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Nucleotide sequence changes in the *MSX1* and *IRF6* genes in Lithuanian patients with nonsyndromic orofacial clefting

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Background. Nonsyndromic orofacial clefing (NS-OFC) is among the most common malformations in humans. It is a complex multifactorial trait with a considerable genetic component. Among numerous candidate genes, those related to syndromic OFC recently have emerged as particularly strong ones. Our study was aimed to investigate whether mutations in the *MSX1* and *IRF6* genes contribute to NS-OFC in the population of Lithuania.

Patients, materials and methods. Patients with NS-OFCs from Lithuania were tested for nucleotide sequence changes in the *MSX1* gene (80 patients) and *IRF6* gene (190 patients). DNA fragments covering *MSX1* gene and exonic parts of the *IRF6* gene were PCR-amplified, and the direct sequencing of the PCR products was performed with a subsequent comparison of sequencing results with reference DNA sequences of the genes.

Results. Eight and 22 different nucleotide sequence changes were revealed in the *MSX1* and *IRF6* genes, respectively. While only already known functionally neutral polymorphisms were detected in the *MSX1* gene, scanning *IRF6* gene resulted in nine novel nucleotide sequence variants. Of them, three were missense mutations (p.S212I, p.L295P, p.R400L), which, together with the p.A61G mutation found in the case of Van der Woude syndrome, might be related to the NS-OFC phenotype in the population of Lithuania.

Conclusions. Failure to find *MSX1* gene mutations potentially related to NS-OFC phenotype of the probands from Lithuania does not reject the necessity to continue investigation on a larger population sample. Our study highlights the *IRF6* gene sequence variability and supports the hypothesis that variation in this gene contributes to NS-OFC phenotype, encouraging further investigations to test whether *IRF6* gene mutations identified in individuals from Lithuania are rare alleles causative for NS-OFC.

Key words: nonsyndromic cleft lip with or without cleft palate, candidate gene, *IRF6*, *MSX1*, nucleotide sequence variant

BACKGROUND

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/CP), which is a group of OFCs considered to be separate from CPO. However, it is recognized that there are many pathways and cellular mechanisms in common. OFCs occur in a wide geographic distribution with an average birth prevalence in the world from 1/300 to 1/2500 births for CL/CP and around 1/500 births for CPO and considerable population differences, with high birth prevalences in Asians and native Americans to intermediate in

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European and low in African populations [2]. The incidence of OFCs in Lithuania, 1.84 for 1000 newborns (1/544 livebirths) [3], appears to be similar to that of other European populations.

Approximately 30% of OFC cases are syndromic and have additional characteristic features that can be subdivided into categories of chromosomal abnormalities, >350 recognisable Mendelian single gene disorders [4], teratogenic effects and various uncategorised syndromes. The majority of OFC cases (~70%) are considered nonsyndromic (NS-OFC) where the clefts are isolated, i.e. occur without other anomalies. NS-OFC arises as a complex multifactorial trait with a considerable genetic component with numerous Mendelian patterns hidden under varying levels of penetrance, sex differences, and stochastic factors [5] as well as environmental overlays acting either independently or in combination [6].

