Phenotypic and molecular genetic analysis of rare diseases associated with dental and maxillofacial manifestations

DOCTORAL (PHD) THESIS

Dr. Gréta Antal

Doctoral School of Interdisciplinary Medicine

Human Molecular Genetics Programme

Doctoral School and Programme Director: Prof. Dr. Ferenc Gallyas

Thesis Supervisor: Dr. Berenténé Dr. Bene Judit

University of Pécs

Medical School

Department of Medical Genetics

Pécs, 2024

1. INTRODUCTION

Rare diseases are diseases with a low prevalence, life-threatening or permanent loss of function, or a rare combination of common diseases.

Rare diseases are characterised by a wide variety of signs and symptoms. The fact that rare diseases may be associated with non-specific symptoms often delays diagnosis and the start of possible treatment. Many rare diseases have dental manifestations. According to some data, nearly 900 genetic syndromes have dental or oro-maxillofacial anomalies in their clinical picture, of which a high percentage are of ectodermal origin.

Dental malformations can result from teratogenic effects during odontogenesis, as well as from genetic disorders, in isolation or as part of a syndrome associated with other symptoms. In some cases, dental abnormalities may be the most prominent symptoms and may be diagnosed first, while other manifestations affecting different organs may appear later. These anomalies may have both functional and aesthetic implications. The presence of dental and oral anomalies together with extraoral symptoms can often facilitate the early diagnosis of rare genetic syndromes. Therefore, it is very important for dentists to be aware of the existence of rare diseases, their clinical presentation and their early detection, which may be underlying a serious disease.

In our study, we performed a phenotypic and molecular genetic analysis of three rare diseases associated with dental and/or maxillofacial anomalies, namely Gardner syndrome, Treacher Collins syndrome and Noonan syndrome. The aim of this study is to determine the type and prevalence of dental manifestations in these patient groups and to identify potential prognostic markers through the analysis of geno-phenotypic data.

2. LITERATURE REVIEW

2.1. Dental disorders

Teeth are a special organ of the maxillofacial region and their development is a long and complex process. Their development is influenced by both genetic and environmental factors. Early diagnosis of rare genetic diseases can be aided by the presence of warning dental orofacial anomalies. These dental anomalies consist of changes in the appearance, internal structure or topography of one or more deciduous or permanent teeth, resulting from congenital or acquired abnormalities of genetic origin. The number, position, structure and shape of teeth are regulated by a complex system of genes, alterations of which can result in dental abnormalities. Depending on the stage of tooth development at which the change occurs, different dental abnormalities can occur, affecting tooth number (anodontia, hypodontia and hyperdontia), structure (amelogenesis imperfecta, dentinogenesis imperfecta and dentin dysplasia) and shape (microdontia, macrodontia and taurodontia). Dental malformations can result from teratogenic effects during odontogenesis, as well as from genetic abnormalities, in isolation or as part of a syndrome (Table 1) in association with other symptoms.

Table 1 Syndromes associated with dental/craniofacial manifestations and their genetic background

Dental/craniofacial abnormality	Syndrome	OMIM number	Genetic variation (gene/locus)
Hyperodontia			
	Cleidocranial dyplasia	119600	*RUNX2* (6p21.1)
	Gardner syndrome	175100	*APC* (5q22.2)
	Down syndrome	190685	Trisomy 21
Hypodontia (oligodontia)			
	Down syndrome	190685	Trisomy 21
	Achondroplasia	100800	*FGFR3* (4p16.3)
Taurodontism			
	Amelogenesis imperfecta type IV	104510	*DLX3* (17q21.33)
	Down syndrome	190685	Trisomy 21
Cleft lip and palate			
	Kabuki syndrome	147920 300867	*KMT2D* (12q13) *KDM6A* (Xp11.3)
	Kallmann syndrome		ANOS1 (Xp22.3)
Gingival hyperplasia and dental disorders	Neurofibromatosis	162200	*NF1* (17q11)
Craniofacial anomalies	Treacher Collins syndrome	154500 613717 248390	*TCOF1* (5q32) *POLR1D* (13q12.2) *POLR1C* (6p21.1)

2.1.1. Genetic background of tooth development and dental anomalies

Tooth development involves a number of different cellular and molecular processes. These processes consist of several stages (Figure 1), ranging from the embryonic stage to the emergence of the remaining teeth. Both deciduous and permanent teeth develop from oral ectoderm and underlying neural mesenchymal cells.

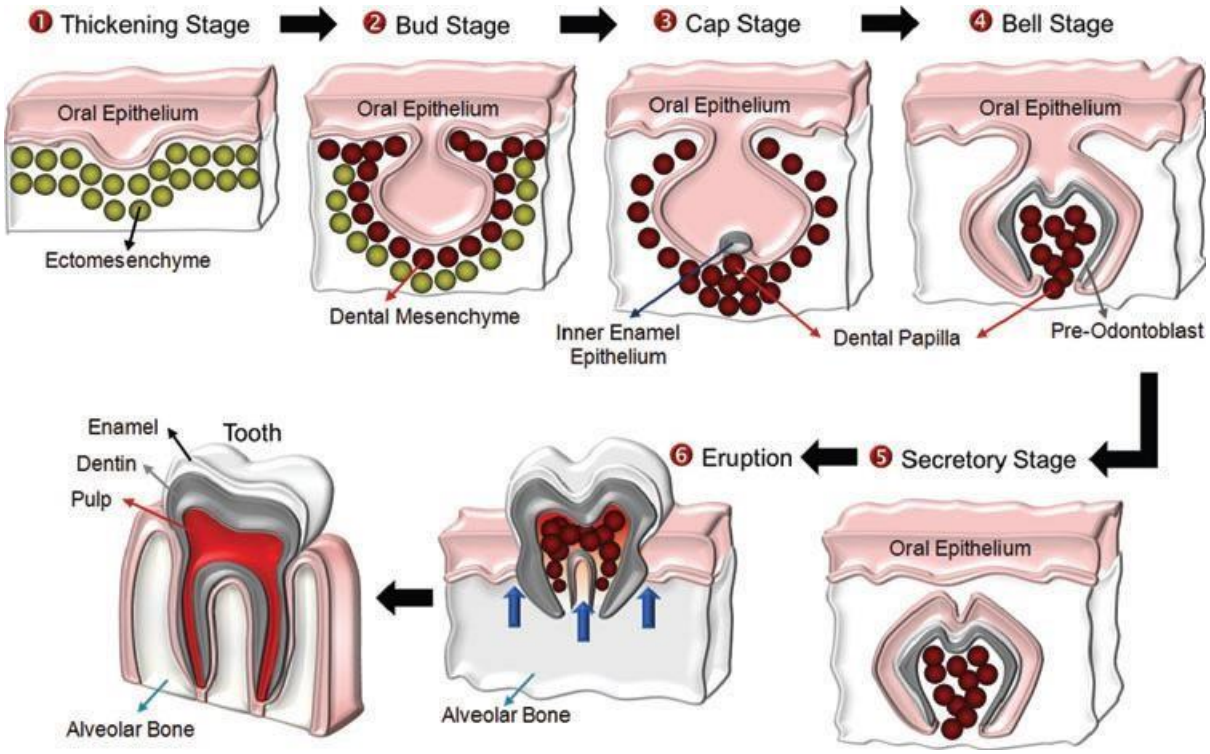

Figure 1 The process of tooth development

Tooth development is initiated by the interaction between the ectomesenchyma and the oral epithelium and consists of six successive stages: thickening, bud, cap, bell, secretion, and eruption (Kim et al 2012)

Facial and dental malformations are caused by mutations in a gene or group of genes, as the expression or function of the encoded protein(s) is altered. In addition to genetic mutations, environmental factors can affect gene expression or interfere with the normal function of protein products. The position, number and shape of teeth determined by more than 300 genes. *HOX* genes are the most commonly studied genes related to tooth development. The genes involved in the development of various dental anomalies are listed in Table 2.

