ORIGINAL ARTICLE

KOREA. J. ORTHOD.
2003. : 33(3) : 185-197

Sequencing analysis of the OFC1 gene on the nonsyndromic cleft lip and palate patient in Korean

Sung-Sik Kim[1], Woo-Sung Son[2]

This study was performed to identify the characteristics of the OFC1 gene (locus: chromosome 6p24.3) in Korean patients, which is assumed to be the major gene behind the nonsyndromic cleft lip and palate. The sample consisted of 80 subjects: 40 nonsyndromic cleft lip and palate patients (proband, 20 males and females, mean age 14.2 years); and 40 normal adults (20 males and 20 females, mean age 25.6 years). Using PCR-based assay, the OFC1 gene was amplified, sequenced, and then searched for similar protein structures. Results were as follows:

1. The OFC1 gene contains the microsatellite marker 'CA' repeats. The number of the reference 'CA' repeats was 21 times, and formed as TA(CA)11TA(CA)10. But,in Koreans, the number of tandem 'CA' repeats was varied from 17 to 26 except 18, and 'CA' repeats consisted of TA(CA)n.
2. Nine allelic variants were found. Distribution of the OFC1 allele was similar between the patients and control group.
3. There was a replacement of the base 'T' to 'C' after 11 tandem 'CA' repeats in Koreans compared with Weissenbach's report. However, the difference did not seem to be the ORF prediction results between Koreans and Weissenbach's report.
4. The BLAST search results showed the Telomerase reverse transcriptase (TERT) and the Nucleotide binding protein 2 (NBP2) as similar proteins. The TERT was a protein product by the hTERT gene in the locus 5p15.33 (NCBI Genome Annotation; NT023089). The NBP2 was a protein product by the ABCC3 (ATP-binding cassette, sub-family C) gene in the locus 17q22 (NCBI Genome Annotation; NT010783).
5. In the Pedant-Pro database analysis, the predictable protein structure of the OFC1 gene had at least one transmembrane region and one non-globular region.

Key words : Nonsyndromic cleft lip and palate, OFC1 gene, Mirosatellite marker, CA repeats

Clinical professor[1], Professor[2].
Department of orthodontics, Dental College, Pusan National Universit.

Reprint requests to : **Sung-sik Kim**
1-10, Seo-Gu, Ami-Dong, Pusan, Korea, 602-739
Department of orthodontics, Dental College, Pusan National University.
82-51-240-7447 / softid@korea.com

A cleft lip and palate is a common birth defect in the head and neck regions.[1,2] Their frequency varies across populations of different geographic origins. In Korea this condition afflicts about 1 in 500 live births, resulting in the need for surgical, speech, psychological, dental, and cosmetic interventions.[3]

There are more than 300 described syndromes that have a cleft lip and palate as associated characteristics.[4,5] If the cleft were genetic in origin and associated with a syndrome (eg, Van der Woude syndrome, Apert syndrome), its etiology could be easily determined by understanding the genetic transmission of the particular syndrome. However, most cleft lip and palate cases are of a nonsyndromic type that do not accompany syndromes, and this nonsyndromic form is known to affect as much as 70% of the whole cleft lip and palate phenotype.[6] Nonsyndromic cleft lip and palate may be defined as complex traits, since they do not exhibit classic Mendelian recessive or dominant inheritance attributable to any single locus, but do show strong familial aggregation and have a substantial genetic component.[7-9] Therefore, a major objective of research into the cleft lip and palate is to develop an under-standing of the etiology of its nonsyndromic form.[10]

Genetic analyses of the nonsyndromic cleft lip and palate have yielded, in recent years, significant success. Ardinger et al.[11] observed a significant association between 2 RFLPs (Restriction Fragment Length Polymorphisms) at the TGFA (transforming growth factor alpha gene) locus and the occurrence of clefting. Chenevix-Trench et al.[12] confirmed signifi-cant associations with the TGFA Taq I and Bam HI RFLPs. In a view of the known teratogenic role of retinoic acid, the nonsyndromic cleft lip and palate had a significant association with a Pst I RFLP of RARA (retinoic acid receptor alpha gene).[13] There was no evidence of interaction between the TGFA and RARA poly-morphisms on risk, but jointly they appeared to account for almost half of the attributable risk of clefting.[14-18] Using a large populationbased casecontrol study, Shaw et al.[19] showed an example of gene-environment interaction that clefting risks were even greater for infants with the TGFA allele previously associated with clefting when the mothers smoked 20 or more cigarettes per day. These risks for white infants ranged from 3-fold to 11-fold across pheno-typic groups.[20] Several studies have demonstrated an association between facial shape in parents and the presence of oral clefts in their offspring. It was assumed that facial shape was one of the predi-sposing components among many in a multifac-torial model of inheritance. By cephalometric analysis of a large family with five generations of affected indivi-duals, Ward et al.[21] concluded that facial shape can be used to identify presumed carriers of a major gene associated with an increased risk for cleft lip and palate. Davies et al.[22] suggested the presence of a locus for cleft lip and palate in chromosome 6 by YAC clones from contigs in 6p25-p23. Stein et al.[23] found that APOC2 (apolipoprotein C-II gene), which is located at 19q13.1 (or 19q13.[2]) and linked to the proto-onco-gene BCL3 (B-cell leukemia/lymphoma 3 gene), gave suggestive evidence for linkage to cleft lip with or with-out palate. Scapoli et al.[24] conducted a linkage study of 38 families using microsatellite markers that mapped to 6p24-p23, and demonstrated the presence of a locus for cleft lip with or without palate in the 6p23 chromo-some region. Lidral et al.[25] suggested that MSX1 (muscle segment homeo box 1 gene) and TGFB3 (transforming growth factor beta 3 gene) are both involved in the pathogenesis of clefting.

