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치의학박사 학위논문

Genetic Effect of Transforming growth factor alpha Gene Variants on the Risk of Nonsyndromic Cleft Lip with or without Palate in Korean Population

한국인에서 transforming growth factor alpha 유전자변이가 비증후군성 구순구개열 발생 위험도에 미치는 영향

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ABSTRACT

Genetic Effect of Transforming growth factor alpha Gene Variants on the Risk of Nonsyndromic Cleft Lip with or without Palate in Korean Population

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Objective: The purpose of the study was to identify the contribution of transforming growth factor alpha (TGFA) gene variants to the risk of nonsyndromic cleft lip with or without palate (NS-CL±P) in Korean families.

Materials and Methods: The samples consisted of 142 Korean NS-CL±P families and 119 control-parents having unaffected children. First, ten single nucleotide polymorphisms (SNPs) were selected by linkage disequilibrium (LD) TAG SNP selection. Minor allele frequency (MAF), heterozygosity, and X^2 test for Hardy-Weinberg equilibrium (HWE) were calculated at each ten selected SNP. Ten SNPs were used to examine

the association with case-parent trios with transmission disequilibrium test (TDT) and conditional logistic regression models (CLRMs). Both allelic and genotypic TDTs for individual SNPs and sliding windows of haplotypes consisting of two to five SNPs were tested using family- and haplotype-based association test programs. Genotypic odd ratios (GORs) were also obtained from CLRMs using STATA software. Finally, parent-of-origin effect was evaluated for all ten SNPs and comparison between 218 case-parents and 119 control-parents was performed to investigate paternal and maternal odds ratios.

Results: The family-based TDT and haplotype analysis exhibited no statistical significance but a relatively meaningful association with rs3771497 (all $P < 0.05$; 2 SNPs, rs3771497 and rs3755377; 5 SNPs, rs3771497, rs3755377, rs3771485, rs11466212 and rs3771475). G/G homozygotes at rs3771497 have significant decreased risk of being NS-CL±P (GOR = 0.30, 95% confidence interval = 0.11 - 0.80, $P < 0.01$). All SNPs did not show parent-of-origin effects. However in comparison between case-parents and control-parents, single marker analysis of maternal line showed significant association with NS-CL±P in rs3771497 ($P < 0.001$, recessive model).

Conclusion: Association of TGFA gene with NS-CL±P in Korean populations is not clearly found. However, the results of this study suggest that the TGFA genotype have an impact on the risk of NS-CL±P in Korean families. Therefore, it is needed to investigate the influence of maternal genotype on the etiologic effect of TGFA gene on NS-CL±P patients.

KEYWORDS: Transforming growth factor alpha; SNP; nonsyndromic cleft; maternal transmission

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Ⅰ. INTRODUCTION

Nonsyndromic oral clefts (NSCLP), which is divided into cleft palate only (CPO) and cleft lip with or without cleft palate (CL±P), have heterogeneous genetic backgrounds and developmental origins.[1-3] Generally, surgery, orthodontic treatment, speech therapy, and psychological consultation are required for the effective improvement of the diverse problems the NSCLP patients encounter.[4,5]

TGFA has been mapped in chromosome 2p13, is comprised of 70 to 100 kilobases of genomic DNA and has six exons.[6] TGFA is known to be involved in growth regulation of the normal tissue and neoplasia. It is secreted by the diversity of cell types, primarily of ectodermal origin, and binds to the epidermal growth factor (EGF) receptor. EGF is expressed at the medial edge epithelium of the palatal shelves during palatal fusion process.[4,7]

In murine models, EGF null mice showed an increased incidence in orofacial clefts.[8] TGFA interacts with the EGF receptor and can promote extracellular matrix synthesis and mesenchymal cell migration, ensuring the strength of the fused palate.[9] Miettinen et al.[10] also found that newborn EGF receptor knock-out mice had a high incidence of cleft palate, implying that TGFA is a likely ligand for EGF receptors. They suggested that it may explain the genetic correlation of human cleft palate with polymorphisms in TGFA gene. On the other hand, Mann et al.[11] and Luetteke et al.[12] reported that the TGFA gene null mutant mice had abnormal skin, hair, and eyes, but not oral clefts.

Case-control and case-parent triad studies in humans have reported

conflicting results. Although several etiological studies have insisted that NSCLP is associated with allelic variations of TGFA gene,[13-15] others have not observed such association.[16-20]

To date, few association studies between the risk of NS-CL±P and TGFA gene polymorphisms have been conducted using a large number of samples in Korean populations. The purpose of the study was to identify the contribution of the TGFA gene to the risk of NS-CL±P in Korean populations.

Ⅱ. REVIEW OF LITERATURE

1. Genetic approach to NS-CL±P

In recent years, advances in genetics and molecular biology have begun to reveal the basis of craniofacial development, and a number of genes associated with oral clefts have been identified.[21] Candidate gene studies have been at the core of cleft research since Ardinger and colleagues[13] suggested a role for TGFA variants in risk for NS-CL±P. Kohli and Kohli[21] reported that TGFA, drosophila msx homeobox homolog-1, 5,10-Methylenetetrahydrofolate reductase, transforming growth factor beta (TGFB) 3, and other genes and loci had been demonstrated for candidate genes.

2. TGFA : Composition and developmental function

TGFA is a protein-coding gene located in chromosome 2 and a 5.5 kDa protein containing 50 amino acid residues and three disulfide bridges.[6] Spliced transcript variants encoding different isoforms have been found for TGFA.[15]

TGFA has a 40% communal sequence identity with EGF. High amounts of TGFA/EGF receptor complexes have been noticed in some neoplasia.[4,10] TGFA and other EGF-like proteins are thought to regulate the proliferation of a wide range of epidermal and epithelial cells.[4,10,13]

In addition, TGFA is known to play a role in the autocrine growth of certain transformed cell and acts synergistically with TGFB to promote anchorage-independent cell proliferation in certain cell lines.[20]

TGFA is a well-recognized candidate gene for NS-CL±P.[15] TGFA, known to localize to the medial edge epithelial (MEE) cells in vivo, can modulate palatal extracellular matrix biosynthesis, particularly by the MEE. TGFA has been immunoloacalized in MEE cells together with its EGF receptor around the time of midline palatal epithelial seam formation and degeneration.[22] Dixon et al.[4] suggested that physiological levels of TGFA and its interaction with the EGF receptor appear to be important for normal palatal development. In immunocytochemical investigation of murine palatogenesis, they found that intense staining for TGFA was seen in the midline epithelial seam and in the subjacent mesenchyme during fusion of palatal shelves and that the regional and temporal differences was appeared in staining for EGF receptor and TGFA.[4]

3. Genetic studies of TGFA for NS-CL±P

(1) Animal study models of TGFA

Miettinen et al.[10] reported that newborn Egfr-deficient mice have facial mediolateral defects, narrow, elongated snouts, underdeveloped lower jaw, and high incidence of cleft palate. However, Luetteke et al.[12] found that TGFA deficiency results in abnormality of hair follicle and eye without oral clefts in targeted and waved-1 mice.

