

Original Article

Association of MSX1 genetic polymorphisms with non-syndromic cleft lip with or without cleft palate in a uyghur population in Xinjiang, China

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Abstract: Background: To study the relationship between the non-syndromic cleft lip with or without cleft palate, (NSCL/P) and Muscle segment homobox gene (MSX1) in Xinjiang Uyghur Population. Methods: Using case-control study, we randomly selected 120 Uyghur's NSCL/P and 100 controls. We tested genotyping and allele through snapshot method. Using the goodness-of-fit chi-square analysis of the frequencies of genotyping and allele whether fit Hardy-Weinberg law of genetic equilibrium. Results: Distribution of rs3821949 genotypes, Minor allele carriers (AG+GG) and Minor allele frequency showed significant difference between NSCL/P and control subjects (P=0.003, P<0.001 and P=0.027, respectively). The frequency of the minor allele carriers (AG+GG) and the minor allele frequency of the rs3821949 was significantly higher in NSCL/P patients than in Uyghur control subjects (90.8% versus 70%; 65.8% versus 55.5% respectively), The odds ratio (OR) for carriers of Minor allele (AG+GG) and the minor allele of the rs3821949 for NSCL/P was 4.247 [95% confidence interval (CI): 2.000~9.020] and 1.545 (95% CI: 1.050~2.273) respectively in Uyghur subjects. There was no significant difference between NSCL/P and control subjects for rs12532 (all P>0.05). In the haplotype-based case-control analysis, haplotypes were established through the use of rs12532 and rs3821949. The overall distribution of the haplotypes established by rs12532 and rs3821949 was no significantly different between the NSCL/P patients and the control subjects. Conclusion: We conclude that the minor allele carriers and the minor allele of rs3821949 of NSCL/P gene may be a genetic maker in Uyghur population.

Keywords: Non-syndromic cleft lip with or without cleft palate (NSCL/P), Muscle segment homobox gene (MSX1), uyghur population, case-control study

Introduction

Non-syndromic cleft lip with/without cleft palate (NSCL/P) is one of the most common birth abnormality in all population. It arising from failure of normal craniofacial developmental processes at early stage of embryogenesis. The prevalence of NSCL/P was estimated to be 1 in 700 live births worldwide ranging from 1 in 500 to 1 in 1,000 [1]. Which varies across geographic origins, socioeconomic level and ethnic groups [2-7]. The etiology of NSCL/P is complex and multifactorial, with both environmental and genetic factors play a role in its development, but neither of these has been fully elucidated [8-10]. Recently studies have identified several susceptibility loci, and the genetic variations of

more than 10 genes have been associated with the risk of NSCL/P [11-15]. However, owing to the heterogeneity of the disease, the genetic determination for NSCL/P remains largely unresolved.

The muscle segment homobox gene 1 (MSX14p16.1), encodes a protein of 297 amino acids that functions as a transcriptional repressor during embryogenesis. MSX1 has emerged as a strong candidate for NSCL/P, based on the complete secondary cleft palate, the complete failure of incisor development. In mice, transgenic MSX1 deletion resulted in cleft palate and facial and dental abnormality [16]. In human, Linkage studies have demonstrated the association between rare missense/non-

MSX1 and NSCL/P

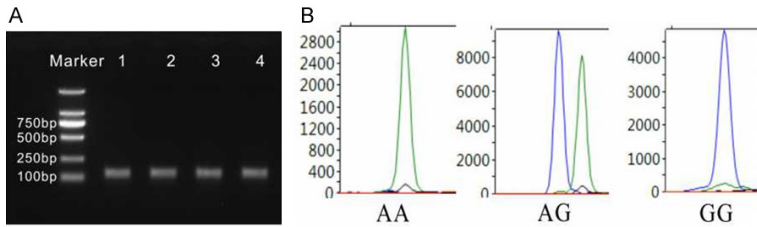


Figure 1. Electrophoresis detection the PCR product of rs3821949. The length is 181 bp (A), snapshot analyses for determination of genotype (B).