The current list of candidate genes for nonsyndromic cleft lip and palate includes OFC1 (orofacial cleft 1 gene), TGFA, RARA, BCL3, MSX1, MTHFR (methy-lenetetrahydrofolate reductase gene), OFC2 (orofacial cleft 2 gene), OFC3 (orofacial cleft 3 gene), and TGFB3.[26-32] This list will continue to evolve as additional candidate loci are identified from genome scans, studies of syndromic forms of cleft lip and palate, transgenic mice, expression assays, and knowledge of gene functions. At the present time, the strongest evidence implicates a primary gene on 6p24.3 (OFC1)

and a role for TGFA as a modifier of nonsyndromic cleft lip and palate genes.[33,34] However, there is not clear evidence while major genetic loci for nonsyndromic cleft lip and palate has not yet been examined closely, and furthermore there has been little research about specifically Korean subjects. This study was performed to identify the characteristics of the OFC1 gene in Koreans.

MATERIALS AND METHODS

Individuals who visited the Department of Orthodontics, Pusan National University, during the period from 1998 to 2002 who had nonsyndromic cleft lip and palate were included in the study. They consisted of 40 probands (20 males and 20 females). The mean age was 14.2 years (12.6 – 18.3 years). The control group consisted of 40 subjects (20 males and 20 females), who did not have any birth defects or syndromes for three generations. The mean age was 25.6 years (24.3 – 28.2 years).

Experimental and control group samples were analyzed for allelic variants of the OFC1 genes, using PCR (Polymerase chain reaction)-based assays. 30 mL of whole blood was collected to yield large amounts of DNA (> 100 ㎍). Genomic DNA was obtained from lymphocyte in these samples with the Wizard® Genomic DNA purification kit (Cat. #A1120, Promega, Madison, WI, USA).

To perform PCR, a reaction mix was prepared with 3 ㎕ of DNA, 1 ㎕ of each primer pairs, and 15 ㎕ distilled water. A total volume of 20 ㎕ mixture was added to the AccuPower PCR PreMix kit (Cat. #K-2016, Bioneer, Korea) tube; then the PCR started. The primer concentrations and reaction are described below.

Forward primers : 5'-AAGGCTAAGTCTCTGTGGGG-3' ; reverse primer: 5'-GCTTAACTCAGCACTGGGAT-3' ; both at 10pmol/㎕. The primer sequence was obtained from microsatellite marker AFM035wc1 (GDB: 161588).[35,36] The 279 base pair region of the OFC1 gene was amplified for 35 cycles (MWG-Biotech AG, Ebersberg, Germany) : The steps of 1 cycle are as follpredenaturation at 94℃ for 5 minutes ; denaturation at 95℃ for 30 seconds ; annealing at 55℃ for 30 seconds ; elongation at 72℃ for 60 seconds ; and postelongation at 72℃ for 10 minutes. The PCR products were digested for 3 hours at 37℃, and visualized by 2% agarose gel electrophoresis (100 V for 3 hours) (Fig. 1).

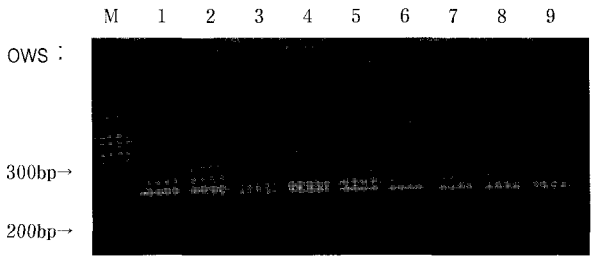


Fig. 1. 2 % agarose gel electrophoresis of OFC1 gene PCR product. M: molecular weight marker ; lane 1,2,3,4 : nonsyndromic cleft lip and palate patients; lane 5,6,7,8,9 : normal controls ; bp : basepair

All PCR products were separated by using 2% agarose gel electrophoresis, stained with ethidium bromide 0.2 g/mL and visualized under ultraviolet light. All PCR screening methods used in this study have been validated.[36]

Sequencing was performed in both directions on DNA samples. Templates included PCR products purified by a QIAEX® II Gel Extraction kit (Cat. No. 20021, QIAGEN, Valencia, CA, USA). Templates were sequenced by means of an ABI 3700 sequencer (Applied Biosystems Inc., Foster City, CA, USA) machine.

Two types of analyses were performed. First, we compared references with the allelic variants by basepair sequence alignment of the experimental and control group. We used the 292 basepair genomic DNA contig of 6p24.3 in The ABI linkage mapping panel 2″ as a reference (taxon : 9606) (Table 1). To assemble the for-ward and reverse sequence alignment, the Contig Express program was used (Contig Express, Vector NTI Suite 8 package, Infomax, Frederick, Maryland, USA) (Fig. 2). All samples were compared by the Align X

Table 1. Sequence alignment of OFC1 gene in reference

Locus Type
: gene with protein product, function known or inferred
: source 1..292 /chromosome="6"
/db_xref="taxon:9606"
/organism="Homo sapiens"

BASE	COUNT	85 a	76 c	64 g	67 t	
ORIGIN	1	ACCTACAACA	GGTAAGGCTA	AGTCTCTGTG	GGGTTGAGGT	CCCTTGCCCC
	51	CAGCGCACAT	ACACACACAC	ACACACACAC	ACATACACAC	ACACACACAC
	101	ACACAGCCAC	CAATGCCATG	CTCATGCTTT	GTATAGTTTT	GGGATTTGTG
	151	ACAACTTTTA	AGTCTGAGGG	GAATGAATGT	ACTCATGAAG	AAAATCTCCA
	201	CCGTTAAGGA	AATGCCCTTG	GTGTGGACTA	GTGCTAGAGG	CACAGACCTT
	251	GAGTAGGGAG	TCCTGAATTC	CTATCCCAGT	GCTGAGTTAA	GC