Extensive attempts to reveal the genetic basis of OFC are being undertaken using different approaches such as mutation screening of specific genes, linkage and association studies, genome-wide scans, chromosome breakpoint mapping, expression analyses, animal models, etc. [5, 7, 8]. As a result, a number of potential candidate loci and candidate genes throughout the human genome were located (some of them were identified in more than one study) [7–10]. Application of modern high throughput genotyping and expression analysis technologies [11, 12] also increases the list of candidate genes. Despite this, researchers have largely failed to reveal the molecular basis of OFC due to its complexity, failure to replicate findings, statistical insignificance of the results from numerous studies. Analysis of the risk of recurrence suggests that three to six (up to 14) may interact contributing to the OFC phenotype [13], making such efforts particularly confounding. On the other hand, some important findings emerged, especially from studies involving syndromic forms of the pathology. These include a number of genes (e.g., *DHCR*, *FGFR1*, *FGFR2*, *IRF6*, *MID1*, *MSX1*, *TP73L*, *TBX22*, *PVRL1*, etc.) [1], which have now been shown to contribute a major genetic effect on the etiology of syndromic OFC. Recent findings suggest that at least some of such genes (*MSX1*, *IRF6*, *PVRL1*, and *TBX22* in the first place [14] can be used to demonstrate a significant overlap between syndromic and nonsyndromic OFCs, because it has been shown that reduced activity of the proteins they encode can affect embryonic orofacial development. It is likely that mutations having a mild effect on the functions of genes causing the syndromes could cause phenotypes not distinct from nonsyndromic CL / CP. Thus, such genes draw attention as candidate genes for at least some instances of NS-OFCs, and a variety of candidate genes initially related to syndromic OFC are currently being analysed in extended cohorts of affected individuals providing an increasing amount of evidence to support this hypothesis.

Van der Woude syndrome (VWS; MIM #119300) provides one of the best models for nonsyndromic CL / CP, since most patients have only minor additional phenotypes of lip pits and occasional hypodontia, while 15% may be clinically indistinguishable from isolated CL / CP [15]. Linkage analysis localized the gene on human chromosome 1 (1q32-41). Investigation of a pair of monozygotic twins who were discordant for the VWS phenotype and had unaffected parents and subsequent studies resulted in recognizing the mutant *IRF6* gene (MIM 607199; length: 18,217 bp; coding part: 9 exons; product: 297 amino acids protein, which is a member of the interferon regulatory transcription factor (IRF) family and is involved in palate formation) being responsible for VWS and popliteal pterygium (PPS; MIM 119500), another syndrome including CL / CP or CPO as a characteristic feature [16]. For *IRF6*, Zucchero et al. [17] estimate the attributable risk of CL / CP of about 12 percent, suggesting a substantial causal role.

MSX1 gene in humans (MIM 142983; location: 4p16.1; length: 4,052 bp; coding part: 2 exons; product: 297 amino acids Msh homeobox 1-like protein MSX-1) has recently been proposed as another strong candidate gene with allelic variants potentially contributing to nonsyndromic forms of CL / CP. Support for this hypothesis comes from human linkage and linkage disequilibrium studies [18], chromosomal deletions resulting in haploinsufficiency [19, 20], a large family with a nonsense mutation that includes clefting as a phenotype [21], and the Msx1 phenotype in a knockout mouse [22]. OFC, selective hypodontia, or dental anomalies associated with other ectodermal features are found with specific coding sequence mutations in the gene [20, 23]. Missense mutations in the *MSX1* gene have been identified in 2% of patients with NS-OFC, predominantly CL / CP [24].

Nevertheless, a number of conflicting reports have recently been published on the presence or absence of association of the sequence variants of the candidate genes, the above-stated ones in the first place, with the NS-OFC phenotype, and the problem of its genetic basis is still unsolved and needs further investigation, including replication of already performed studies in other populations, testing larger samples of affected individuals and applying new approaches.

The main objective of the present study was to test the hypothesis of the contribution of *MSX1* and *IRF6* genes allelic variation to the incidence of nonsynsdomic CL / CP by scanning for and analysis of the changes in their nucleotide sequences in the NS-OFC individuals from the population of Lithuania, which has not been investigated in this field yet. Our initial results presented here support the contribution of the *IRF6* gene in the development of human face and OFC phenotype.

PATIENTS, MATERIALS AND METHODS

Patients

Our study group consisted of 190 unrelated patients with isolated NS-OFCs: cleft lip with cleft palate (CLP), cleft

palate only (CPO) and cleft lip only (CLO) (Table 1). Recruitment and investigation of OFC patients from all regions of Lithuania was performed at the Department of Human and Medical Genetics and Institute of Odontology of the Faculty of Medicine of Vilnius University in the period 2002–2005. The OFC phenotype was evaluated and genealogical analysis was performed by qualified odontologists and geneticists to screen for the presence of associated anomalies or syndromes, and only cases of NS-OFC were included in the study. All individuals investigated were Caucasians, the majority being of Lithuanian origin, with some admixture of individuals with the ancestry of neighbouring Slavic populations (Russians, Poles, Byelorussians), which are known to be genetically close to Lithuanians.

