VILNIUS UNIVERSITY

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DETERMINATION OF GENOMIC VARIANTS OF THE COMPLEX AETIOLOGY CLEFT LIP AND (OR) PALATE IN LITHUANIAN PATIENT GROUP

Summary of doctoral dissertation

Biomedical sciences, biology (01 B)

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VILNIAUS UNIVERSITETAS

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GENOMINIŲ VEIKSNIŲ ĮTAKA DAUGIAVEIKSNĖS ETIOLOGIJOS LŪPOS IR (ARBA) GOMURIO NESUAUGIMAMS LIETUVOS PACIENTŲ GRUPĖJE

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INTRODUCTION

Cleft lip and (or) palate (CL/P) are common birth defects of complex aetiology. CL/P can occur in isolation or as a part of chromosomal, Mendelian or teratogenic syndromes. Although there has been marked progress in identifying genomic and environmental factors for syndromic CL/P, the aetiology of more common (nonsyndromic) forms remains poorly characterised. The incidence of cleft lip and (or) palate varies from 0.4 to 2.0 in 1000 live births across populations. The incidence of this malformation in Lithuania is 1 in 1000 according to the unpublished data. Recently, using a combination of epidemiology, careful phenotyping, genome – wide association studies and analysis of animal models, several distinct genetic and environmental risk factors have been identified and confirmed for (nonsyndromic) CL/P. More and more CL/P candidate loci are being confirmed using novel genome-wide methods of molecular genetics and tools of statistical analysis. The aim of this study was to identify the alleles of the candidate genes for cleft lip with or without cleft palate and isolated cleft palate in the Lithuanian patient group, applying the molecular genotyping of the genomic markers in 42 CL/P candidate genes and association analysis using transmission disequilibrium test approach, integrating case – control analysis data of a bigger investigative group from three closely genetically linked Baltic populations of Lithuania, Latvia, and Estonia. All association analysis tools were applied in two phenotype groups – patients with cleft lip with or without cleft palate and cleft palate only. More detailed subclinical phenotyping holds great promise to enhance the power of family studies and may lead to opportunities for translational research that is relevant for both clinical care of patients and clinical genetics as a science.

Aim of the study:

To identify the strongest (nonsyndromic) cleft lip with or without cleft palate and cleft palate only candidate genes and their alleles in the Lithuanian patient group.

Main tasks of the research study:

1. To select genetic markers in the cleft lip and (or) palate candidate genes and genotype cleft lip and (or) palate patients and their parents triads and individuals from the general population of Lithuania.
2. To perform association analysis by transmission disequilibrium test using family based approach for patients with cleft lip with or without cleft palate.
3. To perform association analysis by transmission disequilibrium test using family based approach for patients with cleft palate only.
4. To evaluate the association analysis results based on case – control study in the Lithuanian patient and control group.
5. To evaluate the association analysis results of the cleft lip and (or) palate patients from Lithuania, Latvia, and Estonia.
6. To evaluate the determination of cleft lip and (or) palate candidate genes' alleles for the phenotype.

Relevance and novelty of the research

Future advances in our understanding of the molecular pathogenesis of CL/P will require strategies that increasingly integrate genetic analysis of precisely phenotyped cohorts of patients, global approaches for the identification and ranking of candidate genes, and improved methods for delineating and analysing functional elements controlling gene expression. Integration of genetic and environmental risk using epigenetics, systems biology, gene expression and epidemiology will all be required to generate a synthesis that

will more completely characterise aetiologies, as well as provide access to better clinical care and prevention.

More and more candidate loci of the complex aetiology phenotype cleft lip and (or) cleft palate (CL/P) is being revealed as novel molecular genetic methods and statistical analysis tools are being introduced. CL/P research is being performed in the Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University since 2002. In this study a specific candidate gene research approach was performed – 579 single nucleotide polymorphisms (SNPs) in 42 selected CL/P genes were included in the study. 106 triads of patients with cleft lip with or without cleft palate and cleft palate only and both his/her parents and 219 individuals of the control group from general population of Lithuania were genotyped for the selected genomic markers. In order to enhance statistically significant association results a bigger research group was organised comprising CL/P patients from three genetically close Blatic populations (Nelis et al, 2009) – Lithuania, Latvia, and Estonia. Thus, two different strategies – family based and case – control studies – were performed which enabled statistically significant associated variants to be identified.