Table 2 Genes involved in the development of dental disorders (Khan et al 2022)

Dental abnormality	OMIM number	Gene(s)
Germ deficiency (agenesia)	106600	*MSX1, PAX9, AXIN2, WNT10A, EDA*
Supernumerary tooth	187100	*RUNX2, APC*
Amelogenesis imperfecta	104530	*AMELX, ENAM, MMP20, KLK4, DLX3*
Dentinogenesis imperfecta	125490	*DSPP*
Taurodontism	272700	*DLX3, MMP2*

2.2. Gardner syndrome

Gardner syndrome (GS) is a rare autosomal dominant (AD) inherited disorder characterized by a triad of gastrointestinal polyposis, multiple osteomas, and hard and soft tissue tumors. It is a rare clinical subtype of Familial Adenomatous Polyposis (FAP), affecting approximately 10% of FAP patients. GS can affect multiple organ systems and its clinical features are classified into a group of intestinal and extraintestinal manifestations. The most common and most severe clinical manifestations are gastrointestinal polyposis, adenomatous polyposis of the colon and rectum.

An ENT specialist, dentist or maxillofacial surgeon can play an important role in the early diagnosis of FAP, potentially saving the patient's life. Dental abnormalities associated with GS are present in 30-70% of cases and can be screened for by routine examination and panoramic radiography. The first clinical signs of GS may be osteomas and other dental anomalies in 53% of cases. As some of the symptoms of GS do not appear at the same time, the role of the dentist/maxillofacial surgeon in early diagnosis is very important, as they are often the first to encounter the first signs of the disease.

2.3. Treacher Collins syndrome

Treacher Collins syndrome (TCS) is a rare, inherited developmental disorder with an estimated incidence of 1 in 50,000. In 40 percent of cases, it is linked to a family history, while in the remaining 60 percent, *de novo* mutations are responsible for the development of the disorder. The literature distinguishes four clinical subtypes of TCS based on genetic background (Table 3).

Table 3 Classification of Treacher Collins syndrome subtypes and their genes (Marszalek-Kruk et al 2021)

Phenotype		Gene					
TCS subtype	OMIM	Gene name	OMIM	Inheritance	Chromosome locus	Pathogenic variant frequency in TCS	Gene product
TCS1	154500	*TCOF1*	606847	AD	5q32-q33	86 %	Treacle protein
TCS2	613717	*POLR1D*	613715	AD,AR	13q12.2	6%	DNA-dependent RNA polymerases I and III, RPAC2
TCS3	248390	*POLR1C*	610060	AR	6p21.1	1,2%	DNA-dependent RNA polymerases I and III, RPAC1 subunit
TCS4	618939	*POLR1B*	602000	AD	2q14.1	1,3%	DNA-dependent RNA polymerases I and III, subunit RPA2

The classic and rare symptoms of TCS and their prevalence are shown in Table 4. TCS is equally common in both sexes, and the severity of deformities does not increase with age. The symptoms of TCS are highly variable, and can vary considerably between individuals and within families.

Table 4 Classic features of Treacher Collins syndrome (Marszalek-Kruk et al 2021, Vincent et al 2016)

	Symptoms	Prevalence in affected individuals
Very frequent	Downslanting palpebral fissures	89-100 %
	Malar hypoplasia/hypoplasia of zygomatic complex	81-97%
	Conductive hearing loss	83-92%
	Mandibular hypoplasia/micrognathia	78-91%
Frequent	Atresia of the external ear canal	68-71%
	Microtia	10-77%
	Lower eyelid coloboma	54-69%
	Delayed speech development	57-63%
	Facial asymmetry	52%
	Preauricular hair displacement	24-49%
Rare	Nasogastric tube or gastrostomy in neonates	28%
	Cleft palate	21-33%
	Intubation or tracheostomy in neonates	12-18%
	Choanal stenosis/atresia	13-25%
	Cardiac malformation	11 %
Very rare	Renal malformation	4%
	Microcephaly	3%
	Intellectual disability/delayed motor development	1,7-10%
	Limb anomaly	1,5%

2.3.1. Dental manifestations in TCS

Patients with TCS have a number of dental abnormalities that result from craniofacial developmental abnormalities. Bite abnormalities are also often observed.

Developmental abnormalities of the mandible and maxilla may result in open bite, cross bite or other bite abnormalities.

2.4. Noonan syndrome

Noonan Syndrome (NS) is a relatively common, mostly autosomal dominant inherited congenital disorder affecting multiple organ systems.

Typical clinical features of NS are mild facial dysmorphic features, short stature, congenital heart defects, including pulmonary stenosis and hypertrophic cardiomyopathy. In addition, skeletal abnormalities (e.g., thoracic deformities and scoliosis), cryptorchidism in males, ophthalmological abnormalities, varying degrees of cognitive deficits and bleeding disorders may also occur.

The genetic background of Noonan syndrome is linked to abnormalities in the RAS-MAPK pathway (Figure 2), which regulates cell growth and differentiation. It is primarily caused by mutations in the RAS/mitogen-activated protein kinase (MAPK) signalling pathway, which is essential for cell cycle differentiation, growth and ageing.

