doi:10.25143/prom-rsu_2017-02_dts

Rīgas Stradiņa universitāte

Kristīne Kadiķe

DENTAL ANOMALIES AND ORAL HEALTH IN CHILDREN WITH NONSYNDROMIC CLEFTS IN LATVIAN POPULATION AND THEIR ASSOCIATION WITH GENETIC CHANGES

Summary of Doctoral Thesis
for obtaining the degree of a Doctor of Medicine
Speciality – Dentistry

Riga, 2017

Kristīne Kadiķe

DENTAL ANOMALIES AND ORAL HEALTH IN CHILDREN WITH NONSYNDROMIC CLEFTS IN LATVIAN POPULATION AND THEIR ASSOCIATION WITH GENETIC CHANGES

Summary of Doctoral Thesis

for obtaining the degree of a Doctor of Medicine

Speciality – Dentistry

Riga, 2017

The Doctoral Thesis was elaborated in: Rīga Stradiņš University Department of Conservative Dentistry and Oral Health.

Scientific supervisors:

Dr. med., Professor **Rūta Care**,
RSU Department of Conservative Dentistry and Oral Health, Latvia
Dr. med., Professor **Ilze Akota**,
RSU Department of Oral and Maxillofacial Surgery, Latvia

Official reviewers:

Dr. biol. **Dace Pjanova**,
Latvian Biomedical Research and Study Center, Latvia
Dr. med., Professor **Gundega Jākobsone**,
RSU Department of Orthodontics, Latvia
Dr. med., Associate Professor **Eva Platkāja**, Latvia

Defence of the Doctoral Thesis will take place at the public session of the Doctoral Council of Medicine on 1 March 2017 at 17.00 in Hippocrates Lecture Theatre, 16 Dzirciema Street, Rīga Stradiņš University.

Doctoral thesis is available in the RSU library and at RSU webpage:
www.rsu.lv

Secretary of Doctoral Council:

Dr. med., Professor **Anda Brinkmane**

ANNOTATION

Cleft lip and palate is one of the most common congenital malformation in maxillofacial region. The etiology of clefts are complex, involving both genetic and environmental factors. Dental anomalies in children with congenital clefts occure more often, they are considered as an additional clinical marker for determining the risk of clefts. One of the most common oral disease is dental caries. There is increasing research into genetics relating to dental caries and genes involved in caries susceptibility.

The aim of this study is to investigate the types of dental anomalies and determine the oral health status of children with nonsyndromic congenital clefts in Latvia and their association with genetic markers rs2240308, rs11867417, rs9929218, rs642961, rs11362, rs1800972.

This study is important as it has provided novel data about dental anomalies and oral health in children with nonsyndromic congenital clefts in Latvia as well as studied specific genetic markers in association with both dental anomalies and dental caries.

The study consisted of three parts; the first, a retrospective analysis of 126 clinical charts for children with nonsyndromic clefts. It shoved that more dental anomalies were seen in children with unilateral (75%) and bilateral (87.5%) cleft lip and palate. The most common dental anomalies were hypodontia (29.37%) and microdontia (28.57%).

The second part was the assesment of oral health evaluating the presence of dental caries, plaque and gum bleeding. Oral health was evaluated in 171 children with nonsyndromic clefts. This was compared to the control group of 196 children in three different age groups ($2-3$, $6-7$ and $11-12$ year olds) according to the bite development. Overall, the prevalence of caries in cleft group children was lower compared to the control group. Caries intensity of $2-3$ year old children with clefts was lower (dmf = 3.49)

compared to control group (4.78). In the 6 – 7 year old group caries intensity was higher and more filled teeth were present in the cleft group. In the 11 – 12 year old group caries intensity was similar in both groups. The plaque and bleeding index in both cleft and control groups was similar, except for plaque index in the 2 – 3 year old children and bleeding index in the 6 – 7 year old group which was less in the cleft group compared to the control group. When assessing oral health influencing behavioural factors using parental questionnaires, no specific factors were identified.

The third part of this study was the identification of genetic markers associated with both, dental anomalies (rs2240308, rs11867417, rs9929218, rs642961), and dental caries (rs11362, rs1800972). The research identified *IRF6* (Interferon Regulatory Factor 6) gene genetic markers rs642961 significant association with dental anomalies and nonsyndromic clefts. This marker's A allele was particularly associated with an increased risk of clefts. *DEFB1* (Beta-defensin 1) gene genetic markers rs11362 studies identified association with caries in children with nonsyndromic clefts. In addition this gene's expression in the oral cavity may be associated with an individual's susceptibility to the dental caries.

The results of this study provides insight in this complex multifactorial pathology and the role of possible genes involved. A multidisciplinary approach between health professionals from Cleft lip and palate centres and other professionals involved in the care is essential in order to improve care for the children with congenital nonsyndromic clefts.

TABLE OF CONTENT

1. TOPICALITY OF THE STUDY

Cleft lips and palates, as well as facial clefts are one of the most common congenital malformations in maxillofacial region. Their prevalence throughout the world varies from 1/500 to 1/2500 of live births (Prescott et al., 2001; Slayton et al., 2003; Yildirim et al., 2012).

Literature data show that incidence of dental anomalies in children with congenital clefts is more common compared to population data in general (Ribeiro et al., 2003). They may include anomalies of the number of teeth (hypodontia – absent teeth, supernumerary teeth), anomalies of dental shapes, dental eruption disorders, enamel mineralization disorders (Ribeiro et al., 2002). Observations show relationship between dental anomalies and types of congenital clefts – the more severe is a form of a cleft, the pronounced is a dental anomaly (Kraus et al., 1966; Dewinter et al., 2003; Menezes and Vieira et al., 2008; Al Jamal et al. , 2010).

The latest data confirm that dental anomalies and congenital nonsyndromic clefts are controlled by the same genes (Vieira, 2003; Vieira et al., 2007a; Vieira, 2008). Studies show that *AXIN2* (axis inhibition protein 2), *CDH1* (E – cadherin), *IRF6* (interferon regulatory factor 6) genes are linked with development of both dental anomalies and nonsyndromic clefts (Zucchero et al., 2004; Vieira et al., 2007; Vieira et al., 2008d; Vieira et al., 2008c; Callahan et al., 2009; Letra et al., 2009; Letra et al., 2012a). Dental anomalies can be as an additional clinical marker of a congenital cleft, therefore it is important to identify them in order to help improvement of assessment of cleft risks (Letra et al., 2007; Menezes and Vieira, 2008; Tannure et al., 2012d).

It is essential to perform treatment of clefts of maxilla-facial area in due time and in a particular order. In order to perform surgical or orthodontic treatment, oral health is vitally important. Healthy primary and permanent teeth

allow performance of optimal orthodontic and surgical treatment with maximum long–term results.

In 2001, the Nationale Institution of Health (NIH) in the USA released a statement of a development program, listing main directions in research of caries. One of them was targeted at the need for genetic researches in order to identify genes and genetic markers in diagnosis, prognosis and treatment of caries. During the last decade, the number of studies on genetic factors influencing an individual's susceptibility to dental caries (NIH, 2001) has rapidly increased. Oral cavity is also proved as an important location of expression of *DEFB1* (Defensin beta 1) gene (Krisanaprakornkit et al., 1998; Mathews et al., 1999; Dunsche et al., 2001), which is associated with resistance to microbial colonization, therefore perhaps this gene is associated with caries predisposition in children with nonsyndromal clefts.

1.1. The aim of the study

To investigate types of dental anomalies and to determine oral health status in children with nonsyndromic congenital clefts in Latvia and their association with genetic markers rs2240308, rs11867417, rs9929218, rs642961, rs11362, rs1800972.

1.2. The tasks of the study

1. To determine dental anomalies in children with nonsyndromic congenital clefts in different types of clefts in cleft and outside cleft areas.
2. To assess oral health status in children with nonsyndromic congenital clefts, namely, their dental caries, oral hygiene, and mucosal condition.

3. To assess behavioural factors influencing oral health in children with nonsyndromic congenital clefts, namely, their eating habits, tooth brushing habits, knowledge or awareness of oral health.
4. To determine the link between dental anomalies and genetic markers (rs2240308 and rs11867417 in *AXIN2* gene; rs9929218 in *CDH1* gene; rs642961 in *IRF6* gene).
5. To determine the link between dental caries and genetic markers (rs1800972 and rs11362 in *DEFB1* gene).

1.3. Hypothesis of the study

1. Dental anomalies are linked with types of congenital clefts – the more severe is the form of a cleft, the higher is the frequency of dental anomalies and their number.
2. Oral health condition is affected by the type of congenital cleft, eating, and oral hygiene habits.
3. Formation of cleft lips and palates, as well as dental anomalies is determined by the same genes.
4. Genetic markers rs11362 and rs1800972 of the *DEFB1* gene are linked with higher predisposition to caries in children with nonsyndromic congenital clefts.

1.4. Scientific novelty

This is the first study in Latvia defining dental anomalies and assessing oral health in children with nonsyndromic congenital clefts. This is the first time in Latvia when the link between genetic markers rs2240308, rs11867417, rs9929218, rs642961, rs11362, rs1800972 is determined in children with nonsyndromic congenital clefts.

2. MATERIALS AND METHODS

The study was conducted from 1 April 2009 to 7 July 2012. Three independent parts of the study were established. The first part was targeted at identification of dental anomalies, the second part dealt with assessment of oral health, and the third part covered establishment of the link between genetic markers and both dental anomalies and dental caries.

2.1. Part one: identification of dental anomalies in children with nonsyndromic congenital clefts

2.1.1. Selection of the study population

The study was retrospective, in which, based on clinical records and X–ray images, dental anomalies were identified in children with nonsyndromic congenital clefts. The study included evaluation of clinical records (n = 289) of children who participated in the grant "Identification of genes involved in craniocephal morphogenesis and predisposing orofacial clefts in the human genome" (2003.–2005.) by the Latvian Council of Science held within the scientific project between Taiwan and Baltics states on research of genetics in children registered at the RSU Institute of Stomatology Cleft Lip and Palate Centre.

Inclusion criteria:

1) age of patients from 6 years of age;
2) entry in a clinical record card on dental anomalies and a clear X–ray image (orthopantomography (OPG), and/or dental (peri–apical, occlusal) X–ray images);
3) absence of other serious congenital diseases or genetically determined syndromes.

Exclusion criteria:

1) patients under 6 years of age (n = 123);
2) patients with unclear x-ray images (n = 4);
3) patients with no affirmative clinical records on dental anomalies (n = 17);
4) patients with genetic syndromes or other severe congenital abnormalities (n = 19).

The obtained data of 126 children were included in the further study and analysed.

2.1.2. Identification of types of clefts and dental anomalies

Information on types of cleft was obtained from clinical records. For further analysis and identification of dental anomalies, children with nonsyndromic congenital clefts were divided into four groups according to the level of severity of the cleft, starting from isolated CP (relatively mild type of cleft) to bilateral CLP (relatively severe type of cleft) (Stahl et al., 2006):

1) isolated CP (n = 21);
2) unilateral CL ± A (n = 25);
3) unilateral CLP (n = 56);
4) bilateral CLP (n = 24).

