Association of *TFAP2A* gene polymorphism with susceptibility to non-syndromic cleft lip with or without palate risk in south Indian population

Venkatesh Babu Gurramkonda [a], Altaf Hussain Syed [b], Jyotsna Murthy [b], Bhaskar V.K.S. Lakkakula [a,c,*]

[a] *Department of Biomedical Sciences, Sri Ramachandra University, Chennai, India*
[b] *Department of Plastic Surgery, Sri Ramachandra University, Chennai, India*
[c] *Sickle Cell Institute Chhattisgarh, Raipur, India*

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ABSTRACT

The aetiology of non-syndromic cleft lip with or without cleft palate (NSCL/P) is complex involving multiple interacting genes and environmental factors. The primary objective of the present study was to investigate the role of *TFAP2A* gene single nucleotide polymorphisms (SNPs) in the pathogenesis of NSCL/P. In this study, 173 unrelated NSCL/P patients and 176 controls without clefts were genotyped with *TFAP2A* rs1675414 (Exon 1), rs3798691 (Intron 1), and rs303050 (Intron 4) variants by allele-specific amplification using the KASPar SNP genotyping system. The method of multifactor dimensionality reduction (MDR) was used to analyze gene-gene interactions. *TFAP2A* polymorphisms are not found to be associated with non-syndromic cleft lip with or without cleft palate (NSCL/P) at either the genotype or allele levels. No linkage disequilibrium (LD) was found between *TFAP2A* variants. MDR analysis did not show a significant effect of the *TFAP2A* gene polymorphisms on susceptibility to NSCL/P ($p > 0.05$). These results suggest that the analyzed variations in *TFAP2A* gene might not be associated with NSCL/P pathogenesis in south Indian population.

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1. Introduction

Orofacial clefts are among the commonest birth defects with a worldwide frequency of 1 in 750 live births. About 70% of clefts are non-syndromic that occur in isolated conditions while the remaining 30% are syndromic clefts that are associated with other cognitive or structural anomalies. Based on different clinical manifestations, non-syndromic cleft lip and palate (NSCL/P) is categorized into two groups - cleft lip with or without cleft palate (CL/P) and cleft palate only (CPO) (Mossey et al., 2009). CPO is less common than CL/P. The incidence of non-syndromic clefts shows variations depending upon economic, geographical and genetic backgrounds. Asians and Native Americans exhibited the highest prevalence rates, followed by Caucasians and Africans (Murthy and Bhaskar, 2009).

Over the last decade, numerous linkage and association studies have been performed to identify the genes that predispose to NSCL/P. The development of lip and palate is known to be dependent on a spectrum of signaling molecules, transcription factors and growth factors. However, the relative contribution of individual susceptibility genes varies across different populations, thereby accentuating the need for replication of association studies in different populations. Transcription factors are DNA-binding proteins capable of activating or repressing gene regulation. The transcription factor AP-2 alpha, encoded by the *TFAP2A* gene, is another important functional candidate for the non-syndromic oral clefts. The gene coding for AP-2 alpha transcription factor (*TFAP2A*) is located on 6p24-p22.3 and composed of seven exons with the last six exons encoding the majority of the protein. AP-2 alpha protein acts as a sequence specific DNA-binding transcription factor, recognizing and binding to the specific GC-rich regions that are present in the cis-regulatory regions of several cellular genes. Thus it regulates gene expression during the embryogenesis of eye, ear, face, body wall and limbs (Ahituv et al., 2004).

The first evidence to prove that *TFAP2A* gene is a risk factor for cleft lip/palate is derived from the breakpoints within 375 to 930 kb of the 5′-end of the *TFAP2A* gene in 3 patients that showed balanced translocations [46,XX,t(6;9)(p23;q22.3)] involving 6p (Davies et al., 1995, 2004). Several genome-wide linkage scans have provided evidence for linkage between 6p23 region that harbor *TFAP2A* gene and NSCL/P in different populations (Carinci et al., 1995; Prescott et al., 2000; Moreno et al., 2004; Schultz et al., 2004). The *TFAP2A* has been identified as a causative gene for Branchio-Oculo-Facial Syndrome (BOFS) that commonly presents with facial clefting (Li et al., 2013). The present study aims to investigate the role of *TFAP2A* gene polymorphisms in the pathogenesis of NSCL/P in south Indian population.

