BIOLOGIJA. 2007. Vol. 18. No. 1. P. 1–6

Are *TGFA*, *TGFB3*, *GABRB3*, *RARA* and *BCL3* loci associated with nonsyndromic orofacial clefts? A Lithuanian study

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Nonsyndromic orofacial clefting (NS-OFC) is a common complex multifactorial trait with a considerable genetic component and a number of candidate genes suggested by different approaches, but the question of the contribution of their sequence variation to the risk of NS-OFC is still open. A set of 21 biallelic and microsatellite DNA markers in the strong candidate loci *TGFA*, *TGFB3*, *GABRB3*, *RARA*, and *BCL3* were analysed for allelic association with the NS-OFC phenotype in 112 nuclear families (child affected with NS-OFC poband + both parents) from Lithuania using the transmission disequilibrium test (TDT). Association was found between the *TGFA* gene marker rs2166975 and nonsyndromic clef palate (CPO) phenotype (P = 0.0455 [df 1]) as well as between the D2S292 marker and isolated cleft lip with or without cleft palate (CL/P) phenotype in allele-wise TDT (P = 0.0053 [df 9]) and genotype-wise TDT (P = 0.0206 [df 24]). A weak association (P = 0.0850 [df 3]) of the BCL3 marker (*BCL3* gene) with the risk of CPO was also shown. Thus, our initial results support the contribution of *TGFA* locus allelic variation in the etiology of CL/P in the population of Lithuania, but do not point to *TGFA* as a major causal gene. Different roles for the *TGFA* and *BCL3* genes in the susceptibility to NS-OFC phenotypes are suggested.

Key words: allelic association, candidate genes, nonsyndromic orofacial clefts, transmission disequilibrium test, TDT

INTRODUCTION

Development of the head and face is one of the most complex and tightly controlled events during embryonic development. Disturbances during the period critical for the formation of the face (4–10 weeks for humans) may lead to orofacial clefts (OFCs) [1] which are among the most common malformations in humans and, although surgically treatable, result in lifelong medical and social consequences. Clinically diverse OFCs are generally classified as cleft palate only (CPO, MIM 119540), cleft lip only (CLO, MIM 119530) and cleft lip with cleft palate (CLP, MIM 119530). The latter two categories are often collectively referred to as cleft lip with or without cleft palate (CL/P), which is a group of OFCs considered to be separate from the CPO group. However, it is recognized that there are many common pathways and cellular mechanisms. OFCs occur in a wide geographic distribution with an average birth prevalence in the world from 1/300 to 1/2500 births for CL/P and around 1/500 births for CPO [2]. The incidence of OFCs in Lithuania, 1.84 for 1000 newborns (1/544 livebirths) [3], appears to be similar to other European populations.

The majority of OFC cases (~70%) are considered nonsyndromic (NS-OFC) where clefts occur without other abnormalities. NS-OFC arises as a complex multifactorial trait with a considerable genetic component together with environmental overlays and stochastic factors [4]. Extensive attempts to reveal the genetic basis of NS-OFC are being undertaken using different approaches [4]. As a result, >30 potential candidate loci and candidate genes throughout the human genome were located (some of them were identified in more than one study). The *MSX1* (4p16.1), *TGFA* (2p13), *TGFB1* (19q13.1), *TGFB2* (1q41), *TGFB3* (14q24), *RARA* (17q12), *MTHFR* (1p36.3) genes are among the strongest candidates [5–7]. Numerous studies were aimed to reveal the allelic association of these loci with the NS-OFC phenotype, but the results often appeared to be conflicting, possibly due to bias, lack of statistical power, population diversity, etc. Therefore, investigations in the field are aimed both to reveal

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new candidate loci, especially by application of modern high throughput technologies [4, 8] and to test already known loci for potentially causal alleles and their association with the NS-OFC phenotype.

The purpose of this study was to test the hypothesis on the relation of the *TGFA*, *TGFB3*, *GABRB3*, *RARA*, and *BCL3* genes to the NS-OFC by evaluating the allelic association of a number of binary and microsatellite markers within or close to these loci with the OFC phenotype in nuclear families from the population of Lithuania.

