ORIGINAL PAPER

DOI: https://doi.org/10.20883/jms.2016.1

Polymorphic variants in the *DLX1* gene and the risk of non-syndromic cleft lip with or without cleft palate

Agnieszka Gaczkowska[1], Kamil K. Hozyasz[2], Piotr Wójcicki[3], Barbara Biedziak[4], Paweł P. Jagodziński[1], Adrianna Mostowska[1]

[1] Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poland
[2] Department of Pediatrics, Institute of Mother and Child, Warsaw, Poland
[3] University Clinic of Medical Academy in Wroclaw and Department of Plastic Surgery Specialist Medical Center in Polanica Zdroj, Poland
[4] Department and Clinic of Dental Surgery, Poznan University of Medical Sciences, Poland

ABSTRACT

Introduction. Non-syndromic cleft lip with or without cleft palate (NSCL/P) is a common developmental anomaly, which etiology is complex and not completely elucidated. Therefore, the aim of the present study was to evaluate whether common polymorphisms in the distal-less homeobox gene 1 (*DLX1*) may contribute to the risk of orofacial clefts in the Polish population.

Materials and Methods. Five single nucleotide variants were genotyped using high-resolution melting curve analysis in a group of 278 patients with NSCL/P and properly matched controls (n = 574).

Results. Statistical analysis revealed that two variants located in the 3' untranslated region of DLX1, rs788172 and rs788173, were associated with a decreased risk of NSCL/P (p_{trend} = 0.041 and p_{trend} = 0.025, respectively). The allelic frequencies for these polymorphisms were significantly lower in patients compared to healthy individuals (p = 0.040 and p = 0.024, respectively). However, all these results did not remain statistically significant after applying the Bonferroni correction for multiple comparisons. The results of single-marker analysis for *DLX1* were confirmed by haplotype analysis. The best evidence of the haplotype association with the risk of NSCL/P was observed for the T-G-G haplotype consisting of rs1047889, rs788172 and rs788173 major alleles. This high risk haplotype was more frequent among cases than controls (p = 0.013, $p_{corrected}$ = 0.026).

Conclusions. We found evidence for the association between *DLX1* gene variants and the risk of NSCL/P in the Polish population. To confirm our preliminary findings further, larger sample size studies are required.

Keywords: NSCL/P; *DLX1*; polymorphism; haplotype.

Introduction

Non-syndromic cleft lip with or without cleft palate (NSCL/P, OMIM %1195130) is one of the most common craniofacial abnormalities. The frequency of NSCL/P varies widely across different regions of the world and depends on racial and ethnic background, and socioeconomic status [1, 2]. The birth prevalence of this malformation in European-derived populations is about 1/1000 live births [3]. Orofacial clefts represents a significant public health burden since their treatment requires long-term and multidisciplinary management strategies. In addition, patients with this developmental anomaly may have a higher incidence of psychological disorders and cancer [4, 5]. Zhu *et al.* have shown that also parents having children with orofacial clefts have an increased risk of developing lymphomas or leukemia [6].

The etiology of NSCL/P is complex and multifactorial, involving the integration of a number of genetic and environmental risk factors [1, 7]. In addition, there are reports showing that maternal genetic and nutritional factors can also be implicated in the pathogenesis of this birth anomaly [8]. Despite a number of molecular and epidemiological studies with different study designs, a definitive statement about causes of NSCL/P still remains obscure. The candidate genes and chromosomal loci identified so far can explain only a fraction of the genetic component of orofacial clefts. The most consistent findings across studies include the association of polymorphic variants of the *IRF6* gene (OMIM *607199) and 8q24.21 locus with the increased risk of NSCL/P [1]. It has been assessed that *IRF6* gene variants may be responsible for about 12% of the genetic contribution to NSCL/P at the population level [9]. In the Polish population, the functional promoter *IRF6* polymorphism (rs642961) and the 8q24.21 gene desert variant (rs987525) have been shown to be associated with an almost two-fold increase in the risk of this developmental defect [10].