Statements to be defended:

1. *TIMP2*, *BMP2*, *FGF1* gene variants are associated with cleft lip with or without cleft palate in the population of Lithuania.
2. *COL11A1*, *COL11A2*, *COL2A1* gene variants are associated with cleft palate only in the population of Lithuania.
3. Different cleft lip and (or) palate candidate genes and their variants are associated with cleft lip with or without cleft palate and with cleft palate only in the Lithuanian patient group.
4. Investigating the association analysis results of cleft lip and (or) palate patients from Lithuania, Latvia, and Estonia, *COL2A1*, *COL11A2* and *IRF6* genes are statistically significantly associated with cleft palate

only, and *FGF1*, *FOXE1* and *TIMP2* genes – cleft lip with or without cleft palate.

PATIENTS, MATERIALS, OBJECTS, AND METHODS

All patients were included in the study collaborating with the biggest orthodontic clinics in Lithuania and after careful examination by the clinical geneticists at the Center for Medical Genetics, Vilnius University Hospital Santariškių Klinikos. Different phenotypes were distinguished and CL/P cases with associated congenital anomalies were excluded according to the data of inner organs and heart scan as well as additional clinical and genetic testing. 106 patients with cleft lip with or without cleft palate and cleft palate only (cleft lip only phenotype is not considered as a different entity thus was included in the cleft lip with or without cleft palate group) and both his/her parents were included in this study (Figure1).

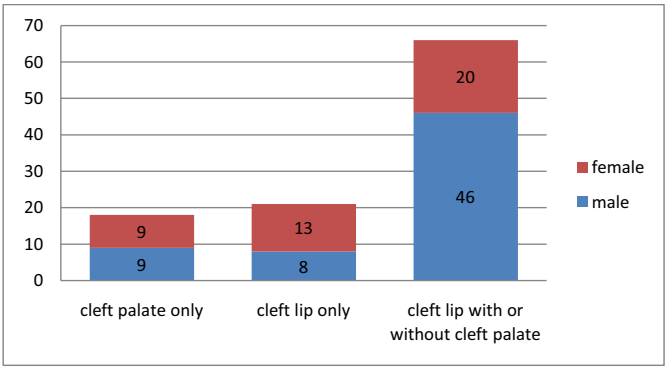

Figure 1. Study group according to the phenotypes.

The Lithuanian population DNA samples were collected from six different ethno linguistic regions in Lithuania (i.e. East Aukštaičiai, West Aukštaičiai, South Aukštaičiai, West Žemaičiai, South Žemaičiai, and North Žemaičiai). The DNA samples of 219 unrelated individuals were genotyped for use as population-based controls.

The study was approved by the Lithuanian Bioethics Committee, and informed consent was obtained from all participating individuals.

Venous blood samples were obtained from each individual participating in the study. Total genomic DNA was extracted from blood using phenol – chloroform extraction according to the established protocols.

Literature analysis and bioinformatic tools were used to select 42 candidate CL/P genes and 814 SNPs in and at the 3' and 5' sequences from them. Genes investigated and number of SNPs in them is shown in table 1.

Table 1. CL/P candidate genes and number of SNPs

No.	Gene	Chr.	Number of SNPs	No.	Gene	Chr.	Number of SNPs
1	*COL11A1*	1	41	22	*BMP4*	14	3
2	*IRF6*	1	8	23	*JAG2*	14	9
3	*MTHFR*	1	9	24	*TGFB3*	14	8
4	*SKI*	1	22	25	*CDH1*	16	9
5	*FN1*	2	26	26	*MMP25*	16	6
6	*TGFA*	2	42	27	*MMP2*	16	22
7	*FGF2*	4	19	28	*RARA*	17	4
8	*MSX1*	4	15	29	*TIMP2*	17	27
9	*FGF1*	5	33	30	*WNT3*	17	7
10	*MSX2*	5	7	31	*WNT9B*	17	14
11	*COL11A2*	6	21	32	*SMAD2*	18	8
12	*EDN1*	6	16	33	*SMAD4*	18	2
13	*FGFR1*	8	10	34	*LOXHD1*	18	10
14	*FOXE1*	9	4	35	*CLPTM1*	19	5
15	*MMP13*	11	16	36	*BCL3*	19	2
16	*MMP3*	11	5	37	*PVRL2*	19	12
17	*PVRL1*	11	19	38	*BMP2*	20	21
18	*TBX10*	11	10	39	*MMP9*	20	6
19	*COL2A1*	12	31	40	*TIMP3*	22	30
20	*LHX8*	12	9	41	*TBX22*	X	4
21	*SPRY2*	13	3	42	*TIMP1*	X	4