SUBJECTS, MATERIALS AND METHODS

Subjects

The study group consisted of 112 unrelated nuclear families (triads: child with NS-OFC – father – mother). Recruitment of the patients and their families from all regions of Lithuania was performed at the Institute of Odontology and the Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University. Written informed consent was obtained from each person (or his/her legal guardian) participating in the study. The OFC phenotype was evaluated and genealogical analysis was performed to screen for the presence of associated anomalies or syndromes, and only the cases of NS-OFC were included in the study. The NS-OFC cases were grouped according to the phenotype: 1) cleft lip with or without cleft palate (CL/P) collectively including CLP and CLO (94 triads), 2) CPO (18 triads).

Material

Venous blood samples (5–10 ml) were obtained from each individual. Total genomic DNA was extracted from blood samples by salting out or phenol–chloroform extraction according to established protocols.

Methods

Selecting DNA markers. Initial bioinformatics analysis of the selected candidate loci *TGFA*, *TGFB3*, *GABRB3*, *RARA*, and *BCL3* was performed to choose a set of biallelic (SNP) and microsatellite markers within the candidate loci or close to them for the analysis of allelic association with the NS-OFC phenotype. Selection of SNPs (except the *Taq*I marker in the *TGFA* locus and 5'UTR.1 marker in the *TGFB3* locus) was performed from public SNP databases dbSNP and *Ensembl* [9] and using the *FastSNP* programme (http://ibms.sinica.edu.tw). Criteria for selecting a SNP: 1) known allelic frequency determined by methods other than *in silico*; 2) if LD had been found between a group of SNPs, only one SNP from the group was selected; 3) potential functional significance: all selected intronic SNPs were located in intronic splicing enhancers. Microsatellite markers in the candidate loci were selected on the basis of maximal known heterozygosity in Caucasian populations according to the *Ensembl* database [9].

Genotyping. Genotyping of the individuals for all selected DNA markers was based on polymerase chain reaction (PCR) amplification of the DNA fragment covering a relevant marker with subsequent identification of its alleles.

To amplify DNA fragments covering selected markers, primer pairs were selected: 1) using *Primer3* software (http://www-genome.wi.mit.edu) for SNPs, 2) retrieving from the *Ensembl* database [9] for microsatellite markers, 3) from other studies for *Taq*I marker in

Table 1. **Selected biallelic markers within the candidate genes**

Candidate gene	SNP		Allele identification method
	Code	Alleles (A_1/A_2)[b]	
TGFA	rs2166975[a] (p.V160V)	C/T	RFLP (*Hpy*188I)[c]
	rs1058213	C/T	RFLP (*Hinf*I)[c]
	rs538118	C/T	RFLP (*Sty*I)[c]
	rs3732253	C/T	RFLP (*Pfl*MI)[c]
	rs473698	C/G	RFLP (*Mfe*I)[c]
	rs503314	C/G	RFLP (*Dde*I)[c]
	*Taq*I	A1/A2	PCR product size (EP-PAA)[d]
TGFB3	rs3917210	A/T	direct sequencing
	rs3917169	A/G	TaqMan technology
	5'UTR.1	B1/B2	PCR product size (EP-PAA)[d]
GABRB3	rs10438462	C/T	RFLP (*Nla*III)[c]
	rs12437487	A/G	RFLP (*Mly*I)[c]
	rs6576605	C/G	RFLP (*Bst*UI)[c]
	rs17117193	C/T	RFLP (*Alu*I)[c]
	rs11634050	C/T	RFLP (*Bsm*AI)[c]
RARA	rs12946680	C/G	RFLP (*HpyCH*4IV)[c]
BCL3	rs1046881	A/T	RFLP (*Bsu*36I)[c]

[a] refSNP ID according to dbSNP in NCBI [24]; [b] A_1, ancient (wild type) allele; A_2, recent (variant) allele; [c] Informative restriction enzyme; [d] EP-PAA, electrophoresis on polyacrylamide gel.

the TGFA locus [10] and for the 5'UTR.1 marker in the TGFB3 locus [11]. PCRs were performed separately for each marker. The annealing temperature was optimized experimentally for each primers pair. Primer pair sequences and PCR protocols are available from the authors upon request.