One of the approaches to search for new candidate genes in the etiology of NSCL/P is the analysis of phenotypes manifest in mice carrying null mutations (knock-out mice) of genes expressed during craniofacial development [11]. It has been shown that *Dlx1/Dlx2* double homozygous null mice have fully penetrant cleft of the secondary palate and agenesis of upper molar teeth [12, 13]. The cleft palate in these mutants is the result of reduced mesenchymal cell proliferation at the initial stages of palatogenesis and severely deficient growth of the posterior palate [13]. Furthermore, mice lacking both *Dlx1* and *Dlx2* die at birth with multiple defects including abnormal development of forebrain [14]. The *Dlx1* and *Dlx2* belong to a highly conserved family of distal-less homeobox genes encoding homeodomain transcription factors, which play crucial roles in various aspects of embryogenesis [15]. It is worth noting that a polymorphic variant located in the 3' untranslated region (3' UTR) of the human *DLX1* gene (OMIM *600029) has been found to be associated with the risk of NSCLP in the Brazilian population [16]. Therefore the aim of the present study was to evaluate whether common nucleotide variants in the *DLX1* locus may contribute to the risk of orofacial clefts also in the Polish population. Five known single nucleotide polymorphisms (SNPs) were selected and analyzed in a group of patients with NSCL/P and properly matched control samples.

Materials and Methods

Study population

The study included 278 patients (57% males) with a diagnosis of NSCL/P recruited from the Department of Pediatrics at the Institute of Mother and Child in Warsaw, the Department of Plastic Surgery at the Specialist Medical Center in Polanica Zdroj and from the Department and Clinic of Dental Surgery at the Poznan University of Medical Sciences. The patient group comprised 238 (86%) individuals with non-syndromic cleft lip with cleft palate (CLP) and 40 (14%) individuals with non-syndromic cleft lip only (CL). Patients with cleft palate only (CPO) were excluded from the study prior to genotyping. Case eligibility was ascertained by clinicians using detailed diagnostic information from medical records. The control group was composed of 574 healthy individuals (50% males) with no family history of clefting or other congenital birth defects. All study participants were unrelated Caucasians of Polish origin. The study was performed according to the rules of the Ethics Committee of the Poznan University of Medical Sciences, Poland. Written and oral consent was obtained from all participants or their legal guardians.

SNP selection and genotyping

SNPs in the DLX1 locus were identified from public databases such as the dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the 1000 Genomes Browser (http://browser.1000genomes.org/index.html), and related literature. A final set of five SNPs was selected based on functional significance, gene-linkage disequilibrium (LD) patterns and association with NSCL/P in previous association studies. LD patterns and the structure of haplotype blocks across the DLX1 locus were determined using genotype data from the HapMap database (http://hapmap.ncbi.nlm.nih.gov/) and the Haploview 4.0 software (http://www.broad.mit.edu/mpg/haploview/). Characteristics of those SNPs that were finally selected are presented in **Table 1** and **Figure 1**. Genomic DNA for molecular analyses was isolated from peripheral blood lymphocytes by a standard salt-out extraction procedure. Genotyping was conducted by high-resolution melting curve analysis (HRM) on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany).

Quality control was ensured by including 10% of the samples as duplicates. Samples that failed genotyping were removed from statistical calculations.

Table 1. Characteristics of polymorphisms genotyped in the *DLX1* locus

rs no.	Location[a]	Alleles[b]	SNP function[c]	MAF[d]
rs1047889	chr2:172946527	C / T	N/A (upstream)	0.47
rs788172	chr2:172953438	A / G	UTR-3	0.35
rs788173	chr2:172953460	A / G	UTR-3	0.35
rs10186317	chr2:172955011	A / G	N/A (downstream)	0.31
rs13390848	chr2:172957643	G / T	N/A (downstream)	0.16

[a] NCBI build 37 / hg19.
[b] Underline denotes the minor allele (based on whole sample).
[c] According to the Single Nucleotide Polymorphism database (dbSNP).
[d] MAF, minor allele frequency calculated from the control samples.