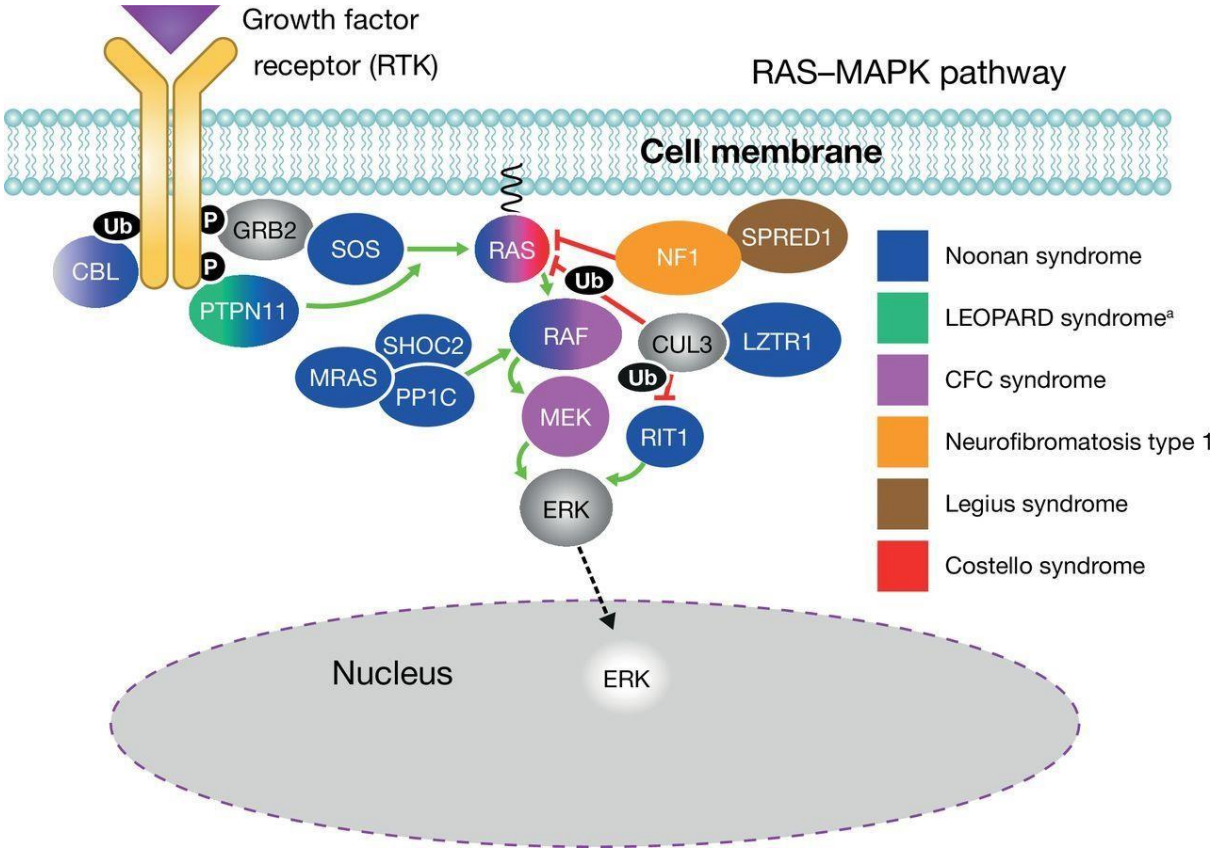

Figure 2 The RAS-MAPK signal transmission path Image source: https://adc.bmj.com/content/107/12/1073

3. OBJECTIVES

It is known from the literature that dental abnormalities can be present in the clinical picture of many rare diseases. In our study we mapped the dental manifestations of three rare diseases, namely Gardner syndrome, Treacher Collins syndrome and Noonan syndrome.
The following objectives were set for the three groups of patients studied:

1. Phenotypic and genetic study of patient with atypical symptoms suffered from Gardner syndrome and their affected family members, identification of dental manifestations in the disease.
2. Exploring the different mutations of the *APC* gene, their intragenic localization and the association between dental and/or bone lesions using literature data.
3. Molecular characterization of mutations identified in a group of patients with Treacher Collins syndrome using a next-generation sequencing technique, phenotypic characterization of patients, and detection of dental and maxillofacial manifestations.
4. Exploration of the types of dental manifestations in a family with Noonan syndrome and comparison with the literature.

4. PATIENTS AND METHODS

4.1. PATIENTS PARTICIPATING IN THE STUDY

4.1.1 Gardner syndrome

4.1.1.1 Patient

The 17-year-old girl was referred from the Department of Dentistry and Oral Surgery at the University of Pécs to the genetic counselling unit of the Department of Medical Genetics, University of Pécs when she was 11 years old. During dental examination, a panoramic radiograph (Figure 3) was taken showing a bone abnormality. A tooth 3.6, which was causing uncertain complaints, was surgically removed and a sample was taken from the abnormal bone area under the tooth socket. Histopathological examination of the jawbones revealed a Paget's disease-like bone metabolism disorder with mixed osteoblastic and osteoclastic activity of remodeling bone tissue. Years later, a new sample was taken for the removal of tooth 3.7 under local anaesthesia due to suspicion of Paget's disease. Cortical and cancellous bone samples were taken from the mandibular corpus corresponding to the root of the previously removed tooth 3.6. At this time, NGS-based genetic testing of the patient was performed.

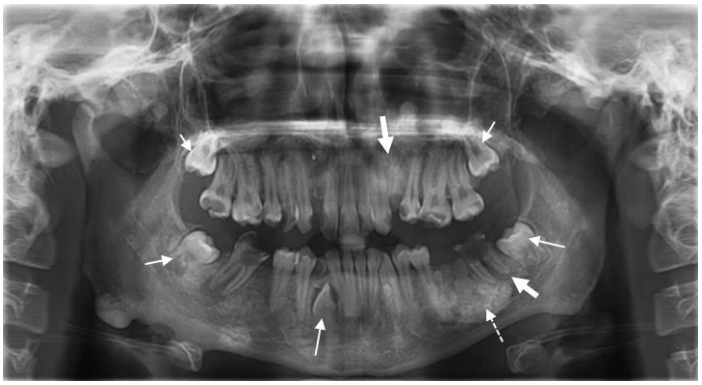

Figure 3 Panoramic radiograph shows impacted teeth (small arrows), radix relicta (large arrows), and the dotted arrow indicates the location of the specimen taken for histopathological examination. Dental caries are present in almost all teeth

4.1.1.2. patient

At the same time as the NGS examination of the 17-year-old girl (proband), the 14-year-old sister of the proband came to our genetic counselling unit with skeletal and soft tissue manifestations. Her medical history included the following: at the age of 11 years, two tumours had been removed from the subcutaneous connective tissue of the head, one from the right parietal region and one from the left occipital region. Histological examination confirmed a dermoid cyst. At 14 years of age, another dermoid cyst was removed from the left occipital region. At the same time, a painful tumour appeared on the left side of the mandible. Radiological findings suggested Gardner's syndrome and the patient was admitted to oncology.

The patient underwent a dental examination at a maxillofacial clinic for multiple mandibular exostoses, age inappropriate eruption status of the teeth. Extaoral examination revealed swelling in the mandibular area of the left angulus-ramus. Exostoses of about 0.5 cm were palpable basally on the right side. Intraoral examination revealed an eruption status of the teeth, canines and premolars were in retention (Figure 4). On CBCT (cone beam computed tomography) scan, exostoses of 18-20 mm cortical consistency were visible in the mandibular ramus region, with no compression of the cancellous bone stock.

