracted from peripheral blood samples with the Puregene kit (Gentra) after obtaining informed consent. The Illumina Trusight One Exome Sequencing Panel (Illumina Inc., San Diego, CA, USA) covering the coding region of 4813 clinically relevant genes was applied using Illumina MiSeq (Illumina Inc., San Diego, CA, USA). Variants were filtered based on severity and frequency against public variant databases including single-nucleotide polymorphism database (dbSNP), ClinVar, Exome Aggregation Consortium (ExAC), Exome Variant Server (EVS) and in-house clinical exome database of 360 unrelated Hungarian persons. The presence of the identified variant was confirmed by Sanger sequencing using primers (Fig. 1).

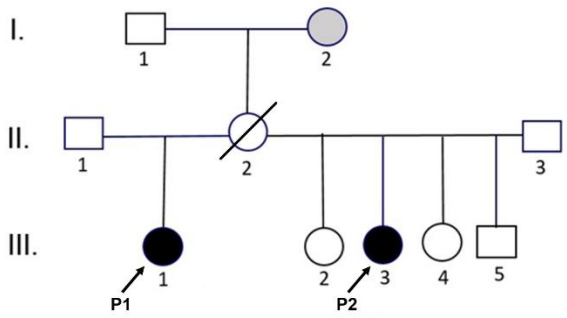
Fig 1:



4. RESULTS and DISCUSSION

A novel mutation of the patched-1 gene was detected in two half-siblings (Fig.2). Detailed clinical, radiographic, and genetic analyses were undertaken in both affected members.

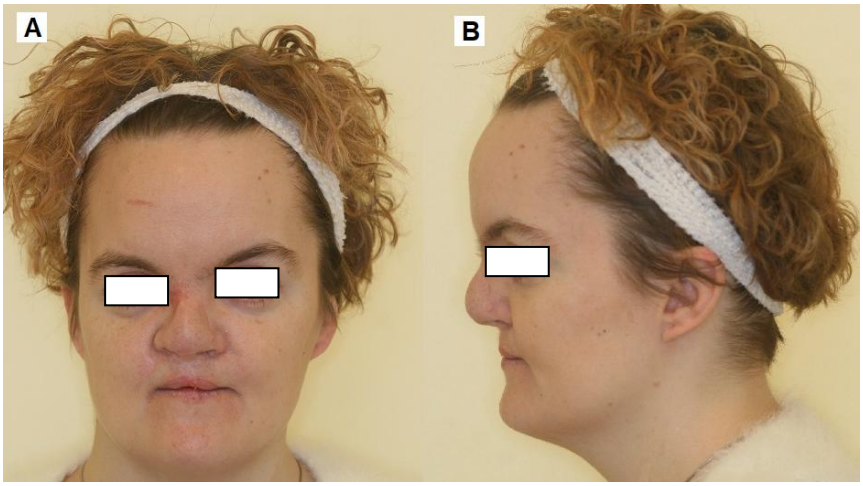
Fig 2:



Patient 1

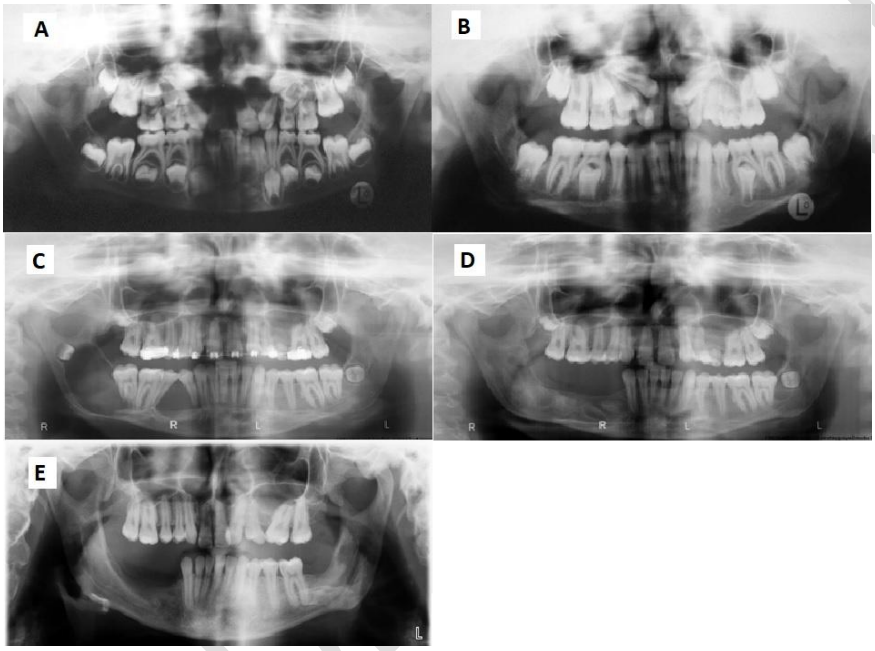
A 15-year-old girl was referred to our maxillofacial department with a huge cyst involving the whole ramus, angle and body of the mandible on the right side. On facial and oral examination, she exhibited previously operated bilateral cleft lip and palate, macrocephaly (head circumference was 59.0 cm, 99th percentile), frontal bossing, and hypertelorism. Canthal index on measurement of inner and outer canthal distance was 42.8 (Fig. 3 A, B).