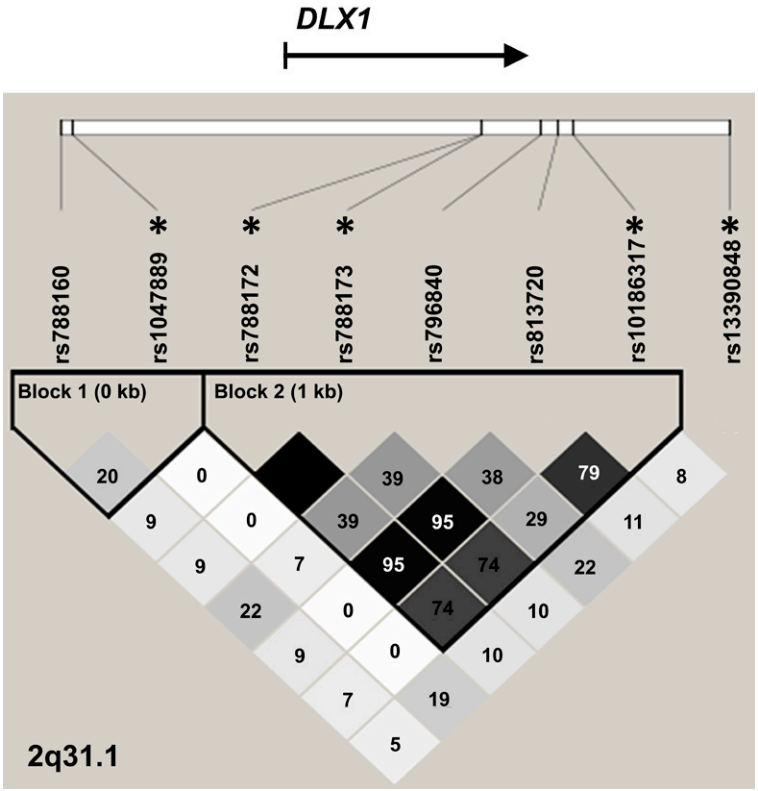

Figure 1. The Linkage Disequilibrium (LD) plot of HapMap SNPs within the DLX1 locus. The plot was generated using the genotype data from HapMap CEU samples and the Haploview 4.0 software (Broad Institute, Cambridge, MA). The examined SNPs are marked with an asterisk (*). The numbers in the squares denote r2 values expressed as a percentage of maximal value (1.0). Square without number corresponds to r2 = 1.0. A black-to-white gradient shows highest (1.0) to lowest (0.0) r2

Statistical analysis

Each SNP was tested for deviation from the Hardy-Weinberg equilibrium (HWE) in both patients and controls using the chi-square (χ^2) test. The differences in allele and genotype frequencies between cases and controls were determined using standard χ^2 or Fisher exact tests. SNPs were tested for association with NSCL/P using the Cochran-Armitage trend test. Odds Ratios (ORs) with 95% Confidence Intervals (95%CIs) were used to assess the strength of the association. The dominant and recessive models were analyzed. The Bonferroni correction was applied to account for multiple comparisons, and the p-values < 0.01 (0.05/5 SNPs) were interpreted as statistically significant. Statistical calculations were performed for the overall phenotype NSCL/P and the NSCLP and NSCL subgroups. The haplotype-based association analysis using a sliding window approach was conducted using Haploview 4.0 software. Significant p values were corrected using the 1,000-fold permutation test.

Results

Single-marker association analysis

None of the tested polymorphisms showed significant deviation from HWE in either NSCL/P patients or healthy individuals (p > 0.05). In controls, the minor allele frequency (MAF) for analyzed nucleotide variants was at least 16%. Statistical analysis revealed that two 3'UTR variants, rs788172 and rs788173, were associated with the risk of NSCL/P (p_{trend} = 0.041 and p_{trend} = 0.025, respectively; **Table 2**). The allelic frequencies of these polymorphisms were significantly lower in affected individuals compared to controls (p = 0.040 and p = 0.024, respectively). The rs788172 and rs788173 were in perfect LD with each other: r^2 = 1.00 and D' = 1.00 (**Figure 1**). Under an assumption of a dominant inheritance, the calculated ORs for these variants were 0.77 (95%CI: 0.579 – 1.030, p = 0.079) and 0.753 (0.564 – 1.005, p = 0.053), respectively. Statistical analysis conducted in the NSCLP subgroup of patients revealed similar results, with the most significant asso-

