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Original Article

Validation the roles of new gene *CDH23* among NSCLP trios from Western Han Chinese

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Abstract: Background: Non-syndromic cleft lip with or without cleft palate (NSCL/P) is the most common craniofacial birth defects with complex etiology, in which numerous genes and environmental modifiers involved. Cadherin 23 (*CDH23*) gene has been shown to play crucial roles in hearing loss, which is often accompanied with NSCL/P. Methods: This study was designed to investigate the possible associations between *CDH23* gene and 235 case-parent trios with NSCL/P and NSCPO in Western Han Chinese. We selected eight SNPs at *CDH23* to make maximum coverage. In order to identify the contribution of *CDH23* gene to the etiology of NSCL/P, we performed several statistical analysis to validate its role from different aspects, including transmission disequilibrium test (TDT), parent-of-origin effects,pairwise linkage disequilibrium (LD) and sliding window haplotype analysis. Results: Allelic TDT analysis showed that minor allele G at rs6480548 exhibited a statistically under-transmitted among NSCLP trios (P=0.0082, $OR_{transmission}$=0.50, 95% CI: 0.30-0.84). Genotypic TDT analysis showed that C/C at rs6480548 was over-transmitted among the NSCLP trios (Z=2.97, P=0.0030). In parent-of-origin effects, allele G at rs6480548 displayed maternal under-transmission for NSCLP trios (P=0.02). Conclusions: In conclusion, we supported that *CDH23* was associated with NSCLP from Western Han Chinese, which will supply scientific evidence for future research and genetic counseling.

Keywords: *CDH23,* single nucleotide polymorphisms, nonsyndromic cleft lip and palate, TDT, parent-of-origin effect

Introduction

Cleft lip with or without cleft palate (CL/P) is complex birth disorder with variable penetrance and genetic heterogeneity across populations. Epidemiologic studies showed that prevalence rate of cleft lip and palate (CLP) for Asian and Amerindian is 1/500, European-derived population is 1/1000 and African derived population is 1/2500 [1]. Based on the developmental model and epidemiological studies, it is divided into two groups: 70% of the cases are isolated cleft lip with or without cleft palate and 30% are chromosomal, mendelian or teratogenic disease related syndromicclefting, of all orofacial clefting related syndrome, mendelian syndromes are over 500 [1]. Non-syndromic cleft lip with or without cleft palate (NSCL/P) is considered a multifactorial disorder results from both genes and environmental factors, but neither has been completely identified [2].

CDH23 (NM_022124.5) is located at Chromosome 10q21-23 region and consists of 69 exons [3]. It is a member of cadherin family and encodes the protein cadherin-23, which has 3354 amino acids and could form 27 extracellular domains (EC), one single transmembrane domain and one short cytoplasmic domain. *CDH23* is critical to the development of inner ear, and it expresses in outer and inner ear hair cell. The mutation of *CHD23* is related with both Usher Syndrome and autosomal recessive non-syndromic hearing loss [3, 4], Usher syndrome is identified by sensorineural hearing loss and visual loss [5].

Table 1. Type of non-syndromic orofacial clefts

	NSCPO	NSCLO	NSCLP	NSCL/P	Total
Male	36	55	59	114	150
Female	31	25	28	53	84
Unknown	0	0	1	1	1
Total	67	80	88	168	235

Note: NSCPO, Nonsyndromic cleft palate only; NSCLO, Nonsyndromic cleft lip only; NSCLP, Nonsyndromic cleft lip with cleft palate; NSCL/P, Nonsyndromic cleft lip with or without cleft palate.

Cleft palate (CP) and cleft lip and palate (CLP) patients often accompanied with otitis media, frequent inflammation causes ossicular chain abnormalities and further results in hearing loss. And more than 50% infants with NSCLP or NSCPO have different degree abnormal audition, 24.4% of them are hearing loss [6]. The rate of abnormal pure tone average would reduce in the several years later after cleft repair operations performed [7]. However, the patients may miss the best time for speech corrections. Therefore, the relationship of CLP or CP and hearing loss could not be ignored.

CHD23 gene belongs to the E-cadherin group. E-cadherin is one type of the cell adhesion reporter in the process [8], as the primary cell adhesion reporter within adheres junction. E-cadherin is expressed in the epithelium of palatal shelves during the palategenesis of mouse embryos [9] and is expressed in lateral and median nasal prominences during the essential stage in lip and palate development [10].