Dental anomalies were identified only in regard to permanent teeth and the information on these malformations was obtained through assessment of dental history provided in clinical records and radiological examinations – orthopantomographs (OPT), dental (peri–apical, occlusal) X–ray images.

There were investigated anomalies of the number of teeth (hypodontia (absent teeth) and supernumerery teeth) and anomalies of dental shapes (microdontia – abnormally small teeth).

2.1.3. Data statistical analysis methods

The obtained data were entered into a Microsoft Office Excel database. Statistical analysis of the data was performed using software such as Microsoft Office Excel and SPSS v.22.

Proportion of dental anomalies in the groups of types of nonsyndromic congenital clefts was identified using frequency tables. Depending on distribution of the data, the analysis was performed using Pearson's Chi-Square Test (Pearson χ^2) or Fisher Exact test. Statistical differences were determined by *p* value below 0.05 ($p < 0.05$).

2.2. Part two: assessment of oral health in children with nonsyndromic congenital clefts

2.2.1. Selection of the study population

The study included and analysed data on children who from April 2009 to April 2012, attended multidisciplinary consultations at The Cleft Lip and Palate Centre (study group, n = 171) and Children's dentistry department of the Institute of Stomatology of RSU (control group, n = 196).

Oral health was assessed in three age groups according to the stage of development of dentition: 2 – 3 years old children (primary dentition); 6 – 7 years old children (mixed dentition); and 11 – 12 years old children (permanent dentition).

Inclusion criteria:

1) age of child corresponds to the age of the study group:
   2 – 3 years of age (study group n = 85, control group n = 90);
   6 – 7 years of age (study group n = 50, control group n = 60);
   11 – 12 years of age (study group n = 36, control group n = 46).
2) no history of severe congenital diseases or syndromes.

Exclusion criteria:

1) age of child did not correspond to the age group of the study;
2) children who were included in the study, but multidisciplinary consultations attended repeatedly;
3) children with severe congenital anomalies;
4) children who refused to participate in the study.

Methodology of the study was confirmed by the RSU Ethics Committee. Written consents were received from all parents of the participants confirming participation in the study.

2.2.2. Oral health assessment

For the purpose of assessment of oral health, children underwent examination of oral cavity in order to determine caries prevalence and indices of intensity of dental plaque and bleeding. Examinations were carried out in a dental chair under standard lighting, using dental mirror and dull probes. The obtained data were entered in clinical records (WHO Oral Health Assessment Form (1987)).

2.2.2.1. Evaluation of caries

Caries prevalence was evaluated as ratio between individuals with decayed teeth and the total number of examined individuals and expressed in percentage.

Caries intensity was assessed using DMF index (D – decayed, M – missing, F – filled) in primary dentition in both teeth (dmft) and surfaces (dmfs); DMF index (D – decayed, M – Missing, F – Filled) in permanent dentition both in teeth (DMFt) and surfaces (DMFs) and mixed dentition dmf + DMF both in teeth and surfaces (WHO, 1997).

2.2.2.2. Assessment of dental plaque and gingival health

Dental plaque was measured by modified plaque index described by Silness & Löe (Silness and Löe, 1964). Plaque was measured using a blunt probe for six individual teeth (maxillar and mandibular first molars sand central incisors) in four dental surfaces (buccal, lingual, mesial, and distal). To calculate plaque index for each individual, all of the indicators were summed up and divided by the number of examined teeth.

Gingival health was assessed using bleeding index described by Löe & Silness – plaque index (Löe and Silness, 1963). Plaque was determined using a blunt probe for six individual teeth (maxillar and mandibular first molars and central incisors) in four dental surfaces (buccal, lingual, mesial and distal). For estimation of the index, all of the indicators of each individual were summed up and divided by the number of examined teeth.

2.2.3. Questionnaire survey

The survey was carried out using validated modified questionnaires from an international Collaborative study carried out by WHO in Latvia in 1993. The questionnaire covered questions about marital status of parents, levels of parents' education, children's eating habits, tooth brushing habits, regularity of dentist and dental hygienist appointments, knowledge of parents on oral health. The questionnaire was completed by parents while children underwent clinical examinations.

2.2.4. Data statistical analysis methods

Caries prevalence was estimated in the study or the cleft group and in the control group. Statistical significance of the prevalence was determined by Pearson's Chi-square Test (Pearson χ^2). In each age group, oral health indicators were calculated for both the cleft and the control group (dmf, dmf + DMF, DMF index for teeth and surfaces; plaque and bleeding index).

Differences in these indicators between the groups were determined using t – test for independent signs or ANOVA univariate analysis with Bonferoni correction. Frequency tables were used for description of the results of the questionnaire. Differences were determined by Pearson's Chi-square Test (Pearson χ^2) or Fisher's Exact test. The impact of eating habits and tooth brushing habits on indicators of oral health was assessed using multivariate regression analysis. *P* value below 0.05 ($p < 0.05$) was selected as a level for statistically significant difference.

2.3. Part three: research of genetic markers

Children with congenital clefts and their relatives underwent sampling of venous blood carried out by a nurse in the Cleft Lip and Palate Centre of the Institute of Stomatology of RSU within the project on scientific research of genetics implemented between Taiwan and the Baltic states (2003.–2005.). Methodology of the blood collection was agreed with the RSU Ethics Committee and the Central Medical Ethics Committee.

The control group was comprised of 190 randomly selected individuals born without structural anomalies and whose relatives were not included in the National Population Genomic databases of the Latvian Biomedical Research and Study Centre.

DNAs of individuals involved in the project and representatives of the control group were isolated from blood samples at RSU Molecular Genetics Laboratory and the Latvian Biomedical Research and Study Centre (BMC) using standard chloroform – phenol method (Sambrook et al., 1989). Dried DNA samples of children with congenital clefts (n = 189) and of the control group (n = 190) were prepared for transportation to a laboratory of the University of Pittsburgh, USA.

Investigation of connection of genetic markers with dental anomalies and caries was carried out from 25 April 2012 to 7 July 2012 at the Vieira Lab of the University of Pittsburgh School of Dental Medicine (USA) by the author.

Genetic markers were selected based on the following four criteria:

1) localization in a gene;
2) function (if known that it changes expression of the gene or sequence of amino acids);
3) frequency in the population (ideal marker has a frequency of 50% in both alleles);
4) linkage disequilibrium (if selected SNPs were within one haplotype block, then one SNP was selected from the haplotype block).

2.3.1. Genetic markers and genotyping

For the research of dental anomalies, four genetic markers were selected according to the criteria described above: rs2240308 and rs11867417 in *AXIN2* gene (Mostowska et al., 2006; Callahan et al., 2009; Letra et al., 2012a); rs9929218 in *CDH1* gene (Frebourg et al., 2006; Letra et al., 2009); rs642961 in *IRF6* gene (Zucchero et al., 2004; Vieira et al., 2007, 2007a; Rahimov et al., 2008; Vieira et al., 2008d).

For the research of dental caries, two genetic markers were selected according to the criteria described above: genetic markers of *DEFB1* gene (Ozturk et al., 2010): rs11362 (– 20G > A); rs1800972 (– 44G > C).

Genotyping was carried out by real-time PCR using the TaqMan chemistry method (Ranade et al., 2001), and performed on an Applied Biosystems 7900 HT Sequence Detection System machine (Applied Biosystems Inc., Foster City, CA, USA), in line with recommendations of the manufacturer.

Reaction of the genotyping was carried out using DNA Engine BIO RAD Tetrad 2 Peltier Thermal Cycler (Alpha™ Unit Block Assembly for DNA Engine Systems BIO RAD, Made in Mexico).

The obtained results were analysed by real-time PCR (AB Applied Biosystems 7900HT Fast Real–Time PCR System, Made in Singapore). All reactions were carried out in line with the standard protocol recommended by the manufacturer.

2.3.2. Research of genetic markers in connection with dental anomalies

2.3.2.1. Selection of the study population

In order to investigate genetic markers in connection with dental anomalies, 189 children with congenital clefts and the control group (n = 190) with DNA samples available were analysed. In the result of application of inclusion and exclusion criteria, 93 children aged 6 to 17 years (mean age 12.3 years) were included in the study.

Inclusion criteria:

1) child's age as from 6 years of age;
2) an entry in the clinical record on dental malformations and clear X–ray images (orthopantomography (OPG) or dental (periapical, occlusal) X–rays);
3) no history of severe congenital diseases or syndromes;
4) available DNA sample.

Exclusion criteria:

1) children under 6 years of age (58 children);
2) no entries in the child's clinical record on dental anomalies and children with unclear X–ray images (22 children);

3) children with genetic syndromes or severe congenital diseases (16 children).

2.3.2.2. Determination of types of clefts and dental anomalies

Information on types of clefts was obtained from clinical records. Clefts were characterized by the side of the cleft (right, left, bilateral) and the presence of dental anomalies outside the cleft area. Children with nonsyndromic congenital clefts were divided into three groups according to the cleft type:

1) cleft lip with or without alveolar bone (CL ± A) (n = 22);
2) cleft lip and cleft palate (CLP) (n = 61);
3) isolated cleft palate (CP) (n = 10).

Presence of dental anomalies was based on clinical records and X–ray imaging. Investigation included anomalies of the number of teeth (hypodontia (absent teeth) and supernumerary teeth) and anomalies of dental shapes (microdontia – abnormally small teeth). Dental anomalies in cleft area (maxillary central incisors, lateral incisors, canines) were excluded from the study because the absence or changes of these teeth can be a development anomaly of the cleft area and/or a result of a surgical treatment (Kraus et al., 1966, Ribeiro et al., 2002)

2.3.2.3. Data statistical analysis methods

Statistical analyses were conducted using the PLINK 1.05 software (http://pngu.mgh.harvard.edu/~purcell/plink/) and Epi Info3.5.3. statistical software package (http://www.cdc.gov/epiinfo/). Odds ratio calculations and chi – square or Fisher's exact tests were used with a level of significance of 0.05 to determine if the distribution of alleles or genotypes was different between individuals born with clefts or born without any structural

abnormalities. Chi – square was also used to test for deviation from Hardy – Weinberg equilibrium.

2.3.3. Investigation of genetic markers in connection with caries

2.3.3.1. Selection of the study population

In order to carry out investigation of genetic markers associated with tooth decay, the same 189 children with congenital clefts of appropriate ages with DNA samples were analysed. The control group did not participate in this part. According to inclusion and exclusion criteria, the study included 69 children with nonsyndromic clefts aged 2 to 12 years (mean age 5.1 years).

Inclusion criteria:

1) child's age (isolated three age groups according to the stage of development of dentition; 2 – 3; 6 – 7; 11 – 12 years old children);
2) child had undergone a clinical examination for caries detection and had an entry in his/her clinical record;
3) child had severe congenital disease or syndrome;
4) child had DNA sample.

Exclusion criteria:

1) children, whose age did not correspond the particular age group (2 – 3 years of age and 6 – 7 years of age ; 11 – 12 years of age) (n = 85);
2) children with a genetic syndrome or a severe congenital disease (n = 16);
3) children without clinical records of examinations (n=19).