(2) Human studies of TGFA

Ardinger et al.[13] performed an association study comparing genetic variation in unrelated clefted individuals with that of controls in Caucasian families. In their study, a significant association was observed between two restriction fragment length polymorphisms (RFLPs) at the TGFA locus and the occurrence of clefting.[13] Therefore, they suggested that either the TGFA gene itself or DNA sequences in an adjacent

region contributed to the development of a portion of CL±P cases in humans.[13]

In a retrospective association study, Shiang et al.[14] found a significant association between alleles of TGFA gene and CPO, which suggested a role for this gene as one of the genetic determinants of craniofacial development.

Beaty et al.[15], in a case-parent trios study from three populations (Maryland, Singapore, and Taiwan), reported that TGFA gene yielded an evidence of LD among CL±P trios in Maryland and Taiwan populations.

However, Lidral et al.[17] evaluated the association data for four candidate genes (TGFB2, MSX1, TGFA, and TGFB3) using a population from the Philippines. In this case-control study, since they did not find any significant association for TGFA gene, they suggested that TGFA gene could play a less role in population from the Philippines than in Caucasians.[17] Rahman et al.[18] also reported that there was no evidence of TGFαTAq1 polymorphism in association with CL±P and CPO in Kelantan, Malaysia.

Zhu et al.[20] performed a case-parent trios study in northern and southern Chinese populations. Since they also did not find an association between the TGFA variant and risk of CL±P, they mentioned that the conflicting results regarding an association between TGFA gene and CL±P might be attributable to heterogeneity in the ethic composition, clinical characteristics, and exposure histories between patient groups.[20]

4. Gene identification and analysis methods

(1) TDT

The TDT, which was proposed by Spielman et al.[23], is a popular methods for a family-based association test. The TDT considers that parents are heterozygous for an allele associated with disease and evaluates the frequency with which allele or its alternate is transmitted to affected offspring.[23,24]

When compared with conventional tests for linkage, the TDT has some advantages because it does not require data either on multiple affected family members or on unaffected siblings. However, the TDT has limitation that it can detect linkage between the marker locus and the disease locus only if association is present.[23,24]

(2) Parent-of-origin test

When affected probands and their biological parents are genotyped at a candidate gene or a marker, the data from case-parents trio make powerful tests for linkage in the presence of association. Therefore, when linkage disequilibrium has been detected in genetic studies, the possible parent-of-origin effects should be examined.[25,26]

Various method of testing for parent-of-origin effects has been proposed; TAT (transmission asymmetry test), TDT (maternal/paternal transmission disequilibrium test), CPG (conditioning on parental genotype), PO-LRT (parent-of-origin likelihood ratio test), and CPEG (conditioning on exchangeable parental genotype).[25,26]

Although the TDT or likelihood-based methods can be used effectively for a diallelic marker, the likelihood ratio test (LRT) has several

advantages over the TDT test.[25,26] LRT can find possible prenatal maternal genetic effects mediated through the maternal phenotype, and efficiently exploit information from incomplete triad (dyad) because of a missing parent.[25,26]

If there is no expression of prenatal maternal genetic effects during gestation, the parental-asymmetry test can provide valid estimation of a parent-of-origin parameter.[25,26] In diseases which could have maternal genetic effects on risk, the PO-LRT provides a strong alternative.[25,26] In a special case where risk is determined by the child's own genotype, the LRT is closely related to the CPG method.[25,26]

Ⅲ. MATERIALS AND METHODS

Sample description

The samples consisted of 142 Korean NS-CL±P families (90 males and 52 females; 9 cleft lip, 26 cleft lip and alveolus, and 107 cleft lip and palate; 76 trios and 66 dyads, Table 1). The control group were composed of 119 Korean parents having unaffected children (60 men and 59 women). The orthodontists examined and diagnosed NS-CL±P patients for the study. Peripheral venous blood samples from the patients and their parents were collected at either Seoul National University Dental Hospital (SNUDH), SAMSUNG Medical Center (SMC), or Hallym University Chuncheon Sacred Heart Hospital with their compliance by informed consent. The study was approved by the Institutional Review Board at each institution (SNUDH IRB CRI-G07002, SMC IRB #2007-08-086, and Hallym University HIRB-2007-001).

SNPs selection and genotyping

We selected SNPs located in a region from 2kb ~ 5' to 2kb ~ 3' of the TGFA gene using the LD TAG SNP selection (TagSNP) of the SNPinfo Web server (http://anpinfo.niehs.nih.gov/snptag.htm).[25] A final set of ten SNP markers which were in strong LD ($r^2 > 0.8$) with published SNPs were chosen. All the SNPs have high heterozygosity (> 0.1) in reference to the HapMap data (JPT) and high design scores (> 0.6) provided by Illumina Inc.(San Diego, CA, USA).

Genomic DNA (gDNA) was extracted from each sample using a commercial DNA extraction kit (Quiagen Inc. Valencia, CA, USA) and quantified at the Samsung Biomedical Research Center. The

concentration of gDNA was verified on a spectrofluorometer (Perkin Elmer Inc., Norwalk, CT, USA) using the PicoGreen dsDNA quantification kit. SNP primers were synthesized with Oligator technology at Illumina Inc. and genotyped using the VeraCode Technology® at the SNP Genetics Inc. (Seoul, Korea). Genotype call rates of all SNPs and sample call rates were more than 95%.

Statistical analysis

The MAF, heterozygosity, and a X^2 test for HWE were calculated at each SNP between case and control groups. Pairwise LD was also computed as both $D'$and r^2 for all SNPs using the Haploview program (http://www.broad.mit.edu/mpg/haploview/index.php/).[24,28-29] The family-based association test program was used with both allelic and genotypic TDT for single SNP. Sliding windows of haplotypes consisting of two to five SNPs were tested by the FBAT program (http://www.biostat.harvard.edu/fbat/default.html).[24,30]

GORs for heterozygotes and homozygotes were calculated separately for single SNP with co-dominant and recessive models. GORs were acquired by match sets which consisted of the case and three pseudo-controls derived from parents and analyzed through conditional logistic regression models of the STATA software package.[24] For individual SNP analysis, P-value less than 0.05 was considered statistically significant.