CDH23 has been proved contributed a lot to hearing loss, but its effect to NSCL/P and NSCPO has not been reported. In the study, we selected eight SNPs of *CDH23* to study the relationship between *CDH23* and non-syndromic cleft lip with or without cleft palate in Western Han Chinese population.

Material and methods

Subjects and ethics statement

Our sample consists of 235 complete case-parent trios (67 NSCPO, 80 NSCLO and 88 NSCLP), all of whom were recruited between 2010 and 2013 from the Cleft Surgery Department of West China Hospital of Stomatology, Sichuan University. Human subject study protocols were reviewed and approved by the Hospital Ethics Committee (HEC) of West China Hospital of Stomatology, Sichuan University. Written informed consent was obtained from each subject before enrollment in the study; for children younger than 16 years old, consents were requested from their parents or guardians, as approved by the local ethics committee. To assess non-syndromic status of cases, all of the probands were screened for the presence of associated anomalies or syndromes by a physician, and only those determined to have an isolated orofacial cleftings were included in this study. All subjects were self-identified as western Han Chinese. **Table 1** shows gender of all cases (**Table 1**).

SNPs selection and genotyping

Genomic DNA of sample was extracted from venous blood by using the protein precipitation method. We chose eight SNPs (rs10999841, rs10740383, rs1867997, rs17531870, rs790-2068, rs1227086, rs6480548 and rs2121-534) from Hapmap CHB & JPT (http://hapmap.ncbi.nlm.nih.gov/cgi-perl/gbrowse/hapmap24_B36/#search) with the MAF>0.24 and try to make the maximum coverage of the *CDH23* gene. All the genotyping experiments were done by the Shanghai BioWing Applied Biotechnology Company (http://www.biowing.com.cn/) using ligase detection reactions (LDR).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) and minor allele frequency at each SNP was assessed among the normal parents. For individual SNPs, allelic Transmission Disequilibrium Test (allelic TDT) and Parent-of-origin effects were performed by PLINK software. Genotypic TDT and Sliding window haplotype analysis were done by FBAT program. Haploview software was used to calculate D' and r^2 of all the SNP of pairwise linkage disequilibrium (LD).

Results

HWE and MAF

Significant deviation from Hardy-Weinberg expectations could reflect genotyping errors or true heterogeneity in the general population and could bias our statistical tests. There were

Table 2. Hardy-Weinberg equilibrium and minor allele frequency

SNP	A1	Hardy-Weinberg equilibrium				Minor allele frequency			
		NSCPO	NSCLO	NSCLP	NSCL/P	NSCPO	NSCLO	NSCLP	NSCL/P
rs10999841	T	0.46	0.24	0.4	0.12	0.37	0.28	0.34	0.31
rs10740383	A	0.17	0.52	1	0.66	0.49	0.47	0.46	0.46
rs1867997	T	0.85	0.72	0.17	0.46	0.34	0.32	0.35	0.34
rs17531870	C	0.73	0.42	0.64	0.37	0.45	0.46	0.44	0.45
rs7902068	G	0.85	0.57	0.36	0.89	0.35	0.3	0.29	0.3
rs1227086	A	0.7	0.31	0.18	0.09	0.34	0.28	0.35	0.32
rs6480548	G	1	0.69	0.7	1	0.28	0.28	0.27	0.27
rs2121534	A	0.68	0.0057	0.031	0.00032	0.29	0.23	0.24	0.24

Note: SNP, Single Nucleotide Polymorphism; A1, Minor allele; NSCPO, Nonsyndromic cleft palate only; NSCLO, Nonsyndromic cleft lip only; NSCLP, Nonsyndromic cleft lip with cleft palate; NSCL/P, Nonsyndromic cleft lip with or without cleft palate.

seven SNPs except rs2121534 (P<0.05 among NSCLO, NSCLP and NSCL/P) were compatible with HWE in unaffected parents. Minor allele frequency was all above 0.24 except rs2121-534 in NSCLO group, which is consistent with the data of CHB & JPT from Hapmap project (**Table 2**).