Fig 3:



She was undergoing orthodontic treatment. All her lower teeth, except the third molars, had erupted. The right premolars and molars were slightly displaced. Previous routine panoramic radiographs at the ages of 6 and 12 failed to show any pathology in the mandible (Fig. 4 A, B, C).

Fig 4:



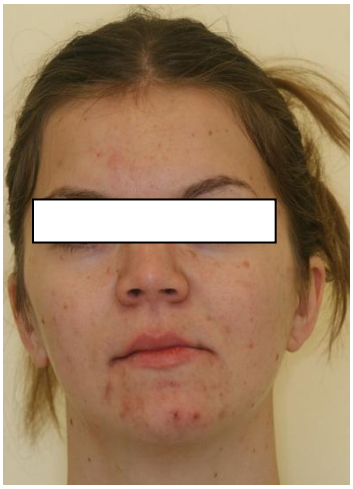
The cyst was removed together with the premolars and molars and the cystic cavity was filled with an autologous bone graft from the iliac crest. Histopathology was consistent with an odontogenic keratocyst. The diagnosis of NBCCS was made upon one major criterion (odontogenic keratocysts) and three minor criteria (cleft lip and palate, macrocephaly, hypertelorism). From her medical history, it should also be mentioned that she was under psychiatric treatment for distress, bipolar affective disorder and schizophrenia. She was diagnosed with hypothyroidism, too. A year later another cyst with the same histology was enucleated along with the left lower third molar (Fig. 4 D). At the same time, impacted upper third molars were also removed as a preventive measure. Following that, the development of further cysts was not noted (Fig. 4 E).

Although at her first visit she was free of dermatological lesions, after the age of 18 she had several basal cell cancers removed from her face.

Patient 2

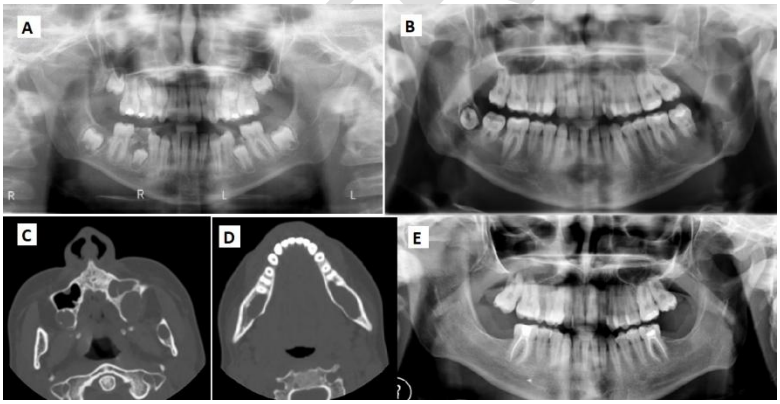
The younger maternal half-sibling of the patient mentioned above was also referred to our department with a unilateral facial swelling at the age of 17. Clinical examination revealed a prominent forehead, hypertelorism (canthal index was 42.2), and macrocephaly (head circumference was 57.0 cm, 99th percentile) (Fig. 5).

Fig 5:



A panoramic x-ray and CT scan showed multiple extended radiolucent lesions bilaterally in both jaws (Fig. 6 B, C, D). The previous X-ray at the age of 9 was normal (Fig. 6 A). Skull radiograph confirmed lamellar calcification of falx cerebri. Dermatological examination revealed pits on the soles of the feet and multiple pigmented nevi. After endodontic treatment of mandibular first molars, all the cysts were removed together with both lower second molars and the lower right third molar. Histological findings were consistent with odontogenic keratocysts from all biopsies. The diagnosis of NBCCS was established on the presence of two major criteria (multiple odontogenic keratocysts, pits on the soles) and of one minor sign (hypertelorism). One year after the first operation, panoramic x-ray revealed recurrence in the left maxillary sinus, which was enucleated (Fig. 6 E).