In order to perform genetic analysis, groups of 6 – 7 years old children (n = 23) and of 11 to 12 year-olds (n = 9) were combined.

2.3.3.2. Caries determination

Data were obtained from the clinical tests that were carried out in a standard dental chair light, dental mirror and using a blunt probe. Caries intensity was determined for each child using dmft and/or DMFt and dmfs and/or DMFs indexes, respectively, for teeth and surfaces.

According to the median caries intensity ratio, children of each age group were divided into subgroups (Vieira et al., 2008a; Ozturk et al., 2010):

1) low and medium caries intensity group (n = 35);

In 2 – 3 years old children (n = 17), dmft ranged from 0 to 1, but in the combined group of 6 – 7 and 11 – 12 years old children, a group of low and medium caries intensity was determined in children (n = 18) with dmft + DMFt from 0 to 7.

2) in the group of high caries intensity (n = 34);

In 2 – 3 years old children (n = 20) dmft was ≥ 2 but in the combined group of 6 – 7 and 11 – 12 years old children (n = 14) dmft + DMFt was ≥ 8.

2.3.3.3. Data statistical analysis methods

Statistical analyses were conducted using the PLINK 1.05 software (http://pngu.mgh.harvard.edu/~purcell/plink/) and Epi Info3.5.3. statistical software package (http://www.cdc.gov/epiinfo/). The statistical analysis was performed between a combination low and moderate caries experience group and the high caries group. Odds ratio calculations and chi – square of Fisher exact tests at the level of significance of 0.05 were used to determine if caries experience was associated with any allele or genotype. The standard chi – square test was used to test for deviation from Hardy – Weinberg equilibrium. ANOVA test was used for dmft + DMFt mean evaluation between cleft types.

3. RESULTS

3.1. Part one: identification of dental anomalies in children with nonsyndromic congenital clefts

3.1.1. Description of the study group

Following certain selection, the study on dental anomalies included 126 children (73 boys and 53 girls) with nonsyndromic congenital clefts. Determining types of clefts, cleft lip with/without alveolar bone (CL ± A) was observed in 19.84% (n = 25); cleft lip and palate (CLP) – in 63.49% (n = 80); isolated cleft palate (CP) in 16.67% (n = 21) of children. A more detailed breakdown by types and sides of clefts are shown in Table 1.

Table 1

Breakdown of children with nonsyndromic congenital lip and palate clefts by types and sides

Cleft type	Right n (%)	Left n (%)	Bilateral n (%)	Total
CL ± A	11 (44.0)	14 (56.0)	-	25
CLP	15 (18.75)	41 (51.25)	24 (30.0)	80
Isolated CP				21
Total:	26 (24.76)	55 (52.38)	24 (22.86)	126

In order to more accurately describe dental anomalies according to types of clefts, CLP were divided into unilateral and bilateral clefts leading to a total of four groups:

1) unilateral CL ± A – 25 (19.84%) children;
2) unilateral CLP – 56 (44.44%) children;
3) bilateral CLP – 24 (19.05%) children;
4) isolated CP – 21 (16.67%) children.

3.1.2. Dental anomalies in children with nonsyndromic congenital clefts

Of all 126 children with nonsyndromic congenital clefts, at least one dental anomaly was observed in 78 (61.91%) children. Dental anomalies were more common in boys 68.49% (n = 50), compared to 52.83% (n = 28) of girls, but these differences were not statistically significant ($p = 0.073$). In general, dental anomalies were more common in children with unilateral and bilateral CLP. The least number of dental anomalies was observed in children with isolated CP ($p < 0.0001$) (Table 2).

Table 2

Number of children with different types of clefts and dental anomalies

Cleft type	Children with dental anomalies		Children without dental anomalies		Total
	n	%	n	%	n
Unilateral CL ± A	12	48.0	13	52.0	25
Unilateral CLP	42	75.0	14	25.0	56
Bilateral CLP	21	87.50	3	12.50	24
Isolated CP	3	14.29	18	85.71	21
Total:	78	61.91	48	38.09	126

Anomaly of one tooth was observed in children with unilateral CLP and bilateral CLP, while anomalies of two teeth were more observed in children with bilateral CLP ($p < 0.0001$) (Figure 1).

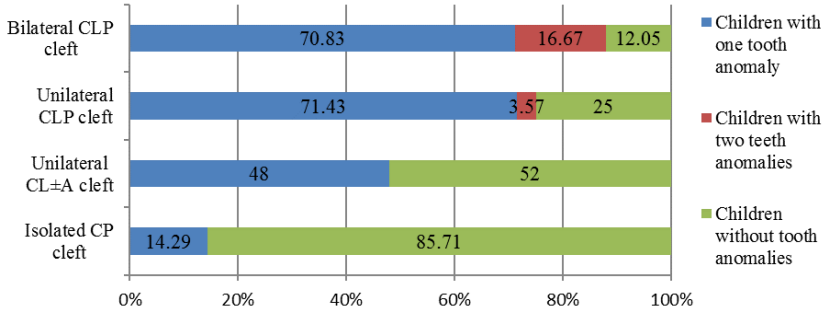

Figure 1. **Dental anomalies in different types of clefts**

Hypodontia and microdontia were the most common dental anomalies. More frequently, hypodontia and microdontia was observed in children with unilateral and bilateral CLP. Supernumerary teeth were more common in children with bilateral CLP. However, these differences were not statistically significant (p = 0.196) (Table 3).

Table 3

Number of various dental anomalies in children with different types of nonsyndromic congenital clefts

Dental anomalies	Cleft type								Total: (n = 126)	
	Unilateral CL ± A (n = 25)		Unilateral CLP (n = 56)		Bilateral CLP (n = 24)		Isolated CP (n = 21)			
	N	%	n	%	n	%	n	%	n	%
Hypodontia	5	20.0	19	33.93	10	41.67	3	14.29	37	29.37
Microdontia	6	24.0	21	37.50	9	37.50	0	0	36	28.57
Supernumerary teeth	1	4.0	3	5.36	6	25.0	0	0	10	7.94
No dental anomalies	13	52.0	14	25.0	3	12.50	18	85.71	48	38.10
Total:	25	100.0	57*	101.79*	28*	116.67*	21	100.0	131*	103.98*

* More than one dental anomaly was observed in children with unilateral and bilateral CLP

3.1.2.1. Hypodontia in children with nonsyndromic congenital clefts

Of all 126 children of the study group, hypodontia was observed in 29.37% (n = 37) of children. Hypodontia of one tooth was observed in 62.16% (n = 23), of two teeth – in 13,51% (n = 5), of three teeth – in 18.93% (n = 7), of four teeth – in 2.70% (n = 1) of children and absence of 5 teeth – in 2.70% (n = 1). Hypodontia was more common in maxilla, namely, lateral incisive hypodontia. In mandible, hypodontia of the second premolar was the most common. Hypodontia was more observed in children with unilateral CLP.

3.1.2.2. Microdontia in children with nonsyndromic congenital clefts

Microdontia was observed in 28.57% (n = 36) of children. Microdontia of one tooth was observed in 94.44% (n = 34), of two teeth – in 2.78% (n = 1) and of three teeth – in 2.78% (n = 1).

Microdontia was observed only in maxilla. Lateral incisive microdontia was observed more frequently. Microdontia of lateral incisors was observed only in the cleft side. No microdontia was observed in children with isolated CP.

3.1.2.3. Supernumerary teeth in children with nonsyndromic congenital clefts

Supernumerary teeth were observed in 7.94% (n = 10) of children. Supernumerary one tooth was observed in 90.0% (n = 9) of children while supernumerary two teeth were observed in 10.0% (n = 1) of children. Supernumerary teeth were observed only in maxilla, the most frequently they were lateral incisors. In children with bilateral CLP, supernumerary teeth were observed in 6 cases, in children with unilateral CLP – in 3 cases and in children with unilateral CL ± A – in one case. In children with isolated CP, no supernumerary teeth were observed.

3.1.2.4. Dental anomalies outside cleft area in children with nonsyndromic congenital clefts

Outside cleft area, only hypodontia was observed. No microdontia and supernumerary teeth were observed outside cleft area. In the study population, of all 126 children, hypodontia outside cleft area was observed in 9.52% (n = 12). Most often it was observed in children with unilateral CLP – in 5.55% (n = 7), in children with bilateral CLP in 2.28% (n = 3), in children with unilateral CL ± A – in 0.79% (n = 1), and in children with isolated CP – in 0.79% (n = 1). Ten children had hypodontia outside cleft area only in mandible, while two children had it both in maxilla and mandible. 22 teeth were affected by hypodontia. Hypodontia of one absent tooth outside cleft area was observed in 5 children, hypodontia of two missing teeth – in 5 children, hypodontia of three and four missing teeth – in one child. Mandibular second premolars were most often affected by hypodontia.

3.2. Part two: oral health of children with nonsyndromic congenital clefts

3.2.1. Description of the study group

The study on oral health was comprised of 367 children (cleft group with n = 171, control group with n = 196). Distribution in age groups between cleft and control groups was similar and these differences were not statistically significant ($p = 0.76$). Although dental caries was observed in both the cleft and the control group, more often it was observed in the control group ($p = 0.006$). The overall description of the study population is summarized in Table 4.

Table 4

Overall description of the cleft group and the control group

	Cleft group n = 171		Control group n = 196		*p* value
	n	%	n	%	
Age:					
2 – 3 years of age	85	49.71	90	45.92	0.76
6 – 7 years of age	50	29.24	60	30.61	
11 – 12 years of age	36	21.05	46	23.47	
Sex:					
Boys	93	54.39	94	47.96	0.22
Girls	78	45.61	102	52.04	
Caries:					
Yes	128	74.85	169	86.22	**0.006**
No	43	25.15	27	13.78	

3.2.1.1. Caries prevalence in children with nonsyndromic congenital clefts

Lower caries prevalence among 2 – 3 year-olds was observed in the cleft group (p = 0.008). Similar caries prevalence in both the cleft and the control group was among 6 – 7 year-olds, while among 11 – 12 year-olds, caries prevalence was slightly lower in the cleft group and this difference was not statistically significant (p = 0.8, p = 0.28). Caries prevalence among children in both the cleft group and the control group is shown in Figure 2.

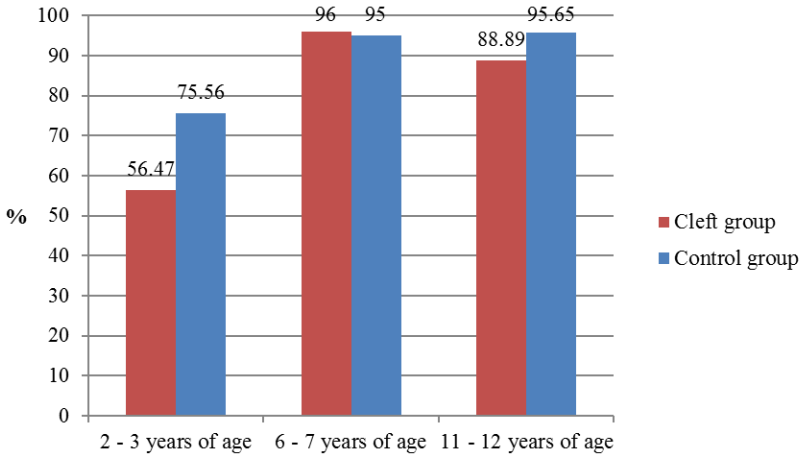

Figure 2. **Percentage of caries prevalence in the cleft and the control group**

3.2.1.2. Caries intensity in 2 – 3 year-olds with nonsyndromic congenital cleft

Although the range of minimum and maximum values of caries intensity in the cleft group was more extensive (0 to 20), overall ratio of dental caries intensity (dmft) in the control group was higher (p = 0.039). Caries intensity (dmf index) in teeth and surfaces in the cleft group and the control is shown in Table 5.