Parent-of-origin effects were executed using STATA/SE 11.0 (StataCorp, College Station, Texas, USA) and STATA package (genassoc). The TAT, CPG analysis, PO-LRT, and CPEG analysis were evaluated

(http://www-gene.cimr.ac.uk/staff/clayton/courses/florence11/lectures/lecture13-4up.pdf).[26]

Comparison between 218 case-parents (76 trios and 66 dyads) and 119 control-parents was performed independently by gender to investigate paternal and maternal odds ratios using publicly available library, 'SNPassoc' in the R software (www.r-project.org). The Bonferroni correction was applied for multiple comparison and P-value less than 0.005 was considered statistically significant.

Ⅳ. RESULTS

MAF, heterozygosity, HWE, and LD analyses

The MAF for rs11466212 was the lowest one (0.094), while other MAFs showed values from 0.175 to 0.452. There was no significant evidence of deviation from HWE for all 10 SNPs in the parents ($P > 0.05$) (Table 2). Since we increased the coverage by choosing tagging SNPs based on data from the HapMap JPT samples with a threshold of $D' > 0.8$. All SNPs were not in high LD ($D' < 0.8$) except for a pair of SNPs, rs765871 and rs3771498 ($D' = 0.9$) (Figure 1).

TDT analyses for individual markers and haplotypes

P-values were calculated by chi-square sum and minimal P-value tests.[31] Although the TDT analysis in the additive model did not show any significant association with single SNPs at the $P < 0.005$ significance level, rs3771497 in the dominant and recessive models appeared to have relatively meaningful P-values ($P = 0.011$, both in the dominant and recessive models, Table 2).

The haplotype analysis in the additive model revealed that the association was not significant at the $P < 0.005$ significance level, but relatively meaningful in the haplotype including rs3771497 consisting of two and five SNPs (2 SNPs, rs3771497 and rs3755377; $P = 0.0491$, minimal P-value; and 5 SNPs, rs3771497, rs3755377, rs3771485, rs11466212 and rs3771475; $P = 0.0470$, chi-square sum).

In the strict sense statistically significant P-value was not found by Bonferroni multiple comparison correction. All P-values were appeared

greater than 0.005 value. And the significance was not increased through haplotype analysis.

GORs (Table 3)

Only the G/G homozygote at rs3771497 shows significantly inverse association in the recessive model at the $P < 0.05$ significance level (GOR = 0.30, 95% confidence interval = 0.11 - 0.80, P = 0.0068) which suggests a trend of protection to NS-CL±P.

Parent-of-origin effects (Table 4)

None out of ten SNPs showed statistically significant values in TAT, CPG analysis, and PO-LRT analysis (all $P > 0.05$). Although rs765871 appeared meaningful values in CPEG analysis, significant differences was not found by Bonferroni correction ($P = 0.0112$, $P > 0.005$).

Results of allelic tests and comparison between 218 case-parents and 119 control-parents (Table 5)

Case-Control group differences for mothers and fathers were separately estimated. Single marker analysis showed significant association between the rs3771497 and NS-CL±P, especially in relation to maternal line (P = 0.0217, Log-Additive model; P = 0.0007, Recessive model; P = 0.0028, Co-dominant model). However, the genetic risks due to the TGFA gene variants were not significant in the fathers'group. Frequencies with the rarer genotype G/G of the rs3771497 were more than 5 individuals in all comparison groups (i.e., 7 cases/23 controls and 18 cases/24 controls in father and mother groups, respectively).

V. DISCUSSION

Debates exists regarding the association between NS-CL±P and TGFA allelic variation. The previous studies have supported a role of TGFA allelic variation in the etiology of NS-CL±P,[4,13,32-34] while Stoll et al.[16] and Lidral et al.[17] reported failures to observe any association of TGFA gene with NS-CL±P. Vieira[35] suggested that TGFA gene is postulated to be a genetic modifier of clefting in humans. On the other hand, Beaty et al.[15], in a case-parent trio study from three populations (74 from Maryland, 64 from Singapore, and 95 from Taiwan), implied that ethnic background might influence the risk of cleft occurrence because TGFA gene showed significant etiological evidence in two of the three populations (Taiwan and Maryland).

Among the ten SNPs used in the present study, only rs3771475 was studied in other association studies between TGFA gene and NS-CL±P. Sull et al.[36] reported that parent-of-origin likelihood ratio test gave significant values with maternal transmission (rs3771475: P = 0.027) in CL±P case-parent trios regardless of ethnicity, though rs3771475 did not show statistical significance in single SNP and haplotype analyses. Their study was based on four populations (76 from Maryland, 146 from Taiwan, 35 from Singapore, and 40 from Korea) and ethnicity was not reflected in this result. However, the present study investigated Korean NS-CL±P patients and did not find any significant association with rs3771475 in single SNP and haplotype analyses as well as in the paternal and maternal case-control analyses.

Association of rs3771497 in the TGFA gene with NS-CL±P in Korean populations had not been reported in previous studies. The present

study, which included 76 Korean NS-CL±P case - parent trios and 66 dyads, showed not significant but relatively meaningful value of linkage disequilibrium for SNPs in the TGFA gene with rs3771497 (P = 0.011, dominant and recessive model, Table 2). The haplotype for rs3771497 also had relatively meaningful evidence of linkage and LD with rs3771497 in the 142 Korean CL±P families (2 SNPs; P = 0.0491, minimal P-value; 5 SNPs; P = 0.0470, chi-square sum, Table 2). These results implied that statistical power might be more increased as the sample size increases compared to the previous study.[36]

The finding that rs3771497 showed a lower odds ratio of 0.30 in the G/G genotype (95% CI = 0.11 - 0.80; P = 0.0068, under the assumption of recessive effect, Table 3) meant that the T allele at rs3771497 was seen more frequently in NS-CL±P patients than was the G allele and the GG genotype is associated with decreased risk of NS-CL±P. In rs3771497, the recessive mode of inheritance was best fit to the current data.

In parent-of-origin effects, the TAT, TDT, CPG, PO-LRT and CPEG analysis did not exhibit any significant maternal and paternal transmission with rs3771497. However in comparison with case and control parents tests, single marker analysis of maternal line showed more significant association with NS-CL±P in rs3771497 (P = 0.0217, Log-Additive model; P = 0.0007, Recessive model; P = 0.0028, Co-dominant model, Table 4). This case-pseudo sib control test of families have to be studied later using population studies with more sample. And in present study the result shows merely the possibility of an association between maternal side of TGFA gene and the risk of NS-CL±P.