Table 2. Association of *DLX1* polymorphisms with the risk of NSCL/P

		Genotype distributionb								
		MAF					Dominant modelc		Recessive modeld	
rs no.	Allelesa	Cases	Controls	ptrend	pallelic	pgeno	OR (95% CI)	p-value	OR (95% CI)	p-value
rs1047889	C / T	46 / 143 / 87	129 / 287 / 158	0.055	0.058	0.122	0.825 (0.603–1.129)	0.229	0.690 (0.475–1.000)	0.050
		0.43	0.47							
rs788172	A / G	25 / 116 / 135	73 / 257 / 244	0.041	0.040	0.123	0.772 (0.579–1.030)	0.079	0.684 (0.423–1.104)	0.118
		0.30	0.35							
rs788173	A / G	24 / 115 / 136	73 / 257 / 243	0.025	0.024	0.080	0.753 (0.564–1.005)	0.053	0.655 (0.403–1.064)	0.086
		0.30	0.35							
rs10186317	A / G	22 / 105 / 143	59 / 234 / 279	0.191	0.183	0.423	0.846 (0.633–1.130)	0.257	0.771 (0.462–1.288)	0.320
		0.28	0.31							
rs13390848	G / T	12 / 78 / 188	16 / 149 / 409	0.188	0.180	0.369	1.187 (0.871–1.617)	0.278	1.573 (0.734–3.374)	0.241
		0.18	0.16							

The p-values < 0.010 (0.05 / 5 SNPs) were interpreted as statistically significant.
[a] Underline denotes the minor allele (based on whole sample).
[b] The order of genotypes: dd / Dd / DD (d is the minor allele).
[c] Dominant model: dd + Dd vs DD (d is the minor allele).
[d] Recessive model: dd vs Dd + DD (d is the minor allele).
MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.

Table 3. Association of *DLX1* polymorphisms with the risk of NSCLP and NSCL

				ORdominant[b]	ORrecessive[c]
SNP	Alleles[a]	Subgroup	ptrend	(95%CI)	(95%CI)
rs1047889	C / T	ALL	0.055	0.825 (0.603–1.129)	0.690 (0.475–1.000)
		NSCLP	0.069	0.848 (0.609–1.181)	0.662 (0.444–0.986)
		NSCL	0.390	0.705 (0.359–1.386)	0.862 (0.388–1.918)
rs788172	A / G	ALL	0.041	0.772 (0.579–1.030)	0.684 (0.423–1.104)
		NSCLP	0.054	0.764 (0.564–1.037)	0.706 (0.427–1.167)
		NSCL	0.357	0.817 (0.430–1.554)	0.557 (0.167–1.852)
rs788173	A / G	ALL	0.025	0.753 (0.564–1.005)	0.655 (0.403–1.064)
		NSCLP	0.032	0.743 (0.548–1.007)	0.672 (0.403–1.121)
		NSCL	0.351	0.814 (0.428–1.547)	0.555 (0.167–1.848)
rs10186317	A / G	ALL	0.191	0.846 (0.633–1.130)	0.771 (0.462–1.288)
		NSCLP	0.188	0.843 (0.620–1.145)	0.738 (0.425–1.282)
		NSCL	0.711	0.862 (0.453–1.637)	0.966 (0.332–2.810)
rs13390848	G / T	ALL	0.188	1.187 (0.871–1.617)	1.573 (0.734–3.374)
		NSCLP	0.087	1.255 (0.908–1.736)	1.852 (0.862–3.977)
		NSCL	0.439	0.826 (0.395–1.729)	0.418 (0.025–7.097)

The p-values < 0.010 (0.05 / 5 SNPs) were interpreted as statistically significant.
[a] Underline denotes the minor allele (based on whole sample).
[b] Dominant model: dd + Dd vs DD (d is the risk allele).
[c] Recessive model: dd vs Dd + DD (d is the risk allele).
OR, Odds Ratio; CI, confidence interval.

ciation with NSCLP found for the rs788173 variant (p_{trend} = 0.032, **Table 3**). However, all the results of single-marker association analysis were not statistically significant after the Bonferroni correction (p > 0.01).

