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## Association between Transforming Growth Factor Alpha TaqI Polymorphism and Susceptibility to the Nonsyndromic Cleft Lip and/or Palate in an Iranian Population: A Case Control Study

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### ABSTRACT

**Background:** The TGF- $\alpha$  TaqI C >T polymorphism is a well-characterized variant for nonsyndromic cleft lip and/or palate (NS CL/P), but it has shown inconsistent results of association with nonsyndromic CL/P across a number of studies. Thus, we have performed this case-control study to clarify the association between the TGF- $\alpha$  TaqI C >T polymorphism and NS CL/P risk.

**Methods:** One-hundred ten cases with NSCL/P and 110 controls were recruited to the current study. We have genotyped the TGF- $\alpha$  TaqI C >T polymorphism using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The odds ratio (OR) and 95% confidence interval (CI) were applied for strength of association TGF- $\alpha$  TaqI C >T polymorphism with NSCL/P.

**Results:** The TGF- $\alpha$  TaqI C >T polymorphism CC, CT and TT genotypes frequencies in the NSCL/P cases were 30.9%, 57.3% and 11.8%, respectively while the corresponding frequencies in the controls were 37.3%, 52.7% and 10.0%, respectively. The frequency of C and T alleles in the case were 59.5% and 40.5%, respectively while the corresponding allelic frequencies in the controls were 63.6% and 36.4%. There was no significant difference in the genotype and allele frequency for TGF- $\alpha$  TaqI C >T polymorphism between cases and controls. The minor allele frequency (MAF) of TGF- $\alpha$  TaqI C >T polymorphism among healthy controls was 0.36.

**Conclusion:** Our study indicates that the TGF- $\alpha$  TaqI C >T polymorphism was not significantly associated with increased risk of NS CL/P in the Iranian population. However, our results still need to be confirmed by further large and well-designed case-control studies.

## Introduction

**N**onsyndromic cleft lip and palate (NSCL/P) is the most common birth defects, with an incidence of 1 to 2 in 1000 live births.<sup>1,2</sup> There are notable differences in prevalence of NSCL/P among different ethnic groups, and it is more common among men, compared with women.<sup>3</sup> Although a combination of factors associated with multiple genetic and environmental factor are important for development of NSCL/P, the exact pathogenesis is still unknown.<sup>4,5</sup>

In recent years, regarding advances in genetics and molecular biology, various efforts have been made to understand the risk and related factors of NSCL/P, to predict its occurrence and prevention.<sup>6,7</sup> There were many candidate gene studies associated with NSCL/P.<sup>8</sup> Although many genes or regions had positive results in one or more of the early studies, few of those findings were consistently positive across all studies. One of the most important genes is transforming growth factor  $\alpha$  (TGFA).<sup>9</sup>

The combined effect of TGFA mutation and environmental influence in NSCL/P has been analyzed by several studies in different populations<sup>8</sup>. This association supports a role for this gene as one of the genetic determinants of craniofacial development.<sup>10</sup> Three common polymorphisms of the TGFA gene, including RsaI, BamHI, and TaqI in intron 5 and in exon 6, have been investigated in association with NSCL/P. Recently, the possible association of BamHI and RsaI polymorphisms in TGFA in developing NSCL/P was identified in an Iranian population. Because individual susceptibility plays an important role in the development of NSCL/P, understanding the genetic background and etiology of these diseases is essential for both risk assessment and finding effective methods for prevention and treatment.<sup>11, 12</sup>

To date, several studies from different ethnicities have been evaluated the association

of TGF- $\alpha$  TaqI C > T polymorphism with NSCL/P risk, but the results remain conflicting. Moreover, there is a study on association of this polymorphism with NSCL/P in the Iranian population.<sup>13</sup> Thus, we performed this case-control study to determine whether TGFA TaqI polymorphism was associated with NSCL/P in Iranian patients.

## Materials and Methods

**Study Population:** The study protocols were approved by the Institutional Ethics Committee of the Kashan University of Medical Science. Informed consent was obtained from parents of children prior to any data collection. A total of 110 children with nonsyndromic CL/P, and 110 age and sex matched children as controls were consecutively enrolled in current study between April 2016 and March 2017. All cases were Iranian who diagnosed with NSCL/P at birth. Excluding criteria were as follow: family history of orofacial clefts or craniofacial anomalies, dental anomalies, congenital anomalies, learning disabilities, attention deficits, hearing impairment and speech deficits.