Allelic and genotypic TDT analysis

In allelic TDT analysis, the minor allele G at rs7902068 exhibited a statistically significant evidence for under-transmission among NSCLP trios (P=0.024, $OR_{transmission}$=0.54, 95% CI: 0.31-0.93) and NSCL/P trios (P=0.02, $OR_{transmission}$=0.65, 95% CI: 0.45-0.93). And minor allele G at rs6480548 also under-transmitted for NSCLP trios (P=0.0082, $OR_{transmission}$=0.50, 95% CI: 0.30-0.84) and NSCL/P trios (P=0.026, $OR_{transmission}$=0.55, 95% CI: 0.45-0.95) (**Table 3**). In genotypic TDT analysis, C/C homozygote at rs7902068 was significantly over-transmitted among NSCLP trios (Z=2.62, P=0.0072) and NSCL/P trios (Z=2.57, P=0.01). C/C homozygote at rs6480547 significantly over-transmitted among NSCLP trios (Z=2.97, P=0.003) and NSCL/P trios (Z=2.65, P=0.0081) (**Table 4**). The associations for NSCL/P might be driven by the NSCLP since the *p*-value in NSCLP group was much lower than that in NSCLP group (**Tables 3** and **4**), which indicated that *CHD23* gene was associated NSCLP from Western Han Chinese population.

Parent-of-origin effects

There was an excess of maternal transmission of the minor allele G at rs7902068 for NSCLP (P=0.04) and NSCL/P (P=0.04); an excess of maternal transmission of the minor allele G at rs6480548 for NSCLP (P=0.02) (**Table 5**). However, we did not find any significant difference between the maternal and paternal transmission for minor alleles of the eight SNPs for each cleft group (data not shown).

Discussion

NSCL/P is a complex trait with no obvious mode of inheritance, and tens of studies failed to identify genes with any major influence on the disease [11]. Till now, only twelve genes/loci can be currently considered confirmed based on replication or the genome wide association studies [12-17]. *IRF6* is still a key element in oral and maxillofacial development, and contribute most to both non-syndromic clefts [19] and syndromic clefts [19]. So, it is very important to search for other main effect causal genes for orofacial clefts.

Many studies have reported the function of *CDH23* with autosomal recessive nonsyndromic hearing loss (ARNSHL) [4] or age-related hearing loss [20] in animal model. Woo et al., 2014 suggested that mutation at *CDH23* in Asians could influence the development of cochlea [4] with which maxillofacial region development is closely related.

The current evidence showed that *CDH23* gene is crucial for the ear related disease, and that CLP and CP patients are often accompanied with ear problems. However, no literature has reported the contribution of *CDH23* gene to NSCLP or CP. In this study, we picked eight SNPs with the MAF>0.24 in CHB & JPT from Hapmap project and try to test the associations