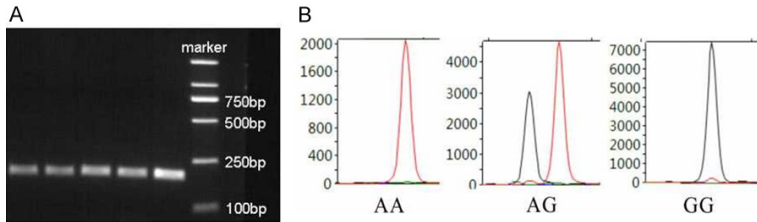


Figure 2. Electrophoresis detection the PCR product of rs12532, the length is 223 bp (A), snapshot analyses for determination of genotype (B).

sense mutations in MSX1 gene and NSCL/P [17-19]. However, the relations between common variations in non-coding areas of MSX1 and NSCL/P remain inconsistent. A study examined eight common single nucleotide polymorphisms (SNPs) of MSX1 gene and reported that rs3821949 and rs12532 were significantly associated with NSCL/P [20]. Rs3821949, which locates in 2 kb upstream of the gene, was further shown to be associated with NSCL/P in a study of Korean population [21] but not in Chinese Uyghur population. Similarly, significant association between rs12532 in 3' untranslated region and NSCL/P was also confirmed in some studies [22-25] but not in others [18, 21, 26]. Inconsistent results suggest that genetic variations of MSX1 may differentially affect NSCL/P across ethnic groups.

According to a national enquiry, The Uyghur, mainly living in Xinjiang Uyghur Autonomous Region, which account for 46% are the largest ethnic group of the area and the second in the country. They also have distinctive living environment, socioeconomic status and lifestyle as compared with other ethnic groups living in the same area. The miscegenation is very rare. Data showed that the prevalence of NSCL/P in Uyghur people was higher than national levels and was the highest among ethnic groups in the area, which makes them excellent subjects for studying the genetic risk factors of the dis-

ease. The goal of the present study was to examine whether similar relationship exists in Uyghur population.

Materials and methods

Ethics statement

The study was reviewed and approved by the Ethics Committee of Hangzhou Normal University. The study complies with the Declaration of Helsinki. Written informed consent was obtained from the guardians of the subjects.

Study population

This case-control study included 120 patients with NSCL/P and 100 normal controls; participants diagnosed with NSCL/P were recruited from 4 major hospitals in Xinjiang, there are Xinhua Hospital in Ili, the First People's Hospital of Urumqi, Kashgar People's Hospital and Khotan People's Hospital in Xinjiang Autonomous Region during 2013 to 2015. All patients were diagnosed independently by two plastic surgeons and only patients with the nonsyndromic form of oral clefts were included. Those with neurological or other major anomalies suggesting the presence of a syndromic form of cleft lip/palate were excluded from the study. For each NSCL/P patient, we selected healthy participants matched for ethnicity, sex, and age as the controls.

DNA extraction and genotyping

Genomic DNA was isolated from the 2 ml blood sample obtained from the antecubital vein of participants using TIANamp Blood DNA Kits (Tiangen Biotech, Beijing, China) and adjusted to a final concentration of 50 ng/L with 10 mmol/L Tris-EDTA (pH 8.0) buffer. All DNA samples were checked for integrity by the electrophoresis on 1.0% agarose gel and OD260/280 ratio (1.7-1.9).