* Corresponding author at: Sickle Cell Institute Chhattisgarh, Genetic Lab, Department of Biochemistry, Pt. JNM Medical College, Raipur (C.G.), India.
E-mail address: lvksbhaskar@gmail.com (B. V.K.S. Lakkakula).

2. Materials & methods

2.1. Subjects

The study included a total of 349 individuals of both sexes, consisting of 176 NSCL/P cases and 173 controls. All the subjects were recruited at Sri Ramachandra Cleft and Craniofacial Centre, Sri Ramachandra University, Chennai, India. The case groups were examined by two surgeons to assess the exact cleft phenotype. Cases with possible specific malformations and those with intellectual disability or other anomalies were excluded from the study. Subjects without family history of clefting were recruited as controls. The study was approved by Institution Ethics Committee of Sri Ramachandra University (Ref:IEC-NI/II/OCT/25/60 dated 12.01.2012) and informed consent was obtained from all the participants. Consent was collected from parent or legal guardian when the subject was a minor.

2.2. Genotyping

3 ml peripheral blood samples were collected from all the subjects. DNA was isolated from the leukocytes using a standard procedure. Three SNPs reported in a previous study (Shi et al., 2011), [rs1675414 (Exon 1), rs3798691 (Intron 1), and rs303050 (Intron 4)] were selected for analysis. Genotyping was performed using KASPar chemistry (KBioscience Ltd., Hoddesdon, UK), which is a competitive allele-specific PCR-SNP genotyping system that uses FRET quencher cassette oligos (Didenko, 2001; Cuenca et al., 2013). On the basis of the fluorescence obtained, the allele call data were viewed graphically as a scatter plot for each marker assayed using the SNPViewer (http://www.lgcgenomics.com).

2.3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) was assessed for all polymorphisms in both cases and controls groups by using chi-square test. Allele frequencies were estimated by the gene counting method (Ceppellini et al., 1955). Comparison of genotype and allele frequencies among cases and the control groups were analyzed by the chi-square test. Odds ratio (OR) and 95% confidence intervals (CI) were calculated using wild type genotypes or allele as reference group. Pairwise linkage disequilibrium (LD) was computed as both of D′ and r^2 for *TFAP2A* gene by using of the Haploview (Barrett et al., 2005) Genotype-genotype interaction was calculated by using Multifactor Dimensionality Reduction (MDR) 3.0.2 software (Hahn et al., 2003).

3. Results

The genotype frequencies of three *TFAP2A* variants were distributed according to the Hardy-Weinberg equilibrium among the controls. There were no significant differences in genotypic distribution between cases and controls of the three variants (Table 1). Odds ratio analysis showed that the variant rs3798691 was associated with decreasing risk trend of oral clefts while the other two variants, namely rs1675414 and rs303050 were associated with an increased risk of oral clefts. However, none of the models mentioned above reached statistical significance (Table 1). Genotype and allele frequency distribution of *TFAP2A* variants by cleft phenotype was depicted in Fig. S1. In subgroup analysis by cleft phenotype, no significant associations were found for all *TFAP2A* polymorphisms (Supplementary Table S1). Measures of pairwise LD presented in Fig. 1 revealed that the *TFAP2A* polymorphisms were in LD as evidenced by lower r^2 values. Haplotype analysis was not informative.

