Meta Gene 9 (2016) 181–184

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

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

ARTICLE INFO

Article history:
Received 28 April 2016
Revised 8 July 2016
Accepted 8 July 2016
Available online 9 July 2016

Keywords:
TFAP2A
Orofacial clefts
NSCL/P
SNP

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.

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).

http://dx.doi.org/10.1016/j.mgene.2016.07.007
2214-5400/

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 ($df = 1$).

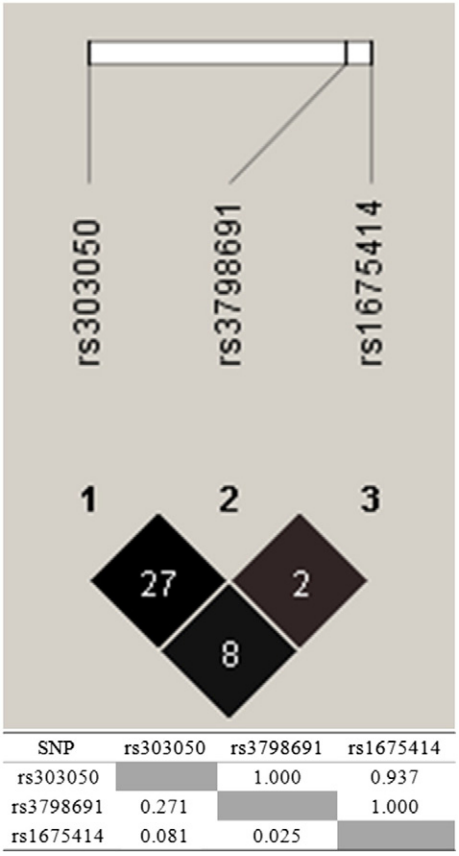

Fig. 1. Paired linkage disequilibrium statistics of *TFAP2A* polymorphisms. Color coding represents the D′/LOD values and the values in cells are r^2 multiplied by 100. Values presented at the bottom of the figure are D′ and r^2, above and below diagonal, respectively.

Table 2
Interaction models by MDR analysis.

Factors	CVC	TA	p value
rs1675414	10/10	0.542	0.119
rs1675414, rs303050	10/10	0.528	0.091
rs1675414, rs3798691, rs303050	10/10	0.528	0.075

CVC: Cross validation consistency; TA: Testing accuracy.

4. Discussion

Analysis of three SNPs within the *TFAP2A* gene did not show any significant association with NSCL/P. No significant linkage disequilibrium was detected between markers. MDR analysis did not show a significant effect of the *TFAP2A* gene polymorphisms on susceptibility to NSCL/P. Similar findings were also observed in the Northern Chinese population (Shi et al., 2011). In contrast to the above mentioned results, both single marker and haplotypes were associated with NSCL/P in a family based association analysis of Italian NSCL/P triads (Martinelli et al., 2011). Earlier studies observed that loss of AP-2 alpha caused severe craniofacial, limb and skin defects (Schorle et al., 1996; Zhang et al., 1996). Further, AP-2 alpha-null chimeras exhibit midline craniofacial clefting, acrania, and a failure of sternal fusion (Nottoli et al., 1998). Furthermore, tissue specific deletion of AP-2 alpha gene revealed that palatal development is independent of neural tube closure, but neural crest-specific expression of AP-2 alpha is essential for particular aspects of craniofacial development (Brewer et al., 2004). Numerous patients with Branchio-Oculo-Facial Syndrome have mutations in the DNA binding domain of *TFAP2A*, suggesting importance of downstream transcriptional targets (Tekin et al., 2009; Milunsky et al., 2011). While some previous studies demonstrated the binding of *TFAP2A* to the IRF6 enhancer (MCS-9.7) and regulate IRF6 expression (McDade et al., 2012), other experiments suggested that the IRF6 binds to upstream of *TFAP2C*, but not *TFAP2A* (Botti et al., 2011). A recent dual-fluorescence transgenic analysis of MCS-9.7, unambiguously demonstrated the in vivo effect of rs642961 SNP on enhancer activity (Bhatia et al., 2015). Although the *TFAP2A* is involved in orofacial development in mice, very few studies have investigated the role of *TFAP2A* in the pathogenesis NSCL/P. There is a definite need for more studies to confirm the role of *TFAP2A* variants in the causation of NSCL/P.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mgene.2016.07.007.