Table 5

Caries intensity in 2 – 3 year-olds

	Cleft group (n = 85) (± SD)	Control group (n = 90) (± SD)	*p* value
dmf of teeth	**3.49 (4.70)**	**4.78 (3.85)**	**0.039**
minimum/ maximum value	0/20	0/15	
d	3.32 (4.52)	4.34 (3.79)	0.104
f	0.08 (0.58)	0.38 (1.10)	**0.028**
m	0.09 (0.50)	0.06 (0.27)	0.526
dmf of surfaces	**7.01 (13.00)**	**8.24 (9.03)**	0.444
minimum/ maximum value	0/65	0/57	
d	6.46 (11.95)	7.34 (8.83)	0.576
f	0.08 (0.58)	0.62 (1.76)	**0.007**
m	0.47 (2.51)	0.28 (1.37)	0.526

Component d (decayed) and f (filled) in the dmf index was larger in the control group, however statistically significant difference was found between filled teeth (p = 0.028) and surfaces (p = 0.007). Component m (missing) of the dmf index was greater in the cleft group, however these differences were not statistically significant (p= 0.526).

3.2.1.3. Caries intensity in 6 – 7 year-olds with nonsyndromic congenital clefts

The broader range of minimum and maximum values in dmf index was among children with clefts (2 to 15) in primary dentition, while in mixed dentition they were almost the same in both groups (0 – 14 in children with clefts; 0 – 15 in the control group). Overall caries intensity in both primary dentition and mixed dentition was higher among children with clefts, while these differences were not statistically significant (p = 0.758, p = 0.142). Caries prevalence and intensity (dmf; dmf + DMF index) in teeth and surfaces of children with clefts and the control group is shown in Table 6.

Table 6

Caries intensity among 6 – 7 year-olds

	Cleft group (n = 50) (± SD)	Control group (n = 60) (± SD)	*p* value
dmf of teeth	**7.50 (4.38)**	**6.75 (2.36)**	0.758
minimum/ maximum value	2/15	5/10	
d	4.62 (2.62)	5.50 (3.70)	0.931
f	2.5 (2.56)	1.25 (1.89)	0.432
m	0.38 (1.06)	0	0.480
dmf of surfaces	**12.38 (8.58)**	**12 (6.78)**	0.941
minimum/ maximum value	2/26	7/22	
d	6.5 (3.82)	9.50 (8.89)	0.732
f	4.00 (4.21)	2.50 (3.79)	0.435
m	1.88 (5.30)	0	0.480
dmf + DMF in teeth	**7.86 (3.91)**	**6.70 (3.78)**	0.142
minimum/ maximum value	0/14	0/15	
d	3.78 (3.02)	3.64 (2.99)	0.808
f	3.10 (2.49)	1.91 (2.23)	**0.011**
m	0.5 (0.97)	0.27 (0.80)	0.100
D	0.36 (0.85)	0.70 (1.26)	0.178
F	0.12 (0.33)	0.18 (0.69)	0.487
M	0	0	
dmf + DMF in surfaces	**15.83 (11.06)**	**12.71 (10.62)**	0.161
minimum/ maximum value	0/39	0/46	
d	6.36 (6.04)	6.29 (6.90)	0.815
f	6.38 (5.59)	4.10 (5.63)	**0.012**
m	2.5 (4.85)	1.34 (3.99)	0.010
D	0.40 (1.01)	0.77 (1.40)	0.177
F	0.19 (0.55)	0.21 (0.85)	0.474
M	0	0	

Carious teeth in primary dentition were more common in children of the control group, while filled and missing (extracted) teeth were more common in children of the cleft group, however these differences were not statistically significant.

In the mixed dentition, all components (d; f; m) of dmf index were greater in children of the cleft group, however statistically significant difference

was found between filled primary teeth (p = 0.011) and surfaces (p = 0.012). In regard to permanent teeth, carious and filled teeth were more found in children of the control group, however these differences also were not statistically significant.

3.2.1.4. Caries intensity in 11 – 12 years old children with nonsyndromic congenital clefts

Caries intensity index in both mixed and permanent dentition was higher in the cleft group, however these differences were not statistically significant (p = 0.427, p = 0.826). Caries intensity (dmf + DMF; DMF index) in teeth and surfaces in children of the cleft group and the control group is shown in Table 7.

Table 7

Caries intensity in 11 – 12 years old children

	Cleft group (n = 36) (± SD)	Control group (n = 46) (± SD)	*p* value
dmf + DMF in teeth	**6.08 (4.09)**	**5.34 (3.53)**	0.472
minimum/ maximum value	0/15	0/17	
d	1.13 (1.60)	0.94 (1.48)	0.69
f	1.25 (1.67)	0.68 (1.12)	0.22
m	0	0	
D	2.38 (2.89)	1.81 (2.69)	0.386
F	1.33 (1.71)	1.91 (1.61)	0.159
M	0	0	
dmf + DMF in surfaces	**9.79 (7.29)**	**9.28 (8.16)**	0.81
minimum/ maximum value	0/25	0/38	
d	2.13 (3.37)	2.16 (5.87)	0.558
f	2.25 (2.69)	1.44 (2.21)	0.245
m	0	0	0.839
D	3.25 (4.52)	2.28 (3.33)	0.564
F	2.08 (3.09)	3.41 (3.18)	0.056
M	0	0	

Continuation of the Table 7

DMF in teeth	**6.5 (3.61)**	**6.21 (2.66)**	0.826
minimum/ maximum value	0/13	1/11	
D	4.17 (2.72)	3.07 (2.43)	0.290
F	1.67 (1.87)	3.00 (2.45)	0.196
M	0.67 (1.15)	0.14 (0.53)	0.100
DMF in surfaces	**11.17 (8.65)**	**9.57 (5.47)**	0.574
minimum/ maximum value	0/29	1/21	
D	4.92 (3.18)	3.57 (3.20)	0.244
F	2.92 (2.94)	5.29 (4.38)	0.191
M	3.33 (5.77)	0.71 (2.67)	0.100

All components of dmf index (d; m; f) in the mixed dentition was higher in primary teeth of children of the cleft group, while among permanent teeth filled teeth were more in children of the control group, however these differences were not statistically significant, too.

In the permanent dentition, children with clefts had more carious and extracted (missing) teeth, while in the control group – filled teeth. However, these differences were not statistically significant.

3.2.1.5. Plaque and bleeding indices in children with nonsyndromic congenital clefts

Plaque index and bleeding index in the cleft group was lower than in the control group. These differences were statistically significant compared to plaque index among 2 – 3 years old children ($p = 0.039$) and bleeding index among 6 – 7 years old children ($p < 0.0001$) (Table 8).

Table 8

Distribution of plaque and bleeding indices between age groups in the cleft and the control groups

Age groups	Plaque index (± SD)		*p* value	Bleeding index (± SD)		*p* value
	Cleft group	Control group		Cleft group	Control group	
2 – 3 years of age	0.62 (0.62)	0.82 (0.65)	**0.039**	0.59 (0.63)	0.67 (0,61)	0.395
6 – 7 years of age	1.12 (0.87)	1.38 (0.69)	0.08	0.91 (0,65)	1.54 (0.85)	**< 0.0001**
11 – 12 years of age	1.37 (0.95)	1.74 (1.29)	0.153	1.21 (0.75)	1.54 (1.06)	0.117

3.2.2. Results of the questionnaire

Socio-demographic indicators of the surveyed children were generally similar, excluding the average age of mothers and educational levels of parents. The average age of mothers in the cleft group was slightly lower.

Statistically significant differences ($p < 0.0001$) were found between educational levels of mothers in the cleft group and the control group. Mothers of children from the cleft group mostly were with higher and secondary education, while in the control group – higher and secondary vocational education, in addition, in the cleft group more mothers were with primary education than in the control group.

Also statistically significant difference was found between educational levels of fathers between the groups ($p = 0.014$). Most fathers of the cleft group were with secondary vocational education, while in the control group – higher and secondary vocational education, in addition, more fathers in the cleft group were with primary education.

When analysing eating habits of children, the majority of children in both groups ate three meals a day and snacked between meals. However, no

statistically significant differences between the cleft and the control groups were found.

In the cleft group, sweets were often given by parents and grandparents, while in the control group – by parents ($p = 0.009$). Responding to a question on what cases children receive sweets most often, the cleft group answered that they give sweets upon a child's request, or for no reason, while the control group indicated that they give sweets upon a child's request or as a reward.

The most common drinks of children in the cleft group were water, juice and then – sugared tea. While in the control group, the most common drink for children was water ($p = 0.0001$).

Teeth were cleaned by almost all children of the study population. In the cleft group were cleaned under parental supervision more frequently, while in the control group children clean their teeth by themselves ($p = 0.004$). In both study groups, children mostly brushed their teeth twice a day or once a day – in the evening ($p = 0.040$) and knew what toothpaste they were using ($p = 0.002$).

No significant differences between the groups were found in regard to usage of dental cleaning aids, except for special toothbrushes that are significantly more used in the cleft group ($p = 0.042$).

Toothbrushes were mostly changed three to four times a year in both the cleft and the control group. Parents of the cleft group more often than parents of the control group answered that their children had used or have been using dental plate for teeth adjustment ($p < 0.0001$).

Parental attitudes towards medical treatment of milk teeth, thoughts about inheritance of good or bad teeth, relationship between diet and dental health, importance of dental care and oral hygiene were similar and no statistically significant differences between the cleft group and the control group were found.

Regardless the fact that certain eating habits (number of meals per day, snacking between meals and the number of snacks, who gives sweets and when; preferred drinks) and tooth brushing habits (who cleans child's teeth, how many times a day they brush their teeth; preference of a toothpaste, preference of dental aids, dentist/hygienist appointments) and parental education statistically significantly differed in some positions between the cleft and the control group, after inclusion of those factors in a multiple regression analysis, none of these factors showed a statistically significant difference in regard to indicators of oral health (caries intensity, bleeding and plaque indices).

3.3. Part Three: investigation of genetic markers in children with nonsyndromic congenital clefts

3.3.1. Investigation of genetic markers in connection with dental anomalies

Of all 93 individuals born with oral cleft included in this study, 53 (56.99%) were males and 40 (43.01%) were females. Among the individuals born without any structural abnormalities, 83 (43.68%) were males and 107 (56.32%) were females. Males were statistically significant more affected by oral cleft than females ($p = 0.035$). Forty – one males and 20 females had CLP, seven males and 15 females had CL ± A, and five males and five females had isolated CP.