Table 3. Allelic TDT results for SNPs in *CDH23* from FBAT

	CHR	SNP	Position (hg19)	A	T/U	OR (95% CI)	CHISQ	P
NSCPO	10	rs10999841	73235808	T	36/31	1.16 (0.72-1.88)	0.37	0.54
	10	rs10740383	73281948	A	32/27	1.19 (0.71-1.98)	0.42	0.52
	10	rs1867997	73328368	T	29/26	1.12 (0.66-1.89)	0.16	0.69
	10	rs17531870	73342512	C	38/31	1.23 (0.76-1.97)	0.71	0.4
	10	rs7902068	73351845	G	28/31	0.9 (0.54-1.51)	0.15	0.7
	10	rs1227086	73447555	A	35/26	1.35 (0.81-2.24)	1.33	0.25
	10	rs6480548	73477949	G	25/27	0.93 (0.54-1.6)	0.08	0.78
	10	rs2121534	73564453	A	32/21	1.52 (0.88-2.64)	2.28	0.13
NSCLO	10	rs10999841	73235808	T	29/40	0.73 (0.45-1.17)	1.75	0.19
	10	rs10740383	73281948	A	36/44	0.82 (0.53-1.27)	0.8	0.37
	10	rs1867997	73328368	T	29/35	0.83 (0.51-1.36)	0.56	0.45
	10	rs17531870	73342512	C	36/30	1.2 (0.74-1.95)	0.55	0.46
	10	rs7902068	73351845	G	28/37	0.76 (0.46-1.24)	1.25	0.26
	10	rs1227086	73447555	A	26/39	0.67 (0.41-1.1)	2.6	0.11
	10	rs6480548	73477949	G	25/28	0.89 (0.52-1.53)	0.17	0.68
	10	rs2121534	73564453	A	20/20	1 (0.54-1.86)	0	1
NSCLP	10	rs10999841	73235808	T	39/37	1.05 (0.67-1.65)	0.05	0.82
	10	rs10740383	73281948	A	37/43	0.86 (0.55-1.34)	0.45	0.5
	10	rs1867997	73328368	T	27/35	0.77 (0.47-1.27)	1.03	0.31
	10	rs17531870	73342512	C	34/42	0.81 (0.52-1.27)	0.84	0.36
	10	rs7902068	73351845	G	20/37	0.54 (0.31-0.93)	5.07	0.024
	10	rs1227086	73447555	A	36/43	0.84 (0.54-1.3)	0.62	0.43
	10	rs6480548	73477949	G	21/42	0.50 (0.30-0.84)	7.00	0.0082
	10	rs2121534	73564453	A	24/21	1.14 (0.64-2.05)	0.2	0.65
NSCL/P	10	rs10999841	73235808	T	68/77	0.88 (0.64-1.22)	0.56	0.45
	10	rs10740383	73281948	A	73/87	0.84 (0.61-1.15)	1.23	0.27
	10	rs1867997	73328368	T	56/70	0.8 (0.56-1.14)	1.56	0.21
	10	rs17531870	73342512	C	70/72	0.97 (0.7-1.35)	0.03	0.87
	10	rs7902068	73351845	G	48/74	0.65 (0.45-0.93)	5.54	0.02
	10	rs1227086	73447555	A	62/82	0.76 (0.54-1.05)	2.78	0.1
	10	rs6480548	73477949	G	46/70	0.66 (0.45-0.95)	4.97	0.026
	10	rs2121534	73564453	A	44/41	1.07 (0.7-1.64)	0.11	0.74

Note: NSCPO, Nonsyndromic cleft palate only; NSCLO, Nonsyndromic cleft lip only; NSCLP, Nonsyndromic cleft lip with cleft palate; NSCL/P, Nonsyndromic cleft lip with or without cleft palate; SNP, Single Nucleotide Polymorphism; T/U, transmitted/untransmitted; OR, Odds Ratio; CI, confidence interval; Chisq, Chi-Square.

among case-parent trios with NSCL/P or CP. The case-parent design has an advantage in researching the transmission and imprint of gene, however, a higher genetic load in the family of probands might cause an aggregation of loci associated with diseases that results in the Hardy-Weinberg disequilibrium (HWD). However, rs2121534 was not significant associated with any cleft group, even if it deviated from HWE.

The SNPs presented weak pair-wise LD, and they did yield some statistical significance for sliding window haplotypes among case-parent trios. So they were not linked together and the adjacent SNPs do not transmitted together from parents to the affect children.

Both of allelic and genotypic TDT analysis showed that rs6480548 was associated with