**DNA extraction and genotyping:** From each participant, five ml of peripheral blood samples were collected in tubes containing  $\mu$ l of 0.5 M EDTA and stored at -20 C. genomic DNA was extracted from peripheral blood using the salting out method. Genotyping of the TGF- $\alpha$  TaqI C > T polymorphism (rs731236) was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) method. The primers sequences were as: forward primer, 5'-TCACTCCCCCTTTTCATCTG-3'; and reverse primer, 5'-CGAGGAGGCTCCTGAGGTG-3'. The PCR cycle of +915 was performed by a stage of pre-denaturation at 95°C for 5 minutes, then 35 cycles of 95°C for 30 seconds, 58.4°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 10 minutes. The pattern of restriction

fragments for homozygote wild-type (CC), heterozygote (CT) and mutant homozygote (TT) were 179 bp, 179+122+53 bp, and 122 + 53 bp, respectively. The PCR products were digested by two TaqI restriction enzymes (Fermentas, Vilnius, Lithuania) at 37°C. The RFLP fragments were detected on 2% agarose gel and stained with ethidium bromide (EtBr). Moreover, we have sequenced 10% of samples to confirm the results of genotyping.

**Statistical Analysis:** A raw genotyping data for PCR-RFLP assays was input into Excel software of Microsoft Office 2013. All statistical analysis of data was calculated by the Statistical Package for the Social Sciences (SPSS) software package version 19.0 (SPSS, Inc., Chicago, IL, USA), which a  $P < 0.05$  was considered as the level for significance. The chi-square test was performed to compare genotype and allele frequency of TGF- $\alpha$  TaqI C > T polymorphism in the cases and controls. Moreover, the odds ratio (OR) and 95% confidence interval (CI) were calculated from CMA software for strength association TGF- $\alpha$  TaqI C > T polymorphism with NSCL/P. Hardy-Weinberg Equilibrium (HWE) was tested for TGF- $\alpha$  TaqI C > T polymorphism for controls using chi-square test.

## Results

The characteristics of cases and controls are shown in Table 1. The mean age of cases and controls at was  $7.41 \pm 4.31$  and  $8.11 \pm 6.31$  years, respectively. The analysis showed that there was no significant difference between cases and controls by age and sex ( $P > 0.05$ ), which indicating that matching was

appropriate. Ninety-seven patients (24.5%) had unilateral NSCL/P and the other 83 (75.5%) had bilateral NSCL/P. Of those 110 cases, 17 (15.4%) and 29 (26.4%) were only cleft lip and only cleft palate, respectively (Table 1).

The genotypes and alleles frequency of TGF- $\alpha$  TaqI C > T polymorphism in cases and controls are shown in Table 2. The distributions of genotype in the control group for TGF- $\alpha$  TaqI C > T polymorphism was in accordance with Hardy-Weinberg equilibrium ( $P = 0.144$ ), and minor allele frequency of this polymorphism in controls was 0.36. As seen in Table 2, The TGF- $\alpha$  TaqI C > T polymorphism wild homozygote (CC), heterozygote (CT) and mutant homozygote (TT) genotypes frequencies in the NSCL/P cases were 34 (30.9%), 63(57.3%) and 13(11.8%), respectively while the corresponding frequencies in the controls were 41 (37.3%), 58 (52.7%) and 11(10.0%), respectively. The frequency of wild and mutant alleles in the case were 131(59.5%) and 89(40.5%), respectively while the corresponding allelic frequencies in the controls were 140(63.6%) and 80 (36.4%). The association of TGF- $\alpha$  TaqI C > T polymorphism with NSCL/P risk is shown in Table 2. We have not found a significant difference in the genotype and allele frequency for TGF- $\alpha$  TaqI C > T polymorphism between cases and controls. Moreover, there was no significant association between TGF- $\alpha$  TaqI C > T polymorphism and susceptibility to the NSCL/P in the Iranian children (Table 2).

**Table 1.** Characteristics of the cases and controls.

	Cases (n = 110)	Control (n = 110)	P
Age ( $\pm$ SD)	7.41 $\pm$ 4.31	8.11 $\pm$ 6.31	0.631
Gender			
Male	64 (58.2)	60 (54.5)	0.587
Female	46 (41.8)	50 (45.5)	
lateral			
Unilateral	28 (25.4)	-	-
Bilateral	83 (75.5)	-	-
$\pm$ CL/CP			
Only CL	17 (15.4)	-	-
Only CP	29 (26.4)	-	-

**Table 2.** A comparison of NSCL/P patients and healthy control samples of allele and genotype distribution of TGF- $\alpha$  TaqI C > T polymorphism.

Polymorphism	Cases (n = 110)	Control (n = 110)	OR (95% CI)	P
Genotype		0.144		
CC	34 (30.9)	41 (37.3)	Ref.	
CT	63 (57.3)	58 (52.7)	1.202 (0.706-2.045)	0.684
TT	13 (11.8)	11 (10.0)	1.206 (0.515-2.823)	0.666
Allele				
C	131 (59.5)	140 (63.6)	0.841 (0.573-1.236)	0.378
T	89 (40.5)	80 (36.4)	1.189 (0.809-1.747)	0.378
Genetic Models				
Dominant (GG+AG vs. AA)			1.407 (0.792-2.501)	0.244
Recessive (GG vs. AG+AA)			2.360 (1.055-5.280)	0.037

OR: Odds Ratio; CI: Confidence Interval.