Table 1. **Distribution of nonsyndromic orofacial clefting phenotype among patients of the present study**

Candidate gene	OFC phenotype groups of the patients tested			Total (n)
	CLO (n)	CPO (n)	CLP (n)	
MSX1 (exon 1)	14	10	56	80
IRF6	22	38	130	190

Written informed consent was obtained from each person (or his / her legal guardian) participating in the study.

Materials

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

Methods

PCR. Intronic primers were used to amplify DNA fragments encompassing exons 1–9 with flanking intronic sequences of the *IRF6* gene. The sequences for primer pairs (Table 2) were selected according to Wang X et al. [25] with some modifications. For PCR amplification, *MSX1* gene sequence (obtained from Ensembl, gene ID ENSG00000163132 [26]) was divided into eight overlapping fragments (A–I) according to Jezewski et al. [24] and fragments covering the 5′-UTR (promoter) region and part of the 1st exon (A), 1st exon and 5′-part of the intron (B) were chosen to scan for nucleotide sequence changes. Primer pair sequences (table 2) were selected on the basis of the *MSX1* and *IRF6* gene sequence using Primer3 software [26]; previously published primer sequences [24] were also used.

PCRs were performed separately for each fragment in 25 µl reaction mixture (50 ng, 0.2 mM each dNTP, 2.5mM $MgCl_2$, 10 pmol each primer and 1 U Taq DNA polymerase). Thermocycling conditions were selected experimentally for each primer pair by varying the annealing temperature (see Table 2).

Direct DNA sequencing. Following DNA amplification, unincorporated PCR primers and deoxynucleotide triphosphates in the sample were removed before sequencing. PCR products were sequenced using a BigDye Terminator protocol with the same set of primers used for PCR using BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 and ABI PRISM 310 and ABI PRISM 3730 Genetic Analyzers (Applied Biosystems, USA). DNA sequencing results were compared with the published sequences in NCBI database.

Sequence analysis. The ABI sequence software (version 2.1.2) was used for lane tracking and analysis of the sequences derived from the patient. The sequences of the *MSX1* and *IRF6* genes were compared to human

Table 2. **Primer pairs for PCR amplification of the *MSX1* and *IRF6* genes**

Gene	Fragment*	Forward primer (5′-3′)	Reverse primer (5′-3′)	Annealing temperature	PCR product size, bp
MSX1	A (5′UTR–Ex 1)	CTTCAGCGCAGAGGAAAGTT	ACACCGAGTGGCAAAGAAGT	57	541
	B (Ex 1–5′-In)	TGGCCAGTGCTGCGGCAGAA	ATTCATCCGCTGGGGTGAA	55	644
	C (In middle-1)	CATGATCCCTCATCTGATCC	CCACTTTAATGGCTCAGTCCT	56	365
IRF6	Ex 1	GCTATCTGGAAAAGGGCGACA	CGCGGAGTGTCATATTTCTTGG	60	480
	Ex 2	AAGACAACTAAAGGTGAATGGGAAT	CAGCCTTTGTCGCCAGTGTT	65	268
	Ex 3	TTCCCATATTTAGTTTCTTTGCTG	ATTACTATGCCTGCTGAGTTTGG	60	454
	Ex 4	ATGGGGCAGTCATGCAAAA	AAGGCTTTCTTGCTTTATCCATC	60	477
	Ex 5	TTCCCTTGATTCTCACTCTTTTT	CTCCCACTTGCTAACAGTCCAG	65	431
	Ex 6	TTGCGTTAGTTATGGGAATCAC	AAGTTAGAAAGCAGGACAGGAAAG	65	392
	Ex 7a	ATGCTGGTTGAAAGGTGGCT	TGGGAGCAACAAGTGATGGG	60	441
	Ex 7b	CAGGGCTGCCGACTCTTCTA	AGGAAAGCAGGAAGGTGAAAGA	65	413
	Ex 8	ATGTGGCTAGTGGCTATTGTAATG	ATGGGCTGATGGATGCTTGA	60	319
	Ex 9a	CCTCAGGGCCTCTTTGGTCT	GAAAAGCAAAGTCTGAAGGGTG	60	516
	Ex 9b	TACCGCATCCTTCAAACCCA	CAATTTCAGGCACTACTCCAATCT	60	459
	Ex 9c	TGTGATTCTCCAAATATGCCTAG	GACTTTGGCACCCATTTCTATT	60	535