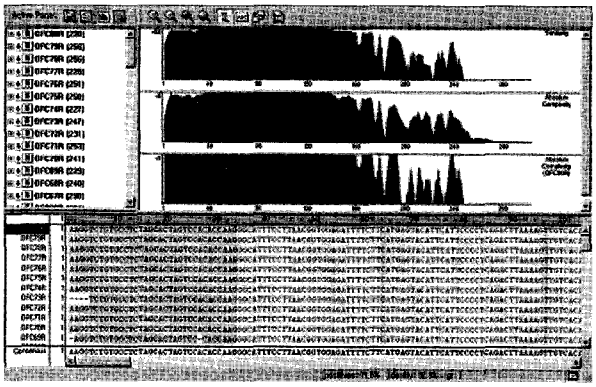


Fig. 2. To find the consensus sequence of OFC1 gene, comparison were performed by Align X program (Vector NTI Suite 8 package, Infomax, Frederick, Maryland, USA)

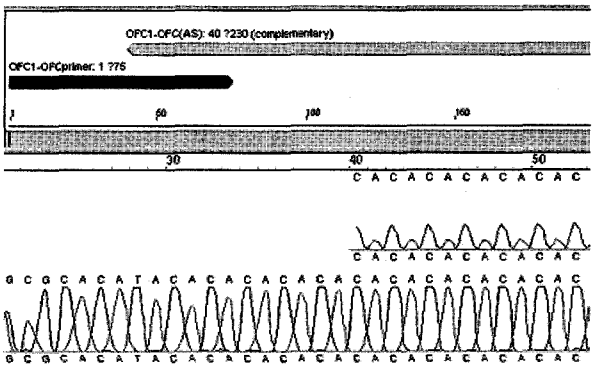


Fig. 3. Assembling of the forward and reverse strands by Contig Express (Vector NTI Suite 8 package, Infomax, Frederick, Maryland, USA); upper sequence: forward directed DNA sequencing result of OFC1 gene; lower sequence: reverse directed DNA sequencing result of OFC1 gene

program (Align X, Vector NTI Suite 8 package, Infomax, Frederick, Maryland, USA) (Fig. 3). Second, we predicted whether similar protein existed: ORF (open reading frame) prediction was performed by Vector NTI Suite 8 program ; PSI-BLAST was used for searching inferred protein against all known sequences contained in SWISS-PROT and EMBLNEW. We included all hits below a PSI-BLAST E-value of 10-2 for more available results, and used the Pedant-Pro database to predict the structure of inferred protein product by the OFC1 gene and confirmed the PSI-BLAST results.

Table 2. Sequence alignment of OFC1 gene allelic variants in Korean

Reference	sequence	---TA(CA)11TA(CA)10---	Ratio in NSCLP (%)	Ratio in control (%)
Allele	Type A	---TA(CA)17 ---	2.5	2.5
	Type B	---TA(CA)19 ---	0	7.5
	Type C	---TA(CA)20 ---	5	5
	Type D	---TA(CA)21 ---	12.5	12.5
	Type E	---TA(CA)22 ---	25	27.5
	Type F	---TA(CA)23 ---	25	15
	Type G	---TA(CA)24 ---	20	25
	Type H	---TA(CA)25 ---	10	0
	Type I	---TA(CA)26 ---	0	5

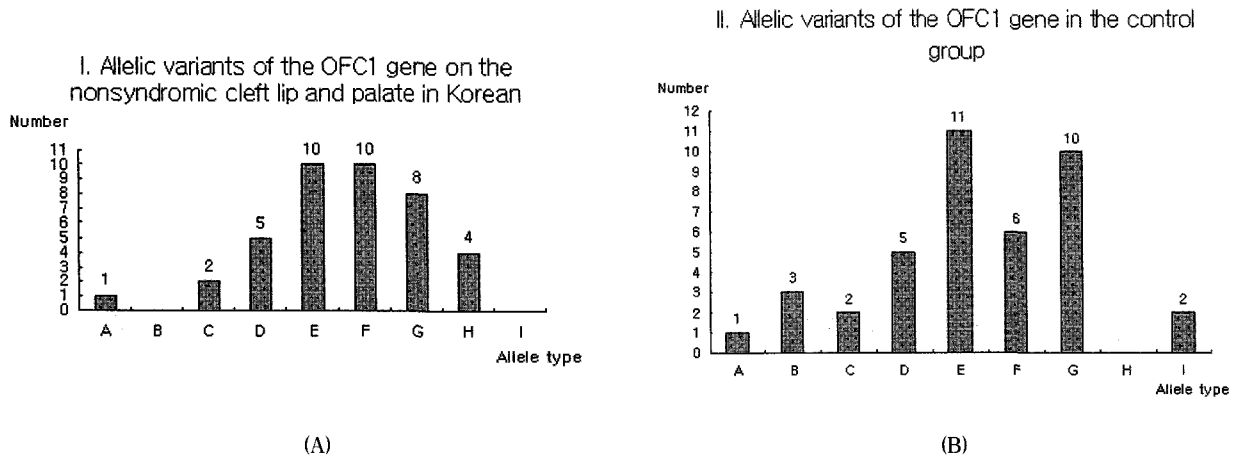
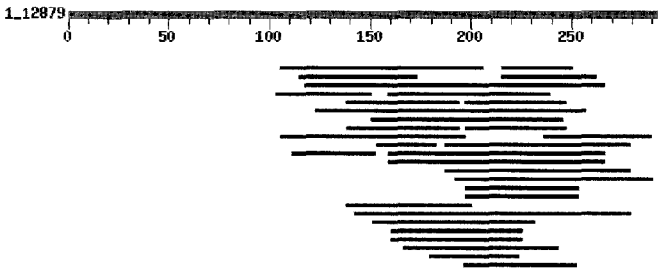