Multi-dimensional reductionality scaling approach was employed to evaluate the epistasis and to characterize the potential interactions of *TFAP2A* gene polymorphisms on NSCL/P risk (Table 2). The best model predicted for 1, 2 and 3 locus interactions were not significant. Both two locus (rs1675414 and rs303050) and three locus (rs1675414,

rs3798691 and rs303050) interaction models were selected 10 out of 10 times by MDR based on a cross-validation consistency (CVC) score of 100% and predictive accuracy of 52.8% (Table 2). Furthermore, we observed a lesser mutual information for the combined effect of any two polymorphisms, in contrast to rs303050 alone (I = 0.84%) or rs1675414 alone (I = 0.50%), as depicted in entropy-based interaction dendrograms (Fig. 2).
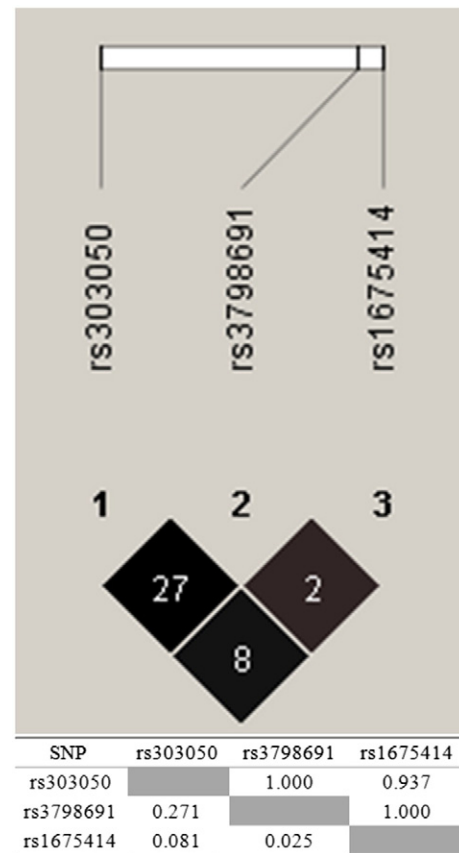
Table 1
Results of association tests with *TFAP2A* gene polymorphisms in NSCL/P and control groups.

SNP	Genotype	Control n (%)	Clefts n (%)	OR (95%CI)	p value
rs1675414	GG	56 (31.8)	46 (26.6)	Reference	0.297[*]
	GA	83 (47.2)	96 (55.5)	1.41 (0.86–2.29)	
	AA	37 (21.0)	31 (17.9)	1.02 (0.55–1.88)	
	GA + AA	120 (68.2)	127 (73.3)	1.29 (0.81–2.04)	0.282
	G	195 (55.4)	188 (54.3)		
	A	157 (44.6)	158 (45.7)	1.04 (0.77–1.40)	0.77
rs3798691	GG	168 (95.5)	167 (96.5)	Reference	0.608[*]
	GC	8 (4.5)	6 (3.5)	0.75 (0.26–2.22)	
	CC	0 (0)	0 (0)	—	
	GC + CC	8 (4.5)	6 (3.5)	0.75 (0.26–2.22)	0.608
	G	344 (97.7)	340 (98.3)		
	C	8 (2.3)	6 (1.7)	0.76 (0.26–2.21)	0.611
rs303050	TT	155 (88.1)	147 (85.0)	Reference	0.194[*]
	TC	19 (10.8)	26 (15.0)	1.44 (0.77–2.72)	
	CC	2 (1.1)	0 (0)	—	
	TC + CC	21 (11.9)	26 (15.0)	0.85 (0.46–1.58)	0.61
	T	329 (93.5)	268 (92.5)		
	C	23 (6.5)	26 (7.5)	1.38 (0.77–2.49)	0.269

[*] p value by χ^2 test ($df = 2$), p value by χ^2 test ($df = 1$).

Fig. 1. Paired linkage disequilibrium statistics of *TFAP2A* polymorphisms. Color coding represents the D′/LOD values and the values in cells are r^2 multiplied by 100. Values presented at the bottom of the figure are D′ and r^2, above and below diagonal, respectively.