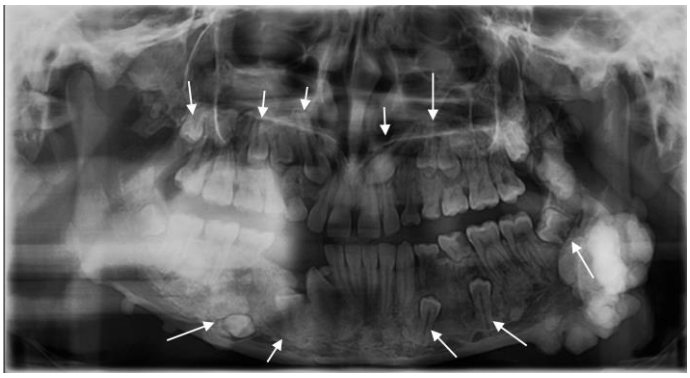

Figure 4 Panoramic radiograph shows multiple impacted teeth

4.1.1.3. patient

The mother also had skeletal symptoms. Agenesis of the teeth was observed in the mandible, her 9th-10th ribs were fused, she complained of jaw pain occasionally. CT scan of the skull showed multiple osteosclerotic lesions on the cranial bones. Gastroenterological and oncological investigations have not yet been performed.

4.1.2 Treacher Collins syndrome

In the second part of our study, we performed a clinical and molecular genetic study of Treacher Collins syndrome. Between 2003 and 2023, six patients were referred to the Genetic Counselling Unit with suspected Treacher Collins syndrome. Genetic testing (NGS-based techniques and targeted Sanger sequencing) identified pathogenic variants in five of the six patients. These five patients (two boys and three girls, age range: 2-29 years) were enrolled into our study.

4.1.3 Noonan syndrome

In the third part of our research, we studied a family with Noonan syndrome. In the family, the mother and two of her three daughters (aged 14 and 12) are affected.

4.2 METHODS

4.2.1 DNA isolation

For molecular genetic analyses, genomic DNA was isolated from EDTA-coagulated peripheral leucocytes using the E.Z.N.A.® Blood DNA Maxiprep Kit (OMEGA®, Bio-tek, Inc., Norcross, GA, USA). The concentration and purity of the isolated DNA were checked using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

4.2.2 New generation sequencing

4.2.2.1 NGS panel sequencing

4.2.2.1.1 Gardner syndrome

The 17-year-old proband underwent a 252-gene containing Sceletal Dysplasia gene panel test at BluePrint Genetics' laboratory, followed by an extended WES test.

4.2.2.2.1.2 Treacher Collins syndrome

Patient 1 underwent a Comprehensive Hearing loss and Deafness NGS gene panel study (including *TCOF1*, *POLR1C* and *POLR1D* genes) with 181 genes at the GENDIA laboratory.

A Facial Dysostosis and Related Disorder NGS gene panel study containing 27 genes (including *TCOF1*, *POLR1C* and *POLR1D*) was performed in patients 3 and 4 at the BluePrint Genetics laboratory.

4.2.2.2 WES

To identify the pathogenic variant in patient 5, whole exome sequencing was performed in our laboratory.

4.2.2.3 Variant classification

For genomic data classification and interpretation of data, we followed the guidelines of the American College of Medical Genetics and Genomics (ACMG).

4.2.3. Statistical analysis

To investigate the association between the localization of *APC* gene mutations and the specific manifestations of dental and/or osseous abnormalities, a descriptive statistical analysis including a chi-square test was performed using the SPSS 27.0 family of programs. The expected values for each cell were five or higher, and $p < 0.05$ was considered the level of significance.

4.2.4 *In silico* analysis of a novel pathogenic variants of *TCOF1* and *POLR1D* genes

To investigate the potential impact of novel variants of *TCOF1* and *POLR1D* on protein structure, 3D modelling was performed using the I-Tasser online server. The *TCOF1* c.1371_1372insT variant resulted in a truncated Treacle protein with an early termination codon at amino acid position 458. This variant dramatically changed the protein structure. The *POLR1D* c.295G>C variant resulted in an amino acid change from glycine to arginine at amino acid position 99. The variant occurs in a highly conserved region of the POLR1D protein, which affects the protein conformation.

5. RESULTS

5.1. Gardner syndrome

5.1.1 Genetic analysis of a patient with atypical skeletal and dental symptoms with Gardner syndrome and his family

NGS-based "Skeletal Dysplasia" gene panel analysis of 252 genes from a DNA sample of a 17-year-old proband with atypical skeletal and dental symptoms, performed in a foreign collaboration (Blueprint Genetics laboratory), did not identify any pathogenic variant associated with the patient's symptoms. Thereafter, a whole exome sequencing (WES) extended genetic analysis was performed, which finally identified a known pathological variant in the *APC* gene [NM_000038.6], c.4700C>G (p.Ser1567*), in a heterozygous form. Targeted mutation analysis by Sanger sequencing in the samples of the proband's younger sibling and mother also detected the mutation in heterozygous form. Based on the phenotype of the patients and their genetic test results, the diagnosis of GS was confirmed.

5.1.2 Association study of dental and skeletal manifestations and *APC* gene mutations in Gardner syndrome

In the second part of our study, we investigated whether in our own patients and in cases with detailed phenotypic and genotypic data published in the literature, we find an association between the intragenic localization of the *APC* mutation carried by the patients and the dental and/or osseous manifestations.

In total, we found 49 different cases (30 different mutations) in the literature, including our own two cases, where patients had a history of various dental and/or osseous abnormalities and were known to carry APC mutations (Table 5). Based on the location of the mutations identified (N-terminal or middle region of the protein), patients were divided into two groups.

In summary, while mutations in the N-terminal domain appear to be predominantly associated with osseous abnormalities, mutations in the middle domain may contribute to a higher incidence of dental abnormalities. These results support a possible role for mutation localization in determining the phenotypic expression of dental and skeletal abnormalities.

Regarding the polyposis phenotype among the presented cases, the same number of patients were diagnosed with classic FAP and Gardner syndrome. The majority of patients with classic FAP carry mutations in the N-terminal region, whereas patients with Gardner syndrome harbor mutations localized in the middle region of the APC protein.

Table 5 APC germline mutations with dento-osseous manifestations and FAP phenotype