Dental anomalies outside the cleft area affected 13 (13.98%) individuals. Tooth agenesis was the most frequent dental anomaly among individuals born with clefts (n = 10; 10.75%). The characteristics of the individuals born with clefts are presented in Table 9.

Table 9

Clinical characteristics of the individuals born with oral clefts

	n	%
Type of clefts		
CL ± A	22	23.66
CLP	61	65.59
CP	10	10.75
CL ± A and CLP	83	89.25
Affected Side		
Right	21	22.58
Left	46	49.46
Both	16	17.21
Associated dental anomalies outside the cleft area		
Yes	13	13.98
No	80	86.02
Tooth agenesis outside the cleft area		
Yes	10	10.75
No	83	89.25
Supernumerary teeth outside the cleft area		
Yes	1	1.08
No	92	98.92
Microdontia outside the cleft area		
Yes	2	2.15
No	91	97.85

All SNPs were in Hardy – Weinberg equilibrium. Table 10 presents the comparison of allele and genotype frequencies among the studied individuals.

Table 10

Genotype and allele distributions

Cleft Subgroups	Genotype n (%)			*p* value	Allele n (%)		*p* value	OR (CI 95%)
***IRF6* rs642961**								
	AA	**AG**	**GG**		**A**	**G**		
Non-Cleft	1 (0.5)	53 (29.0)	129 (70.5)	–	55 (15.0)	311 (85.0)	–	–
All Cleft	4 (4.8)	31 (37.3)	48 (57.8)	**0.016**	39 (23.5)	127 (76.5)	**0.017**	1.74 (1.07–2.82)
CL ± A and CLP	4 (5.4)	29 (39.2)	41 (55.4)	**0.006**	37 (25.0)	111 (75.0)	**0.007**	1.88 (1.15–3.01)
Isolated CP	0	2 (22.2)	7 (77.8)	0.882	2 (11.1)	16 (88.9)	0.483	0.71 (0.11–3.34)
Unilateral	3 (5.1)	24 (40.7)	32 (54.2)	**0.009**	30 (25.4)	88 (74.6)	**0.009**	1.93 (1.13–3.28)
Bilateral	1 (6.7)	5 (33.3)	9 (60.0)	0.065	7 (23.3)	23 (76.7)	0.228	1.72 (0.64–4.48)
All cleft with dental anomalies	1 (7.7)	3 (23.1)	9 (69.2)	**0.045**	5 (19.2)	21 (80.8)	0.365	1.35 (0.42–3.99)
All cleft with tooth agenesis	1 (10.0)	3 (30.0)	6 (60.0)	**0.015**	5 (25.0)	15 (75.0)	0.184	1.88 (0.57–5.84)

OR – odds ratio; CI 95% – confidence interval;

All cleft group were compared to the non–cleft group chi-square or Fisher's exact were used

AXIN2 (rs2240308 and rs11867417) and *CDH1* (rs9929218) were not associated with oral clefts. In *IRF6*, the allele A in the marker rs642961, increased the risk for oral cleft, (OR = 1.74; CI 95% 1.07–2.82; p = 0.017). Similar results was found when only individuals with lip involvement were used in analysis (OR = 1.88; CI 95% 1.15–3.01; p = 0.007).

3.3.2. Investigation of genetic markers in connection with caries

Of all 69 children born with oral cleft included in this study, 46 were males and 23 were females. The definition of caries experience is presented in table 11.

Table 11

Definition of caries experience based on age and dmft + DMFT (Decayed, Missing due to caries, Filled Teeth) scores

Subgroups Caries experience level	Nr. of ind.	Sex		**dmft + DMFT**		**dmfs + DMFS**	
		M	F	Means (± SD)	Average	Means (± SD)	Average
Toddlers (from 2 to 3 yrs of age)	37	28	9	2.97 (3.88)	0/15	5.68 (11.99)	0/65
Low and moderate: dmft = 0–1	17	11	6				
High caries: dmft = 2 or higher	20	17	3				
Children (from 6 to 12 yrs of age)	32	18	14	7.25 (3.2)	0/14	13.12 (9.01)	0/35
Low and moderate: DMFT/dmft = 0–7	18	13	5				
High caries: DMFT/dmft = 8 or higher	14	5	9				

Nr. of ind. ─ Number of individuals; M ─ males; F ─ females; SD ─ Standard Deviation

A statistically difference between sexes and caries experience was no found. The mean dmft (standard deviation) was 3.2 (± 3.9) for male toddlers (2 to 3 year olds) and 2.2. (± 3.8) for toddlers (p = 0.51 for caries distribution by sex), the mean dmft + DMFT was 6.55 (± 3.1) for male children (6 to 12 years olds) and 8.1 (± 3.3) for female children (p = 0.17 for caries distribution by sex). There was also no significant difference in the caries experience between cleft type. The mean dmft + DMFT for CLP was 8.1 (± 8.7), for CL was 8.2 (± 9.5) and for isolated CP was 11.3 (± 15.3) (p = 0.55 for caries distribution by cleft type).

Both analysed SNPs (rs11362, rs1800972) were in Hardy – Weinberg equilibrium. Table 12 shows the distribution of the genotypes of the SNPs between groups.

Table 12

Distribution of *DEFB1* markers based on caries experience

Caries experience groups	Genotypes n (%)			*p*-value	Allele n (%)		*p*-value	OR (CI 95%)
***DEFB1* rs11362**								
	GG	**AG**	**AA**		**G**	**A**		
Low and moderate	7 (20.0)	21 (60.0)	7 (20.0)	**0.047**	35 (50.0)	35 (50.0)	0.224	1.52 (0.7–2.98)
High	15 (44.1)	11 (32.4)	8 (23.5)		41 (60.3)	27 (39.7)		
***DEFB1* rs1800972**								
	CC	**CG**	**GG**		**C**	**G**		
Low and moderate	1 (2.9)	14 (40.0)	20 (57.1)	0.703	16 (22.9)	54 (77.1)	0.910	1.05 (0.4–2.51)
High	2 (5.9)	11 (32.4)	21 (61.8)		15 (22.1)	53 (77.9)		

CI 95% – 95% confidence interval

There were no significant differences between allele distribution and caries experience. A statistically significant difference was found for the genotype distribution of marker rs11362 considering caries experience (p = 0.047). When we analysed the data in a recessive model

(GG vs AG + AA), the genotype GG increased more than 3 times the odds for higher caries experience (p = 0.031; OR = 3.16; CI 95% 0.97–10.62).

4. DISCUSSION

The Cleft Lip and Palate Centre is the only medical institution in Latvia, where children with congenital clefts are provided with an extensive treatment. The Centre has been operating for more than 50 years, however no aggregated information on dental anomalies and oral health of children with congenital clefts is available.

The group of the conducted study does not represent all children with congenital clefts. The study excluded children with syndromic clefts and analysed only children with nonsyndromic or isolated cleft, which, according the literature data, amount to 70%.

Various dental anomalies in children with nonsyndromic congenital clefts are common more often if compared to population data in general (Ranta and Rintala, 1982; Ribeiro et al., 2003). The study found that of all 126 children, anomaly of at least one tooth was observed in 61.91% of children with nonsyndromic congenital clefts. Akcam et al found that at least one dental anomaly was observed more frequently. Although this study, similar to our study, included all types of clefts, , isolated CP were included twice less (only 8.2%), and the studied dental anomalies were distributed in much larger number of types (including enamel hypoplasia, root length, development anomalies), which probably explains why at least one dental anomaly was found in 96.7% (Akcam et al., 2010).

More dental anomalies were found in clinically severe forms of clefts, for example, in children with bilateral (87.5%) and unilateral (75%) CLP. Severity of a cleft also affects development of dental abnormalities in cleft area (Ranta, 1988; Shapira et al., 2000; Dewinter et al., 2003). Dental anomalies are observed both in primary (Kraus et al., 1966; Vastardis, 2000), and permanent dentition (Graber, 1978; Bartzela et al., 2010) and are found in both maxilla and mandible. However, in the permanent dentition they are found significantly

more often, and they are located in the maxillary area of cleft (Schutte et al., 1999; Ribeiro et al., 2002; Ribeiro et al., 2003). Also in this study, dental anomalies were more frequently reported in maxillary cleft area.

Frequency of hypodontia in the population in various studies is mentioned in the range of 3–10% (Graber, 1978; Schutte and Murray, 1999; Arte et al., 2001; Eerens et al., 2001). Results of our study showed that incidence of hypodontia in children with nonsyndromic congenital clefts are 29.37%. Studies described in literature show that absence of maxillary lateral incisor is the most common dental abnormality in children with CLP (Akcam et al., 2010; Bartzela et al., 2010). This could be explained by bone deficit in palate incisor region, therefore hypodontia mostly occurs in cleft area.

Researchers believe that absence of teeth in the opposite side of the cleft means hidden case of bilateral clefts (Menezes and Vieira, 2008; Akcam et al., 2010). In our study, frequency of hypodontia outside cleft area is 9.52%, while other authors show 27.2% (Dewinter et al., 2003) and within the range of 12.5 to 52.8% depending on the location (incisor, premolar or molar region) (Akcam et al., 2010) and clefts types involved in the studied sample.

Literature data on supernumerary teeth in children with nonsyndromic congenital clefts also differ. In various studies, supernumerary teeth are considered to be the second most frequently occurring dental anomaly (Ribeiro et al., 2003). In this study, frequency of supernumerary teeth is 7.94%, while in other studies the frequency is 7.3 (Tortora et al., 2008) to 25.6% (Kraus et al., 1966). These frequency fluctuations also can be explained by different specifics or designs of the study.

Literature data on frequencies of microdontia are also different. Incidence of microdontia in studies of different populations is described from 1.5 to 2.9%. In our study, microdontia in children with nonsyndromic congenital clefts was observed in 28.57%. Most frequently microdontia was observed in cleft area for maxillary lateral incisors, and they are awl-shaped or

reduced (Ranta, 1986). Literature data show that its frequency in children with nonsyndromic congenital clefts is from 4.5 to 37% (Kraus et al., 1966; Tortora et al., 2008) and variations of its frequency could be explained by diversity of study designs.

The second part of the study on oral health included research of a certain-age sample group, however the study results are fully applicable to entire population of children with nonsyndromic clefts in the relevant age group, as they represented 77.7% of all children with nonsyndromic congenital cleft within this age group.

Dental caries is still the most common disease worldwide (Marthaler et al., 1996). Literature data on the intensity of caries in children with nonsyndromic congenital clefts are limited in number and they are different. Most scientists focus on intensity of caries in permanent teeth. It has been described that children with congenital cleft has higher caries intensity than children without cleft (Dahllöf et al., 1989; Bokhout et al., 1996; Ahluwalia et al., 2004; Al-Wahadni et al., 2005; Cheng et al ., 2007; Al-Dajani, 2009). This could be due to a variety of other dental abnormalities such as enamel hypoplasia, dental overcrowding, maxillary bone deficiency, limited access to teeth in the area cleft for hygiene purposes (Menezes and Vieira, 2008; Zhu et al., 2010).

