Orodental, Facial and Clinical Features of Mutation-Positive Noonan Syndrome: A Monocentric Study

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Objective: *To evaluate orodental, facial, clinical and molecular characteristics of the patients with Noonan Syndrome (NS).* ***Study Design:*** *The orodental, clinical and molecular characteristics of 29 mutation-positive patients with NS were recorded. Orodental examination was performed in 17 patients. All exons and exon-intron boundries of PTPN11 and SOS1 genes were analyzed by Sanger sequencing.* ***Results:*** *A total of 29 patients with NS from 27 unrelated families were included in the study. Seventeen patients were examined by a specialist in oral medicine. The most common orodental findings were high-arched palate (n=13), gingivitis (n=6) and severe caries (n=6). Anterior open bite, posterior cross bite, Class II malocclusion, hypodontia, prognathism (maxillary or mandibular), macroglossia and gingival hyperplasia were also detected. Thirteen different mutations were observed in PTPN11 gene and exon 3 was the hotspot region. Hypodontia was detected in two patients who had the same mutation in PTPN11 gene, c.181G>A, p.D61N.* ***Conclusion:*** *This study indicated a high prevalance of orodental problems including high-arched palate, severe dental caries and gingivitis in patients with mutation-positive NS. The mutation in PTPN11 gene, c.181G>A, p.D61N, may be associated with hypodontia in patients with NS.*

KeyWords: *Noonan Syndrome, PTPN11, SOS1, dental caries, gingivitis, orodental*

INTRODUCTION

Noonan syndrome (NS) is a common autosomal dominant genetic disorder with an estimated incidence of 1/1000–2500 live births.[1] The common clinical features include short stature, congenital heart defects (i.e., pulmonary valve stenosis and hypertrophic cardiomyopathy), facial dysmorphic findings, skeletal abnormalities (i.e., chest wall abnormalities, scoliosis), cryptorchidism in males, ophthalmological abnormalities and developmental delay of variable degrees.[2] The craniofacial features may include triangular facial appearance, ptosis, downslanting palpebral fissures, hypertelorism, low set ears, short neck and/or neck webbing.[3]

There is limited data regarding the orodental manifestations of NS. High-arched palate, micrognathia, macrodontia, enamel hypoplasia, hypodontia and taurodontism are some of the features that have been found to be associated with NS.[4] Retroclined mandibular incisors, periodontal problems, supernumerary teeth, malocclusions and multiple odontogenic keratocysts were also described in NS.[5-7] Furthermore, benign multiple giant cell lesions of hard and/or soft tissues which are associated with jaw enlargement, have been reported in patients with NS.[4,8]

The diagnosis of NS is usually made clinically on the basis of characteristic facial and clinical features that were described in a scoring system by van der Burgt. On the other hand, facial appearance of NS shows considerable change with increasing age, which makes the clinical diagnosis more difficult.[9] NS is caused by germline mutations in genes encoding components belonging to the Ras

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and mitogen-activated protein kinase (MAPK) signaling pathway which plays key roles in various cellular processes, including proliferation, survival, differentiation, migration and metabolism.[10] The syndrome is genetically heterogeneous and about half of the cases have a mutation in *PTPN11* gene which encodes protein tyrosine phosphatase SHP-2, a component of several signal pathways involved in embryonic development that modulate cell division. In the remaining patients, mutations in *SOS1, KRAS*, *BRAF, RAF1, MAP2K1, NRAS, RIT1, SOS2, LZTR1, A2ML1, SHOC2* or *CBL* genes have been identified.[11] Phenotypic overlap with other conditions sharing this same pathogenetic mechanism (collectively called RASopathies) leads to a diagnostic challange in some patients.[12] Identification of the causative gene mutations would help in differentiating NS from other RASopathies and understanding genotype-specific features of NS.[13]

In this study, we aimed to evaluate orodental, clinical and molecular characteristics of mutation-positive patients with NS.