	Mutation		Gender/	Polyposis	Colorectal	Extraintestinal manifestations		Ref	Localization
	DNA	Protein	Age	Phenotype	cancer	Dental anomalies	Osseous anomalies		
1	c.481C>T[a]	p.Gln161*	M/54	Classic FAP	+	-	Hs	(75)	APC-N terminal region
	c.481C>T[a]	p.Gln161*	F/20	Classic FAP	-	-	Os, Hs		
	c.481C>T[a]	p.Gln161*	M/18	Classic FAP	-	IT, Od	Dbi		
2	c.532-1G>A[b]	-	M/33	Classic FAP	+	-	Os, Hs		
	c.532-1G>A[b]	-	F/28	Classic FAP	+	Od	Os		
	c.532-1G>A[b]	-	M/7	Classic FAP	-	Od	Os, Hs, Dbi		
3	c.646C>T	p.Arg216*	F/12	ND	ND	IT, Od	-	(36)	
4	c.761C>G	p.Ser254*	-/-	Attenuated FAP	ND	ST	-	(76)	
5	c.839C>G	p.Ser280*	-/39	ND	ND	-	Os	(77)	
6	c.1240C>T	p.Arg414Cys	-/24	ND	ND	-	Os		
7	c.1354_1355delGT	p.Val452SerfsX7	-/-	Classic FAP	ND	-	Os	(76)	
8	c.1370C>G	p.Ser457*	M/39	Classic FAP	+	-	Os, Dbi	(78)	
	c.1370C>G[c]	p.Ser457*	M/32	Classic FAP	-	-	Os, Hs, Dbi		
	c.1370C>G[c]	p.Ser457*	F/34	Classic FAP	+	-	Hs, Dbi		
	c.1370C>G[c]	p.Ser457*	M/11	Classic FAP	-	ST	Hs		
9	c.1495C>T	p.Arg499*	F/27	ND	-	-	Os	(79)	
10	c.2092T>G	p.Leu698*	M/23	Classic FAP	+	Dental anomalies	-	(80)	
	c.2092T>G[d]	p.Leu698*	M/48	Classic FAP	+	-	Os		
	c.2092T>G[d]	p.Leu698*	M/23	Classic FAP	-	-	Os		
	c.2092T>G[d]	p.Leu698*	F/24	Classic FAP	-	-	Os		
	c.2092T>G[d]	p.Leu698*	M/21	Classic FAP	-	-	Os		
11	c.2138C>G	p.Ser713*	-/37	ND	ND	-	Os	(77)	
12	c. 2740T>G	p.Cys914Gly	M/-	ND	ND	Mesiodens	-	(81)	
13	c.3199_3202delCAAT	p.Ser1068Glyfs*57	-/-	Classic FAP	ND	-	Os	(76)	APC-middle region
14	c.3374T>C	p.Val1125Ala	F/-	ND	ND	ST	-	(81)	
	c.3374T>C	p.Val1125Ala	M/-	ND	ND	mesiodens	-	(81)	
15	c.3880_3881delCA	p.Gln1294Glyfs*6	F/30	ND	+	ST, Od, IT	Os	(82)	
16	c.3927_3931delAAAGA	p.Glu1309Aspfs*4	F/18	Gardner sy	-	-	Os		
17	c.4387_4390del	p.Arg1463fs	-/-	Gardner sy	ND	Dental anomalies	Os	(47)	
18	c.4292_4293delGA[e]	p.Ser1465Trpfs*3	M/15	Gardner sy	ND	ST, Od, IT	-	(83)	
	c.4292_4293delGA[e]	p.Ser1465Trpfs*3	M/66	Gardner sy	+	IT	-		
	c.4293_4294delAG	p.Ser1465Trpfs*3	F/31	Gardner sy	-	-	Os	(84)	
	c.4293_4294delAG[f]	p.Ser1465Trpfs*3	F/28	Gardner sy	ND	-	Os		
	c.4293_4294delAG[f]	p.Ser1465Trpfs*3	F/22	Gardner sy	ND	-	Os		
19	c.4510_4513del	p.Ser1505fs	-/-	Gardner sy	ND	-	Os, Dbi	(47)	
20	c.4609dup[g]	p.Thr1537Asnfs*7	F/16	Gardner sy	ND	IT	Os	(85)	
	c.4609dup[g]	p.Thr1537Asnfs*7	M/12	Gardner sy	ND	ST	Os		
21	c.4611_4612delAG	p.Glu1538Ilefs*5	-/-	Gardner sy	ND	ST	-	(47)	
22	c.4621C>T	p.Gln1541*	M/38	Gardner sy	-	IT, missing teeth	Os	(86)	
23	c.4652_4655delAAGA	p.Lys1551Argfs*13	-/-	Attenuated FAP	ND	ST	-	(76)	
24	c.4654_4655del	p.Glu1552Glyfs*6	-/-	Gardner sy	ND	Dental anomalies	Os	(87)	
25	c.4666del	p.Thr1556Leufs*9	M/25	Gardner sy	ND	ST, IT	Os, Dbi	(88)	
26	c.4668_4669insT	p.Ile1557*	-/-	Gardner sy	ND	-	Os	(47)	
27	**c.4700C>G**	**p.Ser1567***	**F/11**	**Gardner sy**	**ND**	**IT**	**-**	**(43)**	
	c.4700C>G	**p.Ser1567***	**F/16**	**Gardner sy**	**ND**	**IT, Od**	**Os**	**(43)**	
28	c.5722A>T	p.Asn1908Tyr	M/-	ND	ND	Mesiodens	-	(81)	
29	c.6127A>G	p.Ile2043Val	F/-	ND	ND	Mesiodens	-	(81)	
30	c.8383G>A[f]	p.Ala2795Thr	M/-	ND	ND	Mesiodens	-	(81)	C terminal region
	c.8383G>A[f]	p.Ala2795Thr	M/-	ND	ND	Mesiodens	-	(81)	

a–g, represents family members; ST, supernumerary teeth; IT, impacted teeth; Os, osteoma; Od, odontoma; Dbi, dense bone island; Hs, hazy sclerosis; M, male; F, female; ND, not mentioned or no straightforward information; +, means the manifestation is present. -, means the manifestation is not present, except in "Gender/Age" column, where no information was available. Blue coloured boldface refers to our cases.

5.2. Treacher Collins syndrome

5.2.1 Molecular characterisation of mutations identified in TCS patients

Mutation screening in an international collaboration has identified two deletions and one insertion in the *TCOF1* gene (NM_001135243), mutations that result in the formation of an early termination codon. In addition, one missense mutation was detected in the *POLR1D* gene (NM_001374407). Following segregation analyses, two of the four mutations identified in the patient population were found to be pathogenic mutations that developed *de novo* (in patients 3 and 4), while two mutations (one pathogenic and one VUS) were passed on by asymptomatic mothers to their affected children (patients 1, 2 and 5).

Sequence analysis of a Comprehensive Hearing loss and Deafness Next Generation Sequencing (NGS) gene panel study in patient 1 revealed a known pathogenic deletion of five bases in length, *TCOF1* c.4369_4373 (p.Lys1457Glufs*12), in the *TCOF1* gene. In patient 2, who was the maternal second cousin of patient 1 and died 15 years ago at the age of two, targeted Sanger sequencing identified the same mutation in the patient's stored DNA sample.

The Facial Dysostosis and Related Disorder NGS panel sequencing identified a novel *TCOF1* c.1371_1372insT (p.Lys458*) insertion in patient 3, which is not yet known in the literature, and a novel *POLR1D* c.295 G>C (p.Gly99Arg) missense variant in patient 5, which is also not known in the literature.

In patient 4, WES analysis identified a pathogenic *TCOF1* c.2103_2106 (p.Ser701Argfs*9) deletion previously described in the literature.

The ACMG classification of the novel insertion *TCOF1* c.1371_1372insT is pathogenic as this mutation is *de novo*, is thought to cause disease, the loss-of-function mutation is a known disease-causing mechanism in TCS and this identified variant is not found in control population databases (neither in the gnomAD genome nor in the gnomAD exome) (PVS1, PM2, PS2). *POLR1D c.295* G>C (p.Gly99Arg) novel missense variant is classified as a VUS according to the ACMG guideline, as this variant is not present in either the gnomAD genomes or the gnomAD exomes and *in silico* analyses indicate an adverse effect of the mutation (PM2, PP3).