## Discussion

NSCL/P is the most common craniofacial malformation.<sup>1</sup> The pathophysiology of NSCL/P is not well understood, but progress has been suggested a multifactorial model of genetic inheritance for NSCL/P based on the interaction of genetic and environmental factors.<sup>3</sup> Studies have showed that infection, drug intake and folic acid supplement during pregnancy might be associated with the occurrence of NSCL/P.<sup>14</sup> By the advances in genetics and molecular biology, several studies have focused on the role of TGF- $\alpha$  TaqI C > T polymorphism in etiology of NSCL/P, as a result of its role in cell proliferation and differentiation during primary palate morphogenesis.<sup>12</sup> However, the exact role of TGF- $\alpha$  TaqI C > T polymorphism played in development of nonsyndromic NSCL/P still remain obscure.<sup>11,12,15</sup>

In the past two decades, different studies have been evaluated the association of TGF- $\alpha$  TaqI C > T polymorphism with risk of NSCL/P. However, only a study by Bagheri et al., have evaluated the association of TGF- $\alpha$  TaqI C > T polymorphism with NSCL/P among Iranian children (113 children with NSCL/P and 209 controls). Their result showed that the TGF- $\alpha$  TaqI C > T polymorphism was not associated with the risk of NS CL/P in the Iranian children.<sup>13</sup> In consistence with their results, the current study showed that the TGF- $\alpha$  TaqI C > T

polymorphism was not significantly associated with increased risk of NS CL/P in the Iranian population. Studies showed that TGF- $\alpha$  TaqI C > T polymorphism is among the few genetic factors that have shown significant interactions with various environmental factors, including maternal smoking and vitamin use. Therefore, the agreement between the current study and the previous study in the Iranian children could be explained by taking into consideration similar environmental influence. Similarly, Souza et al., have evaluated the association of TGF- $\alpha$  TaqI C > T polymorphism with NSCL/P using a transmission disequilibrium test (TDT) in a Brazilian population. Similarly, they have found that TGF- $\alpha$  TaqI C > T polymorphism did not associated with increased risk of NSCL/P.<sup>16</sup> Moreover, their results did not show correlation between exposure to tobacco or alcohol during pregnancy and increased risk of NSCL/P. However, Zhu et al., have reported that the parental smoking may interact with TGF- $\alpha$  TaqI C > T polymorphism of Chinese populations in occurrence of NSCL/P.<sup>17</sup> However, Zhu et al., did not found an association between TGF- $\alpha$  TaqI C > T polymorphism and the increased risk of NSCL/P in Chinese population.<sup>18</sup>

Inconsistent with our results the pooled data showed different role for TGF- $\alpha$  TaqI C > T polymorphism in development NSCL/P. In 2013, Lu et al., in a meta-analysis of 27 case-control studies evaluated the

association between TGF- $\alpha$  TaqI C > T polymorphism and CL/P risk. Their results showed a significant association between TGF- $\alpha$  TaqI C > T polymorphism and CL/P under all five genetic models. Moreover, they have found an increased risk among Caucasians and Asians, but not among mixed populations.<sup>12</sup> In the most previously published meta-analysis based on 26 studies (3,234 cases and 4,348 healthy controls), Yan et al., have reported that the TGF- $\alpha$  TaqI C > T polymorphism was significantly associated with an increased risk of nonsyndromic CL/P. Moreover, their stratifications by ethnicity showed that TGF- $\alpha$  TaqI C > T polymorphism was associated with susceptibility to nonsyndromic CL/P among White and Asian Populations. However, they have reported that the wild allele of TGF- $\alpha$  TaqI C > T polymorphism had a protective effect against NSCL/P in White and Asians populations. They have suggested that the GF- $\alpha$  TaqI C > T polymorphism is likely to be low-penetrance polymorphism with a very weak effect and also another explanation could be the high between-studies heterogeneity of included studies in the pooled data.<sup>11</sup> Therefore, to clarify this finding larger sample sizes and well-designed studies using well-matched controls.

In summary, the current study indicates that the TGF- $\alpha$  TaqI C > T polymorphism was not significantly associated with increased risk of NS CL/P in the Iranian population. However, our results still need to be confirmed by further large and well-designed case-control studies.

### Conclusion

The current study indicates that the TGF- $\alpha$  TaqI C > T polymorphism was not significantly associated with increased risk of NS CL/P in the Iranian population. However, our results still need to be confirmed by further large and well-designed case-control studies.

### Conflict of Interests

Authors have no conflict of interests.

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