Several techniques were used to identify SNPs alleles: 1) denaturing high performance liquid chromatography (DHPLC) using WAVE® System (Transgenomics Limited, UK) and following the manufacturer's directions was applied for SNP verification; 2) for the *Taq*I marker (*TGFA* gene) and 5'UTR.1 marker (*TGFB3*), standard PCR followed by separating products electrophoretically on PAA gel was applied; 3) restriction fragments length polymorphism (RFLP) analysis was used for a number of biallelic SNPs. Informative restriction enzymes (RE) (Table 1) were selected using *NebCutter V2.0* software (http://tools.neb.com). Each PCR product was digested with corresponding RE (protocols are available from the authors upon request), then separated electrophoretically on agarose gel and visualised; 4) ABI TaqMan Protocol following the manufacturer's directions was applied for the rs3917169 marker; 5) direct sequencing on ABI PRISM 3730 Genetic Analyzer (Applied Biosystems, USA) was used for the rs3917210 marker.

Analysis of PCR amplified (using fluorescence-labelled primers) DNA fragments covering selected microsatellite markers were performed by capillary electrophoresis and subsequent analysis on ABI PRISM 310 Genetic Analyzer (Applied Biosystems, USA).

In all 112 OFC triads involved in the present study paternity was confirmed by genotyping four microsatellite loci CSF1PO, TPOX, TH01, vWA using a commercial kit (Promega, USA).

Allelic association analysis. Allelic association analysis between the DNA markers of putative susceptibility alleles and NS-OFC phenotype was performed using the standard transmission disequilibrium test (TDT/S-TDT) [12] and TDT-AE (LRT) statistics [13, 14] for biallelic markers and extended TDT test for multi-allele marker loci [15]. The TDT/S-TDT was carried out entirely by the "z score" approach, not by permutation.

RESULTS AND DISCUSSION

Despite extensive investigations for more than a decade in different populations after loci at 2p13 (*TGFA*), 14q24 (*TGFB3*), 15q11.2-12 *(GABRB3),* 17q21 (*RARA*), and 19q13 (*BCL3*) had emerged as strong potential candidates for the involvement in the etiology of nonsyndromic OFC, the question whether and how allelic variants of these candidate genes could contribute to the risk of NS-OFC is still open. Assessment of different markers within or close to these genes in a number of separate studies has resulted in association with the clefting phenotype as well as in negative results (see a recent review on *TGFA* gene [16] as an example). Here, we present the first results of the allelic association analysis of these candidate genes in the population of Lithuania, which has not yet been investigated in this field.

Table 2. **Selected microsatellite markers within or close to the candidate genes**

Candidate gene	Microsatellite marker		PCR product size (bp)
	ID*	Repeat sequence	
TGFA	*D2S292*	$(CA)_n$	*~180–192*
TGFB3	D14S61	$(CA)_n$	197–227
GABRB3	D15S97	$(CA)_n$	~159
RARA	D17S1335	$(TTTA)_n$	~155
BCL3	BCL3	$(CA)_n$	~127

* Microsatellite ID in *Ensembl* database [9].

On the basis of the initial bioinformatics analysis of the candidate loci *TGFA*, *TGFB3*, *GABRB3*, *RARA*, and *BCL3* a set of 21 DNA markers was selected: 16 SNPs and five microsatellite markers (listed in Tables 1 and 2 (*BCL3* locus marker rs1046881 was not included in Table 1 – see below for the explanation)). Two additional biallelic markers were also chosen on the basis of the studies on other populations: the *Taq*I marker (variant allele is 4 bp deletion in intron V) in the *TGFA* locus [10, 11] and hexanucleotide repeat (AGAGGG) deletion in the 5'UTR.1 region of the *TGFB3* gene [17, 11]. As current knowledge of common genetic variation is not yet complete for the majority of genes including the candidate genes selected for the present study, the first step was validation of the selected biallelic markers. It was performed by genotyping the sample of 48 individuals (96 chromosomes) from the general population of Lithuania, using DHPLC assay of PCR-amplified DNA fragments covering each marker. The results revealed that all selected SNPs (except rs1046881), *Taq*I marker in the *TGFA* locus and 5'UTR.1 marker in the *TGFB3* locus were true polymorphisms with a minor allelic frequency (MAF) >1%. One candidate SNP in the *BCL3* gene (rs1046881) was shown to be monomorphic in Lithuanian population and therefore was excluded from the further investigation.

Genotyping NS-OFC triads Lithuania according to the selected markers in the candidate loci *TGFA*, *GABRB3*, *RARA* and *TGFB3* was performed (although in different numbers of the triads due to technical problems) and allele transmission from parents to affected child was checked for biallelic markers (Table 3). Five selected microsatellite markers appeared to be highly polymorphic (from 9 to 14 alleles – see Table 4) in the NS-OFC families tested. All marker genotype frequencies of the patients and their parents followed the Hardy–Weinberg equilibrium.