Generally, the genetic background in the mother is known to influence the early development of offsprings selectively altering phenotypic traits.[37] And this maternal association also seems to be related with environmental factors and gene-gene interaction. Previous epidemiologic studies have suggested that several environmental factors contribute to NS-CL±P formation including maternal smoking,[34,38-40] health status,[41] drug intake,[38,42] and alcohol intake.[43] Hwang et al.[34] and Shaw et al.[38] insisted that TGFA variation and maternal smoking increased the risk of orofacial clefts. Although multivitamins containing folic acid intake is known to reduce the risk of oral clefts,[44-45] Hayes et al.[46] and Munger et al.[47] suggested that the reduced risk associated with maternal peri-conceptional multivitamin use may be affected by specific genes such as infant's genotype for human muscle segment homeobox 1 (MSX1). In addition, Jugessur et al.[48] reported that the interaction of rare variants of TGFA and MSX-1 could increase the risk of cleft palate and concluded that the gene-gene interaction may influence on the etiology of NS-CL±P.

Since the conflicting results in the previous studies may be partially attributable to differences in the NS-CL±P sample characteristics, it will be necessary to investigate more detailed clinical descriptions of the NS-CL±P patients including the severity and laterality of clefts, the presence of other dental anomalies or missing teeth, and the possible interaction between TGFA gene and environmental factors (especially maternal exposures to smoking, alcohol intake, vitamin supplementation, and etc.). Furthermore, the study should be designed to avoid any bias based on differences in family history, clinical description, genetic markers, and ethnic background.

Ⅵ. CONCLUSION

Association of TGFA gene with NS-CL±P in Korean populations is not clearly found in this study. However, the results of this study suggest that the TGFA genotype have an impact on the risk of NS-CL±P in Korean families. The G/G homozygote at rs3771497 shows significantly inverse association which suggests a trend of protection to NS-CL±P. In comparison with case-parents and control parents, single marker analysis showed significant association with the rs3771497 in relation to maternal line. Therefore, it is needed to investigate the influence of maternal genotype on the etiologic effect of TGFA gene on NS-CL±P patients.

REFERENCES

1. Mossey PA, Little J. Epidemiology of oral clefts: an international perspective. In: Wyszynski DF, ed. Cleft lip and palate: from origin to treatment. New York, NY: *Oxford University Press*, 2002:127-158.
2. Rullo R, Gombos F, Ferraraccio F, Farina A, Morano D, et al. TGF alpha has low protein expression in nonsyndromic clefts. *J Craniofac Surg*. 2007;18:1276-1280.
3. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, et al. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet.* 2010;42:525-529.
4. Dixon MJ, Garner J, Ferguson MW. Immunolocalization of epidermal growth factor (EGF), EGF receptor and transforming growth factor alpha (TGFa) during murine palatogenesis in vivo and in vitro. *Anat Embryol (Berl).* 1991;184:83-91.
5. Sanford LP, Ormsby I, Gittenberger-de Groot AC, Sariola H, Friedman R, et al. TGF*b*2 knockout mice have multiple developmental defects that are nonoverlapping with other TGF*b* knockout phenotypes. *Development.* 1997;124:2659-2670.
6. Brissenden JE, Derynck R, Francke U. Transforming growth factor alpha gene (TGFA) maps to human chromosome 2 close to the breakpoint of the t(2;8) variant translocation in Burkitt lymphoma. (Abstract). *Cytogenet Cell Genet.* 1985;40:589.
7. Lamaroon A, Tait B, Diewert VM. Cell proliferation and expression of EGF, TGF-a, and EGF receptor in the developing primary palate. *J Dent Res.* 1996;75:1534 - 1539.
8. Martinelli M, Scapoli L, Pezzetti F, Spinelli G, Lunardi S, et al. Lack

of association between common polymorphisms of epidermal growth factor receptors and nonsyndromic cleft lip with or without cleft palate. *Int J Pediatr Otorhinolaryngol.* 2009;73:929-931.
9. Dixon MJ, Ferguson MWJ. The effects of epidermal growth factor, transforming growth factor a and b and plateletderived growth factor on murine palatal shelves in organ culture. *Arch Oral Biol.* 1992;37:395 - 410.
10. Miettinen PJ, Chin JR, Shum L, Slavkin HC, Shuler CF, et al. Epidermal growth factor receptor function is necessary for normal craniofacial development and palate closure. *Nat Genet.* 1999;22:69 - 73.
11. Mann GB, Fowler KJ, Gabriel A, Nice EC, Williams RL, et al. Mice with a null mutation of the TGFa gene have abnormal skin architecture, wavy hair, and curly whiskers and often develop corneal inflammation. *Cell.* 1993;73:249 - 261.
12. Luetteke NC, Qiu TH, Peiffer RL, Oliver P, Smithies O, et al. TGFa deficiency results in hair follicle and eye abnormalities in targeted and waved-1 mice. *Cell.* 1993;73:263 - 278.
13. Ardinger HH, Buetow KH, Bell GI, Bardach J, Van Demark DR, et al. Association of genetic variation of the transforming growth factor alpha gene with cleft lip and palate. *Am J Hum Genet.* 1989;45:348-353.
14. Shiang R, Lidral AC, Ardinger HH, Buetow KH, Romitti PA, et al. Association of transforming growth-factor alpha gene polymorphisms with nonsyndromic cleft palate only (CPO). *Am J Hum Genet.* 1993;53:836-843.
15. Beaty TH, Hetmanski JB, Fallin MD, Park JW, Sull JW, et al. Analysis of candidate genes on chromosome 2 in oral cleft case-parent trios from three populations. *Hum Genet.*