MATERIALS AND METHOD

Patient Selection

Twenty-nine patients, who were examined at Department of Pediatric Genetics and Department of Medical Genetics of Behçet Uz Child Disease and Pediatric Surgery Training and Research Hospital between June 2013 and July 2018, were included in the study. The clinical, orodental, molecular findings and medical history of the patients were recorded. Orodental evaluation was performed by a specialist in oral medicine. Severe dental caries included lesions which destroyed a major portion of tooth structure. Presence of marginal gingival redness, gingival bleeding, edema or enlarged gingiva and gingival sensitivity indicated gingivitis. The other dental, mucosal and skeletal pathologies of the oral cavity were also evaluated. Dental radiographic examination was not performed due to lack of a dental radiology clinic in our hospital. The patients were referred to a tertiary center for further radiological investigation when necessary.

The patients with missing data or those who were not followed-up regularly were excluded from the study. The study was conducted by the principles of the Helsinki Declaration and approved by the local Institutional Review Board (approved number: 2018/260). Prior to the study, the nature of the study was explained to the parents and a written/signed informed consent was obtained from all individuals involved.

Molecular Analysis

DNA isolation and Sanger sequencing

Genomic DNA from the peripheral blood lymphocytes of all individuals was extracted with QIAamp DNA Blood Mini Kit (Qiagen GMBH, Hilden, Germany) using standard procedures. All coding exons and exon-intron boundaries of *PTPN11* and *SOS1* genes were amplified by polymerase chain reaction. The sequences were evaluated using CLC Genomics Workbench 3.5.1 (CLC Bio, Aarhus, Denmark). "Ensembl.org" database (GRCh38.p12) with ENST00000351677.6 transcript ID of the *PTPN11* gene and ENST00000402219.6 transcript ID of the *SOS1* gene was used to compare the individual's and the reference sequence. All variations were checked from mutation and SNP databases (Human Genome Mutation Database, National Center for Biotechnology Information, ensembl.org). Each variation was confirmed by bidirectional sequencing. Variation descriptions were done according to the nomenclature recommended by the Human Genomic Variation Society.

RESULTS

The study included 29 patients (17 males, 12 females) with NS from 27 unrelated families who had mutations in *PTPN11* or *SOS1* genes. Two patients inherited the mutation from their affected mother or father. Orodental examination was performed in 17 patients. The median age of the patients was 7 (range 1,5-38 years).

Orodental evaluation

The most common orodental findings of the patients were high-arched palate (n=13, 76.4%; Fig. 1-A), gingivitis (n=6, 35.2%) and severe caries (n=6, 35.2%). Anterior open bite (n=2, 11.7%), posterior cross bite (n=3, 17.6%; Fig. 1B), and prognathism (n=3, 17.6%) were also detected. Maxillary and mandibular prognathism was detected in one and two patients, respectively. Hypodontia was revealed in two patients (11.7%) in orodental evaluation but was not radiologically confirmed. One of these patients, a 15 year-old girl, had 23 teeth and absent bilateral inferior central incisors. The other patient with hypodontia was a 5 year-old boy who had totally 18 teeth in oral examination. The inferior lateral incisors were absent and a shallow groove in the upper segment of inferior central incisors was present, causing the appearance of fused tooth. Class 2 malocclusion was detected in two patients (11.7%) and both of them had also prognathism. One patient did not have any orodental abnormalities, whereas five patients had only high-arched palate. Eleven patients had more than one orodental findings. Table 1 lists the main orodental characteristics of these 17 patients with NS.

Facial and clinical evaluation

The most common dysmorphic features were low-set ears (**Fig. 2-A-B-C**), downslanting palpebral fissures (**Fig. 2-A-B**), ptosis (usually unilateral) (**Fig. 2-B-C**) and pectus excavatum. The major cardiac abnormalities were pulmonary stenosis and atrial septal defect (n=18, 62% and n=10, 34.2%, respectively). Additionally, one patient had hypertrophic cardiomyopathy. Astigmatism was the most common refractive error (n=4; 13.7%). The other ocular findings were hypermetropia (n=2, 6.8%), myopia (n=2, 6.8%), strabismus and nystagmus (n=2, 6.8%). Eleven patients had varying degrees of cognitive and learning disabilities. Furthermore, one patient had atypical autistic features and two patients had attention deficit hyperactivity disorder.