Haplotype analysis

Haplotype analysis of nucleotide variants in the *DLX1* locus revealed 2- and 3-marker haplotypes associated with the risk of NSCL/P (**Table 4**). These results, consistent with results of single-marker analysis, remained statistically significant even after applying the permutation-based correction (p < 0.05). The best evidence of the haplotype association with the risk of NSCL/P was observed for the T-G-G haplotype consisting of rs1047889, rs788172 and rs788173 major alleles. This high risk haplotype was more frequent among cases than controls (p = 0.013, $p_{corrected}$ = 0.026).

Discussion

The *Dlx* family of homeobox genes is crucial for embryonic development in both invertebrates and vertebrates [16, 17]. In mammals, there are six *Dlx* genes arranged

Table 4. Haplotype analysis of SNPs genotyped in the *DLX1* locus

Polymorphisms	Haplotypes	Frequency	Case, Control Ratios	Chi square	p-value	pcorrected-value[a]
2-marker window						
rs1047889_rs788172	**T-G**	**0.362**	**0.402, 0.343**	**5.690**	**0.017**	**0.032**
	C-G	0.303	0.297, 0.306	0.147	0.701	0.975
	T-A	0.179	0.172, 0.182	0.277	0.599	0.918
	C-A	0.156	0.129, 0.169	4.492	0.034	0.069
rs788172_rs788173	**G-G**	**0.665**	**0.701, 0.648**	**4.656**	**0.031**	**0.039**
	A-A	**0.335**	**0.299, 0.352**	**4.656**	**0.031**	**0.039**
rs788173_rs10186317	G-A	0.661	0.697, 0.644	4.601	0.032	0.080
	A-G	0.292	0.265, 0.304	2.745	0.098	0.307
	A-A	0.042	0.030, 0.048	2.827	0.093	0.297
rs10186317_rs13390848	A-G	0.536	0.540, 0.534	0.041	0.839	0.980
	G-G	0.298	0.276, 0.308	1.796	0.180	0.368
	A-T	0.166	0.184, 0.158	1.886	0.170	0.361
3-marker window						
rs1047889_rs788172_rs788173	**T-G-G**	**0.362**	**0.403, 0.342**	**6.118**	**0.013**	**0.026**
	C-G-G	0.304	0.298, 0.307	0.137	0.711	0.978
	T-A-A	0.179	0.171, 0.183	0.341	0.560	0.918
	C-A-A	0.156	0.128, 0.169	4.804	0.028	0.060
rs788172_rs788173_rs10186317	G-G-A	0.661	0.695, 0.645	4.273	0.039	0.111
	A-A-G	0.293	0.269, 0.304	2.270	0.132	0.310
	A-A-A	0.042	0.030, 0.048	2.754	0.097	0.254
rs788173_rs10186317_rs13390848	G-A-G	0.495	0.512, 0.487	0.987	0.321	0.824
	A-G-G	0.292	0.266, 0.304	2.709	0.100	0.348
	G-A-T	0.166	0.184, 0.158	1.886	0.170	0.458
	A-A-G	0.042	0.030, 0.048	2.791	0.095	0.346
4-marker window						
rs1047889_rs788172_rs788173_rs10186317	T-G-G-A	0.362	0.403, 0.342	6.026	0.014	0.062
	C-G-G-A	0.300	0.295, 0.303	0.110	0.740	1.000
	T-A-A-G	0.153	0.153, 0.153	0.000	0.992	1.000
	C-A-A-G	0.139	0.115, 0.151	4.061	0.044	0.129
	T-A-A-A	0.025	0.017, 0.029	2.392	0.122	0.472
	C-A-A-A	0.017	0.014, 0.018	0.465	0.496	0.965
rs788172_rs788173_rs10186317_rs13390848	G-G-A-G	0.495	0.511, 0.488	0.786	0.375	0.848
	A-A-G-G	0.293	0.269, 0.304	2.270	0.132	0.418
	G-G-A-T	0.166	0.185, 0.157	2.066	0.151	0.457
	A-A-A-G	0.042	0.030, 0.048	2.754	0.097	0.338