2006;120:501-518.
16. Stoll C, Qian JF, Feingold J, Sauvage P, May E. Genetic variation in transforming growth factor alpha: possible association of BamHI polymorphism with bilateral sporadic cleft lip and palate. *Am J Hum Genet.* 1992;50:870-871.
17. Lidral AC, Murray JC, Buetow KH, Basart AM, Schearer H, et al. Studies of the candidate genes TGF*b*2, MSX1, TGF*a*, and TGF*b*3 in the etiology of cleft lip and palate in the Philippines. *Cleft Palate Craniofacial J.* 1997;34:1-6.
18. Rahman RA, Ahmad A, Rahman ZA, Mokhtar KI, Lah NA, Zilfalil BA, Samsudin AR. Transforming growth factor-alpha and nonsyndromic cleft lip with or without palate or cleft palate only in Kelantan, Malaysia. *Cleft Palate Craniofac J.* 2008;45:583-586.
19. Ehlers Bertoja A, Sampaio Alho C, De França E, Menegotto B, Miriam Robinson W. TGFA/TAQ I polymorphism in nonsyndromic cleft lip and palate patients from Rio Grande Do Sul, Brazil. *Cleft Palate Craniofac J.* 2008;45:539-544.
20. Zhu J, Hao L, Li S, Bailey LB, Tian Y, et al. MTHFR, TGFB3, and TGFA polymorphisms and their association with the risk of non-syndromic cleft lip and cleft palate in China. *Am J Med Genet A.* 2010;152A:291-298.
21. Kohli SS, Kohli VS. A comprehensive review of the genetic basis of cleft lip and palate. *J Oral Maxillofac Pathol.* 2012;16:64 - 72.
22. Dixon MJ, Carette MJ, Moser BB, Ferguson MW. Differentiation of isolated murine embryonic palatal epithelium in culture: exogenous transforming growth factor alpha modulates matrix biosynthesis in defined experimental conditions. *In Vitro Cell Dev Biol.* 1993;29:51-61.
23. Speilman RS, McGinnis RE, Ewens WJ. Transmission Test for

Linkage Disequilibrium: The Insulin Gene Region and Insulin-dependent Diabetes Mellitus (IDDM). *Am J Hum Genet.* 1993;52:506-516.
24. Lee JK, Park JW, Kim YH, Baek SH. Association between PAX9 single-nucleotide polymorphisms and nonsyndromic cleft lip with or without cleft palate. *J Craniofac Surg.* 2012;23:1262-1266.
25. Weinberg CR, Wilcox AJ, Lie RT. A Log-Linear Approach to Case-Parent - Triad Data: Assessing Effects of Disease Genes That Act Either Directly or through Maternal Effects and That May Be Subject to Parental Imprinting. *Am J Hum Genet.* 1998;62:969-978.
26. Weinberg CR. Methods for detection of paren-of-origin effects in genetic studies of case-parents triads. *Am J Hum Genet.* 1999;65:229-235
27. Xu Z, Taylor JA. SNPinfo: integrating GWAS and candidate gene information into functional SNP selection for genetic association studies. *Nucleic Acids Res.* 2009;37:W600-605.
28. Ardlie KG, Kruglyak L, Seielstad M. Patterns of linkage disequilibrium in the human genome. *Nat Rev Genet.* 2002;3:299 - 309.
29. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005;21:263-265.
30. Rabinowitz D, Laird NM. A unified approach to adjusting association tests for population admixture with arbitrary pedigree structure and arbitrary missing marker information. *Hum Hered.* 2000;50:211-223.
31. Tsui HW, Inman RD, Reveille JD, Tsui FW. Association of a TNAP haplotype with ankylosing spondylitis. *Arthritis Rheum.* 2007;56:234-243.
32. Holder SE, Vintiner GM, Farren B, Malcolm S, Winter RM. Confirmation of an association between RFLPs at the transforming

growth factor-alpha locus and non-syndromic cleft lip and palate. *J Med Genet.* 1992;29:390 - 392.
33. Vintiner GM, Holder SE, Winter RM, Malcolm S. No evidence of linkage between the transforming growth factor-alpha gene in families with apparently autosomal dominant inheritance of cleft lip and palate. *J Med Genet.* 1992;29:393 - 397.
34. Hwang SJ, Beaty TH, Panny SR, Street NA, Joseph JM, et al. Association study of transforming growth factor alpha (TGFa) TaqI polymorphism and oral clefts: indication of gene-environment interaction in a population-based sample of infants with birth defects. *Am J Epidemiol.* 1995;141:629 - 636.
35. Vieira AR. Association between transforming growth factor alpha gene and nonsyndramoc oral clefts: A HuGE review. *Am J Epidemiol.* 2006;163:790-810.
36. Sull JW, Liang KY, Hetmanski JB, Wu T, Fallin MD, et al. Evidence that TGFA influences risk to cleft lip with/without cleft palate through unconventional genetic mechanisms. *Hum Genet.* 2009;126:385-394.
37. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR,DymovS, Szyf M, Meaney MJ. Epigenetic programming by maternal behavior. *Nat Neurosci.* 2004;7:847-54.
38. Shaw GM, Wasserman CR, Lammer EJ, O'Malley CD, Murray JC, et al. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. *Am J Hum Genet.* 1996;58:551 - 561.
39. Wyszynski DF, Duffy DL, Beaty TH. Maternal cigarette smoking and oral clefts: a meta analysis. *Cleft Palate Craniofac J.* 1997;34:206-210.
40. Zeiger JS, Beaty TH, Liang KY. Oral clefts, maternal smoking, and

TGFA: a meta-analysis of gene-environment interaction. *Cleft Palate Craniofac J.* 2005;42:58-63

41. Peteraka M, Tvrdek M, Likousky Z, Peterkova R, Fara M. Maternal hyperthermia and infection as one of possible causes of orofacial clefts. *Acta Chir Plast.* 1994;36:114-118.
42. Edison RJ, Muenke M. Central nervous system and limb anomalies in case reports of fist- trimester statin exposure. *N Engl J Med.* 2004;350:1579-1582.
43. Khoury MJ, Gomez-Farias M, Mulinare J. Does maternal cigarette smoking during pregnancy cause cleft lip and palate in offsprings? *Am J Dis Child.* 1989;143:333-337.
44. Shaw GM, Wasserman CR, Murray JC, Lammer EJ. Infant TGF-Alpha Genotype, Orofacial Clefts, and Maternal Periconceptional Multivitamin Use. *Cleft Palate Craniofacial J.* 1998;35:366-370.
45. Tolarova MM, Harris JA. Reduced recurrence of orofacial clefts after periconceptional supplementation with high-dose folic acid and multivitamins. *Teratology.* 1995;51:71 - 78.
46. Hayes C, Werler MM, Willett WC, Mitchell AA. Case-control study of periconceptional folic acid supplementation and oral clefts. *Am J Epidemiol.* 1996;143:1229 - 1234.
47. Munger R, Lidral A, Basart A, Romitti P, Murray J. The Msx-1 homeobox gene and risk of isolated orofacial clefts: an effect modified by maternal vitamin use? *Am J Epidemiol.* 1995;141;(Suppl.):S74.
48. Jugessur A, Lie RT, Wilcox AJ, Murray JC, Taylor JA, et al. Variants of developmental genes (TGFA, TGFB3, and MSX1) and their associations with orofacial clefts: A case-parent triad analysis. *Genet Epidemiol.* 2003;24:230 - 239.

