

Association between VEGF gene and  
residual ridge resorption of mandible in  
Korean population

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The Master's Thesis submitted to the Department of  
Dentistry of Graduate School of Yonsei university in  
partial fulfillment of the requirements for the degree of  
Master of Dental Science

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June 2013

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2013년 6월

송정현

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# Abstract

## Association between VEGF gene and residual ridge resorption of mandible in Korean population

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**Objective:** To find the genetic association between SNPs and haplotypes of VEGF gene and residual ridge resorption in a Korean population. **Materials and Methods:** One hundred twenty subjects ( $70.93 \pm 9.28$  years) who were treated in Dental clinic of Yonsei university with partially or completely edentulous mandible were recruited. Mandibular bone height was measured following the protocol of the American College of Prosthodontists. Three variants, rs1570360, rs25648, and rs3025039 in VEGF were used as tag-SNPs and genotyped for the Korean population. Student's t-test and ANOVA were used for statistical analysis to examine association between SNPs and height of residual ridge. Haplotypes with frequency rates over 0.05 was selected and was analyzed with same statistical methods. **Results:** Three tag SNPs from VEGF gene were tested and haplotype A-C-C, the second prevalent type, showed statistically significant association with the process of

residual ridge resorption in the dominant group ( $P=0.042$ ). **Conclusion:** Haplotype A-C-C, the second prevalent type, were associated with less atrophied mandibular bone in dominant groups. Other haplotypes might be functionally inferior to type A-C-C. Results of this study may be useful in developing novel genetic diagnostic tests and identifying Koreans susceptible to developing excessive jawbone atrophy after dental extraction.

**Key words:** SNP; VEGF; haplotype; residual ridge resorption; edentulous mandible

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## **I. Introduction**

Tooth extraction is one of the most frequently performed dental procedures. After tooth extraction, residual ridge undergoes process of continual bone resorption due to reduced mechanical loading of the tooth and the dental ligament<sup>1</sup>. With the increase of life expectancy of the elderly population, edentulous patients are increasing as well. The results and satisfactions from complete denture treatments, commonly used for edentulous patients, are dependent upon the preservation of the residual ridge. During denture use, the degree of residual ridge resorption influences masticatory and nutrient intake ability and this greatly affects the quality of life and life expectancy. Therefore, as part of a research on prosthodontic treatment, studies on residual ridge resorption are necessary to give patients more functional satisfaction.

Bone remodeling is a continuous process of osteoclastic and osteoblastic

reaction, and respond to physiological needs and stimulus. For some unknown reason, homeostatic imbalance increases osteoclastic and osteoblastic reaction. Bone resorption is initiated by such reactions and occurs from negative remodeling.

Residual ridge resorption after tooth extraction has been studied extensively in the past <sup>2-5</sup>. After tooth extraction, patient undergoes loss of alveolar process and is viable to mucosa-borne prosthesis. This represents unphysiological occlusal loading which brings reduced masticatory function and lack of forces into the bone <sup>5</sup>. Then mineral content in the bone rapidly decreases leading to bone resorption <sup>1</sup>. Several internal and external factors are involved in the study; however, research on genetic factor has been overlooked.

Once tooth is extracted, blood vessel in the extraction socket gets injured. Then blood supply is blocked and oxygen pressure of the surrounding area decreases, which makes a state of hypoxia within the tissue <sup>6</sup>. Our body tries to maintain homeostasis by expanding blood vessels, increasing vascular development and angiogenesis. During the process of homeostasis, osteocyte works as a biological signal regulating several other mediators. Among such mediators, HIF-1 is the master regulator of genes dependent on oxygen concentration. HIF-1 is closely involved in bone remodeling and osteoclastic bone resorption. Of the 60 or more genes that are expressed by HIF-1, VEGF is involved in angiogenesis and is one of the target genes of bone remodeling <sup>7</sup>. Angiogenesis is the most effective and long-term adaptive response. It has been reported that VEGF is quickly upregulated once HIF-1 expression increases <sup>8</sup>. VEGF is also important for osteoclastic bone resorption and acts as a vital factor for normal bone remodeling.

In the past, studies on HIF-1 alpha have been carried out. Since VEGF is associated with HIF-1 alpha, it can be hypothesized that SNP variations within the gene affects bone resorption in the mandible.

## II. Method and Material

### 1. *Ethics statement*

All research involving human subjects or human data was approved by the Institutional Review Board of Yonsei University College of Dentistry (Yonsei IRB No. 2-2010-0022). All clinical investigation was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before taking part in this study<sup>9</sup>.

