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Association of single nucleotide polymorphisms at 20q12 with nonsyndromic cleft lip with or without cleft palate in a Southern Chinese Han cohort

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Abstract

Background: Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a common congenital malformation in the world. Both environment and genetics are involved with the etiology of the disease. Genome-wide association studies have identified two single nucleotide polymorphisms (SNPs) at chromosome 20q12 to be associated with NSCL/P. The current study aimed to explore the association of the two SNPs at 20q12 with NSCL/P and different subtypes in a Southern Chinese Han cohort.

Methods: A total of 430 NSCL/P patients and 451 controls were recruited in the current study. Two SNPs including rs17820943 and rs6072081 at 20q12 were genotyped in the study cohort using Taqman SNP genotyping analysis. Chi-Square test was used to compare allele and genotype frequencies of NSCL/P patients and control group.

Results: Case–control analysis showed that the allele and genotype of rs17820943 and rs6072081 were significantly associated with NSCL/P ($p < .01$). Comparison between subtypes of NSCL/P and controls showed that frequencies of the G allele and GG genotype of rs6072081 ($p = 4.52 \times 10^{-4}$ and $p = .001$ respectively), and those of the T allele and TT genotype of rs17820943 ($p = 6.7 \times 10^{-5}$ and $p = 1.71 \times 10^{-4}$ respectively) were decreased in cleft lip and palate (CLP). No significant association of the two SNPs with cleft lip only (CLO) and cleft palate only (CPO) was found ($p > .05$).

Conclusion: These results showed that rs17820943 and rs6072081 at 20q12 were associated with NSCL/P, especially with the CLP subtype in a Southern Chinese Han cohort.

KEYWORDS
20q12., nonsyndromic cleft lip with or without cleft palate (NSCL/P), single nucleotide polymorphism (SNP)

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1 | INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (NSCL/P) is a common congenital malformation affecting 1.423/1000 births in China every year (Dai et al., 2010). Multiple environmental and genetic factors are involved with the etiology of the birth defect (Mossey, Little, Munger, Dixon, & Shaw, 2009). NSCL/P can be divided into three clinical subtypes: cleft lip only (CLO), cleft lip and palate (CLP), cleft palate only (CPO).

Recently, genome-wide association studies (GWAS) have found potential NSCL/P-associated genes (e.g., *ACBA4, IRF6, MAFB, MSX1, MYH9,* and *SUMO1*) (Beaty et al., 2010; Liu et al., 2018; Peng et al., 2016; Tang, Wang, Han, Guo, & Wang, 2014; Wu et al., 2019; Zhang et al., 2018). GWAS also identified genetic risk loci of NSCL/P associated with a number of chromosomal loci (e.g., 1p22, 20q12, 1q32, 8q24, 17q22) (Huang, Cheng, Xu, Shu, & Tang, 2012; Salagovic et al., 2017). Venkatesh Babu Gurranmkonda et al. investigated the role of polymorphic variants at 20q11.2 (near *MAFB*) loci in the etiology of NSCL/P specifically in the South Indian population (Gurramkonda, Syed, Murthy, Chaubey, & Lakkakula, 2015). A number of replication studies on these genetic factors, however, have provided different results, probably due to different ethnics and populations. Recently, two NSCL/P susceptibility SNPs (rs6072081 and rs17820943) at chromosome 20q12 were reported in associated with NSCL/P in a Chinese Han population (Huang et al., 2012; Zhang et al., 2018). By far, genetic association of the two SNPs needs to be further replicated in Southern Chinese NSCL/P cohorts. Furthermore, how these two SNPs are associated with different NSCL/P subtypes remain to be elucidated in Southern Chinese cohorts. In the current study, we investigated the two SNPs to explore their association with NSCL/P using a case–control study in a Southern Chinese Han cohort, and conducted genotype–phenotype analysis in different clinical subtypes.

2 | MATERIALS AND METHODS

2.1 | Samples

The study was approved by the ethics committee of the second affiliated hospital of Shantou university medical college. Written consent was collected from all participants before recruitment in the study. In total, 430 NSCL/P patients (age range, 3 to 36 years) and 451 phenotypically normal controls (age range, 20 to 60 years). As for NSCL/P subtype phenotype, 73 were diagnosed as CLO, 256 as CLP, 46 as CPO, and 46 had an unknown sex or subtype. Three controls had an unknown sex (Table 1). All participants were unrelated Han Chinese from Southern China recruited between June 2008 to September 2010 from Department of Plastic Surgery and Burn Center, the second affiliated hospital, Shantou university medical college. All patients and controls were carefully examined by three experienced doctors based on detailed diagnostic information in the medical records. In addition, general characteristics, including sex, ethnicity, birth defects, and age were documented. Ten milliliters of venous blood sample was collected from each participant. DNA was extracted subsequently using a TIANAMP Blood DNA kit (TIANGEN Biotechnology Company, Beijing, China) according to the manufacturer's genetic analysis protocol.