The polymorphisms of rs3821949 and rs12532 were measured using the SNaPshot method according to the manufacturer's instruction (Applied Biosystems, Forest City, CA). For

Table 1. General characteristic between NSCL/P group and control group

Characteristics	Case N=120	Control N=100	T value or χ^2	P value
Age (years, mean \pm SD)	1.98 \pm 0.91	1.81 \pm 0.71	1.396	0.165
Gender (male/female)	62/58	55/45	0.243	0.622

rs3821949 (A>G), a 181-bp fragment was amplified from genomic DNA using the following primers: 5'-GAGTTCTCAAGTCCACACTA-3' (forward) and 5'-AATCGTCAATGAAACACCGAT-3' (reverse) and extended using a SNaPshot primer of 5'-CCTCTCCCCTCCAGGATCAGCG-3'. For rs12532 (A>G), a 223-bp fragment was amplified from genomic DNA using 5'-TGGCTGGAAGAGTCCCTT AGT-3' (forward primer) and 5'-CATTTCCAAAATCGTCAGACA-3' (reverse primer) and extended using a SNaPshot primer of 5'-TTTACTGTCAATTCTGCTGG GGACC-3'. Then DNA products were loaded on an ABI PRISM 3730 XL DNA Sequencer and analyzed for polymorphisms **Figures 1** and **2**.

Statistical analyses

Data analysis was performed with SPSS 16.0.1 (SPSS Inc., Chicago, IL, USA). Case-control statistical analyses, chi-square analyses test was used to examine whether the distributions of polymorphisms in cases and controls fit the Hardy-Weinberg equilibrium. Measurement data are shown as means \pm SD, and the differences between NSCL/P patients and control subjects were assessed by independent-sample t-test. Logistic regression was used to estimate the risk of NSCL/P attributed to SNP genotypes and alleles. Pair-wise linkage disequilibrium (LD) test and haplotype analysis were performed using SHEsis software (<http://analysis.bio-x.cn/SHEsisMain.htm>). A value of $P < 0.05$ was considered statistically significant.

Results

The clinical characteristics of the NSCL/P patients (n=120) and healthy control subjects (n=100) are shown in **Table 1**. There was no significant difference in the age, gender and body mass index (BMI) between the NSCL/P patients and healthy control subjects (all $P > 0.05$). Genotype distribution in cases and controls were in accordance with the Hardy-Weinberg equilibrium (data not shown), The mean age was 1.98 \pm 0.91 years and the male: female

ratio was 1:0.94 in the NSCL/P group. The mean age was 1.81 \pm 0.71 years and the male: female ratio was 1:0.82 in the control group. **Table 2** shows the association between genetic variants and the risk of NSCL/P, we compared MSX1 genotype frequency distributions and their respective alleles

among cases and controls. There was no significant difference between NSCL/P and control subjects for rs12532 (all $P > 0.05$). Distribution of rs3821949 genotypes, Minor allele carriers (AG+GG) and Minor allele frequency showed significant difference between NSCL/P and control subjects ($P = 0.003$, $P < 0.001$ and $P = 0.027$, respectively). The frequency of the minor allele carriers (AG+GG) and the minor allele frequency of the rs3821949 was significantly higher in NSCL/P patients than in Uyghur control subjects (90.8% versus 70%; 65.8% versus 55.5% respectively) (**Table 2**). The odds ratio (OR) for carriers of Minor allele (AG+GG) and the minor allele of the rs3821949 for NSCL/P was 4.247 [95% confidence interval (CI): 2.000~9.020] and 1.545 (95% CI: 1.050~2.273) respectively in Uyghur subjects. There was no significant difference between NSCL/P and control subjects for rs12532 (all $P > 0.05$). In the haplotype-based case-control analysis, haplotypes were established through the use of rs12532 and rs3821949 (**Table 3**). The overall distribution of the haplotypes established by rs12532 and rs3821949 was no significantly different between the NSCL/P patients and the control subjects.

Discussion

NSCL/P is a common congenital craniofacial deformity in humans and the etiology is known to be complex considered to be due to a combination of both environmental and genetic factors [12], The genetic and ethnic variability within the Chilean Population with higher rates in Asians and American Indians, intermediate rates in Caucasians, and lower rates in Africans [27, 28]. Numerous candidate genes have investigated linked to NSCL/P in population-based studies [12, 15, 29-31]. Among these previous studies, few have been replicated in different populations.

