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Polymorphic variants in VAX1 and the risk of nonsyndromic cleft lip with or without cleft palate in a population from northern China

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Abstract

Background: Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common craniofacial birth defects, and the etiology of NSCL/P involves both genetic and environmental factors. Genome-wide association study (GWAS) identified a novel susceptibility locus of ventral anterior homeobox 1 (*VAX1*) in patients with NSCL/P. However, the association of single nucleotide polymorphisms (SNPs) of *VAX1* with NSCL/P is inconclusive due to the differences in the racial and ethnic populations. The aim of this study was to replicate the association between *VAX1* and NSCL/P in a northern Chinese Han population.

Methods: Our study included 186 patients with NSCL/P and 223 healthy individuals from northern China. Five SNPs (rs4752028, rs10787760, rs7078160, rs6585429, and rs1871345) on *VAX1* were genotyped using the SNaPshot method.

Results: Recessive genetic model analysis revealed that homozygous genotype CC of *VAX1* rs4752028 was associated with an increased risk of NSCL/P (odds ratio = 1.89, 95% confidence interval = 1.12–3.19, $P = 0.017$), but the results were not significant after the Bonferroni correction for multiple comparisons. The allele and genotype frequencies of rs10787760, rs7078160, rs6585429, and rs1871345 and the allele frequencies of rs4752028 showed no significant differences between cases and controls. Haplotype and SNP-SNP interaction analyses did not detect any significant association of *VAX1* with the occurrence of NSCL/P.

Conclusion: *VAX1* rs4752028 was weakly associated with NSCL/P development in the studied northern Chinese Han population.

Abbreviations: χ^2 = Chi-square, CIs = confidence intervals, CVC = cross-validation consistency, GWAS = genome-wide association study, HWE = Hardy–Weinberg equilibrium, LD = linkage disequilibrium, MDR = multifactor dimensionality reduction, NSCL/P = nonsyndromic cleft lip with or without cleft palate, OR = odds ratio, SNPs = single nucleotide polymorphisms, TA = testing balanced accuracy, VAX1= ventral anterior homeobox 1.

Keywords: association, nonsyndromic cleft lip with or without cleft palate, single nucleotide polymorphisms, *VAX1* gene

1. Introduction

Nonsyndromic cleft lip with or without cleft palate (NSCL/P), which results from an impaired facial process growth and fusion during embryogenesis, is one of the most common craniofacial birth defects in humans.[1,2] The average incidence of NSCL/P is 1 to 2 of 1000 births globally.[3,4] Moreover, its prevalence varies widely among different geographical regions and ethnic groups.[5] The highest incidence of NSCL/P is observed in the Asians and Native Americans, and the lowest is observed in the Africans. The high prevalence of NSCL/P (1.42/1000) is reported in the Chinese population.[6] Newborns with NSCL/P may have speech and feeding problems, poor nutrition, psychiatric diseases, and infection of the middle ear. Although these defects can be partly corrected by a series of surgical interventions and multidisciplinary treatments, they still bring long-term burdens to the individual, family, and society.[7]

The etiology of NSCL/P is complex and is associated with both the genetic and environment factors.[8,9] Although the specific genetic and environmental risk factors associated with NSCL/P remain unclear, the linkage and association analysis and the genome-wide scanning have provided significant evidence for the potential candidate genes in the development of NSCL/P, such as *MSX1*,[10] *IRF6*,[11] *PVRL1*,[12] *SUMO1*,[13] and *FGF*.[14]

Recently, 2 genome-wide association analyses have confirmed a susceptibility candidate gene that may be involved in NSCL/P, *VAX1* at locus 10q25.3,[15,16] and it was later identified on meta-analysis by Ludwig et al.[17] These studies have revealed that single nucleotide polymorphisms (SNPs) in or near *VAX1* were involved in the risk of NSCL/P, and the analysis of participants of European and Asian origin from multiple populations provided a significant evidence for the susceptibility gene.[15–17] A previous study performed using mouse model suggested that lack of functionally active *VAX1* results in craniofacial deformity, including cleft palate.[18] However, several similar investigations were conducted in the Mesoamericans, Central Africans, Southeast Asians, and southern Chinese population, which

