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Original Article

SATB2 gene variants in non-syndromic cleft lip with or without cleft palate in Indian population

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ABSTRACT

Objectives: Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common craniofacial birth defects and little is known about its aetiology. Initial studies of cytogenetic analysis provided the clues for possible genes involved in the pathogenesis of NSCL/P. This approach led to the identification of SATB2 gene on 2q32-q33. The aim of this study was to determine the association between SATB2 mutations and NSCL/P.

Materials and methods: The rs137853127, rs200074373 and rs1992950 mutations of the SATB2 gene were investigated in 173 patients with NSCL/P and 176 normal controls using Kbioscience KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system.

Results: The mutations in exon 6 (rs137853127 and rs200074373) were monomorphic, the intronic variant (rs1992950) was polymorphic and genotype distribution was in agreement with Hardy–Weinberg equilibrium. The rs1992950 genotype distribution is not statistically significant between NSCL/P and controls.

Conclusion: Our findings suggest that the SATB2 gene variations do not contribute to the development of NSCL/P in the south Indian population.

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1. Introduction

Non-syndromic cleft lip with or without cleft palate (NSCL/P) is one of the most common craniofacial birth defects with complex aetiology, involving both genetic and environmental factors. Non-syndromic clefts are broadly classified into two groups, cleft lip with or without palate (CL/P) and cleft palate only (CPO). During the human embryo development, fusion of the secondary palate is one of the last morphogenetic processes; failure of this fusion process causes the CPO.¹ Both

CL/P and CPO are genetically distinct phenotypes in terms of their inheritance. Cytogenetic analysis of two CPO subjects revealed two de novo translocation breakpoints (5 Mb apart) located between D2S311 and D2S116 markers on 2q32,² which is one of the three regions of the genome for which haploinsufficiency is significantly associated with CPO.³ Furthermore, two de novo chromosomal translocations involving 2q32-q33 were in unrelated individuals with isolated cleft palate.⁴ These breakpoints were located in intron 2 of SATB2 and located 130 kb 3-prime to the SATB2 polyadenylation signal, within a conserved region of non-coding DNA,

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where there is no evidence for transcribed genes.⁴ Whole mount in situ hybridization to mouse embryos shows site and stage-specific expression of SATB2 in the developing palate.⁴ The *Satb2* knock-out mouse embryos showed multiple craniofacial defects that include a significant truncation of the mandible, a shortening of the nasal and maxillary bones, malformations of the hyoid bone and a cleft palate.⁵

SATB2 gene, which is located on chromosome position 2q33.1, encodes AT-rich sequence binding protein composed of 733 amino acids with a molecular weight of 82.5 kDa. *Satb2* is the first cell-type-specific transcription factor that functions as a regulator of the transcription of large chromatin domains.⁶ SATB2 directly interacts with the activity of transcription factors that regulate craniofacial development and cortical neurons differentiation.^{7,8} Furthermore, SATB2 regulates osteoblast differentiation and skeletal development in mice.⁹ The present study aimed to investigate the role of SATB2 gene polymorphisms (rs137853127, rs200074373 and rs1992950) in the pathogenesis of NSCL/P in South Indian population.

2. Materials and methods

2.1. Subjects

The study group consists of 349 individuals, including 176 controls and 173 NSCL/P (144 CL/P and 29 CPO) cases. All the subjects were recruited from Sri Ramachandra cleft and craniofacial centre, Sri Ramachandra University, Chennai, India. All NSCL/P patients underwent a pre-operation examination to diagnose cleft lip and palate, and family history was collected using a questionnaire. The case groups were examined by two plastic surgeons to exclude syndromes known to be associated with any type of orofacial clefting. Cases with possible specific malformations and those with mental retardation or other anomalies were excluded from the study. Age and gender matched individuals and those without family history of clefting were recruited as controls in this study. This study was approved by the ethics committee of Sri Ramachandra University. Written informed consent was obtained from all the adult subjects. Parents or legal guardians provided written consent on behalf of minors.

2.2. Genotyping

A 3-ml peripheral blood sample was collected from all the subjects. DNA was obtained from blood samples using a standard procedure. Genotyping of the 3 SNPs (rs137853127, rs200074373 and rs1992950) was performed by Kbioscience (Hoddesdon, Herts, United Kingdom) by using KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system that uses FRET quencher cassette oligos. 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) assessed cases and control groups by using a chi-square test. Allele frequencies

were estimated by the gene counting method. The genotype frequency of the polymorphic SNP was in agreement with HWE in both cases and controls. Comparison of genotype and allele frequencies among cases and the control group was analyzed by the chi-square test. Odds ratio and 95% confidence interval were calculated using wild type genotypes or allele as reference group.