Table 4. Genotypic TDT results for SNPs in *CDH23* from FBAT

SNP	Genotype	NSCPO			NSCLO			NSCLP			NSCL/P		
		Fam	Z	P	Fam	Z	P	Fam	Z	P	Fam	Z	P
rs10999841	C/C	41	0	1	50	1.61	0.11	54	-0.36	0.72	104	0.87	0.39
	C/T	52	-0.83	0.41	55	-1.48	0.14	59	0.65	0.52	114	-0.56	0.57
	T/T	25	1.29	0.2	19	0	1	24	-0.57	0.57	43	-0.42	0.67
rs10740383	A/A	27	0.93	0.35	38	-1.93	0.054	39	-0.6	0.55	78	-1.83	0.068
	A/T	43	-0.46	0.65	56	1.87	0.061	62	-0.25	0.8	119	1.19	0.23
	T/T	31	-0.29	0.77	40	-0.51	0.61	42	0.9	0.37	83	0.24	0.81
rs1867997	C/C	36	0.86	0.39	45	0.23	0.82	43	1.03	0.31	88	0.88	0.38
	C/T	44	-1.81	0.07	51	0.42	0.67	52	-0.83	0.41	103	-0.3	0.77
	T/T	18	1.78	0.075	19	-1.13	0.26	20	-0.12	0.9	39	-0.87	0.38
rs17531870	C/C	32	1.23	0.22	26	0.63	0.53	33	-0.57	0.57	60	0.14	0.89
	C/T	52	-0.83	0.41	51	-0.14	0.89	56	-0.27	0.79	108	-0.39	0.7
	T/T	37	-0.09	0.93	39	-0.34	0.74	43	0.81	0.42	82	0.35	0.73
rs7902068	C/C	38	0	1	47	0.91	0.36	39	2.69	0.0072	87	2.57	0.01
	C/G	46	0.3	0.77	50	-0.57	0.57	42	-2.16	0.031	93	-1.97	0.049
	G/G	20	-0.49	0.63	17	-0.54	0.59	17	-0.54	0.59	34	-0.77	0.44
rs1227086	A/A	20	0.25	0.81	21	-0.61	0.54	25	-1.46	0.14	46	-1.49	0.14
	A/G	46	0.89	0.38	47	-1.02	0.31	56	1.07	0.29	104	0	1
	G/G	40	-1.16	0.25	43	1.53	0.13	52	-0.22	0.83	96	0.97	0.33
rs6480548	C/C	39	0.5	0.62	36	0.86	0.39	45	2.97	0.0030	82	2.65	0.0081
	C/G	40	-0.63	0.53	42	-0.93	0.35	47	-2.19	0.028	90	-2.11	0.035
	G/G	13	0.32	0.75	16	0.27	0.79	18	-1.07	0.29	34	-0.57	0.57
rs2121534	A/A	18	1.31	0.19	10	0.17	0.87	12	0.16	0.87	22	0.23	0.82
	A/G	37	0.16	0.87	34	-0.34	0.73	37	0.16	0.87	72	0	1
	G/G	33	-1.11	0.27	29	0.29	0.78	34	-0.27	0.79	64	-0.13	0.9

Note: NSCLO, Non-syndromic cleft lip only; NSCLP, Non-syndromic cleft lip with cleft palate; NSCL/P, Non-syndromic cleft lip with or without cleft palate; NSCPO, Non-syndromic cleft palate only; fam, informative family number; Z, vector of the large sample Z statistic.

both of NSCLP and NSCL/P trios (**Tables 3** and **4**), and minor allele G ($OR_{transmission}$=0.50 and 0.66, 95% CI: 0.30-0.84 and 0.45-0.95, respectively) was protective factors for these two groups (**Table 3**), and might reduce the risk of having a NSCLP or NSCL/P affected child; while C/C homozygote at rs6480547 were risk factors for both of NSCLP trios (Z=2.97, P=0.003) and NSCL/P trios (Z=2.65, P=0.0081), which would increase the risk of having a NSCLP or NSCL/P affected child. Minor allele G at rs7902068 were protective factor for both NSCLP trios ($OR_{transmission}$=0.54, 95% CI: 0.31-0.93) and NSCL/P trios ($OR_{transmission}$=0.65, 95% CI: 0.45-0.93), and C/C homozygote at rs7902068 was risk factor for both of NSCLP (Z=2.62, P=0.0072) trios and NSCL/P trios (Z=2.57, P=0.01) (**Table 4**).

After distinguishing the transmission of the minor allele based on their origin, we found that maternal over-transmitted of the allele G at rs6480548 for NSCLP (P=0.02); and the allele G at rs7902068 were also maternal over-transmitted for NSCLP (P=0.04) and NSCL/P (P=0.04). Genomic imprinting is an epigenetic process that silences one parental allele, resulting in mono-allelic expression. Imprinted genes are important in mammalian fetal growth and development [21]. A recent study demonstrated that there are subtle hints of parent-of-origin effects in orofacial clefting [22]. However, we did not find any significant difference between the maternal and paternal transmission for minor alleles (data not shown) (**Table 5**), which might result from the limited size of informative family numbers.