* Ex, exon; In, intron.

sequences available via GENBANK. This included comparisons to *MSX1* and *IRF6* cDNA, BLAST matches from the EST databases and genomic contigs of this region of chromosomes 4 and 1.

Statistical analysis of the results was not performed due to the number of the patient samples insufficient to ensure statistically reliable results.

RESULTS

In 190 DNA samples from subjects with NS-OFC scanned for the *MSX1* and *IRF6* genes (Table 1), 30 variant sites in both candidate genes were identified (summarised in Table 3). Of them, 30 were single nucleotide substitutions comprising 17 transitions (3 in cod-

Table 3. ***MSX1* and *IRF6* gene nucleotide sequence changes[a] identified in Lithuanian probands with nonsyndromic cleft lip with or without cleft palate**

Gene	Gene fragment	Nucleotide position[b]	Nucleotide sequence changes identified	Expected amino acid substitution	Possible relation to NS-OFC phenotype[c]	OFC phenotype and number of cases[d]	Reference
MSX1	5′UTR	-36	G/A	-	P		[24]
	Exon 1	c.101	C>G	p.A34G	P	CPO – 2 het-z, 1 homo-z CLP – 12 het-z	rs36059701[e], [24]
	Intron	IVS1+41-51	delGGGGCCGGGT	-	P		[24]
	Intron	IVS1+590	G/C	-	P		rs3116581, [24]
	Intron	IVS1+405	G/A	-	P		rs33931631, [24]
	Intron	IVS1+428	C/T	-	P		rs34206627, [24]
	Intron	IVS1+520	C/T	-	P		rs35254762, [24]
	Intron	IVS1+432	A/G	-	P		[24]
IFR6	Exon 1	IVS1-*50*	T/A		P		rs34743335
	Exon 1	IVS1-39	A/T		P		rs12403006
	Exon 1	IVS1-27	C/T		P?		This study
	Exon 1	IVS1-23	G/C		P?		This study
	Exon 2	IVS2-4	T/C		P		rs2235377
	Exon 2	-73	A/G		P		rs861019
	Exon 4	IVS4-5	G/C		P		rs7552506
	Exon 4	c.182	C>G	p.A61G	CM?	CLP – 1 het-z	[16, 28]
	Exon 5	IVS5-84	G/A		P?		This study
	Exon 5	c.459	C/A	p.S153S	P	Allele frequency in OFC: C – 0.71, A – 0.29	rs2013162
	Exon 6	IVS6+27	C/G		P		rs2235375
	Exon 6	IVS6+42	A/G		P?		This study
	Exon 6	c.635	G>T	p.S212I	CM?	CLP – 1 het-z	This study
	Exon 7	IVS7+37	C/T		P		rs2235373
	Exon 7	c.820	G/A	p.V274I	P	CLP – 1 het-z CPO – 1 het-z	rs2235371, [17,30]
	Exon 7	c.884	T>C	p.L295P	CM?	CLO – 1 het-z	This study
	Exon 7	c.1013	G>A	p.E338E	CM?	CLO – 1 het-z	This study
	Exon 9	1404+279	T/C		P?		This study
	Exon 9	1404+479	G/T		P?		This study
	Exon 9	1404+411	C/T		P?		This study
	Exon 9	1404+451	G/A		P		rs17317411
	Exon 9	c.1199	G>T	p.R400L	CM?	CPO – 1 het-z	This study

[a] Nomenclature of the nucleotide sequence changes is according to the recommendations at the Human Gene Mutation Database [28].