Statistically significant results are highlighted in bold font.
[a] p value calculated using permutation test and a total of 1,000 permutations.

as three sets of linked gene pairs: *Dlx2* and *Dlx1*, *Dlx5* and *Dlx6*, and *Dlx3* and *Dlx7* [18]. All *Dlx* genes encode DNA-binding regulators that control large number of downstream effector genes. They are implicated in patterning and development of the brain, craniofacial structures and the axial and appendicular skeleton [15, 17]. Mouse model studies showed that *Dlx1* and *Dlx2* activities are critical for the initial outgrowth of the palatal shelves in a region-specific manner, and are essential for the normal expression of other significant regulators of palate development [13]. Therefore the purpose of this study was to evaluate the association between common nucleotide variants in the *DLX1* locus and the risk of orofacial clefts in the Polish population. Molecular analyses were conducted in patients with non-syndromic forms of cleft lip with cleft palate and cleft lip only. Patients with cleft palate only were excluded from the study due to distinct etiology of this subtype of oral clefts [19].

Statistical analysis of genotyping results revealed that two nucleotide variants located in the 3'UTR of the *DLX1* gene were associated with the risk of NSCL/P in a tested group of patients. The data showed that rs788172 and rs788173 minor alleles are more frequent among controls than among affected individuals. We found that variant allele carriers at rs788172 and rs788173 have a 1.3-fold decreased risk of NSCL/P. However, all these results were not statistically significant after applying the correction for multiple comparisons. In order to adjust for multiple testing, we employed the Bonferroni correction, which is the most conservative approach to control for false positives and may lead to the underestimation of weak and moderate genetic effects [20]. It is worth noting that the results of single-marker analysis for *DLX1* were confirmed by haplotype analysis. We found that 2- and 3-marker haplotypes containing the rs788172 and rs788173 variants are associated with the risk of NSCL/P. These results remained statistically significant even after applying the permutation-based correction. Similar results have been obtained by Saboia *et al.* in the Brazilian population, where the rs788173 polymorphism was associated with the risk of NSCLP subphenotype [16]. They have demonstrated that the A allele of this 3'UTR variant is undertransmitted (protective allele) from heterozygote parents to their affected offspring [16]. In the Brazilian study, however, only one marker in the *DLX1* gene was tested for association with orofacial clefts [16]. Interestingly, the common alleles of *DLX1* rs788172 and rs788173 were shown to be significantly correlated with autism [21].

There is an evidence that *Dlx1* and *Dlx2* regulate the initiation of palatogenesis through promoting proliferation of mesenchyme in the posterior palate independently of Sonic hedgehog (Shh) signaling [13]. The loss of *Dlx1* and *Dlx2* function results in down-regulation of a signaling loop involving Shh, Bone morphogenetic protein 4 (Bmp4) and Fibroblast growth factor 10 (Fgf10), which is important for cell proliferation in the epithelium of the middle palate around E13.5 [13]. In addition *Dlx1* and *Dlx2* activity is required for normal expression of several transcription factor genes, including *Lhx6*, *Barx1*, *Sim2*, *Osr1* and *Osr2*, which mutations result in defects of palate growth and morphogenesis [13, 22–24]. In humans, mutations and polymorphisms of *BMP4* (OMIM *112262) and *FGF10* (OMIM *602115) have been found to increase the risk orofacial clefts in various populations [25–28]. It has been suggested that the FGF signaling pathway may contribute to as many as 3 to 5% of NSCLP [25].