5.3. Noonan syndrome

5.3.1 Genetic analysis of the Noonan syndrome family

A Next Generation Sequencing (NGS)-based "Comprehensive Growth Disorders/Skeletal Dysplasias and Disorders" gene panel analysis of 510 genes was performed in the 14-year-old proband's DNA in an international collaboration (Blueprint Genetics laboratory). The test identified a known pathogenic variant, the c.178G>A (p.Gly60Ser) missense variant in the *PTPN11* (NM_002834.4) gene in heterozygous form. Targeted mutation analysis by Sanger sequencing in the samples of the proband's younger sibling and the mother also detected the mutation in heterozygous form. The phenotype of the patients and their genetic test results confirmed the diagnosis of NS.

5.3.2 Dental examination of a family with Noonan syndrome

The dental manifestations of the two female children, together with the literature data, are presented in Table 6.

Table 6 Dental characteristics of our patients with Noonan syndrome compared with literature data

Number of patients tested	P1	P2	n=17	n=4	n=10	n=11	n=42
References			Gürsoy et . al (91)	Mallineni et al (92)	Lutz et al. (70)	Vavetsi et al. (93)	Janas-Naze et al (94)
Dental anomalies							
delayed dental eruption	X	X	n/a	-	10%	-	n/a
high-arched palate	X	X	76.4%	100%	-	90.9%	n/a
open bite	-	X	11.7%	-	10%	36.4%	n/a
micrognathia	-	X	-	75%	10%	n/a	n/a
hypoplastic jaw	-	X	n/a	50%	n/a	n/a	n/a
malocclusion	-	X	11.7%	100%	30%	63.6%	n/a
crowded teeth	-	X	-	100%	-	18.2%	n/a
hypodontia	-	-	11.7%	75%	10%	-	n/a
supernumerary tooth	-	-	-	-	-	-	26.2%
impacted tooth	-	-	-	100%	n/a	-	23.8%
taurodontism	-	-	n/a	100%	-	n/a	n/a
caries	X	X	35.2%	-	-	100%	n/a
enamel hypoplasia	-	-	-	75%	-	n/a	n/a
gingivitis	-	X	35.2%	75%	-	100%	n/a

- = missing; X = present; n/a = not surveyed or data not available

6. DISCUSSION OF RESULTS AND CONCLUSIONS

6.1. Gardner syndrome

6.1.1 Study of a patient with atypical skeletal and dental symptoms with Gardner syndrome and his family

GS is a rare tumour predisposition syndrome characterised by pleiotropy and genetic heterogeneity. The clinical diagnosis of GS is often challenging due to the variable expression of extraintestinal manifestations. Osteomas are a typical feature of GS and their presence is necessary for the diagnosis. They occur mainly in the jaw, but osteomas may also form in the skull, paranasal sinuses and long bones. In our index patient, we observed only dental (impactio and retencio) and atypical bone defects, but no osteomas characteristic of GS, and therefore her symptoms did not suggest a clinical diagnosis of GS. Finally, the diagnosis was established by a comprehensive genetic analysis using state-of-the-art next-generation sequencing technology. WES identified a pathogenic mutation in *the APC* gene. At diagnosis, none of our patients had intestinal symptoms, however, the mother refused colonoscopy for her children and herself, so we have no information on whether our patients have intestinal polyps.

Dental anomalies associated with GS occur in 30-70% of patients. These may include: supernumerary teeth, compound odontomas, hypodontia, tooth impactio or retencio, hypercementosis and caries. As maxillofacial symptoms of the syndrome may occur several years before intestinal polyposis, it is important that dentists are aware of the importance of the syndrome as a precancerous condition. Panoramic radiography can be a useful tool for early detection of GS. Elements of syndrome, such as osteoma, odontoma, supernumerary teeth and impacted teeth, can be identified on routine radiographic examinations.

Many rare diseases have dental manifestations. According to the London Dysmorphology Database, in 2011, approximately 900 out of 5000 genetic syndromes had dental and oro-maxillofacial anomalies in the clinical picture. Dental and oral anomalies, together with extraoral symptoms, can facilitate early diagnosis of rare genetic syndromes.

6.1.2 Association studies between dental and osseous manifestations and *APC* gene mutations in Gardner syndrome

Extraintestinal manifestations, such as various dental and osseous abnormalities that accompany FAP, have the potential to play a role as clinical indicators for early diagnosis. Some dental and osseous anomalies are more common in patients with FAP than in the general population (Table 7).

Table 7 Prevalence of dental and osseous manifestations in patients with FAP and the general population

Clinical features	Prevalence among FAP patients (%)	Prevalence in the general population (%)	Ref
Osteoma	76.1	4.3	(106)
	62	14	(107)
	57.7	2.6	(104)
	46-93	4-16	(34)
	40	6.6	(108)
	60-80	1-2	(85)
Odontoma	26.9	0	(104)
	9.4-83.3	0-4	(34)
Impacted tooth	11,5	3.8	(104)
	4-38	0-4	(34)
Dental abnormality	53.3	0	(108)

Several studies have performed genotype-phenotype association studies in FAP patients to explore the relationship between *APC* mutation localization and extraintestinal manifestations. Septer and colleagues studied pediatric patients with FAP. They found that patients who carried mutations located in codon 1309 or in the region before codon 1309 had a higher frequency of osteomas (77.8%) and jaw bone sclerosis (44.4%), and 77% of these patients also had at least one dental abnormality. In addition, osteomas were observed to develop in the jaw in 42.9% of the genetic variants in the region between codon 5′ UTR and codon 516, 66.7% of the variants between codons 849 and 1309, and 77.8% of the variants between codon 1310 and the 3′ UTR region.

In our study, we analysed the mutations identified in our patients together with 29 previously published *APC* mutations in 47 patients. Patients carrying the c.4700C>G (p.Ser1567*) mutation had impacted teeth, odontomas and osteomas. This mutation has been described previously, but, no dental-osseous abnormality has been associated with it. A detailed clinical evaluation of dental and osseous anomalies in patients with known *APC*

mutations showed that, similar to previous findings, osseous manifestations were more frequent than dental abnormalities (34 of 46 cases of the former and 24 of the latter).

Osteoma was the most common bone lesion, and supernumerary teeth were the most common dental anomaly. Genotype-phenotype correlations between *APC* mutations and these extraintestinal manifestations showed that mutations localized in the N-terminal region of the APC protein (1-1000 amino acids) were more frequently associated with bone-only lesions than mutations localized in the middle region (1000-2100 amino acids) (65% versus 32%). In addition, dental manifestations were more frequently associated with mutations in the middle region than with mutations in the N-terminal region (68% versus 35%). Supernumerary teeth proved to be the most common dental abnormality and were more frequently associated with mutations in the middle region than with mutations in the N-terminal region.

APC is a multifunctional protein that contains several functional regions, including: specific repeats, specific motifs, nuclear localization and export signal sequences (Figure 5).