[b] Source of genomic DNA sequences: Ensembl [26]. Gene ID ENSG00000163132 for *MSX1*; gene ID ENSG00000117595 for *IRF6*.

[c] P, functionally neutral nucleotide sequence polymorphism; CM, causal mutation.

[d] Phenotypes and numbers of cases (this study) are provided for coding sequence changes only; het-z, nucleotide sequence change in heterozygous condition; homo-z, nucleotide sequence change in homozygous condition.

[e] dbSNP ID in NCBI [29]

ing regions) and 13 transversions (6 in coding regions), and one was 10 bp intronic deletion. All identified intronic nucleotide sequence changes showed a considerable heterozygosity with both alleles being present in homozygous conditions in a number of individuals tested (data not shown), while all except one (see below) changes in the coding parts of the genes were unique cases (i.e. found in a single individual on a single chromosome).

Eight different sequence variants were identified in the *MSX1* gene. Six sequence changes were identified in the intron, one SNP was found in the 5′UTR, one sequence change was identified in the 1st exon. No sequence changes were found in the 2nd exon of the *MSX1* gene.

Out of 22 single nucleotide substitutions detected in the *IRF6* gene in NS-OFC patients from Lithuania, 10 sequence changes were identified in the parts of introns flanking exons 1, 2, 4, 5, 6, 7, and coding parts of the exons 2, 4, 5, 6, 7 and 9. No sequence changes were found in the exons 3 and 8 of the *IRF6* gene.

DISCUSSION

Fifteen *MSX1* gene mutations causing Witkop syndrome, tooth agenesis and orofacial clefting (data of October 2006) and a number of functionally neutral nucleotide sequence polymorphisms have already been reported by Jezewski et al. [24], registered in the online databases HGMD [28] and dbSNP [29]. The majority of such mutations have been found in the exon 1 of the gene, suggesting the presence of hidden regulatory elements in this exon thus causing conservation of the sequence [24]. Therefore, exon 1 (together with neighbouring noncoding sequences) was initially scanned for mutations in the present study. All eight different sequence variants identified in the present study in the coding and noncoding parts of the *MSX1* gene (Table 3) have already been described in other populations as functionally neutral polymorphisms [24], including one mutation expected to result in amino acids conversion, i. e. c.101C > G substituting alanine for glycine (p.A34G).

Although missense mutations of the *MSX1* gene (the majority in the exon 1) potentially related to the NS-OFC phenotype have been identified in 2% of patients, predominantly CL/CP [24], we failed to find such mutations in the NS-OFC patients from Lithuania. This failure might result from the different ethnicity of our patients, i.e. the different genetic background providing different susceptibility alleles in other genes. On the other hand, it is likely a bias due to a small sample size (80 probands) tested as well as absence of data on other parts of the *MSX1* gene. Thus, further investigation is necessary, in the first place testing the remaining 110 available NS-OFC probands.

At least 77 *IRF6* gene mutations causing WVWS or PPS (data of September 2006) and a number of functionally neutral nucleotide sequence polymorphisms have already been registered in the online databases HGMD [28] and dbSNP [29], revealing a considerable variability of the gene sequence but a relatively limited spectrum of phenotypic diversity. Out of 22 sequence changes detected in the *IRF6* gene in NS-OFC patients from Lithuania, 10 appeared to be previously reported SNPs available in the dbSNP [29]. Twelve *IRF6* gene sequence alterations identified in the present study are novel (i.e. not yet published by other researchers and not listed in the in the dbSNP [29] and / or HGMD [28]). Of them, four are single nucleotide substitutions in the noncoding parts of the gene (i.e. intronic sequences flanking exons 1, 5 and 6) but do not affect sequences essential for splicing. Thus they are most likely functionally neutral polymorphisms, although their potential effect on intronic enhancers cannot be excluded. Out of seven single nucleotide substitutions in the coding parts of the *IRF6* gene, five are expected to be missense mutations. Of them, four appear to be new (i.e. not yet published by other authors):

1) c.635G>T substituting serine for isoleucine (p.S212I). Serine is a polar uncharged amino acid, and isoleucine is a nonpolar uncharged amino acid with a larger side chain;

2) c.1199G>T substituting arginine for leucine (p.R400L). Arginine is a positively charged polar amino acid with a long side chain, and leucine is a nonpolar uncharged amino acid.