2.2 | SNP genotyping

Two SNPs, including rs17820943 and rs6072081 at 20q12, were genotyped. The sequences of the SNPs locus are given in Table 2. According to the manufacturer's proposal, the SNPs were genotyped (Taqman SNP Genotyping Assay; Applied Biosystems, Inc. [ABI]), Foster, California).

2.3 | Statistical analysis

HaploView 4.2 was used in the control group to analyze the Hardy–Weinberg Equilibrium (HWE) of each SNP. Chi-square test implemented in SPSS 25.0 statistical software was used to compare each SNP genotype and allele frequencies in different groups and subtypes, and calculated the 95% confidence interval (95% CI), odds ratio (OR) and *p*-value.

3 | RESULTS

The two SNPs in this study showed no deviation from HWE in the control subjects (all $p > .05$) (Table 3), and thus were both included in further analysis. Our case–control study showed that the allele and genotype of rs17820943 and rs6072081 were significantly associated with the risk of NSCL/P. Compared with control group, the frequencies of G allele and

TABLE 1 Sex and age comparisons between NSCL/P patients and controls

		Sex		Phenotype			
Group	Age (Mean ± *SD*)	Male	Female	CLO	CLP	CPO	Total
NSCL/P	6.5 ± 7.0	257	128	73	256	56	385
Control	36.7 ± 19.5	208	240	None	None	None	448

Note: Abbreviations: CLO, cleft lip only; CLP, cleft lip and palate; CPO, cleft palate only.

TABLE 2 Sequences of genotyping for the two SNPs in at chromosome 20q12

Locus	SNP (Assay ID)	Sequence	VIC	FAM
20q12	rs6072081	ACAGTGGTAGCCGGTAATCAACCGG[A/G]	A	G
	(C__29493999_10)	TTGGTCACACACGCAGTGCCCTACA		
	rs17820943	GGCAGCATGGCTCAATTCAGTGCTC[C/T]	C	T
	(C__27165669_10)	CTGGCCTAGTCACAGCTTTGGGAAG		

TABLE 3 Hardy–Weinberg equilibrium in controls

Chromosome	SNPs ID	ObsHET	PredHET	HWpval	MAF	Alleles
20q12	rs6072081	0.522	0.493	0.246	0.439	A:G
	rs17820943	0.523	0.495	0.283	0.452	C:T

Note: Abbreviations: HWpval, Hardy–Weinberg equilibrium; ObsHET, Observe the heterozygosity; PredHET, Predict heterozygosity; P-value, MAF: Less allele frequency.

GG genotype of rs6072081 (the allelic, recessive, and dominant models $p = .001$, .008 and .008, respectively), and those of T allele and TT genotype of rs17820943 (the allelic, dominant and recessive model $p = .001$, .007 and .004 respectively) were significantly lower in NSCL/P group. Comparison between subtypes of NSCL/P and controls revealed that frequencies of the G allele and GG genotype of rs6072081 (the allelic, dominant and recessive models $p = 4.52 \times 10^{-4}$, .001, and .015, respectively), and those of the T allele and TT genotype of rs17820943 (the allelic, dominant and recessive models $p = 6.7 \times 10^{-5}$, 1.71×10^{-4}, and .007, respectively) were decreased in the CLP subtype. No significant association with CLO or CPO was found (all $p > .05$) (Tables 4 and 5).

4 | DISCUSSION

The NSCL/P is a complex disease with interaction between environment and genetics. In the past decade, great progress has been made in the identification of these complex birth defects, which provides an unprecedented opportunity to identifying susceptible individuals. Beaty et al. found SNPs near chromosome 20q12 (the significant SNP rs6072081 and rs17820943) associated with NSCL/P (Beaty et al., 2010). In addition, recent findings of Na Mi et al showed that *MAFB* (rs13041247, rs6065259 and rs11696257) at 20q12 was associated with NSCL/P in a population of Heilongjiang, northern China (Mi et al., 2014) and Yongchu Pan et al. found *MAFB* rs13041247 significant in a Chinese Han Population (Pan et al., 2011). Enmin Huang et al. found SNPs at 20q12 (rs13041247, rs6102085, and rs6072081) were associated with NSCL/P in Han Chinese (Huang et al., 2012). Zhang, B. et al. studied six SNPs (rs6072081, rs6065259, rs17820943, rs13041247, rs11698025 and rs6102085) near *MAFB* at 20q12 and strong evidence of an association was found at rs17820943 and rs13041247 in a Western Han Chinese population (Zhang et al., 2018). Our study found that rs17820943 and rs6072081 were associated with NSCL/P, and our results