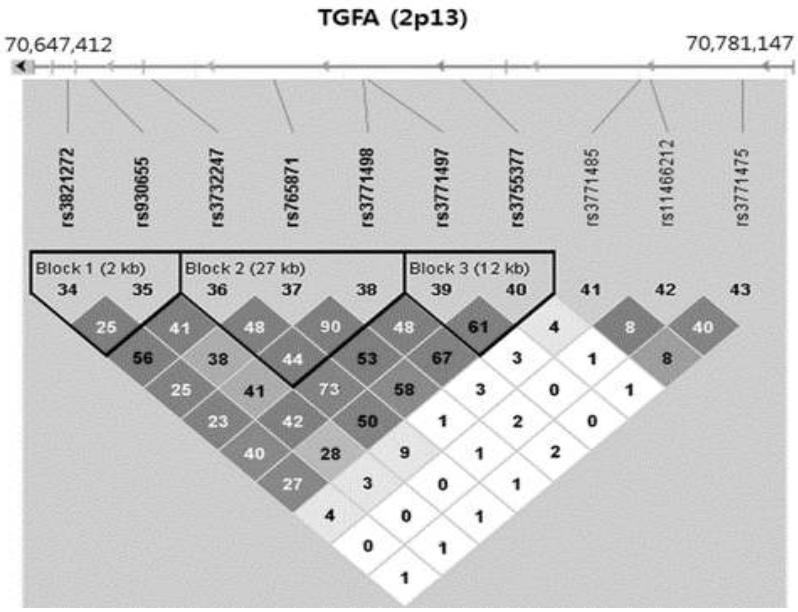

Figure1. Linkage disequilibrium (LD) plot of transforming growth factor alpha (TGFA). The level of pairwise LD is expressed by color [bright red, D′ = 1 and logarithm of odds ratio (LOD) ≥ 2; shades of pink/red, D′ < 1 and LOD ≥ 2; blue, D′ = 1 and LOD < 2; white, D′ < 1 and LOD < 2].

Table 1. Cleft type and gender among 142 Korean non-syndromic cleft lip with or without cleft palate (CL±P) families.

		CL		CLA		CLP		Sum	
		unilateral	bilateral	unilateral	bilateral	unilateral	bilateral		
Trio	Male	2	1	9	2	17	17	48	76
	Female	1	0	2	1	16	8	28	
Dyad	Male	2	0	7	1	19	13	42	66
	Female	3	0	3	1	12	15	24	

CL represents cleft lip only; CLA, cleft lip and alveolus; CLP, cleft lip and palate.

Table 2. Marker information and transmission disequilibrium test (TDT) results for ten single nucleotide polymorphisms (SNPs) in the TGFA gene showing relatively meaningful values of linkage and LD in 142 CL±P families

SNP	M/m[a]	MAF	HWP (p)	T/NT[b]	Allele (p)[c]			Haplotype (p)[c] - Additive model			
					Additive	Dominant	Recessive	2	3	4	5
rs3821272	**T/C**	0.311	0.077	30:22	0.267	0.384	0.439	0.3398	0.6352	0.3799	0.2969
						0.439	0.384	0.3711	0.7437	0.4581	0.3311
rs930655	**G/A**	0.357	0.384	36:25	0.204	0.058	0.729	0.3908	0.3786	0.3033	0.3595
						0.729	0.058	0.3973	0.3872	0.2637	0.3588
rs3732247	**G/A**	0.429	0.443	37:30	0.392	0.732	0.317	0.4262	0.3935	0.5964	0.2776
						0.317	0.732	0.6477	0.6449	0.7612	0.7285
rs765871	C/T	0.399	1.000	38:32	0.553	0.221	0.078	0.5953	0.5520	0.2975	0.3980
						0.078	0.221	0.4531	0.6749	0.5810	0.7998
rs3771498	A/G	0.380	0.832	37:35	0.907	0.173	0.246	0.4946	0.2209	0.3369	0.4808
						0.246	0.173	0.5338	0.1699	0.6367	0.7234
rs3771497	**T/G**	0.452	0.516	40:31	0.285	0.435	**0.011**	0.1053	0.1635	0.1444	**0.0470**
						0.011	0.435	**0.0491**	0.3991	0.3643	0.2067
rs3755377	C/T	0.445	0.195	36:33	0.718	0.131	0.384	0.6212	0.5487	0.1991	
						0.384	0.131	0.5494	0.3650	0.1990	
rs3771485	**G/C**	0.435	0.884	42:36	0.371	0.595	0.389	0.6240	0.3534		
						0.389	0.595	0.6650	0.7246		
rs11466212	**T/C**	0.094	0.822	15:11	0.433	0.574	0.574	0.3123			
								0.5978			
rs3771475	A/G	0.175	0.997	26:18	0.180	0.354	0.354				

[a] Over-transmitted alleles are in bold type.

[b] Transmission/non-transmission counts from heterozygous parents.

[c] Significant *P* values for individual SNP and global *P*-values for sliding windows of haplotypes of two to eight SNPs from TDT analyses. Tests significant after SNPSpD correction (SNP spectral decomposition method; an effective Bonferroni-type http://gump.qimr.edu.au/general/ daleN//) are shown in bold. First-line values on the table shows p-values obtained from chi-square test and second line is minimal p-value test results.

MAF represents minor allele frequency; HWE, Hardy-Weinberg equilibrium; TDT, transmission disequilibrium test; SNP, single nucleotide polymorphism

Table 3. Genotypic ORs for heterozygotes and homozygotes for individual SNPs showing significant evidence of linkage and LD in 142 CL±P families

SNP	Genotype	N[a]	Co-dominant model		Recessive model	
			GOR (95% CI)	P-value[b]	GOR (95% CI)	P-value[b]
		n[a] = 76				
rs3771497	T/T	107	-	0.0137		**0.0068****
	T/G	187	0.48 (0.74 - 2.97)			
	G/G	66	0.43 (0.14 - 1.35)		**0.30 (0.11 - 0.80)**	

LD means linkage disequilibrium; CI, confidence interval; **, P<0.01.

[a] 'N' and 'n' refer to the number of subjects carrying the genotype and the number of case/pseudo-control sets generated, respectively.

[b] P values of X^2 tests for the conditional logistic regression model for each SNP.