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showed inconsistent results.[19–21] To the best of our knowledge, no GWAS examining the association between SNPs of *VAX1* and the risk of NSCL/P in a northern Chinese population has been reported. Therefore, the aim of our current study was to investigate whether the 5 SNPs (rs4752028, rs10787760, rs7078160, rs6585429, and rs1871345) of *VAX1* were associated with the susceptibility of NSCL/P in a northern Chinese Han population.

2. Methods

2.1. Subjects

The current case–control study was approved by the Institutional Ethics Committee of the Harbin Medical University and was a hospital-based study. Diagnosis of the case group (patients with NSCL/P) was done through clinical investigations by 2 experienced dentists to assess individual phenotypic features and cases were identified through medical records; the cases with other major congenital anomaly and syndromes were excluded from the study. The study consisted of 186 cases (101 female subjects and 85 male subjects) who visited the department of Oral and Maxillofacial Surgery, Harbin Medical University Affiliated Stomatological Hospital during the period from March 2006 to April 2011. During the same period, 223 healthy controls (118 female subjects and 105 male subjects), who had no history of congenital malformation or familial history of orofacial clefting, were also selected from the same hospital. A signed informed consent was obtained from each patient, volunteer, or their guardians. About 1-mL peripheral venous blood was withdrawn from each participant.

2.2. Polymorphism selection

To investigate the role of *VAX1* gene polymorphisms on NSCL/P risk in a northern Chinese population. We selected *VAX1* tag SNPs (rs10787760, rs6585429, and rs1871345), which were selected from CHB (Beijing Han population of China) with a minor allele frequency (MAF) >0.05 in the HapMap Project. In addition, on the basis of the genome-wide association studies (GWAS) of Mangold et al,[15] we selected other 2 SNPs (rs7078160 and rs4752028) of *VAX1*.

2.3. Genotyping

DNA was extracted from the peripheral venous blood samples from each participant using the QIAamp DNA Blood Kit (Valencia, CA), according to the manufacturer's protocol. Genotyping for *VAX1* (rs4752028, rs10787760, rs7078160, rs6585429, and rs1871345) polymorphisms was performed using SNaPshot technology. Primers were designed using the Primer 3 software (http://frodo.wi.mit.edu/). PCRs consisting of 10 to 50 ng DNA, 1 × HotStarTaq buffer (Invitrogen, Carlsbad, CA), 3 mM $MgCl_2$, 300 μM of each dNTP, 0.08 μM of each primer, and one unit of HotStarTaq polymerase (Invitrogen, Carlsbad, CA) were set up in a 20 μl reaction volume. A touchdown PCR program was used with the following conditions: initial denaturation at 95°C for 10 minutes, followed by 20 cycles at 94°C for 20 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 40 seconds; the annealing temperature was decreased by 0.5°C per cycle. The extension reaction contained 1 × ABI PRISM SNaPshot Multiplex Ready Reaction Mix (Applied Biosystems, Foster City, CA), 0.5 μM of each primer and 1 μl of each PCR product and was carried out according to the manufacturer's instructions (Applied Biosystems). Further, the PCR products were purified and scanned using 3730 Genetic Analyzer (Life Technologies Corporation, Vancouver, British Columbia, Canada).