3) c.884T>C substituting leucine for proline (p.L295P). Both amino acids are nonpolar uncharged ones, but proline is the only amino acid whose side group cyclises onto the backbone and acts as a structural disruptor in the middle of a regular secondary structure of polypeptide.

The fourth, new nucleotide substitution c.1013G>A is expected to be a silent mutation p.E338E, which, like the already known polymorphism c.722C/A (p.S153S), is unlikely to have a causal relation to NS-OFC phenotype.

On the basis of the features of substituted amino acids the preliminary assumption that three new missense mutations in the *IRF6* gene identified in the patients from Lithuania are potentially related to the NS-OFC phenotype seems reasonable, in the first place for p.L295P and p.R400L, which confers considerably different amino acid properties.

The missense mutation c.182C>G substituting alanine for glycine (p.A61G) has already been identified scanning VWS patients' *IRF6* gene and was not observed in 180 control chromosomes [16], but there are no other data to support its relation to VWS phenotype except its location in the DNA binding domain of the *IRF6* gene product. The corresponding amino acids in normal and mutant alleles (alanine and glycine, respectively) are nonpolar and uncharged, with a small side chain, i.e. with very similar characteristics, and thus the effect of this substitution is most likely negligible, if any. If this mutation appeared to be causative for VWS, it would be

reasonable to expect its relation to the susceptibility for the NS-CL/CP phenotype within a different combination of other genetic and environmental factors.

Further investigations are necessary to test whether missense mutations identified in the NS-OFC probands from Lithuania are indeed causative of this malformation, as well as analysis of their evolutionary conservation, potential effect on the gene product structure and function, transmission of the alleles in the NS-OFC families, prevalence of the alleles in general population, etc., together with increasing the sample under investigation. On the other hand, future studies are necessary to investigate the role of the identified *IRF6* gene mutations and / or polymorphisms on sense or antisense RNA stability, RNA–protein interactions, or promoter activity.

IRF6 polymorphism p.V274I (c.820G>A) within the protein binding domain needs separate discussion as it is still the only *IRF6* gene nucleotide sequence variant associated with the NS-OFC phenotype. It was identified, with p.274V allele being a common variant and p.274I being rare, in the VWS studies and has since been evaluated as a potential modifier in isolated CL / CP [17]. The results of transmission disequilibrium testing have revealed a significant overtransmission of the p.274V allele in a number of populations, particularly from Asia or South America [17, 30], suggesting it as a CL / CP risk allele. On the other hand, its frequency was found to be close or equal to 100% in the individuals of Caucasian, African and Pakistani descent [17, 31], while the prevalence of p.274I allele in the Thai population reached 33% [30]. In Lithuania, the rare p.274I allele was found on two out of 380 tested chromosomes (0.53%) in probands with NS-OFC, which is consistent with the data from other Caucasian populations.

Results of the analysis of a number of polymorphisms at the *IRF6* locus in Italian and American populations [32, 33] support the initial hypothesis of Zucchero et al. [17] about the contribution of variation in this gene to nonsyndromic CL / CP. A strong evidence of linkage disequilibrium between additional SNPs in the vicinity and the NS-OFC (CL / CP) phenotype has been provided suggesting that p.V274I polymorphism itself is hardly etiologic but rather that the p.274V allele resides on a haplotype on which another mutation, yet to be identified, is the specific risk factor for nonsyndromic CL / CP.