Cleft lip with or without cleft palate (CL/P) is considered to be etiologically separate from cleft palate only (CPO). However, it is recognized that there are many pathways and cellular mechanisms in common for those two main OFC phenotype groups. Mutations in some can-

Table 3. **Results of transmission disequilibrium test (TDT) based analysis of the association between individual biallelic markers of selected candidate genes and non-syndromic orofacial clefting in the nuclear families (triads) from Lithuania**

Candidate gene	SNP		Phenotype group	Triads (n)	Transmission of allele A_1 to affected child		TDT/S-TDT		TDT-AE	
	Code	Alleles (A_1/A_2)[a]			Transmitted alleles (n)	Non-transmitted alleles (n)	χ^2	P-value (df[b]1)	LRT[c]	P-value
TGFA	rs2166975[a] (p.V160V)	C/T	OFC[d]	107	48	41	0.551	0.4579	0.0153	0.9015
			CL/P	89	36	37	0.014	0.9058	0.3326	0.5641
			CPO	18	12	4	4.0	0.0455	2.5272	0.1119
	rs1058213	C/T	OFC	107	46	41	0.287	0.5921	0.1400	0.7083
			CL/P	89	35	36	0.014	0.9058	0.0983	0.7539
			CPO	18	11	5	2.25	0.1336	2.3059	0.1289
	rs538118	C/T	OFC	107	47	51	0.163	0.6864	0.3126	0.5761
			CL/P	89	38	43	0.309	0.5782	0.3112	0.5770
			CPO	18	9	8	0.059	0.8080	0.1520	0.6966
	rs3732253	C/T	OFC	107	41	45	0.186	0.6662	0.0180	0.8931
			CL/P	89	37	35	0.056	0.8129	0.3016	0.5829
			CPO	18	4	10	2.571	0.1088	2.6566	0.1031
	rs473698	C/G	OFC	107	51	48	0.091	0.7629	0.1569	0.6920
			CL/P	89	41	41	0	1	0.0118	0.9136
			CPO	18	10	7	0.529	0.4670	0.5322	0.4657
	rs503314	C/G	OFC	107	47	50	0.093	0.7603	0.0016	0.9678
			CL/P	89	39	41	0.05	0.8230	0.0039	0.9501
			CPO	18	9	8	0.059	0.8080	0.0589	0.8083
	*Taq*I	A1/A2	OFC	112	34	28	0.581	0.4459	0.2501	0.8825
			CL/P	91	30	19	2.469	0.1161	1.5651	0.4572
			CPO	21	4	9	1.923	0.1655	2.0523	0.3584
TGFB3	rs3917210	A/T	OFC	107	32	26	0.621	0.4306	0.8325	0.3616
			CL/P	89	26	23	0.184	0.6679	0.3203	0.5714
			CPO	18	3	6	1	0.3173	1.0194	0.3126
	rs3917169	A/G	OFC	107	34	28	0.581	0.4459	0.2916	0.5892
			CL/P	89	29	25	0.296	0.5864	0.0975	0.7548
			CPO	18	5	3	0.5	0.4795	0.5053	0.4772
	5'UTR.1	B1/B2	OFC	118	7	6	0.077	0.7814	No valid alleles	
GABRB3	rs10438462	C/T	OFC	107	54	44	1.02	0.3125	0.8942	0.3443
			CL/P	89	47	38	0.953	0.3289	0.7715	0.3797
			CPO	18	7	6	0.077	0.7814	0.0770	0.7814
	rs12437487	A/G	OFC	107	52	60	0.571	0.4498	0.7176	0.3969
			CL/P	89	44	50	0.383	0.5360	0.5163	0.4725
			CPO	18	8	10	0.222	0.6375	0.2227	0.6370
	rs6576605	C/G	OFC	107	53	49	0.157	0.6919	0.2757	0.5996
			CL/P	89	46	42	0.182	0.6696	0.3238	0.5693
			CPO	18	7	7	0	1	0.0000	1.0000
	rs17117193	C/T	OFC	107	38	38	0	1	0.0000	1.0000
			CL/P	89	34	27	0.803	0.3701	0.7794	0.3773
			CPO	18	4	11	3.267	0.0706	3.3970	0.0653
	rs11634050	C/T	OFC	107	48	56	0.615	0.4329	0.4514	0.5017
			CL/P	89	45	40	0.294	0.5876	0.1058	0.7450
			CPO	18	8	11	0.474	0.4911	0.4757	0.4904
RARA	rs12946680	C/G	OFC	107	16	22	0.947	0.3304	No valid alleles	
			CL/P	89	15	18	0.273	0.6013	No valid alleles	
			CPO	18	1	4	1.80	0.1797	No valid alleles	

[a] See Table 1 for explanation; [b] df, degrees of freedom; [c] LRT, likelihood ratio test; [d] OFC, all NS-OFC triads (CL/P + CPO) tested.