The genotyping was performed using arrayed primer extension (APEX-2) (Krujtškov et al, 2008) method. After initial analysis 679 SNPs were selected for the investigation in the Lithuanian patient group, 100 of those showed poor call rate and was excluded from further analysis. 39 SNPs (table 2) appeared to

be monomorphic in the population of Lithuania and were not included in the association analysis using transmission disequilibrium test (TDT).

Table 2. List of monomorphic SNPs in the population of Lithuania

No.	Gene	Chr.	SNP	1 allele	2 allele	1 allele freq*	2 allele freq*	1 allele freq**	allele freq**
1	*COL11A1*	1	rs17446095	[A]	[C]	0,031	0,969	0,083	0,917
2	*COL11A1*	1	rs12728397	[C]	[T]	0,976	0,024	0,933	0,067
3	*COL11A1*	1	rs1841835	[C]	[G]	0,027	0,973	0,050	0,950
4	*COL11A2*	6	rs213210	[A]	[G]	0,965	0,035	0,097	0,903
5	*FGF1*	5	rs17099096	[G]	[T]	0,950	0,050	0,950	0,050
6	*FGF1*	5	rs6882717	[C]	[T]	0,965	0,035	0,950	0,050
7	*FGF1*	5	rs250201	[G]	[T]	0,983	0,017	0,925	0,075
8	*FGF1*	5	rs33992	[A]	[G]	0,029	0,971	0,092	0,908
9	*FGF1*	5	rs11954586	[C]	[T]	0,042	0,958	0,067	0,933
10	*FGF2*	4	rs17472986	[A]	[G]	0,038	0,962	0,092	0,908
11	*FGF2*	4	rs308438	[C]	[T]	0,967	0,033	0,958	0,042
12	*FGF2*	4	rs17407577	[C]	[T]	0,045	0,955	0,083	0,917
13	*FGFR1*	8	rs2978083	[C]	[T]	0,946	0,054	0,958	0,042
14	*FN1*	2	rs11883812	[C]	[T]	0,038	0,962	0,092	0,908
15	*FN1*	2	rs6716361	[C]	[G]	0,969	0,031	0,950	0,050
16	*FOXE1*	9	rs10984009	[A]	[G]	0,039	0,961	0,142	0,858
17	*IRF6*	1	rs12143281	[C]	[T]	0,969	0,031	0,942	0,058
18	*MMP13*	11	rs11225485	[A]	[G]	0,948	0,052	0,883	0,117
19	*MMP13*	11	rs11826440	[G]	[T]	0,988	0,012	0,950	0,050
20	*MMP13*	11	rs7119194	[C]	[T]	0,965	0,035	0,933	0,067
21	*MMP3*	11	rs629946	[A]	[G]	0,045	0,955	0,058	0,942
22	*MMP9*	20	rs6032619	[C]	[T]	0,952	0,048	0,942	0,058
23	*BMP2*	20	rs235756	[A]	[G]	1,000	0,000	0,650	0,350
24	*MSX1*	4	rs12501827	[C]	[T]	0,953	0,047	0,892	0,108
25	*PVRL1*	11	rs906827	[A]	[C]	0,045	0,955	0,083	0,917
26	*SKI*	1	rs4648625	[G]	[T]	0,026	0,974	0,075	0,925
27	*SMAD2*	18	rs17814648	[C]	[T]	0,969	0,031	0,958	0,042
28	*TGFA*	2	rs13034560	[G]	[T]	0,950	0,050	0,950	0,050
29	*TGFA*	2	rs6709121	[A]	[G]	0,028	0,972	0,075	0,925
30	*CDH1*	16	rs3785076	[A]	[G]	0,967	0,033	0,950	0,050
31	*TGFA*	2	rs540006	[C]	[T]	0,043	0,957	0,042	0,958
32	*TGFB3*	14	rs3917148	[G]	[T]	0,045	0,955	0,067	0,933
33	*TIMP2*	17	rs1531795	[G]	[T]	0,958	0,042	0,875	0,125