Fig. 4. Allelic variants of OFC1 gene in Korean. Ratio of each allele type were similar between patient group (A) and control group (B)

RESULTS

Nine allelic variants were found (Table 2, Fig. 4). But prominent differences were not found in agarose gel electrophoresis. All samples showed a single band in the 270–290 basepair region (Fig. 1). The sequence alignment listed in Table 1. Underlined portion of the reference was different in Korean subjects. The number of the reference's 'CA' repeat were 21 repeats, and formed as $TA(CA)_{11}TA(CA)_{10}$. However, all samples had a different form of 'CA' repeats with the reference sequence in our samples. The number of 'CA' repeats in Korean subjects was varied from 17 to 26 except 18, and 'CA' repeats consisted of TA(CA)n.

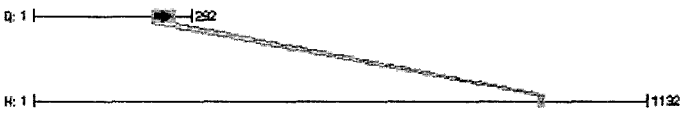
Table 3. Results of BLASTX: hTERT protein (telomerase catalytic subunit)



Sequences producing significant alignments :	Score (bits)	E-Value	
gi|6226781|sp|O70372|TERT_MOUSE Telomerase reverse transcriptase	27	7.5	
gi|6226780|sp|O14746|TERT_HUMAN Telomerase reverse transcriptase	27	9.8	.omitted.

Detail information of best hit

gi|6226780|sp|O14746|TERT_HUMAN (1 element)-Protein: Telomerase reverse transcriptase, Length : 1132




```
Consensus          PWCGL L   TL   S
Query sequence (216) PWCGLVLEAQTLSRES
gi|6226780|sp|O14746(929) PWCGLLLDTRTLEVQS
```

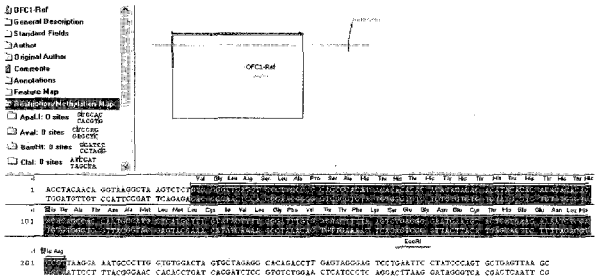


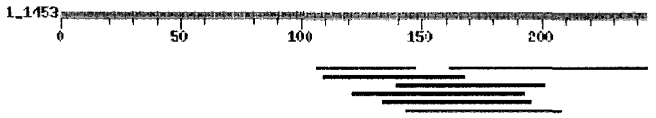
Fig. 3. Assembling of the forward and reverse strands by Contig Express (Vector NTI Suite 8 package, Infomax, Frederick, Maryland, USA); upper sequence: forward directed DNA sequencing result of OFC1 gene; lower sequence: reverse directed DNA sequencing result of OFC1 gene

The results of the ORF (open reading frame) prediction were shown in Fig. 5. The search conditions were established to include an arrangement of more than 50 amino acids. All the same results were derived from sequence in this experiment and the reference sequence.

The PSI-BLAST search results showed 'Telomerase reverse transcriptase (TERT)' and 'Nucleotide binding protein 2 (NBP2)' as similar proteins. The TERT was a protein product by hTERT gene in locus 5p15.33 (NCBI Genome Annotation; NT023089) (Table 3). The NBP2 was a protein product by ABCC3 (ATP-binding cassette, sub-family C) gene in the locus 17q22 (NCBI Genome Annotation ; NT010783) (Table 4).

In the Pedant-Pro database analysis, the predictable protein structure of the OFC1 gene has at least one transmembrane region and one non-globular region (Table 5).

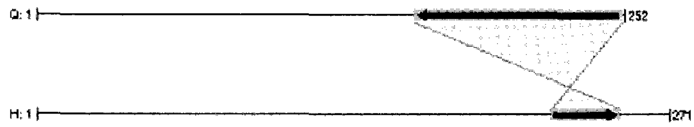
Table 4. Results of BLASTX: NBP2 protein



Sequences producing significant alignments:	Score(bits)	E-Value	
gi|13632176|sp|Q9Y5Y2|NUB2_HUMAN Nucleotide binding protein...	25	23	.omitted.

Detail information of best hit

gi|13632176|sp|Q9Y5Y2|NUB2_HUMAN (1 element)-Nucleotide binding protein 2(NBP 2), Length: 271




```
                  Consensus  P    SVPLD           G DF  E   SE
       Query sequence(251)  PYIRSVPLD*SHQGHFLNGGDFLHEYIHSE
gi|13632176|sp|Q9Y5Y(221)  PFLGSVPLDPALMRTLEEGHDFIQEFPGSE
```

Table 5. Analysis of Pedant-Pro database

Protein function

EMBLNEW : AL008731 Human DNA sequence from clone RP3-514K20 on chromosome 6p22.3-24.3 5e-87

Protein structure

Structural class : Membrane
Low complexity : 18.9%
Transmembrane regions : 1

Structural summary

Sequence
EFRTPYSRSVPLALVHTKGISLTVEIFFMSTFIPLRLKSCHKSQNYTKHEHGIGGCVCVCVCMCVCVCVCMCAGGKGPQPHRDLALPVVG
Structure
..........eeeee.....eeeeeee.....hhhhhhh..............eeeeeeeee..eeeeee.................
Transmembrane
....MMMMMMMMMMMMMMMMM.................
Low complexity
....LLLLLLLLLLLLLLLL.................
Non-globular
...........NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN.................

