Association of Transforming Growth Factor Alpha Polymorphisms with Nonsyndromic Cleft Lip and Palate in Iranian Population

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Abstract

Background: Cleft lip with or without cleft palate (CL/P) is one of the most common congenital anomalies and the etiology of orofacial clefts is multifactorial. Transforming growth factor alpha (TGFA) is expressed at the medial edge epithelium of fusing palatal shelves during craniofacial development. In this study, the association of two important TGFA gene polymorphisms, BamHI (rs11466297) and RsaI (rs3732248), with CL/P was evaluated in an Iranian population.

Methods: The frequencies of BamHI and RsaI variations were determined in 105 unrelated Iranian subjects with nonsyndromic CL/P and 218 control subjects using PCR and RFLP methods, and the results were compared with healthy controls. A p-value of <0.05 was considered statistically significant.

Results: The BamHI AC genotype was significantly higher (p=0.016) in the patients (12.4%) than the control group (5.0%). The BamHI C allele was significantly higher (p=0.001; OR=3.4, 95% CI: 1.6-7.4) in the cases (8.0%) compared with the control group (2.5%).

Conclusion: Our study showed that there was an association between the TGFA BamHI variation and nonsyndromic CL/P in Iranian population.

Keywords: Association Study, Cleft lip/palate, Polymorphism, Transforming Growth Factor Alpha

Introduction

Cleft lip with or without cleft palate (CL/P) is one of the most common birth defects 1. The worldwide prevalence of CL and CL/P is 3.28 and 6.64 per 10,000 cases, respectively 2 4. Genetic factors are thought to contribute to the development of this disorder, because the risk of recurrence of CL/P within a family is approximately 28-40-fold greater for the general population 5 6. Nonsyndromic cleft in humans is most likely due to combination of genetic and environmental factors 7 8. Population based candidate gene studies as well as linkage disequilibrium has been used to identify the etiology of CL/P so as to predict its occurrence and to prevent it from occurring in the future. Identification of the genes involved in the development of the human craniofacial region can serve as a first step towards developing a better understanding of the diagnosis, prevention and treatment of developmental anomalies of this region 9 10.

The association between CL/P and specific alleles in the transforming growth factor alpha (TGFA) gene suggests that TGFA could be a candidate gene for CL/P 11 15.

In 1989, Ardinger et al published the first association study of CL/P with five candidate genes which were involved in palate formation. Analysis of 80 unrelated patients from Iowa showed that there were significant associations of CL/P with TaqI and BamHI RFLPs at the TGFA locus 34. Holder et al in a British
population 24, Tanabe et al in a Japanese population 30 and Stoll et al in the French population 25 indicated that the TGFA gene variant contributes to the occurrence of nonsyndromic CL/P. However, this is contrary to a study done by Lidral et al in the Philippines 36, which may be due to genetic differences in different populations.

TGFA is, both structurally and functionally, similar to Epidermal Growth Factor (EGF), and induces a mitogenic response by binding to and stimulating the tyrosine kinase activity of EGF receptor 16,17. During mitogenic response by binding to and stimulating the epidermal growth factor (EGF), and induces a

Table 1. Primer sequences and their PCR product sizes, restriction enzymes, and RFLP fragments for the TGFA BamHI and RsaI polymorphisms

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Global MAF*</th>
<th>Primer Sequence (5′ → 3′)</th>
<th>Product Size (bp)</th>
<th>RFLP Fragments (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI (rs11466297 A/C)</td>
<td>C=0.0238</td>
<td>F: GCCCGGCTATTTGTTGAGTT R: AAGGGCGAAGGAAACACAGG</td>
<td>174</td>
<td>A allele=120+54 C allele=174</td>
</tr>
<tr>
<td>RsaI (rs3732248 C/T)</td>
<td>A=0.2075</td>
<td>F: TGGGCTTCTGCTGCTATCAGT R: CAGGCGAAATGTCACCAAGT</td>
<td>166</td>
<td>C allele=91+75 T allele=166</td>
</tr>
</tbody>
</table>

* Global Minor Allele Frequency

Subjects
To determine the possible role of BamHI and RsaI polymorphisms in the TGFA gene in developing oral clefts in an Iranian population, a case-control study was performed. A sample of 105 newborns with nonsyndromic CL/P and 218 control subjects were included. A clinical examination to look for dysmorphic features (such as lip pits) was undertaken. The exclusion criteria of this study were evidence of other facial or skeletal malformations (such as lip pits, congenital heart lesion, etc), metabolic or neurologic disorders or anomalies of other organ systems. Samples were recruited from Mofid Hospital, a referral pediatrics center in Tehran, Iran in 2013-15. A control group consisted of 218 Iranian newborns, without cleft, who were born in or around Tehran between the years 2013 and 2015 were selected and their blood samples were stored. Ethical approval for the study was obtained from the Ethics Committee of the Dental Research Center of the University of Shahid Beheshti. Informed consent was obtained from all parents.

Materials and Methods
Statistical analysis
Chi square (χ²) and Fisher’s exact test with Open Epi Version 2.2 (free statistical software) were performed to compare genotype and allele frequencies in the study groups. The p<0.05 were considered statistically significant. Statistical significance was corrected for multiple testing comparisons.