Table 2, continued. List of monomorphic SNPs in the population of Lithuania

No.	*Gene*	Chr.	SNP	1 allele	2 allele	1 allele freq*	2 allele freq *	1 allele freq **	allele freq **
34	*TIMP2*	17	rs4789937	[A]	[G]	0,045	0,955	0,075	0,925
35	*TIMP2*	17	rs12944916	[C]	[G]	0,974	0,026	0,950	0,050
36	*TIMP3*	22	rs4449	[A]	[G]	0,019	0,981	0,042	0,958
37	*TIMP3*	22	rs11287	[C]	[T]	0,988	0,012	0,950	0,050
38	*TIMP3*	22	rs10483166	[C]	[G]	0,054	0,946	0,058	0,942
39	*COL11A1*	1	rs6671691	[C]	[G]	0,959	0,041	0,942	0,058

*- allele frequency in the studied group

**- allele frequency according to the *Ensembl (61 release)*, *dbSNP* using data of *1000Genomes* Project (and *HapMap* data for rs213210 VNP)

In the further stage of the study association analysis using TDT was performed, thus paternity testing was used for all the triads included in the study. Paternity testing was confirmed for all 106 triads using four microsatellite loci CSF1PO, TPOX, TH01, vWA (*Gene Print Fluorescent CTTv Quadriplex kit, Promega, USA*).

Association analysis using family based approach was performed by TDT (http://genomics.med.upenn.edu/spielman/TDT.htm) (Spielman, 1993, Spielman, Ewens, 1998). Association analysis using case – control study approach for the combined three population research part was conducted using *PLINK version 1.06* (http://pngu.mgh.harvard.edu/purcell/plink) (Purcell et al., 2007). This part of the statistical analysis was performed by Tiit Nikopensius (Tartu University, Department of Biotechnology, Institute of Molecular and Cell Biology).

RESULTS AND DISCUSSION

Association analysis using transmission disequilibrium test in the 88 Lithuanian patients with cleft lip with or without cleft palate and their parents' triads, 68 SNPs in 25 CL/P candidate genes showed statistically significant results ($p<0.05$) (table 3).

Table 3. Statistically significant SNPs in cleft lip with or without cleft palate group

No.	*Gene*	*Chr.*	*SNP*	*Locus*	*χ2*	*p value*
1	*MMP3*	11	rs629946	102226906	15.385	8.76816E-05
2	*TGFB3*	14	rs7156293	75493336	12.302	0.0005
3	*BMP2*	20	rs235756	6715111	10	0.0016
4	*FGF2*	4	rs308439	123993029	10	0.0016
5	*PVRL1*	11	rs906827	119019339	9.981	0.0016
6	*TIMP2*	17	rs4789936	74409569	9.72	0.0018
7	*PVRL1*	11	rs10892421	119003613	8.67	0.0032
8	*TIMP1*	X	rs723556	47317691	8.395	0.0038
9	*MTHFR*	1	rs9651118	11784801	8.018	0.0046
10	*COL11A1*	1	rs7543626	103350922	8	0.0047
11	*TIMP2*	17	rs6501266	74418948	7.839	0.0051
12	*COL2A1*	12	rs1635550	46664237	7.563	0.0060
13	*EDN1*	6	rs1476046	12401207	7.353	0.0067
14	*COL11A1*	1	rs7556513	103325111	7.078	0.0078
15	*EDN1*	6	rs9357336	12392025	7.043	0.0080
16	*BMP2*	20	rs2206916	6682650	7.024	0.0080
17	*FGF1*	5	rs33992	141937322	7	0.0082
18	*BMP2*	20	rs6085682	6719211	6.914	0.0086
19	*COL11A2*	6	rs213208	33285988	6.811	0.0091
20	*COL2A1*	12	rs12721428	46655160	6.081	0.0137
21	*TIMP3*	22	rs242082	31554439	6.081	0.0137
22	*PVRL1*	11	rs10750161	119003828	6.05	0.0139
23	*COL11A1*	1	rs1841835	103211618	6	0.0143
24	*TIMP3*	22	rs4449	31608202	6	0.0143
25	*PVRL1*	11	rs4354701	118994022	5.944	0.0148
26	*TBX10*	11	rs2514027	67162878	5.769	0.0163
27	*MTHFR*	1	rs1801131	11777063	5.73	0.0167
28	*BMP2*	20	rs235757	6714019	5.628	0.0177
29	*EDN1*	6	rs2071943	12403800	5.586	0.0181
30	*FGF2*	4	rs308442	123994363	5.565	0.0183