2.4. Statistical Analyses

Hardy–Weinberg equilibrium (HWE) of the genotype distributions of cases and controls was examined by using Chi-square (χ^2) test. The differences in genotype and allele frequencies of the tested SNPs between cases and control groups were evaluated using standard χ^2 and Fisher tests. The association between SNPs and risk of NSCL/P was evaluated by calculating the odds ratios (ORs) and 95% confidence intervals (CIs). Bonferroni correction of $P < 0.01$ (0.05/5) was used to note the statistical significance and solve the problem of multiple comparisons. Statistical analyses were performed using PLINK (a free open-source whole genome association analysis toolset), and R. Linkage disequilibrium (LD) was evaluated using Haplotype 4.2 software[22] depending on D' and r^2 values. SNP–SNP interactions in *VAX1* were evaluated using the R package of Multifactor Dimensionality Reduction (MDR).[23] A result with P value of <0.05 was considered as a statistically significant result.

3. Results

All the SNPs in the cases and control groups were observed to be consistent with the Hardy–Weinberg equilibrium ($P > 0.05$) (Table 1). The allele and genotype frequencies of rs10787760, rs7078160, rs6585429, and rs1871345 and the allele frequencies of rs4752028 in the NSCL/P cases were not significantly different from those in the controls ($P > 0.05$) as summarized in Table 2. Further, analyses of the dominant and recessive genetic models revealed that *VAX1* rs4752028 was differently distributed between the cases and control groups ($P = 0.017$). The results of the recessive genetic model showed that the homozygous genotype CC was associated with an increased risk of NSCL/P (OR = 1.89, 95% CI = 1.12–3.19), combining the TT and CT genotype of *VAX1* rs4752028. However, the results did not show significance after the Bonferroni correction for multiple

Table 1

The 5 studied *VAX1* SNPs.

Gene	SNP	Position	HWE *P*	Call rate (%)	MAF*	Alleles
VAX1	rs7078160	118817550	0.7738	100	0.472	G:A
	rs4752028	118824981	0.4263	100	0.401	T:C
	rs10787760	118880683	0.7961	100	0.274	C:T
	rs6585429	118883221	0.372	100	0.405	A:G
	rs1871345	118895368	0.6094	100	0.467	G:A

* MAF = minor allele frequency calculated for the cases and controls.

Table 2

Frequencies and ORs of genotypes and alleles of *VAX1* SNPs in controls and cases.

SNPs	Genotype/allele	Controls (%)	Cases (%)	OR (95% CI)	P*
rs4752028				1.21 (0.92–1.60)	0.168
	TT	83 (37.22)	68 (36.56)	1	—
	CT	111 (49.78)	77 (41.40)	0.85 (0.55–1.31)	0.451
	CC	29 (13.00)	41 (22.04)	1.73 (0.97–3.06)	0.062
	Dominant	TT vs (CT + CC)		1.03 (0.69–1.54)	0.890
	Recessive	(TT + CT) vs CC		1.89 (1.12–3.19)	0.017
	MAF	37.89	42.74		
rs10787760				0.88 (0.65–1.21)	0.439
	CC	112 (50.22)	102 (54.84)	1	—
	CT	95 (42.60)	71 (38.17)	0.82 (0.55–1.23)	0.342
	TT	16 (7.17)	13 (6.99)	0.89 (0.41–1.95)	0.774
	Dominant	CC vs (CT + TT)		0.83 (0.56–1.23)	0.352
	Recessive	(CC + CT) vs TT		0.97 (0.46–2.08)	0.942
	MAF	28.48	26.08		
rs7078160				1.05 (0.80–1.39)	0.727
	GG	59 (26.46)	53 (28.49)	1	—
	GA	120 (53.81)	88 (47.31)	0.82 (0.51–1.30)	0.389
	AA	44 (19.73)	45 (24.19)	1.14 (0.65–1.99)	0.648
	Dominant	GG vs (GA + AA)		0.90 (0.58–1.40)	0.646
	Recessive	(GG + GA) vs AA		1.30 (0.81–2.08)	0.277
	MAF	46.64	47.85		
rs6585429				0.89 (0.67–1.18)	0.417
	AA	71 (31.84)	69 (37.10)	1	—
	GA	118 (52.91)	89 (47.85)	0.78 (0.50–1.19)	0.249
	GG	34 (15.25)	28 (15.05)	0.85 (0.47–1.54)	0.589
	Dominant	AA vs (GA + GG)		0.79 (0.53–1.19)	0.265
	Recessive	(AA + GA) vs GG		0.99 (0.57–1.70)	0.957
	MAF	41.70	38.98		
rs1871345				1.09 (0.82–1.44)	0.541
	GG	61 (27.35)	52 (27.96)	1	—
	GA	120 (53.81)	90 (48.39)	1.09 (0.82–1.44)	0.585
	AA	42 (18.83)	44 (23.66)	1.23 (0.70–2.16)	0.472
	Dominant	GG vs (GA + AA)		0.97 (0.63–1.50)	0.892
	Recessive	(GG + GA) vs AA		1.34 (0.83–2.15)	0.234
	MAF	45.74	47.85		