DISCUSSION

The nonsyndromic cleft lip and palate may be defined as a multifactor threshold model, and there is a genetic heterogeneity within and between populations, environments, and geographic regions.[3] Through genetic analysis, at the present time, the strongest evidence implicates a primary gene on 6p24.3 (OFC1) and a role for TGFA as a modifier of nonsyndromic cleft lip and palate genetics.[33,34]

Eiberg et al.[37] investigated 42 non-DNA polymorphic marker systems with linkage analysis in nonsyndromic cleft lip and palate patients. He first found a suggestive locus on the distal portion of 6p, a major locus for nonsyndromic cleft lip and palate. Marazita et al.[38] confirmed that locus 6p was associated with nonsyndromic cleft lip and palate, and concluded that the best-fitting most parsimonious model for 6p is that of an autosomal recessive major locus. In 1995, Davis et al.[22] used YAC clones from contigs in 6p25-23 to investigate cleft lip and palate patients who showed abnormalities on 6p, and these clones mapped to 6p 24.3. The presence of a locus for cleft lip and palate is suggested in that region. Weissenbach et al.[35,36] first described the sequence of 6p24.3 by using BAC (M13 mp18 cloning vector) cloning linkage mapping, and locus 6p24.3 was named by OFC1 (orofacial cleft 1) gene.

The OFC1 gene includes microsatellite markers. The microsatellite markers are dispersed regions of the genome composed of variable numbers of dinucleotides repeated in tandem.[39] The most common type is a repeat of 'CA'. Biologically, the etiology of microsatellite markers was explained as a concept of 'selfish DNA'.[40] The selfish DNA theory (repeated elements without obvious cellular function) is thought to be an important factor in genome evolution. Extragenic interspersed repeats of DNA basepairs are responsible for gene shuffling and duplication, as well as regulatory changes. Experimentally, DNA fragments containing high-copy-number tandem repeats are difficult to maintain stably in clones. When tandemly repeated sequences are grown

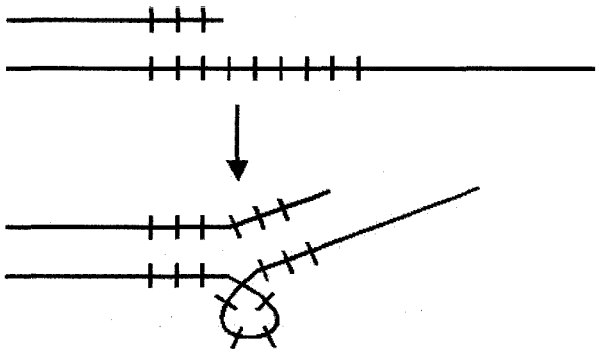


Fig. 6. When tandemly repeated sequences are replicated in meiosis, the repeat units, which are homologous to one another, tend to recombine asymmetrically with one another, leading to changes in the number of copies of the tandem repeat in the clone

in bacterial clones, the repeat units, which are homologous to one another, tend to recombine asymmetrically, leading to changes in the number of copies of the tandem repeat in the clone (Fig. 6). Because smaller clones often replicate faster, the clone DNA inserts that have lost many of their tandem repeat copies replicate fastest and become the predominant DNA molecules in the clone. Such clones are obviously not representative of the portion of the genome from which they originate. A correct consensus sequence for these regions of the genome is extremely difficult, or often impossible, to reconstruct.

In this research, it was difficult to succeed in correct sequencing alignment because of the 'CA' repeats region (Fig. 7). So, we performed the experiment two times for forward and reverse directed sequencing analysis, and then obtained the assembled contig alignment by computer program (Contig express, Vector NTI Suite 8 package, Informax, Frederick, Maryland, USA) (Fig. 3). Obscure sequence alignment was decided upon by three investigators.

The results showed a variable number of 'CA' repeats regions and sequence alignment patterns compared with the reference ; 'TA(CA)11TA(CA)10' vs 'TA(CA)n'.

Both groups of Korean subjects did not show the

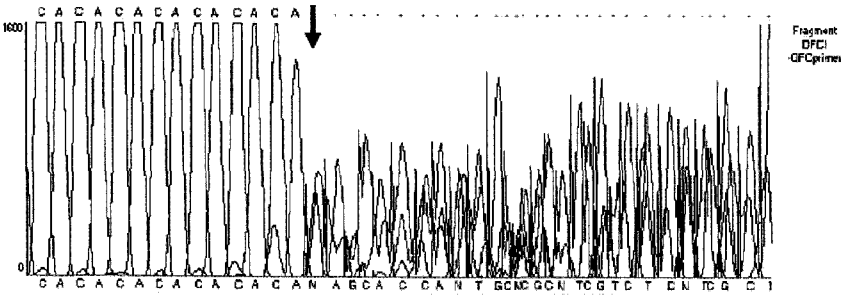


Fig. 7. It is difficult to analyze sequence after DNA fragments containing high-copy-number tandem repeats. Vertical arrow: End of CA tandem repeats