TABLE 4 Comparison of genotypes and alleles between NSCL/P patients and controls

SNP		NSCL/P ($n = 430$)	Control ($n = 451$)	P-value	OR (95% CI)
rs6072081	AA	165	135	0.008[a]	0.686 (0.519–0.908)[a]
	AG	216	236	0.008[b]	0.596 (0.407–0.875)[b]
	GG	49	80	0.001[c]	0.731 (0.604–0.885)[c]
	A	547	506		
	G	313	396		
rs17820943	CC	160	129	0.007[a]	0.676 (0.510–0.897)[a]
	CT	218	236	0.004[b]	0.584 (0.402–0.848)[b]
	TT	52	86	0.001[c]	0.722 (0.597–0.873)[c]
	C	540	494		
	T	322	408		

[a]The dominant model.
[b]Recessive model.
[c]Allelic model.

TABLE 5 Comparison of genotypes and alleles between NSCL/P with clinical subtypes and controls

Subtype	Genotype	Allele	P[a]	P[b]	P[c]	OR (95% CI)[a]	OR (95% CI)[b]	OR (95% CI)[c]
rs6072081	AA/AG/GG	A/G						
CLO ($n = 73$)	21/43/9	85/61	0.813	0.245	0.638	0.936 (0.543–1.616)	1.546 (0.739–3.235)	1.089 (0.764–1.552)
CLP ($n = 255$)	108/119/28	335/175	0.001	0.015	4.52×10^{-4}	0.587(0.426–0.808)	0.567 (0.358–0.900)	0.669 (0.534–0.838)
CPO ($n = 56$)	17/31/8	65/47	0.973	0.507	0.703	1.011(0.552–1.849)	0.767 (0.349–1.683)	0.925 (0.622–1.378)
Control ($n = 448$)	135/233/80	503/393						
rs17820943	CC/CT/TT	C/T						
CLO ($n = 73$)	19/45/9	83/63						
CLP ($n = 255$)	109/117/29	335/175	1.71×10^{-4}	0.007	6.7×10^{-5}	0.542 (0.393–0.747)	0.54 (0.344–0.849)	0.633 (0.506–0.793)
CPO ($n = 56$)	14/33/9	61/51	0.553	0.573	0.946	1.213 (0.641–2.297)	0.806 (0.380–1.708)	1.014 (0.683–1.504)
Control ($n = 448$)	129/233/86	491/405						

[a]The dominant model
[b]Recessive model
[c]Allelic model.

were consistent with previous reports in another Chinese Han population (Zhang et al., 2018).

Moreover, we divided NSCL/P patients into three subgroups to improve the phenotype–genotype analysis and the accuracy of genetic counseling risk assessment. However, the allele and genotype frequencies were similar between some of the subgroups (i.e. CPO) and controls. It could probably due to the complexity and genetic heterogeneity of NSCL/P (Carinci et al., 2003). However, our further analysis of the CLP subgroup revealed lower frequencies of the G allele and GG genotype of chromosome 20q12 rs6072081, and the T allele and TT genotype of rs17820943, with similar but even larger effect size compared to the total NSCL/P cohort. Therefore, it is possible that rs17820943 and rs6072081 at 20q12 may have a more important role in the incidence of CLP compared to that of other subtypes in our Chinese Han cohort.

In summary, we successfully replicated the significant effect of rs17820943 and rs6072081 variants on the susceptibility of chromosome 20q12 and NSCL/P in our Southern Chinese Han cohort, although our study was limited by the sample size. The results of our study may be attributed to the relatively genetically homogeneous population structure of the study cohort in Chaoshan area of Southern China. A limitation of a relatively genetically homogeneous population is that, it may not be able to replicate all genetic factors that are found in other populations with different genetic origins.

In the future, other well-designed studies with larger sample sizes from different Chinese Han populations will be needed to validate these findings. In addition, investigation of interaction between the genetic polymorphisms at 20q12 and environment in different Chinese Han populations could better elucidate the role of the locus in NSCL/P.

ETHICS STATEMENT

The study was reviewed and approved by the Ethics Committee of The Shantou University Medical College. It complies with the Declaration of Helsinki. Informed consent was obtained from all participants before the study began.

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CONFLICT OF INTEREST

All the authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Jianhuan Chen and Shijie Tang contributed to the study design. Yunpu He and Yuqian Zheng performed the genotyping experiment. Yunpu He and Liheng Huang collected the data and performed the data analysis. All authors prepared the manuscript. Yunpu He and Jian-Huan Chen revised the manuscript.

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