Table 4. The results of parent-of-origin test for ten SNPs in the TGFA gene

	TAT (Transmisson Asymmetry Test)	TDT		CPG	PO-LRT		CPEG
		Maternal transmission	Paternal transmission		Test of parental origin effects	Test of parental origin effects with maternal genotype	
rs3821272	0.5495	0.6547	0.2008	0.5359	0.3200	0.5200	0.0686
rs930655	0.9028	0.4142	0.2207	0.7354	1.0000	0.7100	0.2380
rs3732247	0.9181	0.4652	0.4913	0.9680	0.5900	0.8900	0.3311
rs765871	0.2401	0.1936	0.8185	0.2481	0.4000	0.2200	0.0112*
rs3771498	0.6456	0.5775	0.8273	0.5388	0.3800	0.6600	0.3300
rs3771497	0.1880	0.8474	0.0593	0.1335	0.7400	0.1500	0.0823
rs3755377	0.4779	0.4497	0.8084	0.4581	0.6000	0.4900	0.1481
rs3771485	0.2447	0.2230	0.8474	0.2435	0.5900	0.2400	0.0544
rs11466212	0.3575	1.0000	0.2059	0.2830	0.7400	0.2800	0.3830
rs3771475	0.2607	0.8273	0.1083	0.2453	0.0760	0.0960	0.6696

TAT represents transmission asymmetry test; conditioning on parental genotype, CPG; PO-LRT, parent-of-origin likelihood ratio test; conditioning on exchangeable parental genotype, CPEG.

*represents $P < 0.05$

Table 5. Paternal and maternal odds ratio (OR) of ten SNPs in the TGFA gene in comparison with 218 case-parents and 119 control-parents

		Gender	MM/Mm/mm		log-Additive		Dominant		Recessive		Co-dominant	
			Case	Control	OR (CI: L95 - U95)	P-value	OR (CI: L95 - U95)	P-value	OR (CI: L95 - U95)	P-value	OR (CI: L95 - U95)	P-value
		Both	49.8/37.8/12.4	43.7/45.4/10.9	0.69 (0.32-1.48)	0.3359	0.57 (0.19-1.69)	0.3088	0.68 (0.15-3.09)	0.6115	0.53 (0.11-2.68)	0.5915
rs3821272	T/C	Paternal	46.8/38.3/14.9	56.7/35.0/8.3	1.39 (0.49-3.96)	0.5408	1.62 (0.36-7.29)	0.5254	1.50 (0.16-13.95)	0.7220	1.75 (0.17-17.58)	0.8145
		Maternal	52.0/37.4/10.6	30.5/55.9/13.6	0.34 (0.09-1.30)	0.08948	0.20 (0.03-1.29)	0.0720	0.37 (0.03-4.07)	0.3893	0.16 (0.01-2.28)	0.1934
		Both	43.3/42.4/14.3	36.1/53.8/10.1	1.16 (0.52-2.59)	0.7243	1.45 (0.47-4.51)	0.5178	0.84 (0.18-4.03)	0.8295	1.10 (0.20-6.21)	0.7435
rs930655	G/A	Paternal	43.6/42.6/13.8	21.7/68.3/10.0	0.78 (0.25-2.44)	0.6626	0.49 (0.09-2.66)	0.4018	1.28 (0.17-9.84)	0.8118	0.72 (0.07-7.83)	0.6299
		Maternal	43.1/42.3/14.6	50.8/39.0/10.2	1.54 (0.43-5.46)	0.5061	3.29 (0.55-19.82)	0.1738	0.13 (0.00-12.76)	0.3129	0.22 (0.00-48.58)	0.1556
		Both	33.6/46.1/20.3	30.3/51.3/18.5	0.81 (0.39-1.66)	0.5603	0.79 (0.26-2.44)	0.6843	0.68 (0.19-2.51)	0.5657	0.64 (0.15-2.77)	0.8306
rs3732247	G/A	Paternal	36.2/39.4/24.5	36.7/51.7/11.7	1.63 (0.56-4.75)	0.3614	1.23 (0.28-5.47)	0.7882	4.57 (0.53-39.25)	0.1546	4.11 (0.41-41.04)	0.3519
		Maternal	31.7/51.2/17.1	23.7/50.8/25.4	0.43 (0.13-1.41)	0.1484	0.48 (0.07-3.20)	0.4454	0.19 (0.02-1.63)	0.0957	0.17 (0.01-2.10)	0.24707
		Both	35.9/48.8/15.2	40.3/47.1/12.6	1.17 (0.54-2.52)	0.6930	1.86 (0.62-5.61)	0.2631	0.54 (0.12-2.49)	0.4277	0.83 (0.16-4.33)	0.23338
rs765871	C/T	Paternal	37.2/43.6/19.1	26.7/56.7/16.7	0.62 (0.20-1.93)	0.4055	0.87 (0.18-4.13)	0.8561	0.24 (0.03-2.02)	0.1858	0.27 (0.03-2.82)	0.4070
		Maternal	35.0/52.8/12.2	54.2/37.3/8.5	2.02 (0.62-6.56)	0.2301	3.88 (0.62-24.37)	0.1242	1.43 (0.14-14.79)	0.7655	2.70 (0.20-36.36)	0.2840
		Both	37.8/48.8/13.4	42.9/47.1/10.1	1.40 (0.61-3.23)	0.4208	2.17 (0.72-6.54)	0.1624	0.60 (0.10-3.50)	0.5675	0.95 (0.14-6.22)	0.2170
rs3771498	A/G	Paternal	40.4/43.6/16.0	28.3/58.3/13.3	0.81 (0.24-2.72)	0.7306	0.83 (0.18-3.91)	0.8144	0.64 (0.05-7.99)	0.7321	0.59 (0.04-8.90)	0.9310
		Maternal	35.8/52.8/11.4	57.6/35.6/6.8	2.14 (0.62-7.34)	0.2214	5.17 (0.82-32.44)	0.0605	0.55 (0.03-10.45)	0.6896	1.17 (0.04-32.27)	0.0896
		Both	30.9/47.5/21.7	29.4/49.6/21.0	0.81 (0.40-1.64)	0.5561	1.21 (0.39-3.71)	0.7434	0.40 (0.11-1.46)	0.1593	0.57 (0.13-2.43)	0.2282
rs3771497	T/G	Paternal	33.0/42.6/24.5	36.7/51.7/11.7	2.50 (0.80-7.79)	0.0958	2.40 (0.50-11.55)	0.2650	6.06 (0.71-52.06)	0.0857	7.91 (0.73-85.62)	0.1965
		Maternal	29.3/51.2/19.5	22.0/47.5/30.5	0.25 (0.07-0.93)	**0.0217***	0.56 (0.08-3.75)	0.5461	0.01 (0.00-0.50)	**0.0007*****	0.02 (0.00-0.96)	**0.0028****
		Both	33.2/45.2/21.7	35.3/48.7/16.0	1.27 (0.61-2.64)	0.517	1.67 (0.55-5.04)	0.3607	1.05 (0.27-4.13)	0.9436	1.43 (0.31-6.50)	0.6305
rs3755377	C/T	Paternal	35.1/39.4/25.5	26.7/53.3/20.0	0.83 (0.30-2.25)	0.708	0.54 (0.11-2.69)	0.4454	1.18 (0.21-6.72)	0.8533	0.74 (0.10-5.66)	0.6595
		Maternal	31.7/49.6/18.7	44.1/44.1/11.9	1.80 (0.56-5.77)	0.3214	4.40 (0.74-26.17)	0.0860	0.52 (0.04-7.09)	0.6188	1.24 (0.07-22.48)	0.1157
		Both	31.8/49.8/18.4	35.3/53.8/10.9	1.60 (0.73-3.53)	0.2348	1.45 (0.47-4.46)	0.5163	2.79 (0.64-12.25)	0.1674	3.02 (0.59-15.57)	0.3763
rs3771485	G/C	Paternal	38.3/42.6/19.1	40.0/53.3/6.7	2.08 (0.69-6.29)	0.1802	1.97 (0.41-9.60)	0.3944	4.21 (0.52-33.83)	0.1584	5.17 (0.51-52.64)	0.3406
		Maternal	26.8/55.3/17.9	30.5/54.2/15.3	1.13 (0.33-3.81)	0.8497	1.01 (0.19-5.34)	0.9928	1.56 (0.14-17.08)	0.7177	1.48 (0.11-19.88)	0.9320
		Both	81.6/18.0/0.5	79.8/18.5/1.7	1.10 (0.39-3.15)	0.8535	0.95 (0.28-3.30)	0.9411	2.93 (0.17-50.15)	0.4720	2.80 (0.16-48.55)	
rs1146621	T/C	Paternal	85.1/14.9/0.0	78.3/20.0/1.7	0.38 (0.06-2.46)	0.2994	0.38 (0.06-2.50)	0.3084	0.00 (0.00-)	0.7036	0.00 (0.00-)	
		Maternal	78.9/20.3/0.8	81.4/16.9/1.7	2.72 (0.66-11.31)	0.1634	2.93 (0.46-18.69)	0.2486	8.04 (0.29-219.43)	0.2244	10.12 (0.34-302.49)	
		Both	67.3/30.0/2.8	63.9/31.9/4.2	1.31 (0.58-2.99)	0.5168	1.46 (0.49-4.33)	0.4953	1.35(0.19-9.40)	0.7628	1.53 (0.21-11.11)	0.7344
rs3771475	A/G	Paternal	71.3/26.6/2.1	58.3/36.7/5.0	0.68 (0.22-2.12)	0.5074	0.63 (0.14-2.82)	0.5465	0.53(0.04-7.26)	0.6299	0.46 (0.03-6.76)	0.5763
		Maternal	64.2/32.5/3.3	69.5/27.1/3.4	2.74 (0.77-9.75)	0.1206	3.43 (0.63-18.63)	0.1455	4.95(0.27-92.52)	0.3063	6.99 (0.35-138.51)	0.3591