31	*COL11A1*	1	rs17446095	103145363	5.538	0.0186
32	*PVRL2*	19	rs11667640	50071631	5.452	0.0195
33	*EDN1*	6	rs16872608	12416007	5.444	0.0196
34	*BMP2*	20	rs173107	6713841	5.4	0.0201
35	*COL11A2*	6	rs2744507	33256856	5.4	0.0201
36	*TBX10*	11	rs2447587	67168406	5.261	0.0218
37	*MMP13*	11	rs685286	102347662	5.233	0.0222
38	*COL2A1*	12	rs12821733	46641450	5.143	0.0233
39	*MTHFR*	1	rs17376328	11799249	4.923	0.0265
40	*EDN1*	6	rs4714351	12384844	4.898	0.0269
41	*COL11A1*	1	rs2229783	103125039	4.813	0.0282
42	*FGF1*	5	rs1860230	142058047	4.8	0.0285
43	*COL11A1*	1	rs12026245	103364053	4.762	0.0291
44	*FGF1*	5	rs250092	141958427	4.667	0.0307
45	*BCL3*	19	rs2965174	49936855	4.629	0.0314
46	*SMAD2*	18	rs1792658	43636603	4.571	0.0325
47	*SKI*	1	rs4648625	2190201	4.455	0.0348
48	*TIMP3*	22	rs11287	31588777	4.455	0.0348
49	*FOXE1*	9	rs973473	99660551	4.414	0.0356
50	*FN1*	2	rs2289200	215941223	4.378	0.0364
51	*MSX1*	4	rs10002530	4900504	4.349	0.0370
52	*COL11A1*	1	rs4908291	103345324	4.313	0.0378
53	*MMP2*	16	rs7187242	54057495	4.267	0.0389
54	*COL11A1*	1	rs11164663	103321085	4.263	0.0390
55	*COL11A2*	6	rs213210	33283802	4.261	0.0390
56	*WNT3*	17	rs199497	42221762	4.245	0.0394
57	*COL2A1*	12	rs17122565	46698677	4.129	0.0422
58	*EDN1*	6	rs16872612	12416068	4.122	0.0423
59	*PVRL2*	19	rs519825	50058619	4.07	0.0437
60	*EDN1*	6	rs521824	12381526	4.05	0.0442
61	*PVRL2*	19	rs3745150	50077599	3.967	0.0464
62	*WNT3*	17	rs11653738	42242117	3.959	0.0466
63	*COL11A1*	1	rs6671691	103347365	3.947	0.0470
64	*FGF2*	4	rs308438	123992337	3.903	0.0482
65	*BMP2*	20	rs1005464	6704148	3.814	0.0508
66	*TIMP2*	17	rs7211674	74410660	3.753	0.0527
67	*COL11A1*	1	rs12123966	103354338	3.667	0.0555
68	*FN1*	2	rs17518731	216026905	3.658	0.0558

Association analysis using transmission disequilibrium test in the 18 Lithuanian patients with cleft palate only and their parents' triads, 33 SNPs in