Molecular Findings

Twenty-six patients (89.6%) had a mutation in *PTPN11* gene, and the mutations were detected in exons 3, 7, 8 and 13. Thirteen different mutations were observed in the patients and nine of these mutations were clustered in exon 3. The most frequently detected mutation in *PTPN11* gene was c.188A>C (p.Y63C) (n=6, 20.6%). Hypodontia was detected in two patients who had the same mutation in *PTPN11* gene, c.181G>A, p.D61N. Additionally, three patients had a mutation in *SOS1* gene. The details of the mutations are shown in Table 2.

Table 1: The orodental manifestations of the mutation positive NS patients (n=17).

Orodental Findings	No. of participants (n)	Percent of participants (%)
Skeletal		
High-arched Palate	13	76.4
Anterior Open Bite	2	11.7
Prognathism	3	17.6
Dental		
Malocclusion	2	11.7
Posterior Cross Bite	3	17.6
Hypodontia	2	11.7
Severe Caries	6	35.2
Oral soft tissue		
Gingivitis	6	35.2
Gingival hyperplasia	1	5.8
Periodontitis	1	5.8
Macroglossia	2	11.7

Table 2: The details of the mutations in *PTPN11* and *SOS1* genes.

Gene	Exon	cDNA	Protein	Number of patients
PTPN11	Exon 3	c.179_181delGTG	p.G60del	1
		c.181G>A	p.D61N	3
		c.184T>G	p.Y62D	3
		c.188A>G	p.Y63C	6
		c.205G>C	p.E69Q	1
		c.214G>T	p.A72S	1
		c.228G>T	p.E76D	1
		c.236A>G	p.Q79R	1
	Exon 7	c.317A>C	p.D106A	1
		c.836A>G	p.Y279C	2
		c.844A>G	p.I282V	1
	Exon 8	c.922A>G	p.N308D	3
	Exon 13	c.1510A>G	p.M504V	2
SOS1	Exon 11	c.1644T>G	p.S548R	1
	Exon 11	c.1655G>A	p.R552K	1
	Exon 17	c.2536G>A	p.E846K	1

Figure 1–Orodental findings of two study patients.

Intraoral photograph showing high-arched palate in the roof of the mouth (arrow).

Intraoral photograph showing posterior cross bite on the right molar side (arrow).

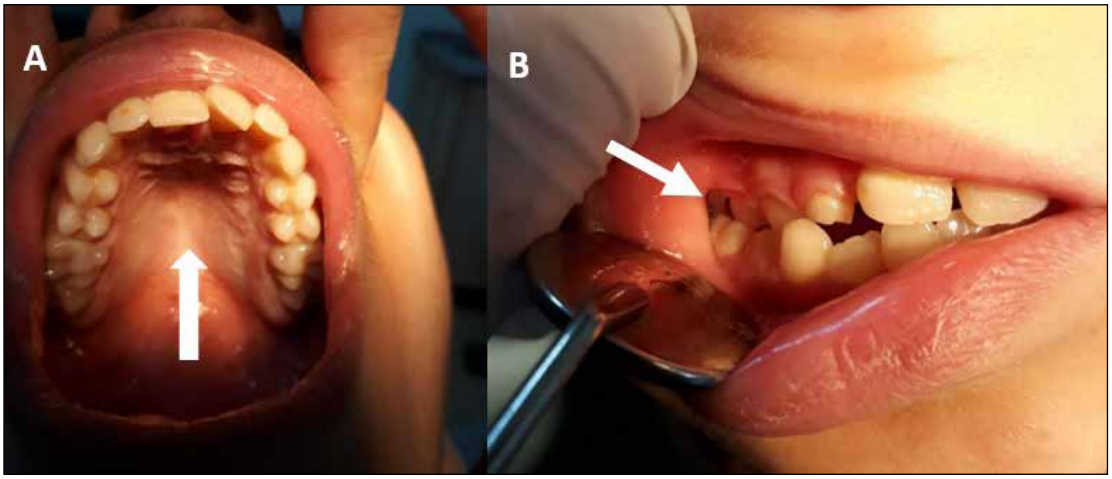

Figure 2–The most common facial features in the study patients.

A five and a half year-old boy who had low-set ears and downslanting palpebral fissures.

Prominent low-set ears, downslanting palpebral fissures and unilateral ptosis demonstrated in a patient who was four and a half years old.