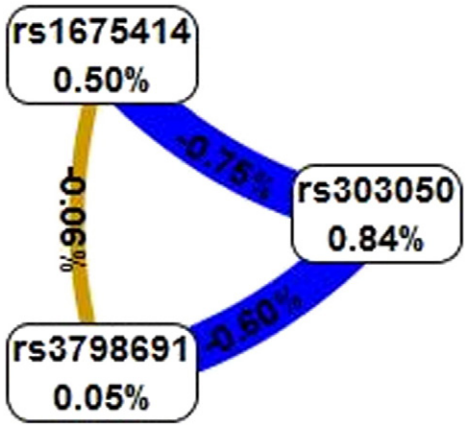

Fig. 2. Entropy graph for gene–environment interaction and NSCL/P. The blue color indicating highest level of redundancy, and gold represents the independence and a midway point between synergy and redundancy.

Conflict of interest

There are no conflicts of interests.

Authors contribution

LVKSB, SAH and JM defined the research theme. LVKSB and GVB designed methods and experiments, carried out the laboratory experiments. LVKSB and GVB analyzed the data, interpreted the results and wrote the paper. All authors have contributed to, seen and approved the manuscript.

Acknowledgements

L.V.K.S. Bhaskar acknowledges funding from the Indian Council of Medical Research (ICMR), Government of India (Project Ref. No. 56/15/2007-BMS and No. 45/3/2013-Hum/BMS).

References

Ahituv, N., Erven, A., Fuchs, H., Guy, K., Ashery-Padan, R., Williams, T., de Angelis, M.H., Avraham, K.B., Steel, K.P., 2004. An ENU-induced mutation in AP-2alpha leads to middle ear and ocular defects in Doarad mice. Mamm. Genome 15, 424–432.
Barrett, J.C., Fry, B., Maller, J., Daly, M.J., 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21, 263–265.
Bhatia, S., Gordon, C.T., Foster, R.G., Melin, L., Abadie, V., Baujat, G., Vazquez, M.P., Amiel, J., Lyonnet, S., van Heyningen, V., Kleinjan, D.A., 2015. Functional assessment of disease-associated regulatory variants in vivo using a versatile dual colour transgenesis strategy in zebrafish. PLoS Genet. 11, e1005193.
Botti, E., Spallone, G., Moretti, F., Marinari, B., Pinetti, V., Galanti, S., De Meo, P.D., De Nicola, F., Ganci, F., Castrignano, T., Pesole, G., Chimenti, S., Guerrini, L., Fanciulli, M., Blandino, G., Karin, M., Costanzo, A., 2011. Developmental factor IRF6 exhibits tumor suppressor activity in squamous cell carcinomas. Proc. Natl. Acad. Sci. U. S. A. 108, 13710–13715.
Brewer, S., Feng, W., Huang, J., Sullivan, S., Williams, T., 2004. Wnt1-Cre-mediated deletion of AP-2alpha causes multiple neural crest-related defects. Dev. Biol. 267, 135–152.
Carinci, F., Pezzetti, F., Scapoli, L., Padula, E., Baciliero, U., Curioni, C., Tognon, M., 1995. Nonsyndromic cleft lip and palate: evidence of linkage to a microsatellite marker on 6p23. Am. J. Hum. Genet. 56, 337–339.
Ceppellini, R., Siniscalco, M., Smith, C.A., 1955. The estimation of gene frequencies in a random-mating population. Ann. Hum. Genet. 20, 97–115.
Cuenca, J., Aleza, P., Navarro, L., Ollitrault, P., 2013. Assignment of SNP allelic configuration in polyploids using competitive allele-specific PCR: application to citrus triploid progeny. Ann. Bot. 111, 731–742.
Davies, A.F., Stephens, R.J., Olavesen, M.G., Heather, L., Dixon, M.J., Magee, A., Flinter, F., Ragoussis, J., 1995. Evidence of a locus for orofacial clefting on human chromosome 6p24 and STS content map of the region. Hum. Mol. Genet. 4, 121–128.
Davies, S.J., Wise, C., Venkatesh, B., Mirza, G., Jefferson, A., Volpi, E.V., Ragoussis, J., 2004. Mapping of three translocation breakpoints associated with orofacial clefting within 6p24 and identification of new transcripts within the region. Cytogenet. Genome Res. 105, 47–53.
Didenko, V.V., 2001. DNA probes using fluorescence resonance energy transfer (FRET): designs and applications. Biotechniques 31, 1106–1121.
Hahn, L.W., Ritchie, M.D., Moore, J.H., 2003. Multifactor dimensionality reduction software for detecting gene-gene and gene-environment interactions. Bioinformatics 19, 376–382.
Li, H., Sheridan, R., Williams, T., 2013. Analysis of TFAP2A mutations in Branchio-Oculo-Facial Syndrome indicates functional complexity within the AP-2alpha DNA-binding domain. Hum. Mol. Genet. 22, 3195–3206.
Martinelli, M., Masiero, E., Carinci, F., Morselli, P.G., Palmieri, A., Girardi, A., Baciliero, U., Scapoli, L., 2011. Evidence of an involvement of TFAP2A gene in non-syndromic cleft lip with or without cleft palate: an Italian study. Int. J. Immunopathol. Pharmacol. 24, 7–10.
McDade, S.S., Henry, A.E., Pivato, G.P., Kozarewa, I., Mitsopoulos, C., Fenwick, K., Assiotis, I., Hakas, J., Zvelebil, M., Orr, N., Lord, C.J., Patel, D., Ashworth, A., McCance, D.J., 2012. Genome-wide analysis of p63 binding sites identifies AP-2 factors as co-regulators of epidermal differentiation. Nucleic Acids Res. 40, 7190–7206.
Milunsky, J.M., Maher, T.M., Zhao, G., Wang, Z., Mulliken, J.B., Chitayat, D., Clemens, M., Stalker, H.J., Bauer, M., Burch, M., Chenier, S., Cunningham, M.L., Drack, A.V., Janssens, S., Karlea, A., Klatt, R., Kini, U., Klein, O., Lachmeijer, A.M., Megarbane, A., Mendelsohn, N.J., Meschino, W.S., Mortier, G.R., Parkash, S., Ray, C.R., Roberts, A., Reardon, W., Schnur, R.E., Smith, R., Splitt, M., Tezcan, K., Whiteford, M.L., Wong, D.A., Zori, R., Lin, A.E., 2011. Genotype-phenotype analysis of the branchio-oculo-facial syndrome. Am. J. Med. Genet. A 155A, 22–32.
Moreno, L.M., Arcos-Burgos, M., Marazita, M.L., Krahn, K., Maher, B.S., Cooper, M.E., Valencia-Ramirez, C.R., Lidral, A.C., 2004. Genetic analysis of candidate loci in non-syndromic cleft lip families from Antioquia-Colombia and Ohio. Am. J. Med. Genet. A 125A, 135–144.