In our study, prevalence of caries is generally higher in the control group. It can be explained by the fact that the control group was comprised of selected children who attended the Children's dentistry department of the Institute of Stomatology at random. Often this department is attended by children with various oral diseases from all over Latvia. However, comparing the results with other studies conducted in Latvia on prevalence of dental caries, prevalence of caries in groups of 2 – 3 year-olds and 6 – 7 year–olds was higher in cleft group, while the prevalence of caries in the group of 11 – 12

year–olds was similar (Bjarnason et al., 1995; Bērziņa and Care, 2003; Henkuzena et al., 2004; Gudkina and Brinkmane, 2010; Skrīvele et al., 2013).

As shown by studies previously conducted in Latvia (Henkuzena and Care, 2002; Henkuzena et al., 2004), primary teeth are little treated. In our study, lower caries intensity was observed in 2 – 3 years old children, compared with the control group. This could be explained by delayed eruption of teeth. According to literature data, children with nonsyndromic congenital clefts show delayed eruption of teeth from 3 to 7 months, but in case of more severe clefts – even longer (Ranta, 1986). Comparing with the study conducted by Skrīvele and co-authors (Skrīvele et al., 2013) on 2 – 3 years old children in Riga pre-school educational institutions, dmf index of teeth (dmft = 1.16) was two times lower compared to children with nonsyndromic congenital clefts (dmft = 3.49).

In our study, caries intensity in permanent dentition was generally similar between the cleft group and the control group, while in primary dentition, intensity of caries in the cleft group was lower compared to the control group. In a study conducted by Tannure et al (Tannure et al., 2012a) in Rio de Janeiro, Brazil on caries intensity observed similar trends, although the design of the study was different (they studied primary and permanent teeth from 5 to 19 years of age; dmft 1.68; DMFt 1.20).

Although caries intensity in our study in comparison with other countries was higher, it can be explained by differences in study designs because of different age groups used or merging of higher age groups (Zhu et al., 2004;), or primary and permanent teeth were studied in different age groups together (Tannure et al., 2012a).

Researching oral health habits, in general no differences between the cleft group and the control group were observed (Zhu et al., 2004; Tannure et al., 2012a). In our study, these data compared to the control group, were similar.

Craniofacial development is a very complex phenomena, which involves many biological processes. Alterations in tooth germ development and occurrence of oral clefts have a close embryological relationship in terms of timing, anatomical position, and genetic involvement (Stahl et al., 2006; Letra et al., 2007; Vieira et al., 2008c). The *AXIN2* gene is a negative regulator of the Wnt signalling pathway that regulates embryogenic development and organogenesis (Letra et al., 2009). Mutations in *AXIN2* have been associated with familial and sporadic tooth agenesis (Lammi et al., 2004; Mostowska et al., 2006), and tooth agenesis itself has been reported in association with oral clefts (Letra et al., 2007; Vieira et al., 2007a; Vieira et al., 2008d; Letra et al., 2009). *CDH1* is involved in cell-cell adhesion. This gene plays a major role in craniofacial morphogenesis and palatal fusion (Letra et al., 2009; Song and Zhang, 2011). Although *AXIN2* and *CDH1* were previously suggested to be associated with oral clefts and tooth agenesis, we did not find association in the studied cohort. However we cannot exclude that the capacity of the study group was too small to be able to assess importance of these gene markers.

Although numerous studies have demonstrated that variants in *IRF6* are associated with oral clefts (Zucchero et al., 2004; Park et al., 2007; Rahimov et al., 2008; Birnbaum et al., 2009; Pan et al., 2010; Wu et al., 2010; Letra et al., 2012b) just few have focused in SNP rs642961. Rahimov et al. (Rahimov et al., 2008) first identified this variant as associated with oral clefts, in which the A allele was significantly overtransmitted in families of European background. This marker alters function of *IRF6* gene, which has been tested in various studies (*Birnbaum* et al., 2009; Pan et al., 2010), while populations of other studies were this association was studied, did not prove this (Paranaiba et al., 2008). This polymorphism in *IRF6* gene is located between *IRF6* and *DIEXF* (digestive organ expansion factor homolog) genes, we suggested to cause disruption of the binding site of the transcription factor AP-2. This in turn disrupts proper expression of the *IRF6* gene. Here, we found an association

between the rs642961 marker and oral clefts, which corroborated previous studies (Rahimov et al., 2008; Birnbaum et al., 2009; Pan et al., 2010; Mostowska et al., 2010; Wu et al., 2010).

Vieira et al. (Vieira et al., 2007a; Vieira et al., 2008c; Vieira et al., 2008d) previously showed that *IRF6* was associated with tooth agenesis. Also, they showed a trend for association between certain palatine rugae patterns and *IRF6* variants (Murdoch et al., 2009). In present study, the *IRF6* rs642961 genotype AA is 20 times more frequent in individuals presenting oral clefts associated with tooth agenesis when compared to unaffected unrelated individuals.

One of the challenges of studying a complex, multifactorial disease, such as caries, is the fact that many genetic and environmental factors contribute to the susceptibility of the disease. It is important to determine a phenotype in detail for research and large sets of data purposes (Vieira et al., 2007a). Investigating caries in clinical and epidemiological studies, caries intensity is determined (DMF index). It provides information on intensity of the disease, identifying decayed teeth and surfaces. Caries intensity groups according to the number of decayed teeth are often identified for further genetic researches (Vieira et al., 2008; Al-Dajani, 2009; Vieira et al., 2014). Such a division in regard to connection of genetic markers and caries was also chosen.

A trial by Ozturk et al (Ozturk et al., 2010) emphasized an importance of a gene *DEFB1* in development of caries which is associated with host-microbial interactions and their contribution to the development of caries. A part of our study also confirms this point of view in results: changes in promoter's region of *DEFB1* play an important role in ethology of dental caries.

DEFB1 is an oral antimicrobial peptide which provides the first defence against a wide spectrum of pathogens (Dunsche et al., 2001; Ouhara et al., 2005; Dale et al., 2006; Rivas-Santiago et al., 2009; Gursoy and Könönen,

2012). The great variations of defensin concentrations in saliva and in oral tissues could be attributed to the genetic variations in the host (Dale et al., 2006; Rivas-Santiago et al., 2009).

Previous epidemiological studies showed high caries experience in Latvian children (Berzina and Care, 2003; Henkuzena et al., 2004; Gudkina and Brinkmane, 2008). Although the prevalence of caries free children between 2 to 6 years of age in the group we studied was similar to the general population, the mean dmft was slightly higher in the children born with oral clefts we studied. However, it is important to emphasize that while the comparison group is from kindergartens in Riga, the capital of Latvia, our sample ir from the only referral unit for cleft children in Latvia, and represents the whole country. In the study performed by Bērziņas and Cares (Bērziņa and Care, 2003) for 11 and 13 years old children the mean values for DMFT and DMFS were significantly lower in Riga than in the rest of Latvia.

Briefly, our results demonstrated that the polymorphism rs11362 G–20–A in the promoter region of *DEFB1* was associated with caries experience in children born with oral clefts. Variation in the expression of *DEFB1* within the oral cavity can be associated with individual susceptibility for caries (Chung et al., 2007). In our study we did not analyse the gene and/or protein expressions in saliva, however, future studies should consider including these analyses in order to clarify the correlation between genetic variation in the promoter region of *DEFB1*, defensin concentration in saliva, and susceptibility to caries.

This is the first study on dental anomalies and oral health in children with nonsyndromic congenital clefts in Latvia. Importantly, this was the first study in Latvia identifying connection of dental anomalies and dental caries with specific genetic markers. It allows us to understand these complex pathologies more widely.

5. CONCLUSIONS

1. Hypodontia and microdontia are the most common dental anomalies in children with nonsyndromic congenital clefts. Hypodontia and microdontia of maxillary lateral incisors is the most common. Dental anomalies were more observed in children with unilateral and bilateral clefts, and most often these anomalies were hypodontia and microdontia. Outside cleft area, only one dental anomaly was reported – hypodontia, and mandibular second premolars were the most frequently affected teeth.

2. Dental caries was observed in children with nonsyndromic congenital clefts in all age groups. The intensity of caries in children with nonsyndromic congenital clefts in the group of 2 – 3 year–olds was lower; in the group of 6 – 7 years–olds it was higher and in the group of 11 – 12 year–olds it was similar compared to the data of the control group. Plaque and bleeding indices in children with clefts was lower than in the control group.

3. Oral health influencing behavioural factors in children with nonsyndromic congenital clefts was generally similar to the control group. Mostly children brush their teeth twice a day or once a day in the evening. Children in the cleft group more frequently brushed their teeth under parental supervision and those children were more likely to have or had dental plates for adjustment of teeth. Children in both groups ate three meals a day and snacked between meals. Knowledge of parents about oral health was generally similar.

4. *IRF6* gene's marker rs642961 was linked with dental anomalies and nonsyndromic clefts. No link between *AXIN2* gene (rs2240308 and rs11867417) and *CDH1* gene (rs9929218) and nonsyndromic congenital clefts and dental anomalies was confirmed.

5. *DEFB1* gene's marker rs11362 showed a connection with development dental caries in children with nonsyndromic clefts.

6. PRACTICAL RECOMMENDATIONS

1. Early provision of recommendations for parents of children registered at the Cleft Lip and Palate Centre on a cleft type, course of treatment, potential dental anomalies, oral care, and healthy eating habits will allow improvement of children's oral health in general.

2. A co-ordinated multidisciplinary work of the team of the Cleft Lip and Palate Centre will help various care professionals (oral and maxillo-facial surgeons, orthodontists, paediatricians, otorhinolaryngologists, cardiologists, therapist geneticists, paediatric dentists, dental hygienists, and other specialists) to effectively provide the necessary treatment for children with congenital clefts.

3. For dentists and dental hygienists, it is important to provide regular dental check-ups, preventive measures, as well as measures for oral rehabilitation and improvement of oral health. Surgical and orthodontic treatment is more effective and less complicated if good oral health has been maintained. Scars after surgeries in cleft are can limit movements of upper lip, therefore medical practitioners have to be careful when handling this area. Bone support in cleft area is insufficient and often teeth in this area are misshapen and more susceptible to caries development. A special care should be paid to brushing teeth in this area.

4. Expression of *DEFB1* gene in an oral cavity may be associated with an individual susceptibility to caries, it is therefore necessary to carry out studies determining the defensin concentration in saliva and caries susceptibility. Perhaps in the future, during dentist appointments children will undergo genetic testing in order to estimate caries risks. Identification of genetic risk factors will help to evaluate and identify high-risk patients and to better understand genetic contribution to caries etiopathogenesis. If risks can be determined prior to formation of defects in teeth, it would help to save

resources (time, costs) to avoid dental caries, as well as to ease patient's pains and sufferings.

7. ACKNOWLEDGEMENTS

I would like to express my greatest and the most sincere gratitude to my supervisor Prof. *Rūta Care* and Prof. *Ilze Akota* for their work, advice, patience, understanding and support in preparation and elaboration of the thesis. Many thanks to Prof. *Alexandre Rezende Vieira* from the University of Pittsburgh (USA) for his great input in elaboration of this study in conjunction with genetic markers and for his assistance in writing articles.
I would like to thank *Baiba Lāce* for her encouragements, advices, understanding and an opportunity to carry out the research of genetic markers for the purpose of the study.