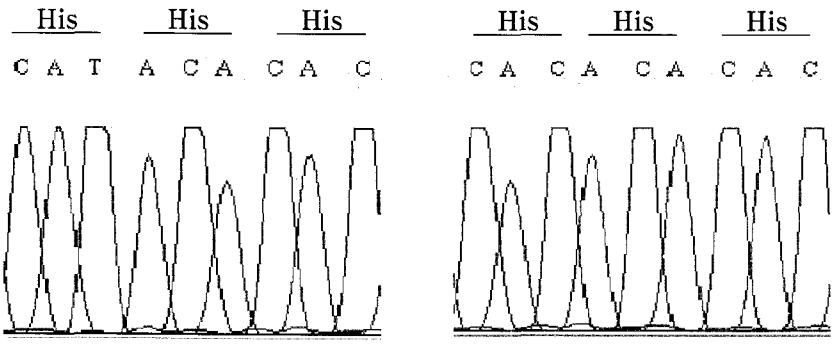


Fig. 8. Direct DNA sequencing of OFC1 gene showing a single nucleotide substitution (T to C) ; Left : reference sequence of The ABI linkage mapping panel 2 (taxon : 9606) ; Right : sequence alignment in Korean

To find the effect of replacement of base 'T' to 'C' in the 'CA' repeats region, we predicted ORF of the OFC1 gene (Vector NTI Suite 8 package, Infomax, Frederick, Maryland, USA) (Fig. 5). The differences were not found. All amino acid sequences of the 'CA' repeats region were ththththththt (Thr-his-Thr-his-Thr-his-Thr-his-Thr-his-Thr-his-Thr-his-Thr-his-Thr), because the amino acid Histidine is equally synthesized from codon 'CAC' and 'CAU' of RNA strands (Fig. 8). Other sequence alignments except for the 'CA' repeats region were all the same.

The most important function of genes is involved with protein production. However, inferred protein of the OFC1 gene had not been studied yet. Therefore, we tried to find out similar sequences by running the PSI-BLAST searches against all known sequences contained in the SWISS-PROT and the EMBLNEW databases. As well, the Pedant-Pro database search tool was used to compare the results of the PSI-BLAST search.

Predictable proteins were proved by the 'Telomerase reverse transcriptase (TERT)' and 'Human nucleotide-binding proteins 2 (NBP2)' in PSI-BLAST search results (Table 3 and 4). Telomeres, which define the ends of chromosomes, consist of short, tandemly repeated DNA sequences loosely conserved in eukaryotes. The telomerase is a ribonucleoprotein which in vitro recognizes a single-stranded G-rich telomere primer and adds multiple telomeric repeats to its 3-prime end by using an RNA template. The telomerase may have a role in the de novo formation of telomeres.[41] Many human cells progressively lose terminal sequence with cell division, a loss that correlates with the apparent absence of the telomerase in these cells. The telomerase reverse transcriptase (TERT) is an enzyme that adds simple sequence repeats to chromosome ends by copying a template sequence within the RNA component of the enzyme. Recently, Leem et al.[42] reported that the hTERT BAC clone mapping using a shot gun approach-the sequence was deposited in the

GenBank/EMBL ; Accession No. AY007685-showed a perfect match to cisplatin resistance related protein (Cleft lip and palate transmembrane protein 1 ; GenBank accession No. AB045223). This result seems to indicate a relationship between inferred protein of the OFC1 gene and hTERT gene. Also, Human nucleotide-binding proteins 2 (NBP2) are concerned with signaling the transduction of thrombin receptor of microvascular endothelial cells. It is protein that achieves the role of controlling calcium sign and the rearrangement of cell frame. The genes hTERT and NBP2 are associated with cell division and proliferation.

In the Pedant Pro search result, however, there were no similar proteins, and only contained at least one inferred transmembrane region and non-globular region (Table 5). The transmembrane region included protein that spans the plasma membrane of a cell, with the extracellular part of the protein having the ability to bind to a ligand and the intracellular part having an activity (such as protein kinase) that can be induced on ligand binding. Thus, the correct action of this transmembrane region is difficult to know accurately because it is so various. Also, non-globular region is not important because most of the proteins like enzymes and antibodies are globular proteins with compact structures.

Therefore, it is difficult to conclude that there is a relation between the PSI-BLAST search result and the OFC1 gene. And, it could not be known about any information of protein structure included transmembrane region in this study. It is essential that a 50% pairwise identical residues fraction of entire protein is needed to predict structure by comparative modeling, but the known basepair size of the OFC1 gene in this study was too small to predict a similar protein.[43]

Identification of the molecular abnormalities underlying human developmental disturbances by the gene sequencing analysis provides insight into the mechanisms of normal morphogenesis, and may suggest strategies for the improvement or prevention of such defects. In a study of the nonsyndromic cleft lip and palate, many investigators have been exploring coupled studies of craniofacial development and candidate gene expression. In this study, however, it was difficult to clear the causal-result relationship between OFC1 gene and nonsyndromic cleft lip and palate phenotype. To assay the influence of OFC1 gene toward the development of the nonsyndromic cleft lip and palate in Koreans, further studies are needed to perform a wider sequencing analysis of the OFC1 gene including neighboring alignments and genetic markers.

CONCLUSION

This study was performed to identify the characteristics of the OFC1 gene (locus : chromosome 6p24.3) in Korean subjects, which is assumed to be major gene acting as a causal factor to nonsyndromic cleft lip and palate. Using PCR-based assay, the OFC1 gene was amplified, sequenced, and searched for inferred protein product. The result of this study showed that the OFC1 gene contains the microsatellite marker 'CA' repeats. However, the number and the form of tandem 'CA' repeats were not similar between Weissenbach's report and the Korean subject findings. As well, nine allelic variants were found in Koreans. There was a replacement of base 'T' to 'C' after 11 tandem 'CA' repeats in Korean subjects compared with Weissenbach's report. However, a difference did not exist in the ORF prediction results.

As a conclusion, there was no clear causal-result relationship between the OFC1 gene and nonsyndromic cleft lip and palate in Korean subjects. To assay the influence of the OFC1 gene toward the development of nonsyndromic cleft lip and palate in Koreans, further studies are needed to perform a wider sequencing analysis of the OFC1 gene including neighboring alignments and genetic markers.