Table 4. **Results of transmission disequilibrium test (TDT) based analysis of the association between candidate gene individual microsatellite markers and non-syndrosmic orofacial clefting in the nuclear families (triads) from Lithuania**

Candidate gene: microsatellite marker	Different alleles detected (n)	Phenotype group [a]	Triads (n)	Allele-wise		Genotype-wise	
				χ^2	P-value (df [a])	χ^2	P-value (df [a])
RARA:		OFC	106	4.1713	0.3834 (4)	9.7915	0.2010 (7)
D17S1335	13	CL/P	87	7.0505	0.1334 (4)	10.8271	0.0941 (6)
		CPO	19	2.5666	0.46345 (3)	3.1791	0.5284 (4)
GABR3:		OFC	110	5.3989	0.9432 (12)	37.9942	0.3793 (36)
D15S97	13	CL/P	91	8.9417	0.6273 (11)	39.9036	0.1911 (33)
		CPO	19	13.1443	0.2161 (10)	15.4235	0.4940 (16)
TGFB3:		OFC	107	9.0457	0.7693 (13)	34.5867	0.4882 (35)
D14S61	14	CL/P	89	11.9361	0.5330 (13)	41.6890	0.1437 (33)
		CPO	18	13.0157	0.1116 (8)	16.6984	0.0817 (10)
TGFA:		OFC	110	11.5124	0.2425 (9)	25.3847	0.4413 (25)
D2S292	9	CL/P	91	23.4704	0.0053 (9)	40.2274	0.0206 (24)
		CPO	19	10.9124	0.2821 (9)	18.8719	0.1276 (13)
BCL3:		OFC	110	3.3055	0.6530 (5)	6.5281	0.5883 (8)
BCL3	9	CL/P	91	7.8525	0.1648 (5)	9.5765	0.2141 (7)
		CPO	19	6.6242	0.0850 (3)	6.6275	0.1571 (4)

[a] See Table 3 for explanation.

didate genes were found in both syndromic CL/P and CPO groups (reviewed by D.P.C. Rice [1]), while syndromic genes have recently been shown to contribute to NS-OFC [18]. Therefore, it was reasonable in the present study to perform TDT-based allele association analysis for each OFC phenotype group (CL/P and CPO) separately and for the whole NS-OFC group (Tables 3 and 4). For 5'UTR.1 marker in the *TGFB3* locus, variant (B2) allele appeared to be relatively rare in the parental group (allele frequency was 0.03), resulting in the heterozygosity in the patients' group as low as 0.056. Therefore, transmission disequilibrium was calculated for the whole OFC group only. Two methods of allele-wise calculations, TDT/S-TDT [12, 15] and TDT-AE [13, 14], were applied for biallelic markers (Table 3) to achieve a better sensitivity. For highly polymorphic microsatellite markers, allele-wise (attempts to establish a pattern of preferential transmission of certain alleles across genotypes) and genotype-wise (considering every heterozygous parental genotype separately and examined whether each allele of the genotype was transmitted to affected offspring on 50% of occasions) calculations were made.

Out of 21 markers tested, only rs2166975 (*TGFA* locus) C allele showed a considerable association with the risk for CPO phenotype (P = 0.0455 [df 1]) in the population of Lithuania. No association of *Taq*I marker alleles and the risk of both NS-OFC phenotypes was shown in this study, although this marker has been shown to yield a positive association in Caucasian populations [19, 20]. Our finding is consistent with the assumption that much of the variation in the allele frequencies of this marker could be due to a chance [16]. For microsatellite markers, an association was found between the D2S292 marker (*TGFA* gene) and CL/P in allele-wise TDT (P = 0.0053 [df 9]) and genotype-wise TDT (P = 0.0206 [df 24]). In the CL/P group, significant excess in transmission of allele 6 (182 bp) of this marker (P = 0.0104) was found. Thus, our results support an association between *TGFA* and CL/P, but do not support the hypothesis that *TGFA* is a major causal gene of NS-OFC, implying that this gene is probably a genetic modifier of clefting in humans.