CI = confidence interval, MAF = minor allele frequency, OR = odds ratio.

* Two-sided χ^2 test for the genotype and allele distributions between cases and controls.

comparisons was applied (corrected $P = 0.05/5 = 0.01$). The LD pattern among these 5 SNPs is depicted in Fig. 1. A haplotype block was constructed in this region with the D' and r^2 values (Table 3). In the haplotype analysis, the haplotype distributions between cases and control groups were compared and it was noted that no haplotype was associated with the risk of NSCL/P ($P > 0.05$; Table 4). The results of MDR analyses of SNP–SNP interactions are summarized in Table 5 and Fig. 2. The 1-locus based model of rs4752028 revealed the highest cross-validation consistency (CVC) of 10/10 and testing balanced accuracy (TA) of 0.53, but it not reached statistical significance ($P = 0.127$). However, no SNP–SNP interactions were found to be associated with the risk of NSCL/P.

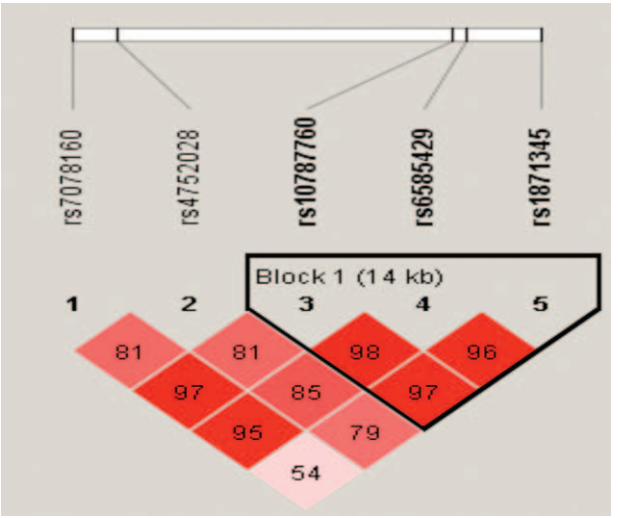

Figure 1. Linkage disequilibrium (LD) blocks for the *VAX1* haplotype analysis.

4. Discussion

The current study investigated the association of *VAX1* with the risk of NSCL/P in a northern Chinese Han population. In this study, we successfully genotyped 5 SNPs of *VAX1*, and the allele and genotype frequencies of rs10787760, rs7078160,

Table 3

Pairwise linkage disequilibrium measures for *VAX1*.

SNPs	rs7078160	rs4752028	rs10787760	rs6585429	rs1871345
rs7078160		0.81	0.97	0.95	0.54
rs4752028	0.49		0.81	0.85	0.79
rs10787760	0.32	0.16		0.98	0.97
rs6585429	0.55	0.33	0.53		0.96
rs1871345	0.28	0.48	0.31	0.55	

D' values are located above the diagonal; r^2 values are below the diagonal.