For NSCPO trios, we did not find any significant associations. Firstly, some epidemiological and embryological studies suggest that NSCLP and NSCPO have different genetic backgrounds

Table 5. Parent-of-Origin effects for SNPs in *CDH23*

Cleft type	SNP	A1	Paternal			Maternal			Z	P
			T/U	CHISQ	P	T/U	CHISQ	P		
NSCPO	rs10999841	T	17.5/14.5	0.28	0.6	18.5/16.5	0.11	0.74	0.15	0.88
	rs10740383	A	15/13	0.14	0.71	17/14	0.29	0.59	-0.1	0.92
	rs1867997	T	12/12	0	1	17/14	0.29	0.59	-0.36	0.72
	rs17531870	C	20.5/15.5	0.69	0.4	17.5/15.5	0.12	0.73	0.33	0.74
	rs7902068	G	14/17	0.29	0.59	14/14	0	1	-0.37	0.71
	rs1227086	A	19.5/15.5	0.46	0.5	15.5/10.5	0.96	0.33	-0.3	0.76
	rs6480548	G	12/14	0.15	0.69	13/13	0	1	-0.28	0.78
	rs2121534	A	16.5/8.5	2.56	0.11	15.5/12.5	0.32	0.57	0.79	0.43
NSCLO	rs10999841	T	15.5/22.5	1.29	0.26	13.5/17.5	0.52	0.47	0.82	0.82
	rs10740383	A	15/18	0.27	0.6	21/26	0.53	0.47	0.95	0.95
	rs1867997	T	16.5/17.5	0.03	0.86	12.5/17.5	0.83	0.36	0.58	0.58
	rs17531870	C	16.5/14.5	0.13	0.72	19.5/15.5	0.46	0.5	0.84	0.84
	rs7902068	G	13/17	0.53	0.47	15/20	0.71	0.4	0.97	0.97
	rs1227086	A	13.5/16.5	0.3	0.58	12.5/22.5	2.86	0.09	0.45	0.45
	rs6480548	G	13/15	0.14	0.71	12/13	0.04	0.84	0.91	0.91
	rs2121534	A	10/10	0	1	10/10	0	1	1	1
NSCLP	rs10999841	T	23/19	0.38	0.54	16/18	0.12	0.73	0.5	0.5
	rs10740383	A	17.5/23.5	0.88	0.35	19.5/19.5	0	1	0.51	0.51
	rs1867997	T	16.5/20.5	0.43	0.51	10.5/14.5	0.64	0.42	0.84	0.84
	rs17531870	C	17.5/21.5	0.41	0.52	16.5/20.5	0.43	0.51	0.98	0.98
	rs7902068	G	11.5/17.5	1.24	0.27	8.5/19.5	4.32	0.04	0.46	0.46
	rs1227086	A	18/21	0.23	0.63	18/22	0.4	0.53	0.92	0.92
	rs6480548	G	12/20	2	0.16	9/22	5.45	0.02	0.48	0.48
	rs2121534	A	11.5/12.5	0.042	0.84	12.5/8.5	0.76	0.38	0.44	0.44
NSCL/P	rs10999841	T	38.5/41.5	0.11	0.74	29.5/35.5	0.55	0.46	0.74	0.74
	rs10740383	A	32.5/41.5	1.1	0.3	40.5/45.5	0.29	0.59	0.69	0.69
	rs1867997	T	33/38	0.35	0.55	23/32	1.47	0.22	0.6	0.6
	rs17531870	C	34/36	0.057	0.81	36/36	0	1	0.86	0.86
	rs7902068	G	24.5/34.5	1.7	0.19	23.5/39.5	4.06	0.04	0.63	0.63
	rs1227086	A	31.5/37.5	0.52	0.47	30.5/44.5	2.61	0.11	0.55	0.55
	rs6480548	G	25/35	1.67	0.2	21/35	3.5	0.06	0.65	0.65
	rs2121534	A	21.5/22.5	0.023	0.88	22.5/18.5	0.39	0.53	0.58	0.58

Note: NSCPO, Nonsyndromic cleft palate only; NSCLO, Nonsyndromic cleft lip only; NSCLP, Nonsyndromic cleft lip with cleft palate; NSCL/P, Nonsyndromic cleft lip with or without cleft palate; SNP, Single Nucleotide Polymorphism; A1, Minor allele; T/U, transmitted/untransmitted; Chisq, Chi-Square.

[23, 24]; Secondly, the *CDH23* gene was very large, only eight SNPs were included in our study would not fully test its association with NSCL/P. Gene sequencing may help us discover more in further study; Thirdly, the sample size was limited that could not represent the population totally and should be expand since we only have 67 nuclear families of NSCPO and the informative family is less than 40 in this study.

In conclusion, this study showed that *CDH23* associated with NSCLP from Western Han Chinese population. The result of our study provides new insights to search for causal genes for clefts.

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Disclosure of conflict of interest

None.

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