REFERENCES

1. Bixler D, Fogh-Andersen P, Conneally PM. Incidences of cleft lip and palate in offspring of cleft parents. Clin Genet 1971 : 2 : 155-9.
2. Kim S, Kim WJ, Oh C, Kim JC. Cleft lip and palate incidence

among the live births in the Republic of Korea. J Korean Med Sci 2002 : 17 : 49-52.
3. Thornton JB, Nimer S, Howard PS. The incidence, classification, etiology, and embryology of oral clefts. Semin Orthod 1996 : 2 : 162-8.
4. Hecht JT, Wang Y, Blanton SH, Daiger SP. Van der Woude syndrome and nonsyndromic cleft lip and palate(Letter). Am J Hum Genet 1992 : 51 : 442-4.
5. Schutte BC, Bjork BC, Coppage KB, Malik MI, Gregory SG et al. A preliminary gene map for the Van der Woude syndrome critical region derived from 900 kb of genomic sequence at 1q32-q41. Genome Res 2000 : 10 : 81-94.
6. Murray JC. Face facts: genes, environment, and clefts. Am J Hum Genet 1995 : 57 : 227-32.
7. Chung CS, Bixler D, Watanabe T, Koguchi H, Fogh-Andersen P. Segregation analysis of cleft lip with or without cleft palate: a comparison of Danish and Japanese data. Am J Hum Genet 1986 : 39 : 603-11.
8. Hecht JT, Yang P, Michels VV, Buetow KH. Complex segregation analysis of nonsyndromic cleft lip and palate. Am J Hum Genet 1991 : 49 : 674-81.
9. Ray AK, Field LL, Marazita ML. Nonsyndromic cleft lip with or without cleft palate in West Bengal, India: evidence for an autosomal major locus. Am J Hum Genet 1993 : 52 : 1006-11.
10. Wilkie Ao, Morriss-kay GM. Genetics of craniofacial development and malformation. Nat Rev Genet 2001 : 2 : 458-68.
11. Ardinger HH, Buetow KH, Bell GI et al. Association of genetic variation of the transforming growth factor-alpha gene with cleft lip and palate. Am J Hum Genet 1989 : 45 : 348-53.
12. Chenevix-Trench G, Jones K, Green AC, Duffy DL, Martin NG. Cleft lip with or without cleft palate: associations with transforming growth factor alpha and retinoic acid receptor loci. Am J Hum Genet 1992 : 51 : 1377-85.
13. Qian JF, Feingold J, Stoll C, May E. Transforming growth factor-alpha: characterization of the BanHI, RsaI, and TaqI polymorphic regions. Am J Hum Genet 1993 : 53 : 168-75.
14. Farrall M, Buetow KH, Murray JC. Resolving an apparent paradox concerning the role of TGFA in CL/P(Letter). Am J Hum Genet 1993 : 52 : 434-7.
15. Hecht JT, Wang Y, Blanton SH, Michels VV, Daiger SP. Cleft lip and palate: no evidence of linkage to transforming growth factor alpha. Am J Hum Genet 1991 : 49 : 682-6.
16. Mitchell LE. Transforming growth factor alpha locus and nonsyndromic cleft lip with or without cleft palate: a reappraisal. Genet Epidemiol 1997 : 14 : 231-40.
17. Mitchell LE, Risch N. Mode of inheritance of nonsyndromic cleft lip with or without cleft palate: a reanalysis. Am J Hum Genet 1992 : 51 : 323-32.
18. 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 : 393-7.
19. Shaw GM, Wasserman CR, Lammer EJ. et. al. Orofacial clefts, parental cigarette smoking, and transforming growth factor-alpha gene variants. Am J Hum Genet 1996 : 58 : 551-61.
20. Wyszynski DF, Duffy DL, Beaty TH. Maternal cigarette smoking and oral clefts:a meta-analysis. Cleft Palate Craniofac J 1997 : 34 : 206-10.
21. Ward RE, Bixler D, Jamison PL. Cephalometric evidence for a dominantly inherited predisposition to cleft lip-cleft palate in a single large kindred. Am J Med Genet 1994 : 50 : 57-63.
22. Davies AF, Stephens RJ, Olavesen MG, Heather L, Dixon MJ et al. Evidence of a locus for orofacial clefting on human chromosome 6p24 and STS content map of the region. Hum Molec Genet 1995 : 4 : 121-8.
23. Stein J, Mulliken JB, Stal S, Gasser DL, Malcolm S et al. Nonsyndromic cleft lip with or without cleft palate: evidence of linkage to BCL3 in 17 multigenerational families. Am J Hum Genet 1995 : 57 : 257-72.
24. Scapoli L, Pezzetti F, Carinci F, Martinelli M, Carinci P, Tognon M. Evidence of linkage to 6p23 and genetic heterogeneity in nonsyndromic cleft lip with or without cleft palate. Genomics 1997 : 43 : 216-20.
25. Lidral AC, Romitti PA, Basart AM, Doetschman T, Leysens NJ et al. Association of MSX1 and TGFB3 with nonsyndromic clefting in humans. Am J Hum Genet 1998 : 63 : 557-68.
26. Hwang SH, Beaty TH, Panny SJ, Street NA, Joseph JM et al. Association study of transforming growth factor alpha(TGF)TaqI polymorphism and oral clefts: indication of gene-environment interaction in a population-based sample of infants with birth defects. Am J Epidermiol 1995 : 141 : 629-36.
27. 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 : 390-2.
28. Marazita ML, Cooper ME, Tobias R, Maher BS, Peanchitlertkajorn S et. al. Nonsyndromic cleft lip with or without cleft palate in China:assessment of candidate regions. Cleft Palate Craniofac J 2002 : 39 : 149-55.
29. Mitchell LE, Beaty TH, Lidral AC, Munger RG, Murray JC et. al. Guidelines for the design and analysis of studies on nonsyndromic cleft lip and cleft palate in humans:summary report from a workshop of the international consortium for oral clefts genetics. Cleft Palate Craniofac J 2002 : 39 : 93-100.
30. Tanabe A, Taketani S, Endo-Ichikawa Y. et. al. Analysis of the candidate genes responsible for non-syndromic cleft lip and palate in Japanese people. Clin Sci 2000 : 99 : 105-11.
31. Wyszynski DF, Beaty TH, Maestri NE. Genetics of nonsyndromic oral clefts revisited. Cleft Palate Craniofac J 1996 : 33 : 406-17.
32. Wyszynski DF, Mitchell LE. Report of newly formed international consortium for oral clefts genetics. Cleft Palate Craniofac J 1999 : 36 : 174-8.
33. McInnes RR, Michaud J. Gene/environment causes of cleft lip and/or palate. Clin Genet 2002 : 61 : 248-56.
34. Prescott NJ, Lees MM, Winter RM, Malcolm S. 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 2000 : 106 : 345-50.
35. Gyapay G, Morissette J, Vignal A, Dib C, Fizames C et al. The 1993-1994 Genethon human genetic linkage map. Nat Genet