18 CL/P candidate genes showed statistically significant results ($p<0.05$) (table 4).

Table 4. Statistically significant SNPs in cleft palate only group

No.	*Gene*	*Chr.*	*SNP*	*Locus*	*χ2*	*p value*
1	*MMP2*	16	rs837533	54052135	9	0.0027
2	*MMP2*	16	rs1005913	54062022	7.364	0.0067
3	*FGFR1*	8	rs2956724	38411423	6	0.0143
4	*FGF1*	5	rs34013	141978564	5.444	0.0196
5	*TIMP2*	17	rs7213204	74343395	5.444	0.0196
6	*MMP25*	16	rs7188573	3051204	5.4	0.0201
7	*RARA*	17	rs482284	35757767	5.4	0.0201
8	*WNT3*	17	rs12452064	42223353	5.4	0.0201
9	*COL11A2*	6	rs213209	33284936	5.333	0.0209
10	*FGFR1*	8	rs2411256	38412620	5.333	0.0209
11	*TBX22*	X	rs6621543	79156787	5.333	0.0209
12	*FGF1*	5	rs33995	141945942	5	0.0253
13	*MSX1*	4	rs4689953	4904370	4.571	0.0325
14	*COL2A1*	12	rs11168359	46702290	4.5	0.0339
15	*MMP13*	11	rs685286	102347662	4.5	0.0339
16	*MMP2*	16	rs243864	54069823	4.5	0.0339
17	*TIMP2*	17	rs7218237	74383233	4.5	0.0339
18	*COL11A1*	1	rs12722976	103118332	4.455	0.0348
19	*MMP2*	16	rs243865	54069307	4.455	0.0348
20	*PVRL1*	11	rs12361680	118994420	4.455	0.0348
21	*TIMP2*	17	rs4789921	74451214	4.455	0.0348
22	*CDH1*	16	rs12597188	67372327	4	0.0455
23	*COL11A2*	6	rs756441	33230149	4	0.0455
24	*LHX8*	12	rs17622436	75362169	4	0.0455
25	*MSX1*	4	rs2087868	4900020	4	0.0455
26	*PVRL1*	11	rs4938713	119074204	4	0.0455
27	*TIMP3*	22	rs130554	31513583	4	0.0455
28	*TIMP3*	22	rs4447	31605140	4	0.0455
29	*WNT9B*	17	rs11079740	42304099	4	0.0455
30	*MMP25*	16	rs2526275	3024110	3.857	0.0495
31	*FGF1*	5	rs10070885	142000564	3.769	0.0522
32	*MMP2*	16	rs1005912	54062328	3.769	0.0522
33	*TIMP3*	22	rs137487	31589104	3.769	0.0522

Case – control association analysis using *PLINK* program for Lithuanian patients and control group individuals showed 10 statistically significant or borderline *p values* (table 5).

Table 5. Statistically significant results for case – control study

No.	*Gene*	*Chromosome*	*SNP*	*p value*
1	*COL11A1*	1	rs17446095	**0.038**
2	*FGF2*	4	rs11737764	0.076
3	*FN1*	2	rs4673990	0.078
4	*IRF6*	1	rs9430018	0.060
5	*COL11A2*	6	rs9277928	0.063
6	*COL2A1*	12	rs6580647	**0.046**
7	*COL2A1*	12	rs1541408	0.057
8	*COL2A1*	12	rs3829736	0.092
9	*COL2A1*	12	rs12821733	0.078
10	*FOXE1*	9	rs973473	0.089
11	*PVRL1*	11	rs10892421	0.095
12	*PVRL1*	11	rs4354701	0.070
13	*TIMP2*	17	rs4789936	**0.042**
14	*TIMP2*	17	rs7502916	**0.013**
15	*TIMP2*	17	rs7213204	0.059
16	*TIMP2*	17	rs6501266	0.066
17	*TIMP2*	17	rs7211674	**0.043**
18	*BMP2*	20	rs235750	0.078
19	*BMP2*	20	rs6085682	**0.040**
20	*BMP2*	20	rs6117432	0.070
21	*BMP2*	20	rs235757	**0.042**
22	*BMP2*	20	rs2206916	0.069
23	*BMP2*	20	rs2206917	0.066
24	*BMP2*	20	rs1005464	**0.037**
25	*BMP2*	20	rs173107	**0.051**
26	*MMP9*	20	rs3918278	**0.018**

When performing the joint (Lithuanian, Latvian, and Estonian CL/P patients and control group individuals) case – control association analysis two different groups were analysed (as throughout the whole research study): cleft lip with or without cleft palate and cleft palate only. SNPs in *FGF1*, *FOXE1* and

TIMP2 genes showed statistically significant values (Nikopensius et al., 2011), indicating their contribution to cleft lip with or without cleft palate. As well SNPs in *COL2A1*, *COL11A2* and *IRF6* genes showed statistically significant values (Nikopensius et al., 2010), confirming their contribution to cleft palate only.

Evaluating the strongest CL/P candidate genes in the Lithuanian patient group the following genes should be pointed out: *BCL3* (B cell lymphoma 3), *RARA* (retinoic acid receptor α), *TGFα* (transforming growth factor α), *TGFβ3* (transforming growth factor β 3), BMP (bone morphogenetic proteins), including MSX1 and MSX2 (Msh homeobox), FGFs (fibroblast growth factors family) and their receptors, *FN1* (fibronectin 1), *COL11A1* (collagen, type XI, α1), *COL11A2* (collagen, type XI, α1), *COL2A1* (collagen, type II, α1), WNT (wingless-type MMTV integration site family), MMP (matrix metalloproteinases).