I also want to thank Prof. *Ilga Urtāne*, Chair of the Board of the Institute of Dentistry of RSU, for financial support for my participation in conferences in order to introduce with my work. Many thanks to all team of the Children's dentirtry department – colleagues, doctors, and nurses for their help and support (especially *Inga Briede*, *Jūlija Kalniņa*, *Dace Lieldaudziete*). I also want to thank the team of the Dental Treatment and Oral Health Department for support. Many thanks *Irēna Rogovska* for assistance in statistical processing of data, as well as to *Ieva Greitāne* from The Cleft Lip and Palate Centre, *Inga Kempe* and *Linda Piekuse* from RSU Molecular Genetics Lab for their help in writing this thesis.

Lots of tanks to reviewers of the thesis Prof. *Gundega Jākobsone*, Assoc. Prof. *Eva Platkāja*, Dr. Biol. *Dace Pjanova* for advices on improvement of the work.

The biggest and warmest thanks to my family, especially to my mother, who always accepted and supported me throughout the study period, to my friends and to my husband for support, understanding, help and stamina. I would like to warmly thank all those people who participated in making of this work.

8. PUBLICATIONS AND PRESENTATIONS

International publications

1. Krasone K., Lāce B., Akota I., Care R., Deeley K., Küchler E.C., Vieira A.R. *IRF6* AP-2a binding site promoter polymorphism is associated with oral clefts in Latvia. // Stomatologija, Baltic Dental and Maxillofacial Journal, 2014; 16: 132-6.
2. Krasone K., Lāce B., Akota I., Care R., Deeley K., Küchler E.C., Vieira A.R. Genetic variation in the promoter region of beta-defensin 1 (*DEFB1*) is associated with high caries experience in children born with cleft lip and palate. // Acta Odontol Scand 2014 Apr; 72(3): 235-40.

Publications in RSU research articles

1. Krasone K., Care R., Akota I. Zobu anomāliju sastopamības biežums bērniem ar iedzimtām lūpu un aukslēju šķeltnēm Latvijā. // RSU Research Articles, 2012; 2: 138-143.
2. Krasone K., Care R., Akota I. Kariesa intensitāte 2-3 gadus veciem bērniem ar iedzimtām lūpu un aukslēju šķeltnēm. // RSU Research Articles, 2011; 1: 276-280.

Thesis and presentations

1. Krasone K., Care R., Akota I. Caries experience in children with cleft lip and palate in Latvia. // 25th Congress of IAPD, Glasgow, UK, 2015. 1-4.July, thesis, 214. p.
2. Krasone K., Care R., Akota I. Kariesa izplatība un intensitāte bērniem ar iedzimtām šķeltnēm Latvijā. // Annual RSU Scientific Conference, Riga, Latvia, 2015, 26., 27.March, thesis, 308. p.
3. Kalniņa J., Krasone K., Care R., Akota I. Kariesa intensitāte maiņas un pastāvīgajā sakodienā bērniem ar orofaciālām šķeltnēm Latvijā.// Annual RSU Scientific Conference, Riga, Latvia, 2013, 21., 22.March, thesis, 296. p.
4. Krasone K., Lāce B., Kempa I., Piekuse L., Akota I., Barkāne B., Care R. Hipodontijas ģenētiskie pētījumi indivīdiem ar lūpu un/vai aukslēju šķeltni Latvijā.// Annual RSU Scientific Conference, Riga, Latvia, 2012, 29., 30.March, thesis, 320. p. and presentation.
5. Krasone K., Care R., Akota I. Caries experience of 2-3 year-old children with cleft lip and palate in Riga cleft lip and palate centre. // 2011, 15-18., June, thesis.

6. Krasone K., Care R., Akota I. Kariesa izplatība bērniem ar iedzimtām šķeltnēm Latvijā. // Annual RSU Scientific Conference, Riga, Latvia, 2011, 14., 15. April, thesis, 327. p.
7. Krasone K., Care R., Akota I. Tooth agenesis in patients with orofacial clefts.// 7th congress of BAMPS, 48-49.lpp. 2010, 20-22. May, thesis, 48–49. p.
8. Krasone K., Care R., Akota I. Biežākās zobu patoloģijas bērniem ar iedzimtām šķeltnēm Latvijā.// Annual RSU Scientific Conference, Riga, Latvia, 2010, 18., 19. March, thesis, 309. p.

Thesis and presentations

1. Krasone K., Care R., Akota I. Prevalence of dental anomalies in children with clefts in Latvia. // IADR/PER Congress in Dubrovnik, Croatia 2014, 9-13.September, presentation.
2. Krasone K., Lāce B., Kempa I., Piekuse L., Akota I., Barkāne B., Care R. Hipodontijas ģenētiskie pētījumi indivīdiem ar lūpu un/vai aukslēju šķeltni Latvijā.// Annual RSU Scientific Conference, Riga, Latvia, 2012, 29., 30.March, thesis, 320. p. and presentation
3. Krasone K., Care R., Akota I. .Tooth agenesis in patients with orofacial clefts.// 7th Congress of the Baltic Association for Maxillofacial and Plastic Surgery, Riga, Latvia 2010, 20-22., May, thesis, 48–49. p. and presentation.

9. REFERENCES

1. Ahluwalia M, Brailsford SR, Tarelli E, Gilbert SC, Clark DT, Barnard K, Beighton D. Dental caries, oral hygiene, and oral clearance in children with craniofacial disorders // J Dent Res, 2004; 83(2): 175-179.
2. Akcam MO, Evirgen S, Uslu O, Memikoğlu UT. Dental anomalies in individuals with cleft lip and/or palate // Eur J Orthod. 2010 Apr; 32(2): 207-213.
3. Al Jamal GA, Hazza'a AM, Rawashdeh MA. Prevalence of dental anomalies in population of cleft lip and palate patients // Cleft Palate Craniofac J, 2010 Jul; 47(4): 413-420.
4. Al-Dajani M. Comparison of dental caries prevalence in patients with cleft lip and/or palate and their sibling controls // Cleft Palate-Craniofacial Journal, 2009 September, Vol.46 No.5: 529-531.
5. Al-Wahadni A, Alhaija EA, Al-Omari MA. Oral disease status of a sample of Jordanian people ages 10 to 28 with cleft lip and palate // Cleft Palate-Craniofacial Journal, 2005 May; 42(3): 304-308.
6. Arte S, Nieminen P, Apajalahti S, et al. Characteristics of incisor-premolar hypodontia in families // J Dent Res, 2001 May; 80(5): 1445-1450.
7. Bartzela TN, Carels CEL, Bronkhorst EM, Rønning E, Rozell S, Kuijpers-Jagtman AM.Tooth agenesis patterns in bilateral cleft lip and palate // Eur J Oral Sci, 2010; 118: 47-52.
8. Berzina S, Care R. Dental health in 11 and 13 year old children in Latvia // Stomatologija 2003; 5(2): 62-64.
9. Birnbaum S, Ludwig KU, Reutter H, Herms S, de Assis NA, Diaz-Lacava A, et al. IRF6 gene variants in Central European patients with non-syndromic cleft lip with or without cleft palate // Eur J Oral Sci, 2009 Dec; 117(6): 766-769.
10. Bjarnason S, Care R, Berzina S, Brinkmane A, Rence I, Mackevica I, Paeglite I, Senakola E. Caries experience in Latvia nursery school children // Community Dent Oral Epidemiol, 1995 Jun; 23(3): 138-141.
11. Bokhout B, Hofman FXWM, van Limbeek J, Kramer GJC, Prahl-Andersen B. Increased caries prevalence in 2.5-years-old children with cleft lip and/or palate // Eur J Oral Sci, 1996; 104: 518-522.
12. Callahan N, Modesto A, Meira R, Seymen F, Patir A, Vieira AR. Axis inhibition protein 2 (AXIN2) polymorphisms and tooth agenesis // Arch Oral Biol, 2009; 54(1): 45-49.
13. Cheng LL, Moor SL, Ho CTC. Predisposing Factors to dental caries in children with cleft lip and palate: a review and strategies for early prevention // Cleft palate- Craniofacial Journal, 2007 Jan; 44 (1): 67-72.
14. Dahllof G, Ussisoo-Joandi R, Ideberg M, Modeer T. Caries, gingivitis, and dental abnormalities in preschool children with cleft lip and/or palate // Cleft Palate J, 1989; 26(3): 233─237; discussion 37─38.
15. Dale BA, Tao R, Kimball JR, Jurevic RJ. Oral antimicrobial peptides and biological control of caries // BMC Oral Health, 2006; 6 Suppl 1:S 13.

16. Dewinter G, Quirynen M, Heidbüchel K, et al. Dental abnormalities, bone graft quality, and periodontal conditions in patients with unilateral cleft lip and palate at different phases of orthodontic treatment // Cleft Palate-Craniofacial Journal, 2003; 40: 343-350.
17. Dunsche A, Acil Y, Siebert R, Harder J, Schroder JM, Jepsen S. Expression profile of human defensins and antimicrobial proteins in oral tissues // J Oral Pathol Med, 2001; 30: 154-158.
18. Eerens K, Vlietinck R, Heidbuchel K, et al. Hypodontia and tooth formation in groups of children with cleft, siblings without cleft, and nonrelated controls // Cleft Palate Craniofac J, 2001; 38: 374-378.
19. Graber LW. Congenital absence of teeth: a review with emphasis on inheritance patterns // J Am Dent Assoc, 1978 Feb; 96(2); 266─275.
20. Gudkina J, Brinkmane A. Impact of salivary mutans streptococci and sugar consumption on caries experience in 6-year olds and 12-year olds in Riga // Stomatologija, 2010; 12(2): 56-59.
21. Gursoy UK, Könönen E. Understanding the roles of gingival beta-defensins // Journal of Oral Microbiology, 2012,4:15127- Dol: 10.3402/jom.v4i0.15127.
22. Henkuzena I, Care R, Rogovska I. Dental status among 2-6 year old children in Riga city, Latvia // Stomatologija, 2004; 6(1): 28-30.
23. Henkuzena I, Care R. Kariesa intensitāte un izplatība piena sakodienā Rīgas pilsētas 2─7 gadus veciem bērniem // Rīgas Stradiņa universitāte. Zinātniskie raksti. ─ Rīga: RSU, 2002. 346-348.
24. Kraus BS, Jordan RE, Pruzansky S. Dental abnormalities in the deciduous and permanent dentitions of individuals with cleft lip and palate // J Dent Res, 1966; 45: 1736-1746.
25. Krisanaprakornkit S, Weinberg A, Perez CN, Dale BA. Expression of the peptide antibiotic human ß-defensin 1 in cultured gingival epithelial cells and gingival tissue // Infect Immun, 1998 Sept; 66(9): 4222-4228.
26. Lammi L, Arte S, Somer M, Jarvinen H, Lahermo P, Thesleff I, et al. Mutations in AXIN2 cause familial tooth agenesis and predispose to colorectal cancer // Am J Hum Genet, 2004 May; 74(5): 1043-1050.
27. Letra A, Bjork B, Cooper ME, Szabo-Rogers H, Deleyiannis FWB, Field LL, et al. Association of AXIN2 with nonsyndromic oral clefts in multiple populations // J Dent Res, 2012a; 91(5): 473-478.
28. Letra A, Fakhouri W, Fonseca RF, Menezes R, Kempa I, Prasad JL, et al. Interaction between IFR6 and TGFA genes contribute to the risk of nonsyndromic cleft lip/palate // PLoS One, 2012b; 7(9):e45441.
29. Letra A, Menezes R, Granjeiro JM, Vieira AR. AXIN2 and CDH1 polymorphisms, tooth agenesis, and oral clefts // Birth Defects Res A Clin Mol Teratol, 2009 Feb; 85(2): 169-173.
30. Letra A, Menezes R, Granjeiro JM, Vieira AR. Defining subphenotypes for oral clefts based on dental development // J Dent Res, 2007 October; 86(10): 986-991.
31. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity // Acta Odontol Scand, 1963; 21:533─551.