Table 4

Haplotype association between SNPs rs10787760, rs6585429, and rs1871345.

Haplotypes*	Total frequency	Case frequency (%)	Control frequency (%)	*P*
Block1 CAA	0.46	175.7 (47.2)	200.5 (45.0)	0.5193
Block1 TGG	0.27	94.4 (25.4)	124.6 (27.9)	0.4102
Block1 CAG	0.13	50.2 (13.5)	58.3 (13.1)	0.8594
Block1 CGG	0.13	48.2 (13.0)	57.9 (13.0)	0.9933

* Haplotypes present in more than 3% of the study population.

rs6585429, and rs1871345 and the allele frequencies of rs4752028 showed no significant differences between cases and controls. We found that the homozygous genotype CC of rs4752028 was associated with an increased risk of NSCL/P (OR = 1.89, 95% CI = 1.12–3.19, $P = 0.017$, using a recessive model). However, the association disappeared after the Bonferroni correction that controlling for multiple comparisons.

These results seem to be a little disappointing; however, given the complicated heterogeneous nature of NSCL/P and a number of other confounding factors, this is an expected result.[8]Studies from animal models suggested that *VAX1* has played a crucial role in the process during craniofacial development. *VAX1* was widely expressed in the craniofacial structures in rats, while *VAX1*-knockout mice exhibit phenotype of cleft palate. In humans, the *VAX1* mutation could result in an uncharacterized syndrome with bilateral lip, which was one of the clinical features of the patients.[18,24] Several studies have analyzed *VAX1* polymorphisms in NSCL/P and showed controversial results. Some previous studies have identified that *VAX1* rs7078160 was associated with the risk of NSCL/P in the Estonians and Mesoamericans.[19,25] However, these studies have not found association of *VAX1* rs7078160 with NSCL/P in populations from Poland,[26] Brazil,[27] and southern China.[21] Consistent with results of the study conducted by Pan et al[21] in a southern Chinese population, we did not found an association between rs7078160 and NSCL/P. Moreover, the MAF of *VAX1* rs7078160 in our cases was similar to which in Pan's research in the southern Chinese population (0.47 vs 0.48).[21] These results suggested that rs7078160 may not be associated with the susceptibility of NSCL/P in Chinese population. Recently, de Aquino et al[28] conducted a study and revealed NSCL/P risk was associated with rs10787760, rs6585429, and rs1871345 of *VAX1* in Brazilian population. In their research, none of the alleles and genotypes showed statistical significance between cases and controls. They found that the frequency of *VAX1* GAC haplotype was higher in patients than that in the controls, though the differences were not significant after the Bonferroni correction. However, inconsistent with findings from de Aquino et al,[28] haplotype analyses of rs10787760, rs6585429, and rs1871345 in our study showed no significant differences between the cases and controls. The inconsistent results may be due to the genetic heterogeneity among various populations and different causative variants in different haplotypic backgrounds.

In the current study, we replicated the result reported in the study by Mangold et al.[15] The association of *VAX1* rs4752028 with NSCL/P risk was weak in our study, and the reasons for the discrepancy may be attributed to the existing allelic heterogeneity at this locus in various populations. The MAF for *VAX1* rs4752028 in our controls was 0.43, which is distinctly different from that of 0.16 in the European population. Nevertheless, the MAF of *VAX1* rs4752028 in our controls was similar to those observed in HapMap CHB populations (0.43 vs 0.35). However, the interpretation of our results has some limitations; after the Bonferroni correction for multiple comparisons, the association was found to be nonsignificant. Fundamentally, multiple markers (comparisons) were used for testing multiple corrections, correcting for spurious associations, and it may be stringent to our research and could lead to a loss of the significant finding. Moreover, our work was a hospital-based case–control study and the selection bias was unavoidable, where the subjects may not be a representative of the general population.