1994 : 7 : 246-339.
36. Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J et al. A second-generation linkage map of the human genome. Nature 1992 : 29 : 777-8.
37. Eiberg H, Bixler D, Nielsen LS, Conneally PM, Mohr J. Suggestion of linkage of a major locus for nonsyndromic orofacial cleft with F13A and tentative assignment to chromosome 6. Clin Genet 1987 : 32 : 129-32.
38. Marazita ML, Hu DN, Spence MA, Liu YE, Melnick M. Cleft lip with or without cleft palate in Shanghai, China: evidence for an autosomal major locus. Am J Hum Genet 1992:51:648-53.
39. Griffiths AJF, Lewontin RC, Gelbart WM, Miller JH. Modern genetic analysis: Integrating genes and genomes. 2nd ed. WH Freeman and Co, New York. 2002 : pp 274-5.
40. Ogata H, Audic S, Barbe V, Artiguenave F, Fournier PE et al. Selfish DNA in Protein-Coding Genes of Rickettsia. Science 2000 : 290 : 347-50.
41. Artandi SE, DePinho RA. Mice without telomerase: what can they teach us about human cancer? Nature Medicine 2000 : 6 : 852-5.
42. Leem SH, Londono-Vallejo SA, Kim JH, Bui H, Tubacher E et al. The human telomerase gene: complete genomic sequence and analysis of tandem repeat polymorphisms in intronic regions. Oncogene 2002 : 21 : 769-77.
43. Liu J, Rost B. Target space for structural genomics revisited. Bioinformatics 2002 : 7 : 922-33.

국문초록

한국인 비증후군성 구순구개열 환자의 OFC1 유전자의 서열 분석

김 성 식, 손 우 성

부산대학교 치과대학 교정학교실

비증후군성 구순구개열을 발생시키는 주요유전자로 추측이 되는 OFC1 유전자(위치: 염색체 6p24.3)의 한국인에서 나타나는 특성을 연구하였다. 3대에 걸쳐서 처음으로 비증후군성 구순구개열이 나타난 40 명의 환자(남자 20명, 여자 20명, 평균 나이 : 14.2 세)와 3 대에 걸쳐서 비증후군성 구순구개열을 포함한 어떤 선천성 기형도 나타나지 않았던 정상 성인 40명(남자 20명, 여자 20명, 평균 나이 : 25.6세)을 연구 대상으로 하였다. 중합효소 연쇄 반응법을 이용하여 OFC1 유전자를 분리 증폭한 후, 염기 서열 분석을 통해서 대립유전자형을 밝히고, BLAST 와 Pedant-Pro 데이터베이스를 이용하여 단백질의 상동성 검색을 수행하였으며, 그 결과는 다음과 같다.

1. OFC1 유전자는 'CA' 연쇄반복서열을 가진 극소위성 표지자로 밝혀졌다.
2. 환자군과 대조군의 OFC1 유전자의 특별한 차이는 발견되지 않았다.
3. 한국인에서 나타난 'CA' 연쇄반복서열의 형태는 'ABI linkage map 2'의 TA(CA)11TA(CA)10 과는 달리, TA(CA)n의 형태를 띄었으며, 연쇄반복의 수는 17 회에서 26 회로 다양하게 나타났다.
4. 'CA' 연쇄반복서열의 횟수에 따라서, 9 가지의 대립유전자형이 발견되었으며, 나타나는 빈도는 환자군과 대조군에서 유사하였다.
5. 'ABI linkage map 2'의 'CA' 연쇄반복서열 사이의 염기서열 T 가 한국인에서는 C 로 치환되어 있었지만, ORF 예측을 하였을 때 예상되는 아미노산의 배열 차이는 관찰되지 않았다.

6. 한국인 OFC1 유전자의 염기서열로 예측되는 단백질을 알아 보기 위하여 BLAST 검색을 한 결과, Telomerase reverse transcriptase(TERT, locus 5p15.33, NCBI Genome Annotation ; NT023089)와 Nucleotide binding protein 2(NBP2, locus 17q22, NCBI Genome Annotation; NT010783)가 유사한 구조를 가지는 단백질로 밝혀졌다.
7. Pedant-Pro 데이터베이스로 단백질 구조의 상동성 검색을 한 결과, OFC1 유전자는 적어도 하나의 transmembrane region과 non-globular region을 가지는 구조로 밝혀졌다.

주요 단어 : 비증후군성 구순구개열, OFC1 유전자, 극소위성 표지자, CA 연쇄반복서