Results
The samples consisted of 105 patients with cleft lip with or without cleft palate and 218 healthy controls. The CL/P samples consisted of 65 males (62.0%) and 40 females (38.0%). A positive family history of cleft was observed in 38 CL/P cases (36.19%). There were 34 (32.3%) patients with unilateral CL/P, 27 (25.7%) with bilateral CL/P, 15 (14.2%) cleft lip only and 29 (27.6%) with cleft palate only. The distributions of genotypes using chi-square showed that in both case...
and control groups, for the TGFA BamHI polymorphism, they were in Hardy-Weinberg equilibrium (p>0.05). For the TGFA Rsal polymorphism, the distributions of genotypes in the case group were in Hardy-Weinberg equilibrium (p=0.625). The genotype distributions and allele frequencies of the TGFA BamHI and Rsal polymorphisms are shown in table 2. The results of the genotyping for the BamHI and Rsal RFLP are shown in figures 1 and 2. Our results showed that there was a significant difference in the genotype distribution and allele frequency of the BamHI polymorphism between the case and control groups. The BamHI AC genotype was significantly higher (p=0.016; OR=2.1, 95% CI:1.2-6.3) in the patients (12.4%) than the control group (5.0%). The BamHI C allele was significantly higher (p=0.001; OR=3.4, 95% CI:1.6-7.4) in the CL/P patients (8.0%) compared with the control group (2.5%). This result suggests that the C allele may be a risk factor for CL/P in Iranian population. In contrast, no significant difference in the genotype and allele frequencies of the Rsal polymorphism was found between the case and control groups.

**Discussion**

TGFA was chosen as a candidate gene in the preliminary association studies of CL/P, because it is expressed in palatal tissue in culture. It subsequently revealed that TGFA was present at high levels in epithelial tissue of the medial edge of the palatal shelves at the time of shelf fusion. The role of TGFA in lip and palate development was then evaluated in different populations.

This study was performed to examine whether the TGFA BamHI (rs11466297 A/C) and Rsal (rs3732248 C/T) variations are associated with the increased risk of CL/P in an Iranian population including 105 CL/P patients and 218 controls. Our results showed that TGFA BamHI polymorphism was associated with the CL/P in Iranian population. The frequency of the BamHI AC genotype in the patients (12.4%) was approximately twice more than that of control group (5.0%). The BamHI C allele was significantly higher (p=0.001; OR=3.4, 95% CI:1.6-7.4) in the cases (8.0%) compared with the control group (2.5%). In contrast, no significant difference in the genotype and allele frequencies of the Rsal polymorphism was found between the case and control groups.
spective, which are very close to the global minor allele frequencies (0.024 and 0.208, respectively).

Ardinger et al. (1989) investigated the possible association of five candidate genes including TGFA, Nuclear Receptor subfamily 3 group C member 1 (NR3C1), Epidermal Growth Factor (EGF), Epidermal Growth Factor Receptor (EGFR) and estrogen receptor (ESR) in an American population with nonsyndromic CL/P. They found a significant association between the TGFA BamHI and TaqI polymorphisms and the occurrence of cleft. Their results suggest that TGFA gene or adjacent DNA sequences may contribute to the development of a portion of cases with CL/P 33. Holder et al. (1992) studied the three variations of TGFA (BamHI, TaqI and Rsal) in a British population with CL/P, and they found a significant association between the TaqI polymorphism and occurrence of cleft 24. Stoll et al. (1992) detected a significant association with BamHI and not with TaqI in a French population of Alsatian ancestry with CL/P. They concluded that TGFA may be a modifier gene, not a major gene that may play a role in the development of bilateral cleft in some individuals 25. Chenevix-Trench et al. (1992) studied the two polymorphisms of TGFA in unrelated Australians with CL/P and a significant association between the TGFA TaqI and BamHI polymorphism and CL/P was confirmed 36. Lidral et al. (1997) evaluated the association of four candidate genes TGFA, TGFB2, TGFB3, homeobox 7 (MSX1) variations in a population from Philippines; however, no evidence for association of TGFA with nonsyndromic CL/P was found in non-Caucasian population 35. Tanabe et al. (2000) assessed the association of polymorphisms of candidate genes TGFA, TGFB and gamma-aminobutyric acid type A receptor beta3 (GABRB3) with nonsyndromic CL/P in Japanese patients, and they found that the TGFA and TGFB2 polymorphisms were associated with CL/P 30.

Conclusion

In conclusion, our study showed that there was an association between the TGFA BamHI variation and nonsyndromic CL/P in Iranian population. Since common environmental exposures especially maternal smoking could play a role in the CL/P etiology, it is suggested that further works be done to explore the role of possible gene-environment interaction in the etiology of CL/P.

Acknowledgement

We would like to thank Dr Roozrokh (Dean of Mofid Hospital) and Mofid hospital staff for their kind helps in recruiting study subjects. Moreover, this study was carried out as a part of a master of sciences thesis in Shahid Beheshti University of Medical Sciences, Tehran, Iran and Genetic Research Centre, University of Social Welfare and Rehabilitation Sciences.

Conflict of Interest

The authors report no conflicts of interest.

References