### 2. *Study population*

The study population consisted of 120 unrelated Korean individuals, 41 men and 79 women who were recruited between January 2011 and February 2013 from Yonsei University Dental Hospital. Each subject was completely or partially edentulous for at least two years. The partially edentulous subjects were missing both maxillary and mandibular premolars and molars either in unilateral or bilateral sides. Those subjects that met the following criteria were included in the study: 1) no known systemic conditions that could affect bone conditions such as osteoporosis, other metabolic bone disease, or pituitary disease; 2) no history of bone transplantation; and 3) presenting a panoramic dental radiograph less than 2 years old. After informed consent was obtained, an oral examination was performed to confirm their edentulous condition<sup>9</sup>.

### 3. *Measurement of mandibular residual ridge height*

The panoramic dental radiograph of each subject was digitized with an anonymously assigned identification number and stored in a secured

computer with password protection. Following the Prosthodontic Diagnosis Index from the American College of Prosthodontists, the lowest height of the edentulous mandible was measured. The mean of mandibular bone height was  $15.03 \pm 4.07$  mm (n=120) and varied from 6.55 mm to 25.73 mm.

#### *4. Genomic DNA extraction*

Each edentulous subject was asked to collect 2 ml of saliva in the tube of an Oragene DNA Self-collection Kit that contained 2 ml of DNA-preserving solution (DNA Genotek Cat. #OG-250). When the saliva was collected, the lid was closed and the liquid in the lid was released into the tube to mix with the saliva. The sample with a total volume of 4 ml was stored at 25°C for no longer than 2.5 weeks. Genomic DNA was collected using the Puregene DNA purification kit from Qiagen (Valencia, CA, USA) according to the manufacturer's protocol. DNA extractions and further analysis were done by DNA Link Inc. (Seoul, South Korea). The concentration of DNA was measured with a Nanodrop Spectrophotometer-1000 (Thermo Scientific, Delaware, USA)<sup>9</sup>.

#### *5. Use of Tag SNPs*

Seven SNPs of VEGF gene were already identified in previous study<sup>10</sup>. Therefore whole sequencing was not needed for the study. Three of seven SNPs were selected based on allele frequency and linkage disequilibrium(LD) and were used as tag SNP in this study . Three tag SNPs that were used are following: rs1570360 in chr6:43737830, rs25648 in chr6:43738977, rs3025039 in chr6:43752536

## *6. Statistical analysis*

All data analysis was completed using the statistics software SAS 9.1.3 (SAS Institute Inc. in Cary, NC, US). Hardy-Weinberg equilibrium (HWE) tests were evaluated to ensure genotyping quality.

The association of SNPs with the mandibular jawbone atrophy was evaluated using Student's t-test for dominant and recessive groups, and ANOVA for the codominant group. The frequencies and association of haplotypes is evaluated by using Haploview program.

### III. Result

Measured mean height of mandibular bone height was  $15.03 \pm 4.07$ mm. Frequencies and association of three tag SNPs are listed on Table 1, 2.

**Table 1. Frequencies of VEGF polymorphisms in 120 Koreans**

<i>Position</i>	<i>SNP Number</i>	<i>Genotype</i>				<i>MAF</i>	<i>HWE</i>
Chr6: 43737830	rs1570360	GG	GA	AA	N	0.175	1
81		36	3	120			
Chr6: 43738977	rs25648	CC	CT	TT	N	0.063	0.370
106		13	1	120			
Chr6: 43752536	rs3025039	CC	CT	TT	N	0.154	0.727
85		33	2	120			

Three individual tag SNPs did not show statistically significant association with RRR. rs1570360 showed p-value of 0.051, which is very near to 0.05. Another two SNPs did not show any genetic association. On the other hand, Haplotype A-C-C, the second most prevalent type showed association with RRR in the dominant group. (Table 3, p value=0.042)

**Table 2. Association of VEGF polymorphisms in 120 Koreans**

<i>Loci</i>	<i>SNP number</i>	<i>Dominant P value</i>	<i>Recessive P value</i>	<i>Codominant P value</i>
chr6:43737830	rs1570360	0.051	0.688	0.15
chr6:43738977	rs25648	0.767	0.293	0.576
chr6:43752536	rs3025039	0.644	0.766	0.88

**Table 3. Association of VEGF haplotypes in 120 Koreans**

<i>Haplotype</i>	<i>Frequency</i>	<i>Dominant P value</i>	<i>Recessive P value</i>	<i>Codominant P value</i>
G-C-C	0.678	0.485	0.092	0.678
A-C-C	0.111	<b>0.042*</b>	0.95	0.111
A-C-T	0.069	0.636	-	0.069

\*p-value<0.05

In Haplotype A-C-C dominant group, mean of mandible height was 20.72mm showing less atrophied bone level than in other types. (Table 4)