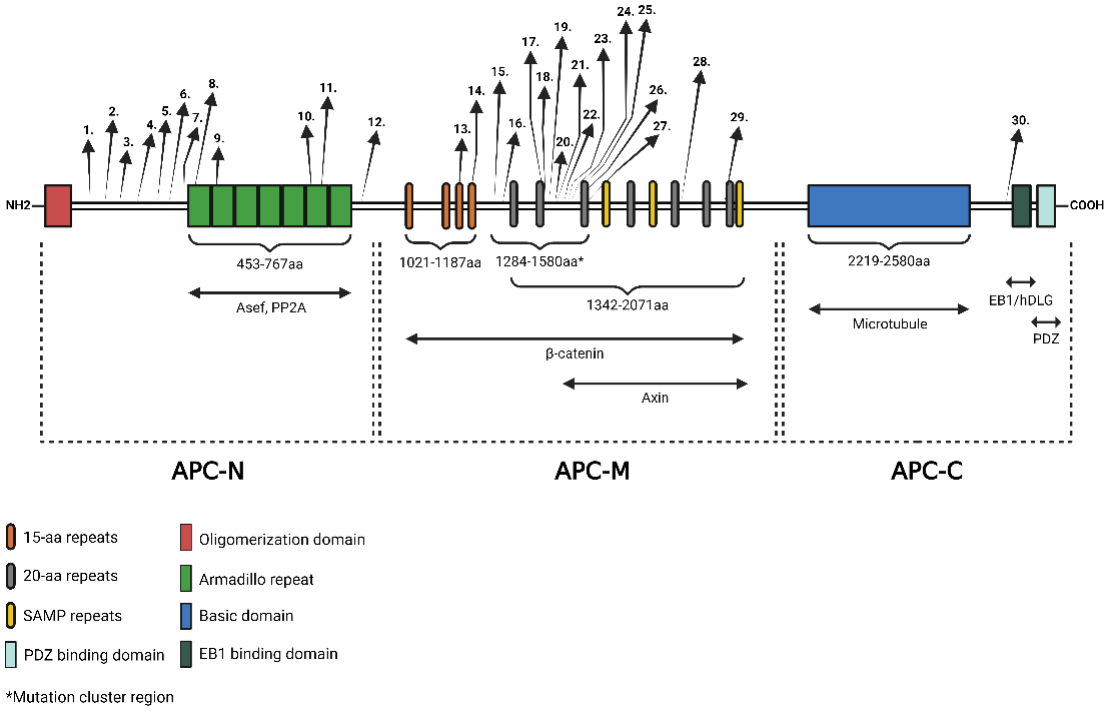

Figure 5 Structure of the APC protein and the localization of known *APC* mutations within the protein. APC contains the regions required for oligomerization, as well as armadillo repeats, a β-catenin-binding domain, an AXIN-binding domain, a basic domain, an EB1-binding domain and an HDLG-binding domain. The names of the three main regions and the protein interaction partners are shown below the APC diagram. APC-N represents the N-terminal region, APC-M the middle region and APC-C the C-terminal region. The location of the mutations is indicated by arrows. The numbers correspond to the numbering in Table 6. The figure was generated using BioRender.com (accessed 29 March 2024).

The role of Wnt in odontogenesis and bone development has been addressed previously. Previous experiments in mouse models have found that balanced Wnt/β- catenin activity is essential for normal tooth development. Perturbation of the Wnt pathway leads to either impaired tooth formation or supernumerary teeth. Normally, β- catenin is phosphorylated and degraded by a destructive complex. The so-called β- catenin destructive complex consists of axin, β-catenin and APC and phosphorylates β-catenin via CK1α and GSK-3β. Previous studies have found that β- catenin accumulation in the cytoplasm and nucleus of cells can occur in various situations, including as a result of direct mutation of β-catenin, Wnt signaling, or inactivation of APC. Truncation of APC is thought to disrupt the interaction of the destructor complex and thus the degradation of β-catenin. The resulting increased amount of β-catenin in the nucleus forms a complex with TCF/LEF and activates gene expression of target genes (Figure 6).

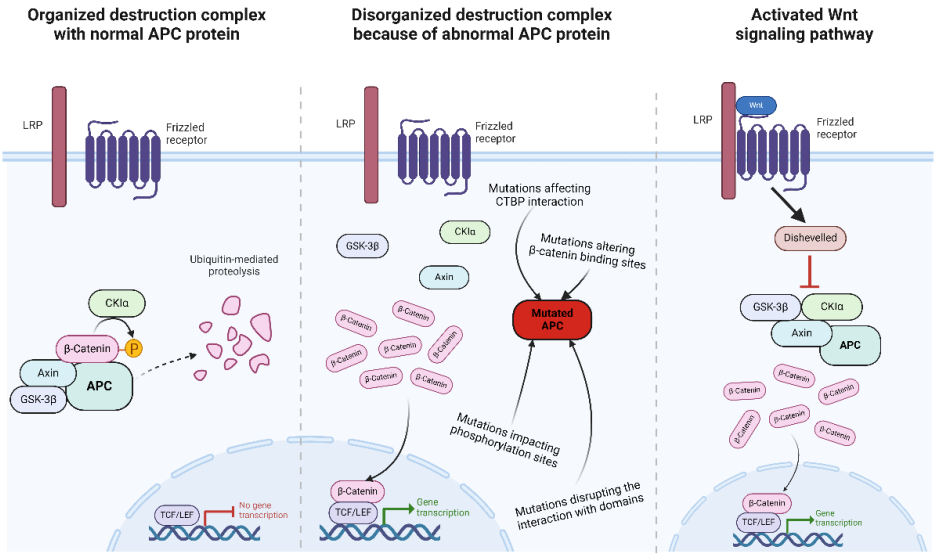

Figure 6. Mutations in APC protein mimic the effects of Wnt signaling pathway. The normal APC protein inactivates the transcriptional role of β-catenin through phosphorylation leading to ubiquitinylation and degradation (left), whereas mutated APC protein allows β-catenin to enter the nucleus (middle), similar to the result of an active Wnt signal transduction pathway (right). The figure was adapted from "Wnt Signaling Pathway Activation and Inhibition", by BioRender.com (accessed on 29 March 2024). Retrieved from https://app.biorender.com/biorender-templates.

Our statistical analysis showed that patients carrying a mutation in the middle domain of the protein had a higher incidence of dental abnormalities. In addition, supernumerary teeth were more frequently associated with mutations in the central region. The majority of mutations in this region are frameshift or nonsense mutations, resulting in loss of translation of the protein, which can lead to a non-functional APC protein or nonsense-mediated degradation of the transcript.

6.2. Treacher Collins syndrome

Several studies have suggested that the inhibition of ribosome biogenesis is one of the underlying causes of TCS. Four genes, namely *TCOF1*, *POLR1C*, *POLR1D* and *POLR1B*, which are thought to be involved in the pathogenesis of TCS, are closely related to ribosome biogenesis.

A crucial factor in the development of TCS is the defective function of the *TCOF1* gene, which is located on chromosome 5q32-q33.1, contains 27 exons and encodes a 152kDa protein. To date, more than 200 pathogenic variants have been described in the *TCOF1* gene. Most of these are deletions between 1 and 40 nucleotides in size, most of which result in an early termination codon and thus lead to truncated treacle protein formation or nonsense-mediated mRNA degradation.