The MDR approach used to explore gene–gene interactions for orofacial clefting has been confirmed.[29] Our study failed to find SNP–SNP interactions of *VAX1*. The discrepancy may be impacted by the sample size, which was insufficient to detect a modest effect of the tested SNP variants.

In conclusion, our study has demonstrated that SNP rs4752028 was involved with the risk of NSCL/P in a northern Chinese Han population, although weak, which to some extent, revealed an association between *VAX1* and the risk of NSCL/P. Discrepancy in results may be due to a complex genetic background and environmental exposure among different populations. Therefore, further studies are required to confirm the current data in a larger sample and with various ethnic groups

Table 5

Interaction models by MDR analysis.

Model	Prediction accuracy	Cross-validation consistency	OR (95% CI)	*P*
rs4752028	53.14	10/10	1.21 (0.92–1.60)	0.127
rs4752028 - rs1871345	54.26	7/10	1.27 (0.86–1.89)	0.566
rs1871345 - rs7078160 - rs6585429	48.99	6/10	0.75 (0.32–1.76)	0.148
rs1871345 - rs10787760 - rs7078160 - rs1871345	49.51	5/10	0.61 (0.04–9.00)	0.366

CI = confidence interval, OR = odds ratio.

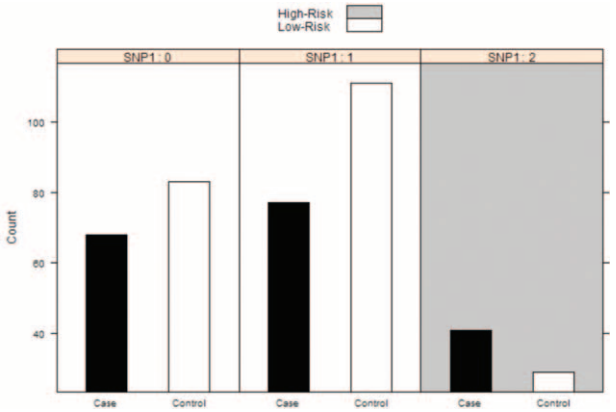

Figure 2. SNP1: rs4752028. Interaction analysis between SNPs in NSCL/P using MDR. The dark and light bars in each cell are the combined genotypes of cases and controls, respectively. Each cell color represents the risk degree used—gray-high risk, light-low risk.

and to determine the association between *VAX1* and the risk of NSCL/P.