The present study has certain limitations including (a) the number of selected nucleotide variants that do not cover the *DLX1* gene fully and extensively, (b) the lack of information about functional relevance of tested SNPs (c) the relatively small number of available NSCL/P patients and healthy individuals and (d) the lack of association analysis of polymorphisms in the *DLX2* gene (OMIM *126255), which is located head-to-head with *DLX1* on chromosome 2q32. It has been demonstrated that *Dlx1* and *Dlx2* have functionally redundant roles during the embryonic development. The *Dlx1/Dlx2* double mutant mouse embryos had a cleft of the secondary palate, also seen in 80% of the *Dlx2-/-* embryos, while only 10% of *Dlx1-/-* embryos had a mild cleft palate [12]. In addition, further studies should focus not only on analysis of common variants in *DLX1* and *DLX2*, but also on identification of rare variants and etiological mutations of these genes in patients with orofacial clefts. Recently, a novel single nucleotide deletion in the *DLX4* gene was described in a family with bilateral CL/P and minor dysmorphic features [29]. In addition, craniofacial abnormalities, including cleft palate, are common clinical features observed in patients with 2q31.1 microdeletions encompassing both *DLX1* and *DLX2* [30, 31].

In conclusion, our case-control study contributes to a better understanding of the role of genetic factors in the etiology of orofacial clefts. We found evidence for the association between *DLX1* gene variants and the risk of NSCL/P in the Polish population. To confirm our findings further, larger sample size studies in different populations are required.

Acknowledgements

Conflict of interest statement
The authors declare no conflict of interest.

Funding sources
The study was supported by the Polish National Science Centre, grant no. 2012/07/B/NZ2/00115.