In our patient cohort, we identified small deletions and one insertion in *the TCOF1* gene using NGS-based methods, consistent with the literature. Two patients carried the most common deletion (c.4369_4373del), which deletion was inherited from their asymptomatic mothers based on the segregation analysis performed. In patient P4, we identified a known deletion (c.2103_2106del) in exon 12 of the gene, resulting in a frameshift of the protein reading frame, and in patient P3, a novel single nucleotide insertion (c.1371_1372insT) in exon 9, leading to the formation of an immediate termination codon.

3D modelling of Treacle protein revealed that the new c.1371_1372insT variant encodes a protein with a dramatically altered structure. However, this new variant results in an immediate termination codon. The transcribed mRNA is likely to be degraded by nonsense-mediated decay, leading to a reduction in the amount of functional protein, so that eventually this new variant expresses its function through haploinsufficiency.

Several studies have been conducted to investigate the phenotype-genotype association of TCS patients, but to date no association has been found. In addition, no

association has been found between disease severity and parental origin of the pathogenic mutation, sporadic or familial cases.

Our four patients carrying the *TCOF1* mutation had the very frequent clinical features of TCS, such as maxillary and mandibular hypoplasia, downslanting palpebral fissures and bilateral conductive hearing loss in three of our patients, but variability was observed in the other clinical features. Common clinical features were atresia of the external auditory canal in all patients, microtia, coloboma of the lower eyelid and speech delay in two patients, and facial asymmetry in one patient. Among rare clinical features, cleft palate was noted in one patient.

Clinical data related to dental status were available for three out of five of our patients. In one patient (P4), no dental abnormality in the primary dentition was detected. In one other patient (P3), delayed dentition was observed in the primary dentition. He has small, spaced teeth with slight plaque. He has no difficulty in brushing his teeth, which may be related to the fact that he does not have a marked craniofacial abnormality. Dental caries, malocclusion, limited mouth opening and irregular tooth changes were observed in a six-year-old male patient (P1). During tooth changes, the left central incisor erupted first in the maxilla, followed by the left lateral incisor also in the maxilla. Due to limited mouth opening, this patient has difficulties in brushing his teeth. The occurrence of cleft palate was not frequent in our patient group, only one patient showed this disorder. It is worth mentioning that these patients are young children between 2.5 and 6 years old, unlike the patients studied by Dalben et al.

6.3. Noonan syndrome

Noonan syndrome is characterized by a triad of short stature, craniofacial dysmorphism and congenital heart defects. Facial features are most prominent in infancy and early-mid childhood, but become more subtle in adulthood. It has been suggested that the orofacial features of NS may be due to oedema of the face and neck as a consequence of developmental disturbances of the 3rd and 4th pharyngeal arches.

Consistent with the literature, delayed dentition, high-arched palate and caries were observed in our patients (Table 6). In addition, malocclusion, open bite, crowded teeth, micrognathia, gingivitis and hypoplastic jaw were also observed in one of our patients.

However, hypodontia, supernumerary teeth, impacted teeth, taurodontitis and enamel hypoplasia were not observed. Surprisingly, in our presented family, the affected family members showed different orodental anomalies, although they carried the same *PTPN11* gene mutation. Intrafamilial clinical heterogeneity is a well-known phenomenon in many rare diseases, including Noonan syndrome. However, to our knowledge, this is the first study to report intrafamilial variability in the dental features of Noonan syndrome.

A preventive strategy and regular dental check-ups are essential for patients with NS, and it is therefore important that professionals involved in oral and maxillofacial care are aware of the specificities of NS, while paediatricians and geneticists should pay attention to the management of oral and maxillofacial abnormalities in patients with NS.

7. SUMMARY OF RESULTS

1. We identified the *APC* gene mutation responsible for the disease in a family with atypical skeletal and dental symptoms of Gardner syndrome using NGS-based modern genetic testing. We conclude that NGS is an effective tool for early diagnosis of patients with atypical symptoms and that dental abnormalities may be early indicators of GS.

2. In an association study of *APC* gene mutation localization and dental and skeletal manifestations in Gardner syndrome, we found that mutations localized in the N-terminal region of the APC protein (1-1000 amino acids) are more frequently associated with bone lesions only than mutations localized in the middle region (1000-2100 amino acids). Furthermore, dental manifestations were more frequently associated with mutations in the middle region than with mutations in the N-terminal region.

3. In our group of patients with Treacher Collins syndrome, we identified two novel genetic abnormalities in the *TCOF1* and *POLR1D* genes that have not been reported in the literature. Using 3D modelling, we found that the novel variants [*TCOF1* c.1371_1372insT (p.Lys458*) and *POLR1D* c.295 G>C (p.Gly99Arg)] dramatically alter the normal structure of the proteins. Among our patients, we observed inter- and intrafamilial phenotypic variability consistent with the literature. Using a segregation study of a large family carrying the common *TCOF1* 4369_4373delAAGAA mutation, we are the first to describe in the literature that the pathogenic mutation is also transmitted between asymptomatic family members.

4. Genetic testing of the Noonan syndrome family identified a mutation in the *PTPN11* gene in affected patients. During the dental examination, we observed similar variability in our patients as in the literature, but for the first time in the literature, we observed intrafamilial variability in dental symptoms.

8. LIST OF PUBLICATIONS

Publications on which the PhD thesis is based

Antal G, Zsigmond A, Till Á, Orsi E, Szanto I, Büki G, Kereskai L, Herbert Z, Hadzsiev K, Bene J. Case report: Initial atypical skeletal symptoms and dental anomalies as first signs of Gardner syndrome: the importance of genetic analysis in the early diagnosis. *Pathol Oncol Res*. 2024 May 14;30:1611768. doi: 10.3389/pore.2024.1611768. eCollection 2024.

IF: 2.3 (2024)

Büki G, **Antal G**, Bene J. Rare Germline Variants in the Adenomatous Polyposis Coli Gene Associated with Dental and Osseous Anomalies. *Int J Mol Sci*. 2024 Jul 26;25(15):8189. doi: 10.3390/ijms25158189.

IF: 4.9 (2024)

Antal G, Zsigmond A, Till Á, Szabó A, Maász A, Bene J, Hadzsiev K. Molecular and Clinical Heterogeneity in Hungarian Patients with Treacher Collins Syndrome-Identification of Two Novel Mutations by Next-Generation Sequencing. *Int J Mol Sci*. 2024 Oct 23;25(21):11400. doi: 10.3390/ijms252111400.

IF: 4.9 (2024)

Antal G, Csabai L, Zsigmond A, Szanto I, Hadzsiev K, Bene J. Heterogeneity of orodental features in a family with Noonan syndrome a rare genetic disorder

Under publication

Other publications

Büki G, Zsigmond A, Czakó M, Szalai R, **Antal G**, Farkas V, Fekete G, Nagy D, Széll M, Tihanyi M, Melegh B, Hadzsiev K, Bene J. Genotype-Phenotype Associations in Patients With Type-1, Type-2, and Atypical NF1 Microdeletions. Front Genet. 2021 Jun 8;12:673025.

IF: 4,772

Total impact factor: 16,87