References

[1] Kurosaka H, Iulianella A, Williams T, et al. Disrupting hedgehog and WNT signaling interactions promotes cleft lip pathogenesis. J Clin Invest 2014;124:1660–71.
[2] Ray HJ, Niswander L. Mechanisms of tissue fusion during development. Development 2012;139:1701–11.
[3] Lidral AC, Murray JC. Genetic approaches to identify disease genes for birth defects with cleft lip/palate as a model. Birth Defects Res A Clin Mol Teratol 2004;70:893–901.
[4] Birnbaum S, Ludwig KU, Reutter H, et al. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. Nat Genet 2009;41:473–7.
[5] Dixon MJ, Marazita ML, Beaty TH, et al. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet 2011;12:167–78.
[6] Dai L, Zhu J, Mao M, et al. Time trends in oral clefts in Chinese newborns: data from the Chinese National Birth Defects Monitoring Network. Birth Defects Res A Clin Mol Teratol 2010;88:41–7.
[7] Stanier P, Moore GE. Genetics of cleft lip and palate: styndromic genes contribute to the incidence of non-syndromic clefts. Hum Mol Genet 2004;13 Spec No 1:R73–81.
[8] Carinci F, Pezzetti F, Scapoli L, et al. Recent developments in orofacial cleft genetics. J Craniofac Surg 2003;14:130–43.
[9] Carinci F, Pezzetti F, Scapoli L, et al. Genetics of nonsyndromic cleft lip and palate: a review of international studies and data regarding the Italian population. Cleft Palate Craniofac J 2000;37:33–40.
[10] Jezewski PA, Vieira AR, Nishimura C, et al. Complete sequencing shows a role for MSX1 in non-syndromic cleft lip and palate. J Med Genet 2003;40:399–407.
[11] Rahimov F, Marazita ML, Visel A, et al. Disruption of an AP-2alpha binding site in an IRF6 enhancer is associated with cleft lip. Nat Genet 2008;40:1341–7.
[12] Sozen MA, Suzuki K, Tolarova MM, et al. Mutation of PVRL1 is associated with sporadic, non-syndromic cleft lip/palate in northern Venezuela. Nat Genet 2001;29:141–2.
[13] Pauws E, Stanier P. FGF signalling and SUMO modification: new players in the aetiology of cleft lip and/or palate. Trends Genet 2007; 23:631–40.
[14] Alkuraya FS, Saadi I, Lund JJ, et al. SUMO1 haploinsufficiency leads to cleft lip and palate. Science 2006;313:1751.
[15] Mangold E, Ludwig KU, Birnbaum S, et al. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. Nat Genet 2010;42:24–6.
[16] Beaty TH, Murray JC, Marazita ML, et al. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. Nat Genet 2010;42:525–9.
[17] Ludwig KU, Mangold E, Herms S, et al. Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. Nat Genet 2012;44:968–71.
[18] Hallonet M, Hollemann T, Pieler T, et al. a novel homeobox-containing gene, directs development of the basal forebrain and visual system. Genes Dev 1999;13:3106–14.
[19] Rojas-Martinez A, Reutter H, Chacon-Camacho O, et al. Genetic risk factors for nonsyndromic cleft lip with or without cleft palate in a Mesoamerican population: evidence for IRF6 and variants at 8q24 and 10q25. Birth Defects Res A Clin Mol Teratol 2010;88:535–7.
[20] Figueiredo JC, Ly S, Raimondi H, et al. Genetic risk factors for orofacial clefts in Central Africans and Southeast Asians. Am J Med Genet A 2014;164A:2572–80.
[21] Pan Y, Ma J, Zhang W, et al. Replication of two novel susceptibility loci for non-syndromic orofacial clefts in a Chinese population. Oral Dis 2011;17:304–8.
[22] Barrett JC, Fry B, Maller J, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
[23] Hahn LW, Ritchie MD, Moore JH. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 2003;19:376–82.
[24] Slavotinek AM, Chao R, Vacik T, et al. VAX1 mutation associated with microphthalmia, corpus callosum agenesis, and orofacial clefting: the first description of a VAX1 phenotype in humans. Hum Mutat 2012; 33:364–8.
[25] Nikopensius T, Birnbaum S, Ludwig KU, et al. Susceptibility locus for non-syndromic cleft lip with or without cleft palate on chromosome 10q25 confers risk in Estonian patients. Eur J Oral Sci 2010;118:317–9.
[26] Zawislak A, Wozniak K, Jakubowska A, et al. Polymorphic variants in VAX1 gene (rs7078160) and BMP4 gene (rs762642) and the risk of non-syndromic orofacial clefts in the Polish population. Dev Period Med 2014;18:16–22.
[27] Bagordakis E, Paranaiba LM, Brito LA, et al. Polymorphisms at regions 1p22.1 (rs560426) and 8q24 (rs1530300) are risk markers for nonsyndromic cleft lip and/or palate in the Brazilian population. Am J Med Genet A 2013;161A:1177–80.
[28] de Aquino SN, Messetti AC, Bagordakis E, et al. Polymorphisms in FGF12, VCL, CX43 and VAX1 in Brazilian patients with nonsyndromic cleft lip with or without cleft palate. BMC Med Genet 2013;14:53.
[29] Mostowska A, Hozyasz KK, Wojcicki P, et al. Associations of folate and choline metabolism gene polymorphisms with orofacial clefts. J Med Genet 2010;47:809–15.