3. Results

Analysis of three SNPs (rs137853127, rs200074373 and rs1992950) of SATB2 gene revealed that the rs137853127 and rs200074373 were monomorphic in both cases and controls. Distribution of rs1992950 genotypes and alleles in control and NSCL/P groups is presented in Table 1. The rs1992950 genotype distribution in both case and control groups followed HWE ($p = 0.469$). The proportions of genotypes were 29.5% GG, 26.0% AA and 44.5% GA in cases, while 28.4% GG, 24.4% AA and 47.1% GA in controls. The genotype distribution was not significantly different between NSCL/P cases and controls ($p = 0.880$). The minor allele frequency is almost similar in both case (48.0%) and control groups (48.0%). Allelic, genotypic and dominant model-based associations of the rs1992950 revealed no association with NSCL/P, and no appreciable risk was observed on the respective associations (Table 1). In subgroup analysis, this SNP did not show significant association with CLP and CPO groups in all three models (Table 1).

4. Discussion

In the present study, we have investigated the impact of SATB2 gene polymorphisms and susceptibility to NSCL/P in a sample of the South Indian population. The rs137853127 is a germline mutation (p.R239X) and rs200074373 is a non-synonymous mutation in the exon 6 of SATB2. These two mutations were monomorphic in the present study. The rs1992950 SNP is located in intron 3 and found to be polymorphic in both cases and controls. No association was found between rs1992950 and NSCL/P in our population.

The *Satb2* is the first cell-type-specific transcription factor that specifically binds nuclear matrix attachment regions (MARs) and is involved in transcriptional regulation and chromatin remodelling. It plays an important role in tooth and craniofacial development.^{5,10,11} Previous studies showed that complete functional loss of *Satb2* leads to increased apoptosis in the developing jaw primordia and subsequent down-regulation of the expression of genes (*Pax9*, *Alx4* and *Msx1*) involved in craniofacial development in humans and mice.^{12,13} Although initial cytogenetic studies showed two translocation break breakpoints on 2q32, which harbours SATB2 gene,² a meta-analysis of genome scans of cleft lip and palate indicated 2q32-q35 region as a clefting susceptibility locus.¹⁴ Sequencing of 184 cleft lip and palate cases revealed T190A mutation in a single Philippine case, which was not found in CEPH controls.¹⁵ Screening of 962 SNPs belonging to 104 genes on chromosome 2 failed to show significant association between SATB2 SNPs and NSCL/P.¹⁶ Screening

Table 1 – Results of association tests with SATB2 gene rs1992950 polymorphism in NSCL/P groups.

Genotype	Control	NSCL/P	OR (95% CI)	p value
GG	50 (28.41)	51 (29.48)	Reference	0.88*
GA	83 (47.16)	77 (44.51)	0.91 (0.56–1.50)	
AA	43 (24.43)	45 (26.01)	1.03 (0.58–1.82)	
GA+AA	126 (71.59)	122 (70.52)	0.95 (0.6–1.51)	0.825
G	183 (51.99)	179 (51.73)	Reference	
A	169 (48.01)	167 (48.27)	1.01 (0.75–1.36)	0.946
MAF	0.48	0.48		
HWp	0.462	0.152		
Genotype	Control	CL/P	OR (95% CI)	p value
GG	50 (28.41)	41 (28.47)	Reference	0.991*
GA	83 (47.16)	67 (46.53)	0.98 (0.58–1.66)	
AA	43 (24.43)	36 (25.00)	1.02 (0.56–1.87)	
GA+AA	126 (71.59)	103 (71.53)	1.0 (0.61–1.62)	0.99
G	183 (51.99)	149 (51.74)	Reference	
A	169 (48.01)	139 (48.26)	1.01 (0.74–1.47)	0.949
MAF	0.48	0.48		
HWp	0.462	0.412		
Genotype	Control	CPO	OR (95% CI)	p value
GG	50 (28.41)	10 (34.48)	Reference	0.444*
GA	83 (47.16)	10 (34.48)	0.60 (0.23–1.55)	
AA	43 (24.43)	9 (31.03)	1.0 (0.39–2.81)	
GA+AA	126 (71.59)	19 (65.52)	0.75 (0.33–1.73)	0.505
G	183 (51.99)	30 (51.72)	Reference	
A	169 (48.01)	28 (48.28)	1.01 (0.58–1.76)	0.97
MAF	0.48	0.48		
HWp	0.462	0.095		

MAF: minor allele frequency; HWp: Hardy–Weinberg p value.
* p value by χ^2 test (df = 2).

of Thai patients with craniofacial dysmorphisms showed presence of a germline mutation (R239X) in one patient that had cleft palate.⁷ However, none of the subjects in the present study showed this mutation. An individual with developmental delay and cleft palate showed a 4.5 Mb deletion of 2q33.1, which includes SATB2.¹⁷ Analysis of two intronic SNPs (rs4673313 and rs17199393) in Irish NSCL/P showed no significant association between NSCL/P and SATB2.¹⁸ Both TRIMM and HAPLIN methods that were used to detect multi-marker effects on oral clefts of Norway and Denmark showed significant maternal effects of SATB2 gene variants for isolated cleft palate in Danish but not in Norwegian samples.¹⁹ The expression of SATB2 during mid-facial development and palatogenesis in mouse, chick and zebrafish is highly conserved, and suggests that the SATB2 gene is under extreme evolutionary pressure.²⁰ SATB2 pathogenic mutations were not identified in unrelated isolated CPO cases and also no evidence of association was found using intragenic intronic SNPs in case and control study.⁴ Although we cannot rule out variations outside the genomic regions studied, our results do not support a significant role for SATB2 in the pathogenesis of NSCL/P in south Indian populations.