Our results are in consistence with the assumption that homozygosity for the p.274V allele increases the risk of NS-OFC, although the presence of the p.274I allele in different types of NC-OFCs (CL/CP and CPO) suggests that homozygosity for the p.274V allele is not an essential risk factor for this malformation. At the same time, our results also confirm the assumption that p.V274I polymorphism itself is unlikely etiologic for nonsyndromic CL / CP, the p.274V allele being in linkage disequilibrium with another causative mutation [32]. Anyway, our results support the importance of investigating the variability of the *IRF6* gene potentially related to NS-OFC.

CONCLUSIONS

Our study failed to reveal *MSX1* gene sequence variants other than already known functionally neutral polymorphisms, but these findings do not exclude the possibility of positive results in a larger NS-OFC sample. On the other hand, results of the investigation highlight the *IRF6* gene sequence variability and support the hypothesis that variation in this gene contributes to the NS-OFC phenotype. Three new missense mutations (p.S212I, p.L295P, p.R400L) were identified in the *IRF6* gene, which, together with the p.A61G mutation found in the case of Van der Woude syndrome, might be related to the NS-OFC phenotype in the population of Lithuania.

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MSX1 IR *IRF6* GENŲ NUKLEOTIDŲ SEKŲ POKYČIAI, NUSTATYTI LIETUVOS PACIENTAMS SU VEIDINĖS SRITIES ĮSKILUMAIS

S a n t r a u k a

Įvadas. Nesindrominiai burnos ir veido įskilumai (NS-BVĮ) yra viena iš dažniausių žmogaus įgimtų raidos anomalijų. Tai daugiaveiksnis požymis, kurio patogenezėje svarbūs genetiniai veiksniai. Tarp daugelio genų kandidatų neseniai ypač išryškėjo su sindrominiais BVĮ susiję genai. Šio darbo tikslas buvo ištirti, ar *MSX1* ir *IRF6* genų mutacijos turi ryšį su NS-BVĮ Lietuvos populiacijoje.

Pacientai, medžiaga ir metodai. NS-BVĮ turintys Lietuvos pacientai buvo tiriami *MSX1* geno (80 pacientų) ir *IRF6* geno (190 pacientų) nukleotidų sekos pokyčiams nustatyti. Visą *MSX1* geną ir *IRF6* geno egzonus apimantys DNR fragmentai buvo pagausinti PGR būdu, vėliau buvo tiesiogiai nustatyta PGR produktų nukleotidų seka ir palyginta su referentine šių genų nukleotidų seka.

Rezultatai ir jų aptarimas. *MSX1* ir *IRF6* genuose buvo nustatyti atitinkamai aštuoni ir 22 nukleotidų sekos pokyčiai. Jei šiame darbe nustatyti *MSX1* geno pokyčiai jau buvo žinomi nukleotidų sekos polimorfizmai, tai skenuojant *IRF6* geną nustatyti devyni nauji sekos variantai. Trys iš jų buvo aminorūgštį keičiančios mutacijos (p.S212I, p.,L295P p.R400L), kurios kartu su jau žinoma p.A61G mutacija, anksčiau nustatyta Van der Vudo sindromo atveju, galėtų būti susijusios su NS-BVĮ fenotipu Lietuvos populiacijoje.

Išvados. Tai, kad tiriant pacientus iš Lietuvos nepavyko rasti *MSX1* geno mutacijų, tikriausiai susijusių su NS-BVĮ fenotipu, neatmeta būtinumo toliau tirti didesnę Lietuvos populiacijos pacientų grupę. Šio darbo rezultatai išryškina *IRF6* geno nukleotidų sekos įvairovę ir paremia hipotezę apie šio geno sekos variantų indėlį į NS-BVĮ fenotipą skatindami tolesnius tyrimus siekiant išaiškinti, ar Lietuvoje nustatytos *IRF6* geno mutacijos yra NS-BVĮ lemiantys retieji aleliai.

Raktažodžiai: nesindrominis lūpos įskilumas ir(arba) gomurio įskilumas, genai kandidatai, *IRF6*, *MSX1*, nukleotidų sekos variantas