32. Marthaler TM, Omullane DM, Vrbie V. The prevalence of dental caries in Europe 1990─1995 // Caries Res,1996; 30: 237-255.
33. Mathews M, Jia HP, Guthmiller JM, Losh G, Graham S, Johnson GK, et al. Production of ß-defensin antimicrobial peptides by the oral mucosa and salivary glands // Infect Immun, 1999; 67: 2740-2745.
34. Menezes R, Vieira AR. Dental anomalies as part of the cleft spectrum // Cleft Palate Craniofac J, 2008 Jul; 45(4): 414-419.
35. Mostowska A, Biedziak B, Jagodzinski PP. Axis inhibition protein 2 (AXIN2) polymorphisms may be a risk factor for selective tooth agenesis // J Hum Genet, 2006; 51(3): 262-266.
36. Mostowska A, Hozyasz KK, Wojcicki P, Biedziak B, Paradowska P, Jagodzinski PP. Association between genetic variants of reported candidate genes or regions and risk of cleft lip with or without cleft palate in the polish population // Birth Defects res A Clin Mol Teratol, 2010 Jul; 88(7): 538-545.
37. Murdoch AM, Patir A, Seymen F, Vieira AR. Studies of palatine rugae and interferon refulatory factor 6 variations in a group of families with sporadic hypodontia // J Oral Sci, 2009 Dec; 51(4): 521-526.
38. National Institute of Health Consensus Development Panel. National Institutes of Health Development Conference statement. Diagnosis and management of dental caries throughout life, March 26-28, 2001 // J Am Dent Assoc, 2001 Aug; 132(8): 1153-1161.
39. Ouhara K, Komatsuzawa H, Yamada S, Shiba H, Fujiwara T, Ohara M, et al. Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, ß-defensins and LL37, produced by human epithelial cells // Journal of Antimicrobial Chemotherapy, 2005 Jun; 55(6): 888-896.
40. Ozturk A, Famili P, Vieira AR. The Antimicrobial peptide DEFB 1 is associated with caries // J Dent Res, 2010; 89(6): 631-636.
41. Pan Y, Ma J, Zhang W, Du Y, Niu Y, Wang M, et al. IRF6 polymorphisms are associated with nonsyndromic orofacial clefts in a Chinese Han population // Am J Med Genet A, 2010 Oct; 152A(10): 2505-2511.
42. Paranaiba LM, Martelli-Junior H, Oliveira Swerts MS, Line SR, Coletta RD. Novel mutations in the IRF6 gene in Brazilian families with Van der Woude syndrome // Int J Mol Med, 2008 Oct; 22 (4): 507-511.
43. Park JW, McIntosh I, Hetmanski JB, Jabs EW, Vander Kolk CA, Wu-Chou YH, et al. Association between IRF6 and nonsyndromic cleft lip with or without cleft palate in four populations // Genet Med, 2007 Apr; 9(4): 219-227.
44. Prescott NJ, Winter RM, Malcolm S. Nonsyndromic cleft lip and palate: complex genetics and environmental effects // Ann Hum Genet, 2001 Nov; 65(Pt6): 505-515.
45. Rahimov F, Marazita ML, Visel A, Cooper ME, Hitchler MJ, Rubini M, et al. Disruption of anAP-2α binding site in an IRF6 enhancer is strongly associated with cleft lip // Nat Genet, 2008 Nov; 40(11): 1341-1347.
46. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, et al. High-throughput genotyping with single nucliotide polymorphisms // Genome Res, 2001 Jul; 11(7): 1262-1268.

47. Ranta R, Rintala A. Tooth anomalies associated with congenital sinuses of the lower lip and cleft lip/palate // Angle Orthod, 1982 Jul; 52(3): 212-221.
48. Ranta R. A review of tooth formation in children with cleft lip/ palate // Am J Orthod Dentofacial Orthod, 1986; 90: 11-18.
49. Ranta R. Numeric anomalies of teeth in concomitant hypodontia and hyperdontia // J Craniofac Genet Dev Biol, 1988; 8(3): 245-251.
50. Ribeiro LL, DasNeves LT, Costa B, Gomide MR. Dental anomalies of the permanent lateral incisors in complete unilateral cleft lip and palate // Cleft Palate Craniofac J, 2002; 39: 193-196.
51. Ribeiro LL, DasNeves LT, Costa B, Gomide MR. Dental anomalies of the permanent lateral incisors and prevalence of hypodontia outside the cleft area in complete unilateral cleft lip and palate // Cleft Palate Craniofac J, 2003 Mar; 40(2): 172-175.
52. Rivas-Santiago B, Serrano CJ, Enciso-Moreno JA. Susceptibility to infectious diseases based on antimicrobial peptide production // Infection and Immunity, 2009 Nov; 77(11): 4690-4695.
53. Sambrook J, Firtsch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor: Cold Sping Harbor Laboratory Press, 1989;6.4.
54. Schutte BC, Murray JC. The many faces and factors of orofacial clefts // Hum Mol Genet, 1999; 8: 1853-1859.
55. Shapira Y, Lubit E, Kuftinec MM. Hypodontia in children with various types of clefts // Angle Orthodontist, 2000; Vol.70, No.1: 16-21.
56. Silness J, Löe H. Periodontal disease in pregnancy II. Correlation between oral hygiene and periodontal condition // Acta Odontol Scand, 1964; 22: 121-135.
57. Skrīvele S, Care R, Bērziņa S, Kneist S, de Moura-Sieber V, de Moura R, Borutta A, Maslak E, Tserekhava T, Shakovets N, Wagner M. Caries and its risk factors in young children in five different countries // Stomatologija, 2013; 15(2): 39-46.
58. Slayton RL, Williams L, Murray JC, Wheeler JJ, Lidral AC, Nishimura CJ. Genetic association studies of cleft lip and/or palate with hypodontia outside the cleft region // Cleft Palate Craniofac J, 2003 May; 40(3): 274-279.
59. Song Y, Zhang S. Association of CDH1 promoter polymorphism and the risk of non-syndromicorofacial clefts in a Chinese Han population // Arch Oral Biol, 2011 Jan; 56(1): 68-72.
60. Stahl F, Grabowski R, Wigger K. Epidemiology of Hoffmeister's "genetically determined predisposition to disturbed development of the dentition" in patients with cleft lip and palate // Cleft Palate Craniofac J, 2006; 43: 457-465.
61. Tannure PN, Costa MC, Küchler EC, Romanos HF, Granjeiro JM, Vieira AR. Caries experience in individuals with cleft lip and palate // Pediatric Dentistry, 2012a Mar/Apr; Vol.34 No.2: 127-131.
62. Tannure PN, Oliveira CA, Maia LC, Vieira AR, Granjeiro JM, de Castro Costa M. Prevalence of dental anomalies in nonsyndromic individuals with cleft lip and palate: a systematic review and meta-analysis // Cleft Palate Craniofac J, 2012d Mar; 49(2): 194-200.
63. Tortora C, Meazzini MC, Garattini G, Brusati R. Prevalence of abnormalities in dental structure, position, and eruption pattern in a population of unilater

and bilateral cleft lip and palate patients // Cleft Palate Craniofac J, 2008 Mar; 45(2): 154-162.
64. Vastardis H. The genetics of human tooth agenesis: New discoveries for understanding dental anomalies // American Journal of Orthodontics and Dentofacial Orthopedics, 2000; 117(6): 650-656.
65. Vieira AR, Cooper ME, Marazita ML, Orioli IM, Castilla EE. Interferon regulatory factor 6 (IRF6) is associated with oral-facial cleft in individuals that originate in South America // Am J Med Genet A, 2007 Sep 1; 143A(17): 2075-2078.
66. Vieira AR, Marazita ML, Goldstein-McHenry T. Genome- wide scan finds suggestive caries loci // J Dent Res, 2008a; 87(5):435-439.
67. Vieira AR, McHenry TG, Daack-Hirsch S, Murray JC, Marazita ML. Candidate gene/loci studies in cleft lip/palate and dental anomalies finds novel susceptibility genes for clefts // Genet Med, 2008c Sept; 10(9): 668-674.
68. Vieira AR, Modesto A, Marazita ML. Caries: review of human genetics research // Caries Res, 2014: 48(5): 491-506.
69. Vieira AR, Modesto A, Meira R, Barbosa AR, Lidral AC, Murray JC. Interferon regulatory factor 6 (IRF6) and fibroblast growth factor receptor 1 (FGFR1) contribute to human tooth agenesis // Am J Med Genet, 2007a Mar 15; 143(6): 538-545.
70. Vieira AR, Seymen F, Patir A, Menezes R. Evidence of linkage disequilibrium between polymorphisms at the IRF6 locus and isolate tooth agenesis, in a Turkish population // Arch Oral Biol, 2008d Aug: 53(8): 780-784.
71. Vieira AR. Oral clefts and syndromic forms of tooth agenesis as models for genetics of isolated tooth agenesis // J Dent Res, 2003 Mar; 82(3): 162-165.
72. Vieira AR. Unraveling human cleft lip and palate research // J Dent Res, 2008 Feb; 87(2): 119-125.
73. World Health Organization. Oral Health Surveys: Basic Methods. 4rd ed. Geneva; 1997.
74. Wu T, Liang KY, Hetmanski JB, Ruczinski I, Fallin MD, Ingersoll RG, et al. Evidence of gene-environment interaction for the IRF6 gene and maternal multivitamin supplementation in controlling the risk of cleft lip with/without cleft palate // Hum Genet, 2010 Oct; 128(4): 401-410.
75. Yildirim M, Seymen F, Deeley K, Cooper ME, Vieira AR. Defining predictors of cleft lip and palate risk // J Dent Res, 2012; 91(6): 556-561.
76. Zhu WC, Xiao J, Liu Y, Wu J, Li JY. Caries experience in individuals with cleft lip and/or palate in China // Cleft Palate Craniofac J, 2010 January; 47(1): 43-47.
77. Zucchero TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate // N Engl J Med, 2004 Aug 19; 351(8): 769-780.