Frontal photograph of a three-year-old patient showing low-set ears, downslanting palpebral fissures and prominent unilateral ptosis.

DISCUSSION

The current research shows that orodental problems are common in patients with NS and molecular diagnosis may suggest possible genotype-phenotype correlation in these patients. NS is a genetically heterogeneous disorder and the diagnosis of NS can be usually made clinically on the basis of characteristic features.[9,14] Germline mutations are responsible for the syndrome and more than 10 genes have been found to be associated with this syndrome.[11]

The clinical spectrum in patients with NS is well described, but the orodental characteristics have not been extensively evaluated in the literature. The most common findings in previous case reports were micrognathia, high-arched palate, hypodontia, dental malocclusion, impacted teeth and giant cell lesions in the maxilla and mandible.[4,15] Consistent with the existing literature, high-arched palate (n=13, 76.4%), gingivitis (n=6, 35.2%) and severe caries (n=6, 35.2%) were the most frequently observed features in our study. Additionally, maxillary or mandibular prognathism and posterior cross bite were detected in 17.6% (n=3 for each) of the patients. The other uncommon orodental findings were hypodontia (n=2, 11.7%), anterior open bite (n=2, 11.7%), macroglossia (n=2, 11.7%) and gingival hyperplasia (n=1, 5.8%). In a recent study authored by Mallineni et al., class I or III malocclusion was reported in four

patients.[4] In the present study, only 2 (11.7%) patients had class II malocclusion and both of them had prognathism. All patients were followed-up clinically and the patients were referred for further radiological evaluation in case of necessity.

Recently, more severe orodental features such as multiple unerupted permanent teeth, multiple submerged and retained deciduous teeth and supernumerary teeth have been reported.[15] These oral manifestations may result in feeding difficulties and facial growth problems, which may require management by a multidisciplinary team.[16] In the present study, there was not any patient that suffered severe orodental disorders. Scully et al. reported that the oral manifestations of NS might occur separately or concurrently with the general features.[17] In our study, the patients had orodental abnormalities in addition to clinical and dysmorphic features. Only one patient, who was one and a half years old, did not have any dental problem.

PTPN11, as the causative gene for NS, accounts for up to 50% of the cases. Mutation analysis is essential in making an accurate diagnosis and providing prenatal diagnosis. Exon 3 and exon 8 in *PTPN11* gene were identified as mutation hotspots in previous studies.[18,19] In our study, 13 different mutations were detected in *PTPN11* gene which were localized in exons 3,7,8 and 13. Additionally, 9 of these mutations were clustered in exon 3. The most common mutation in *PTPN11* gene was c.188A>C (p.Y63C) (n=6) which was also located in exon 3. Of 17 patients, who underwent orodental examination, 5 had this mutation and 3 of them had only high-arched palate. One of them had macroglossia and another subject had prognathism in addition to high palate. The other common mutation, c.181G>A, p.D61N, was detected in 3 patients and 2 of them had hypodontia. We suggest that hypodontia may be associated with this mutation in patients with NS. On the other hand, there was no detectable abnormalities in the third patient who had the same mutation. But, this patient was 1.5 years old and development of the primary teeth was not completed.

According to management guidelines for NS, first dental assessment should be made between 1 and 2 years of age, and then yearly dental examination is recommended for the patients.[20] It is important to monitor the oral health of the patients with NS, because they are prone to severe dental caries, gingival problems and other orodental abnormalities. Prevention of early childhood caries is important to avoid subsequent problems during the eruption of the permanent teeth.[4,21] Therefore, the patients with suspicion or definitive diagnosis of NS should undergo orodental examination. We have examined 17 patients in our study group and all of them are under regular follow-up.

The study has several limitations. The number of the study patients is relatively low. Furthermore, the patients were evaluated by clinical examination in outpatient and oral diagnosis clinic, but dental radiological investigation could not be performed due to lack of a dental radiology clinic in our center.

CONCLUSION

This study indicated that orodental abnormalities such as high-arched palate, severe dental caries and gingivitis are common in patients with NS and the mutation in *PTPN11* gene, c.181G>A, p.D61N, might be associated with hypodontia in patients with NS. Potential correlations between orodental phenotype and genotype should be confirmed in future large-scaled studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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