Mossey, P.A., Little, J., Munger, R.G., Dixon, M.J., Shaw, W.C., 2009. Cleft lip and palate. Lancet 374, 1773–1785.
Murthy, J., Bhaskar, L., 2009. Current concepts in genetics of nonsyndromic clefts. Indian J. Plast. Surg. 42, 68–81.
Nottoli, T., Hagopian-Donaldson, S., Zhang, J., Perkins, A., Williams, T., 1998. AP-2-null cells disrupt morphogenesis of the eye, face, and limbs in chimeric mice. Proc. Natl. Acad. Sci. U. S. A. 95, 13714–13719.
Prescott, N.J., Lees, M.M., Winter, R.M., Malcolm, S., 2000. Identification of susceptibility loci for nonsyndromic cleft lip with or without cleft palate in a two stage genome scan of affected sib-pairs. Hum. Genet. 106, 345–350.
Schorle, H., Meier, P., Buchert, M., Jaenisch, R., Mitchell, P.J., 1996. Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature 381, 235–238.
Schultz, R.E., Cooper, M.E., Daack-Hirsch, S., Shi, M., Nepomucena, B., Graf, K.A., O'Brien, E.K., O'Brien, S.E., Marazita, M.L., Murray, J.C., 2004. Targeted scan of fifteen regions for nonsyndromic cleft lip and palate in Filipino families. Am. J. Med. Genet. A 125A, 17–22.
Shi, J., Song, T., Jiao, X., Qin, C., Zhou, J., 2011. Single-nucleotide polymorphisms (SNPs) of the IRF6 and TFAP2A in non-syndromic cleft lip with or without cleft palate (NSCLP) in a northern Chinese population. Biochem. Biophys. Res. Commun. 410, 732–736.
Tekin, M., Sirmaci, A., Yuksel-Konuk, B., Fitoz, S., Sennaroglu, L., 2009. A complex TFAP2A allele is associated with branchio-oculo-facial syndrome and inner ear malformation in a deaf child. Am. J. Med. Genet. A 149A, 427–430.
Zhang, J., Hagopian-Donaldson, S., Serbedzija, G., Elsemore, J., Plehn-Dujowich, D., McMahon, A.P., Flavell, R.A., Williams, T., 1996. Neural tube, skeletal and body wall defects in mice lacking transcription factor AP-2. Nature 381, 238–241.