Data were analyzed using'SNPassoc' in the R software (www.r-project.org)

MM/Mm/mm denotes major homozygous-/heterozygous-/minor homozygous genotype frequencies.

* represents $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

국문초록

한국인에서 transforming growth factor alpha 유전자 변이가 비증후군성 구순구개열 발생 위험도에 미치는 영향

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목 적 : 본 연구의 목적은 한국인에서 transforming growth factor alpha (TGFA) 유전자 변이가 비증후군성 구순구개열 발생 위험도에 미치는 영향을 규명하기 위함이다.

방 법 : 연구대상은 142명의 한국인 비증후군성 구순구개열 환자와 그 부모 218명 (76 trios, 66 dyads) 및 119명의 정상인 자녀를 가진 대조군 부모였다. 말초혈액으로부터 DNA를 추출하였고, 총 10개의 TGFA 단일염기다형성 (SNP) 을 선택하였다. 각 SNP 에 대한 minor allele frequency, heterozygosity 및 X2 test for Hardy-Weinberg equilibrium 값을 계산하였고, transmission disequilibrium test (TDT) 와 conditional logistic regression models (CLRMs) 을 사용하여 case-parent trios의 연관성을 분석하였다. 단일 SNP 과 2개에서 5개의 SNP 으로 이루어진 haplotype 분석을 위해서 family- and haplotype-based association test program의 allelic and genotypic TDT 를 시행하였다. Genotypic odds ratios (GORs) 결과는 STATA software의 CLRMs 분석을 통해 얻었다. 마지막으로 각 SNP 의 parent-of-origin effect를 확인하기 위해 transmission asymmetry test (TAT), conditioning on parental genotypes

(CPG) analysis, parent-of-origin likelihood ratio test (PO-LRT), conditioning on exchangeable parental genotypes (CPEG) analysis를 사용하였으며, 218명의 환자군 부모와 119명의 대조군 부모와의 비교 분석을 위해 부계와 모계의 odds ratio를 검사하였다.

결 과 :

1. 단일 SNP 와 haplotype 에 대한 family-based TDT 분석 결과, 1개의 SNP (rs3771497)에서 multiple comparison correction 시행한 경우 통계적으로 유의하지는 않았지만 의미있는 연관성을 얻었다 (2 SNPs, rs3771497 and rs3755377; 5 SNPs, rs3771497, rs3755377, rs3771485, rs11466212 and rs3771475; all $P < 0.05$).

2. GORs 분석에서는 rs3771497의 G/G homozygote에서 비증후군성 구순구개열 발생 위험성 감소의 유의한 연관성이 나타났다 (GOR = 0.30, 95% confidence interval = 0.11 - 0.80, $P < 0.01$).

3. Parent-of-origin effects test에서 모든 SNP는 유의한 결과를 보이지 않았다. 그러나 환자군 부모와 대조군 부모를 비교한 결과, 모계의 단일 마커 분석시 rs3771497에서 비증후군성 구순구개열과 유의한 연관성을 나타내었다 ($P < 0.001$, recessive model).

결 론 : 한국인에서 비증후군성 구순구개열과 TGFA 유전자 간의 명확한 연관성은 확인하지 못했다. 그러나 이 연구 결과 TGFA 유전자형이 한국인 가계의 구순구개열 발생 위험도에 영향을 주는 것을 발견하였다. 향후 TGFA 유전자가 구순구개열의 발생에 미치는 효과를 모계 유전자형의 영향력 측면에서 연구할 필요가 있다.

주요어 : Transforming growth factor alpha; 단일염기다형성; 비증후군성 구순구개열; 모계유전

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