Conflicts of interest

The authors have none to declare.

Authors contribution

LVKSB, SAH and JM defined the research theme. LVKSB and GVB designed methods and experiments, and 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.

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REFERENCES

1. Luke DA. Development of the secondary palate in man. *Acta Anat (Basel)*. 1976;94:596–608.
2. Brewer CM, Leek JP, Green AJ, et al. A locus for isolated cleft palate, located on human chromosome 2q32. *Am J Hum Genet*. 1999;65:387–396.
3. Brewer C, Holloway S, Zawalnyski P, Schinzel A, FitzPatrick D. A chromosomal deletion map of human malformations. *Am J Hum Genet*. 1998;63:1153–1159.

4. FitzPatrick DR, Carr IM, McLaren L, et al. Identification of SATB2 as the cleft palate gene on 2q32-q33. *Hum Mol Genet.* 2003;12:2491–2501.
5. Dobrev G, Chahrouh M, Dautzenberg M, et al. SATB2 is a multifunctional determinant of craniofacial patterning and osteoblast differentiation. *Cell.* 2006;125:971–986.
6. Britanova O, Akopov S, Lukyanov S, Gruss P, Tarabykin V. Novel transcription factor Satb2 interacts with matrix attachment region DNA elements in a tissue-specific manner and demonstrates cell-type-dependent expression in the developing mouse CNS. *Eur J Neurosci.* 2005;21:658–668.
7. Leoyklang P, Suphapeetiporn K, Siriwan P, et al. Heterozygous nonsense mutation SATB2 associated with cleft palate, osteoporosis, and cognitive defects. *Hum Mutat.* 2007;28:732–738.
8. Gyorgy AB, Szemes M, de Juan Romero C, Tarabykin V, Agoston DV. SATB2 interacts with chromatin-remodeling molecules in differentiating cortical neurons. *Eur J Neurosci.* 2008;27:865–873.
9. Dobrev G, Dambacher J, Grosschedl R. SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. *Genes Dev.* 2003;17:3048–3061.
10. Chen HC, Lee YS, Sieber M, et al. MicroRNA and messenger RNA analyses of mesenchymal stem cells derived from teeth and the Wharton jelly of umbilical cord. *Stem Cells Dev.* 2012;21:911–922.
11. Mao XY, Tang SJ. Effects of phenytoin on Satb2 and Hoxa2 gene expressions in mouse embryonic craniofacial tissue. *Biochem Cell Biol.* 2010;88:731–735.
12. Britanova O, Depew MJ, Schwark M, et al. Satb2 haploinsufficiency phenocopies 2q32-q33 deletions, whereas loss suggests a fundamental role in the coordination of jaw development. *Am J Hum Genet.* 2006;79:668–678.
13. Park JW, Cai J, McIntosh I, et al. High throughput SNP and expression analyses of candidate genes for non-syndromic oral clefts. *J Med Genet.* 2006;43:598–608.
14. Marazita ML, Murray JC, Lidral AC, et al. Meta-analysis of 13 genome scans reveals multiple cleft lip/palate genes with novel loci on 9q21 and 2q32-35. *Am J Hum Genet.* 2004;75:161–173.
15. Vieira AR, Avila JR, Daack-Hirsch S, et al. Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. *PLoS Genet.* 2005;1:e64.
16. Beaty TH, Hetmanski JB, Fallin MD, et al. Analysis of candidate genes on chromosome 2 in oral cleft case-parent trios from three populations. *Hum Genet.* 2006;120:501–518.
17. Urquhart J, Black GC, Clayton-Smith J. 4.5 Mb microdeletion in chromosome band 2q33.1 associated with learning disability and cleft palate. *Eur J Med Genet.* 2009;52:454–457.
18. Carter TC, Molloy AM, Pangilinan F, et al. Testing reported associations of genetic risk factors for oral clefts in a large Irish study population. *Birth Defects Res A: Clin Mol Teratol.* 2010;88:84–93.
19. Jugessur A, Shi M, Gjessing HK, et al. Maternal genes and facial clefts in offspring: a comprehensive search for genetic associations in two population-based cleft studies from Scandinavia. *PLoS ONE.* 2010;5:e11493.
20. Sheehan-Rooney K, Palinkasova B, Eberhart JK, Dixon MJ. A cross-species analysis of Satb2 expression suggests deep conservation across vertebrate lineages. *Dev Dyn.* 2010;239:3481–3491.