A close to borderline association (P = 0.0850 [df 3]) of the BCL3 marker (*BCL3* gene) was found with the risk of CPO in the population of Lithuania. The TDT test showed an excess transmission of allele 3 (128 bp) to the affected children (P = 0.0523), similarly to the findings in the U.S. population [21]. Thus, the observed TDT results imply a different role for the *TGFA* and *BCL3* genes in CL/P and CPO. On the other hand, other genetic data on the role of the 19q13 locus in NS-OFC, including evidence for the *CLPTM1* [22], *PVR* and *PVRL2* [23] genes, suggest that a regulatory element in this region in linkage disequilibrium with the allele 3 may be affected in some families.

Transmission disequilibrium was not detected (P >> 0.05) for other alleles of the tested DNA markers, providing no evidence for the association of the *TGFB3*, *GABRB3* and *RARA* loci with the NS-OFC phenotype in the population of Lithuania. Nevertheless, this does not exclude the potential for other alleles in these loci as well as the necessity to increase the number of NS-OFC triads to achieve more statistically significant results.

CONCLUSION

Initial results from the population of Lithuania support the contribution of the *TGFA* locus allelic variation in the etiology of CL/P, but do not point to *TGFA* as a major causal gene. Different roles for the *TGFA* and *BCL3* genes in the susceptibility to NS-OFC phenotypes are suggested.

ACKNOWLEDGEMENTS

The authors are grateful to all donors of DNA samples for participation in the study. The study was supported by Ministry of Education and Science of the Republic of Lithuania, Taiwan National Council of Science (grant of the Taiwan–Baltic Programme of Mutual Funds).

Received 24 October 2006
Accepted 17 January 2007

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AR *TGFA*, *TGFB3*, *GABRB3*, *RARA* IR *BCL3* GENAI SUSIJĘ SU NESINDROMINIAIS BURNOS IR VEIDO ĮSKILUMAIS? LIETUVOS TYRIMAI

Santrauka

Nesindrominiai burnos ir veido įskilumai (NS-BVĮ) yra dažna daugiaveiksnė įgimta žmogaus raidos anomalija, kurios patogenezėje svarbūs genetiniai veiksniai. Įvairiais metodais atlikti tyrimai jau atskleidė daugelį genų kandidatų, bet vis dar neaiškus jų nukleotidų sekų variantų ryšys su NS-BVĮ rizika. Šiame darbe taikant perdavimo nepusiausviros testą (*transmission disequilibrium test*, TDT) buvo analizuojama 21 bialelinių ir mikrosatelitinių DNR žymenų, pasirinktų ypač svarbiose kandidatinėse *TGFA*, *TGFB3*, *GABRB3*, *RARA*, ir *BCL3* srityse, alelių asociacija su NS-BVĮ fenotipu 112-oje šeimų (NS-BVĮ turintis vaikas + abu tėvai) iš Lietuvos. Remiantis rezultatais, buvo nustatyta *TGFA* geno rs2166975 žymens asociacija su nesindrominio gomurio įskilumo (NS-GĮ) fenotipu (P = 0,0455 [df 1]) ir D2S292 žymens asociacija su nesindrominiu lūpos ir(arba) gomurio įskilumu (NS-L/GĮ) TDT alelių atžvilgiu (P = 0,0053 [df 9]), taip pat TDT testas genotipų atžvilgiu (P = 0,0206 [df 24]). Taip pat buvo nustatyta nedidelė (P = 0,0850 [df 3]) BCL3 žymens (*BCL3* genas) asociacija su NS-GĮ rizika. Taigi pradiniai mūsų rezultatai paremia hipotezę apie *TGFA* genetinės srities alelių įvairovės indėlį į NS-L/GĮ etiologiją Lietuvos populiacijoje, bet nerodo, kad *TGFA* gali būti pagrindinis priežastinis genas. Tikėtina, kad *TGFA* ir *BCL3* genų vaidmuo nesindrominių burnos ir veido įskilumų polinkiui yra skirtingas.

Raktažodžiai: alelių asociacija, genai kandidatai, nesindrominiai burnos ir veido įskilumai, perdavimo nepusiausviros testas, TDT