Fig 6:

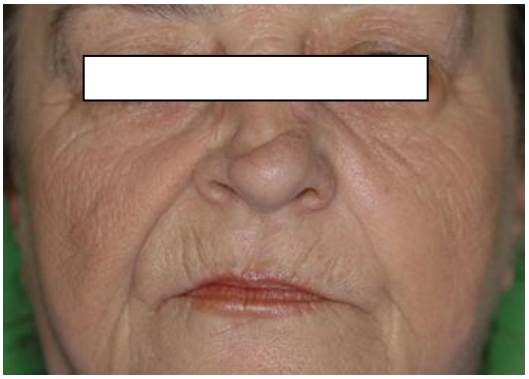


The patients' mother and grandmother

The girls' mother passed away when they were young. They were often accompanied by their maternal grandmother who had hypertelorism and reported that she had been operated with basal cell carcinoma several times (Fig. 7). Medical records of the patient's mother were not available, but according to the

grandmother, she had had a number of jaw cysts removed.

Fig 7:



Genetic investigations

Sequence analysis of SUFU was carried out without identifying any mutation. A yet to be known PTCH1 gene heterozygous mutation has been validated, and its causative role in NBCCS has been confirmed with bioinformatic analysis of the molecular genetic investigation of family members with and without clinical signs of the syndrome.

Clinical exome (Trusight One panel) sequencing revealed a heterozygous variant (NM_000264.3:c.1737_1745delAGTAGTGGT, NP_000255.2:p.Val580_Val582del) in exon 13 of the *PTCH1* gene. This variant has not been found in either dbSNP (database of Single Nucleotide Polymorphisms), ClinVar, ExAC (Exome Aggregation Consortium), HGMD or EVS (Exome Variant Server) public databases or a cohort of 360 unrelated Hungarian controls. Sanger sequencing confirmed the heterozygous mutation in the patient and revealed that her stepsister was heterozygous for this mutation, while the maternal grandmother was not a carrier. Samples were not available from the deceased mother of the girls. The confirmed genetic variation was submitted into ClinVar Database (Submission ID: SUB5336561).

5. CONCLUSIONS

In the above, two maternal half-siblings with NBCCS are presented. In both cases, signs and symptoms in the maxillofacial region raised suspicion of the syndrome, and this was confirmed by further clinical, radiological and genetic investigations. Molecular genetic analysis identified a deletion mutation in exon 13 of the PTCH1 gene in both half-siblings that has not previously been reported in the literature. Although the half-siblings have an identical mutation, their phenotypes are quite different as described by other authors [19,20]. Their mother passed away, so her genetic analysis was not possible. Clinical examination of the maternal grandmother showed one major sign (multiple basal cell carcinomas) and one minor sign (hypertelorism). Although it can be assumed that the girls inherited the disease from their mother and maternal grandmother, interestingly, the defective gene could not be detected in the grandmother's genome. If the mother was alive, we could probably set up her and the grandmother's diagnosis of NBCCS. The deceased mother is the missing link, and although she most probably had NBCCS, it cannot be proved. Without a "first degree relative with NBCCS" as a major criterion, the grandmother's diagnosis of NBCCS cannot be made either. This paradox situation highlights the inaccuracy of the present diagnostic system.

Consent: All authors declare that 'written informed consent was obtained from the patients for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office.

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FIGURES

Fig. 1. DNA sequence chromatograms show the heterozygous deletion mutation (purple arrowhead) in exon 13 of PTCH1

Fig. 2. Genealogy tree of the affected family. Patient 1 and Patient 2 (individuals III-1, and III-3, respectively) carry the PTCH1 mutation with clinical manifestations of NBCCS. Symbol filled in grey (individual I-2) indicates the maternal grandmother with multiple basaliomas and hypertelorism.

Fig. 3. (A) Frontal profile with increased inner canthal distance and scars of previous cleft lip surgery; (B) Side profile with frontal bossing and moderate macrocephaly.

Fig. 4. Panoramic radiograph (A) at the age of 6 without signs of abnormality; (B) at the age of 12 with normal mandibular anatomy; (C) at the age of 15 shows a huge cystic lesion with inferior alveolar nerve canal displacement; (D) at the age 16 reveals development of a cyst around the left lower wisdom tooth; (E) at the age of 22.

Fig. 5. The maternal half sibling's frontal profile photograph with hypertelorism.

Fig. 6. Radiological findings in the half-sibling (A) normal panoramic radiograph at the age of 9; (B) at the age of 17 with multiple expansile radiolucencies associated with impacted teeth; (C, D) CT image shows large cystic lesions on both sides of the maxilla and the mandible (E) check-up after one year.

Fig. 7. The maternal grandmother's photograph reveals hypertelorism and scars on the nose after basal cell carcinoma removals.