Using family based studies and case – control studies approach CL/P candidate genes *TIMP2, BMP2, FN1* variants were confirmed for cleft lip with or without cleft palate and *COL11A1*, *COL11A2*, *COL2A1* for cleft palate only in the population of Lithuania. Estimating the association analysis results of the CL/P patients of Lithuania, Latvia and Estonia, *FGF1, TIMP2* were identified as candidate genes for cleft lip with or without cleft palate and *COL2A1*, *COL11A2* for isolated cleft palate in the North East European populations. Different genomic risk variants have influence for cleft lip with or without cleft palate and for cleft palate only, which confirms the hypothesis of different mechanisms for these two phenotypes.

CONCLUSIONS

1. Using literature data and bioinformatics analysis results genomic markers in 42 cleft lip and (or) palate candidate genes were selected, and Lithuanian patients' triads and individuals from the control group were genotyped according to them.
2. Association analysis results based on transmission disequilibrium test and on case – control study confirm *TIMP2, BMP2, FN1* genes' susceptibility to cleft lip with or without cleft palate in the population of Lithuania.
3. Association analysis results based on transmission disequilibrium test and on case – control study confirm *COL11A1*, *COL11A2*, *COL2A1* genes' susceptibility to cleft palate only in the population of Lithuania.
4. *FGF1, TIMP2* genes confirm susceptibility to cleft lip with or without cleft palate as *COL2A1*, *COL11A2* genes confirm to cleft palate only in the North East European populations according to the association analysis data of Lithuanian, Latvian, and Estonian cleft lip and (or) palate patients.
5. Different genetic risk variants are responsible for cleft lip with or without cleft palate and for cleft palate only in the Lithuanian patient group.
6. Confirmed cleft lip and (or) palate genes in the population of Lithuania code for signalling molecules that attend/act in the early stages of morphogenesis.

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SANTRAUKA

Kaukolės veidinės dalies anomalijos, ypač lūpos ir (arba) gomurio nesuaugimai (L/GN), yra vienos dažniausių žmogaus įgimtų ydų. Įvairių autorių duomenimis jų dažnumas svyruoja nuo 0,4 iki 2,0 1000 gimusiųjų.

Sparčiai tobulėjant molekulinės genetikos metodams ir statistinės analizės įrankiams vis daugiau kandidatinių L/GN genetinių sričių identifikuojama plataus masto viso genomo tyrimais. Šio darbo tikslas buvo nustatyti genų kandidatų alelius, lemiančius polinkį (nesindrominiam) lūpos nesuaugimui su arba be gomurio nesuaugimo ir izoliuotam gomurio nesuaugimui Lietuvos pacientų grupėje, tiriant 42–jų genų galimai lemiančių L/GN genominius žymenis molekuliniais genetiniais metodais ir taikant statistinę asociacijos analizę nepusiausviro perdavimo testu.

Taikant šeimų tyrimo ir atvejo – kontrolės tyrimo asociacijos analizės strategijas pacientų su lūpos nesuaugimu su arba be gomurio nesuaugimo grupėje, patvirtinti *TIMP2, BMP2, FN1* genų kandidatų variantai lemiantys lūpos nesuaugimus su arba be gomurio nesuaugimo ir gomurio nesuaugimus Lietuvos populiacijoje, *COL11A1*, *COL11A2*, *COL2A1* genų kandidatų variantai lemiantys gomurio nesuaugimus Lietuvos populiacijoje. Įvertinus Lietuvos, Latvijos ir Estijos pacientų su L/GN asociacijos analizės rezultatus, buvo nustatyta, kad Šiaurės Rytų Europos populiacijose polinkį lūpos nesuaugimui su arba be gomurio nesuaugimo lemia *FGF1, TIMP2* genų variantai ir polinkį gomurio nesuaugimui *COL2A1*, *COL11A2* genų variantai. Lūpos nesuaugimus su arba be gomurio nesuaugimo lemia skirtingi genetiniai veiksniai nei gomurio nesuaugimą Lietuvos pacientų grupėje.

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