MSX1 is reported to encode some of the transcription factors required during various phases of dental development, such as, patterning,

MSX1 and NSCL/P

Table 2. Frequencies and ORs of MSX1 genotypes and alleles in controls and case

MSX1 Genotypes and Alleles	Control N (%)	Case N (%)	OR (95% CI)	P Value
rs3821949				
AA	30 (30.0)	11 (9.2)	1	0.003
AG	29 (29.0)	60 (50.0)	5.643 (2.483~12.822)	
GG	41 (41.0)	49 (40.8)	3.259 (1.456~7.296)	
Total	100	120	-	
Minor allele carriers (AG+GG)	70 (70.0)	109 (90.8)	4.247 (2.000~9.020)	0.000
Minor allele frequency	111 (55.5)	158 (65.8)	1.545 (1.050~2.273)	0.027
rs12532				
AA	22 (22.0)	25 (20.8)	1	0.896
AG	46 (46.0)	59 (49.2)	0.954 (0.510~1.785)	
GG	32 (32.0)	36 (30.0)	1.077 (0.679~1.708)	
Total	100	120	-	
Minor allele carriers (AG+GG)	78 (78.0)	95 (79.2)	0.945 (0.551~1.620)	0.833
Minor allele frequency	110 (55.0)	131 (54.6)	1.023 (0.691~1.514)	0.930

Table 3. Haplotype association between polymorphisms rs12532 and rs3821949

Haplotype	Case	Control	χ^2	P value	Odds ratio (95% CI)
AA	0.049	0.076	1.376	0.241	0.628 (0.287~1.374)
AG	0.405	0.374	0.446	0.504	1.140 (0.776~1.676)
GA	0.288	0.274	0.114	0.726	1.075 (0.707~1.632)
GG	0.258	0.276	0.195	0.659	0.909 (0.595~1.389)

morphogenesis, and histogenesis [32-36]. Furthermore, MSX1 mutation has been considered in 2% of cases of NSCL/P and should be considered for genetic counseling. Based on the biological evidence composed of expression studies and a knockout mouse model as well as association studies, complete sequencing and linkage studies [37-43].

Numerous previous studies have investigated the role of the MSX1 gene in the etiology of NSCL/P in different human populations [18, 20, 42-45]. Fallin observed eight single-nucleotide polymorphisms (SNPs) in the MSX1 gene, the rs3821949 and rs12532 were association with NSCL/P [20]. The samples consisted of 142 NSCL±P families of Kim NY' study identified that the A allele at rs3821949 appears to increase the risk of NSCLP, while the G allele is under-represented. And Huang et al [18] suggest no significant association between non-syndromic oral cleft and rs3821949 or rs12532 in MSX1 gene in Han Chinese in Western China. This difference could be attributed to genetic differences in ethnic.

In our study the distribution of rs3821949 genotypes, Minor allele carriers (AG+GG) and Minor allele frequency showed significant difference between NSCL/P and control subjects in Uyghur control subjects in Xinjiang, the minor allele carriers and minor allele of rs3821949 appears to increase the risk of NSCL/P. There was no significant

difference between NSCL/P and control subjects for rs12532. The result is consistence with Fallin and partly consistence with Kim NY, suggest that the Non syndromic cleft lip and/or palate (NSCL/P) is a complex congenital anomaly with varying incidence among patients of different geographical origins. Several genes involved in the aetiology of NSCL/P and they are different in different populations. The Uyghur ethnic distinct with Han in the living environment, socioeconomic status and lifestyle in the same area.

Considering that haplotype analysis is more powerful than single-marker analysis, we investigated the LD between the MSX1 SNPs and performed a haplotype analysis between the case and control groups. The analyzed haplotypes were built by rs3821949 and rs12532 was not associated with NSCL/P.

In conclusion, our study demonstrated the rs3821949 genotypes, Minor allele carriers (AG+GG) and Minor allele frequency showed significant difference between NSCL/P and control subjects in Uyghur subjects in Xinjiang,

the G allele of rs3821949 appears to increase the risk of NSCL/P.

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Disclosure of conflict of interest

None.

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