References

1. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet. 2011;12(3):167–178.
2. Carmichael SL, Nelson V, Shaw GM, Wasserman CR, Croen LA. Socio-economic status and risk of conotruncal heart defects and orofacial clefts. Paediatr Perinat Epidemiol. 2003;17(3):264–271.
3. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. Lancet. 2009;374(9703):1773–1785.
4. Christensen K, Juel K, Herskind AM, Murray JC. Long term follow up study of survival associated with cleft lip and palate at birth. BMJ. 2004;328(7453):1405.
5. Bille C, Winther JF, Bautz A, Murray JC, Olsen J, Christensen K. Cancer risk in persons with oral cleft--a population-based study of 8,093 cases. Am J Epidemiol. 2005;161(11):1047–1055.
6. Zhu JL, Basso O, Hasle H, Winther JF, Olsen JH, Olsen J. Do parents of children with congenital malformations have a higher cancer risk? A nationwide study in Denmark. Br J Cancer. 2002;87(5):524–528.
7. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. Am J Med Genet C Semin Med Genet. 2013;163C(4):246–258.
8. Krapels IP, Vermeij-Keers C, Müller M, de Klein A, Steegers-Theunissen RP. Nutrition and genes in the development of orofacial clefting. Nutr Rev. 2006;64(6):280–288.
9. 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;351(8):769–780.
10. 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;88(7):538–545.
11. Juriloff DM, Harris MJ. Mouse genetic models of cleft lip with or without cleft palate. Birth Defects Res A Clin Mol Teratol. 2008;82(2):63–77.
12. Qiu M, Bulfone A, Ghattas I, Meneses JJ, Christensen L, Sharpe PT, et al. Role of the Dlx homeobox genes in proximodistal patterning of the branchial arches: mutations of Dlx-1, Dlx-2, and Dlx-1 and -2 alter morphogenesis of proximal skeletal and soft tissue structures derived from the first and second arches. Dev Biol. 1997;185(2):165–184.
13. Jeong J, Cesario J, Zhao Y, Burns L, Westphal H, Rubenstein JL. Cleft palate defect of Dlx1/2-/- mutant mice is caused by lack of vertical outgrowth in the posterior palate. Dev Dyn. 2012;241(11):1757–1769.
14. Zhou QP, Le TN, Qiu X, Spencer V, de Melo J, Du G, et al. Identification of a direct Dlx homeodomain target in the developing mouse forebrain and retina by optimization of chromatin immunoprecipitation. Nucleic Acids Res. 2004;32(3):884–892.
15. Merlo GR, Zerega B, Paleari L, Trombino S, Mantero S, Levi G. Multiple functions of Dlx genes. Int J Dev Biol. 2000;44(6):619–626.
16. Sabóia TM, Reis MF, Martins ÂM, Romanos HF, Tannure PN, Granjeiro JM, et al. DLX1 and MMP3 contribute to oral clefts with and without positive family history of cancer. Arch Oral Biol. 2015;60(2):223–228.
17. Kraus P, Lufkin T. Dlx homeobox gene control of mammalian limb and craniofacial development. Am J Med Genet A. 2006;140(13):1366–1374.
18. Panganiban G, Rubenstein JL. Developmental functions of the Distal-less/Dlx homeobox genes. Development. 2002;129(19):4371–4386.
19. Wen Y, Lu Q. Risk prediction models for oral clefts allowing for phenotypic heterogeneity. Front Genet. 2015;6:264.
20. Goeman JJ, Solari A. Multiple hypothesis testing in genomics. Stat Med. 2014;33(11):1946–1978.
21. Liu X, Novosedlik N, Wang A, Hudson ML, Cohen IL, Chudley AE, et al. The DLX1and DLX2 genes and susceptibility to autism spectrum disorders. Eur J Hum Genet. 2009;17(2):228–235.
22. Shamblott MJ, Bugg EM, Lawler AM, Gearhart JD. Craniofacial abnormalities resulting from targeted disruption of the murine Sim2 gene. Dev Dyn. 2002;224(4):373–380.
23. Denaxa M, Sharpe PT, Pachnis V. The LIM homeodomain transcription factors Lhx6 and Lhx7 are key regulators of mammalian dentition. Dev Biol. 2009;333(2):324–336.
24. Lan Y, Ovitt CE, Cho ES, Maltby KM, Wang Q, Jiang R. Odd-skipped related 2 (Osr2) encodes a key intrinsic regulator of secondary palate growth and morphogenesis. Development. 2004;131(13):3207–3216.
25. Riley BM, Mansilla MA, Ma J, Daack-Hirsch S, Maher BS, Raffensperger LM, et al. Impaired FGF signaling contributes to cleft lip and palate. Proc Natl Acad Sci U S A. 2007;104(11):4512–4517.
26. Wang H, Zhang T, Wu T, Hetmanski JB, Ruczinski I, Schwender H, et al. The FGF and FGFR Gene Family and Risk of Cleft Lip With or Without Cleft Palate. Cleft Palate Craniofac J. 2013;50(1):96–103.
27. Chen Q, Wang H, Schwender H, Zhang T, Hetmanski JB, Chou YH, et al. Joint testing of genotypic and gene-environment interaction identified novel association for BMP4 with non-syndromic CL/P in an Asian population using data from an International Cleft Consortium. PLoS One. 2014;9(10):e109038.
28. Hu YY, Qin CQ, Deng MH, Niu YM, Long X. Association between BMP4 rs17563 polymorphism and NSCL/P risk: a meta-analysis. Dis Markers. 2015;2015:763090.
29. Wu D, Mandal S, Choi A, Anderson A, Prochazkova M, Perry H, et al. DLX4 is associated with orofacial clefting and abnormal jaw development. Hum Mol Genet. 2015;24(15):4340–4352.
30. Theisen A, Rosenfeld JA, Shane K, McBride KL, Atkin JF, Gaba C, et al. Refinement of the Region for Split Hand/Foot Malformation 5 on 2q31.1. Mol Syndromol. 2010;1(5):262–271.

31. Dimitrov B, Balikova I, de Ravel T, Van Esch H, De Smedt M, Baten E, et al. 2q31.1 microdeletion syndrome: redefining the associated clinical phenotype. J Med Genet. 2011;48(2):98–104.

Acceptance for editing: 2016-03-15
Acceptance for publication: 2016-03-31

Correspondence address:
Adrianna Mostowska, PhD
Department of Biochemistry and Molecular Biology
Poznan University of Medical Sciences
6 Swiecickiego Street, 60-781 Poznan, Poland
phone: +48 618546511
fax: +48 618546510
e-mail: amostowska@wp.pl