**Table 4. Bone height of Haplotype A-C-C**

<i>Model</i>	<i>p-value</i>	<i>Genotype</i>	<i>N</i>	<i>Mean</i>	<i>Std</i>
Dominant	0.041693067	A-C-C	23	20.727	5.170
		Other	97	17.857	6.187
Recessive	0.950018963	A-C-C	1	18.790	
		Other	119	18.404	6.117
Codominant	0.120173625	A-C-C A-C-C	1	18.790	
		A-C-C Other	22	20.815	5.274
		Other Other	97	17.857	6.187

## IV. Discussion

Bone remodeling is continuous throughout life and is an essential part of normal physiology and homeostasis to adapt to new circumstances. In the homeostasis point, body will regulate the deposition and resorption of bone as necessary, which may be controlled by angiogenesis gene such as HIF(Hypoxia inducible factor) and VEGF(Vascular Endothelial Growth Factor). After tooth extraction, bone tissues in residual ridge become hypoxic by reduced mechanical loading which is delivered by periodontal ligament. Bone tissues in residual ridge face a new situation that requires glycolytic ATP generation due to hypoxia, which is very expensive from an energetic viewpoint. Therefore new blood vessel formation is inevitable to increase oxygen delivery to the bone tissue. HIF is the fundamental hypoxia-response protein and over 70 genes have been identified to be HIF-dependent. Among those genes, VEGF-induced angiogenesis is one of the several keys to hypoxia adaptation. Such process increases oxygen delivery by boosting angiogenesis. Angiogenesis and bone remodeling are closely linked. VEGF not only increases oxygen pressure in the bone tissue but also directs autocrine in osteoclast differentiation. VEGF is a proangiogenic cytokine that has been shown to promote osteoclast differentiation and survival. Studies have also revealed that VEGF acts as a macrophage-colony stimulating factor (M-CSF) during osteoclastogenesis in the osteopetrotic mouse model. By enhancing osteoblast and osteoclast activity in the bone remodeling process of the residual ridge, VEGF might be the regulating gene consistently increasing bone resorption in order to adapt to hypoxic circumstances, which will eventually reduce the consumption of unnecessary oxygen. Therefore it

is possible to assume that genetic variation in VEGF directly or indirectly affect level in residual ridge resorption.

VEGF gene is highly polymorphic; hundreds of polymorphisms are annotated in dbSNP. These SNPs are grouped in three major linkage disequilibrium (LD) blocks.<sup>11</sup> The first one is located upstream of the transcription start point; the second one includes the promoter and the first six exons; the third one covers the last two exons and the 3' UTR<sup>12</sup>. Only a small number of reports are available concerning SNPs on the first block. The second block, however, consists of well known SNPs associated with many diseases. rs1570360 and rs25648 are typical SNPs in the promoter region and is often found from direct sequencing of the Korean population. rs1570360 may also be representative of other well known SNPs in the promoter region as it shows strong LD with rs699947 and rs2010963. 3' UTR of the well known polymorphism rs3025039 should be tested to discriminate the major genotypes that are located in the third block. Therefore the three SNPs studied in this research cover the major functional SNPs of recent VEGF studies.

The second block has at least four major common haplotypes, as well as several minor haplotypes, resulting in more than 10 common promoter genotypes in humans. There have been several studies that have assessed the effect of differing haplotypes on the promoter activity. These common promoter genotypes are inherited as part of a larger haplotype, and thus individuals grouped according to genotype at a single polymorphism are actually mixtures of individuals who bear different haplotypes of the VEGF promoter. Although each of three SNPs did not show any statistically significant association, haplotype A-C-C from this study, which is the second

prevalent type containing the 2nd and the 3rd LD block, showed association with residual ridge resorption. Haplotype generally present more strong association evidence than individual SNP genotype. Because the effect of genotype on VEGF expression is cell-type and stimulus specific <sup>11</sup>, this seems to be a well adaptive process in the physiological point of view since residual ridge lost its function to support the tooth and advanced to the process of atrophy. Other haplotypes might be less functional to this degeneration.

## V. Conclusion

Haplotype A-C-C, the second prevalent type, were associated with less atrophied mandibular bone in dominant groups. Other haplotypes might be functionally inferior to type A-C-C. Among three tag SNPs only rs1570360 showed remarkable result, but this association was not significant. Therefore we can suggest that results of this study may be useful in developing novel genetic diagnostic tests and identifying Koreans susceptible to developing excessive jawbone atrophy after dental extraction. Large scaled, controlled and other genetic factors related further study is needed for more accurate prospect of jawbone atrophy.

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## 국문요약

한국인에서 VEGF 유전자와 하악 잔존치조제 흡수와의 연관성

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**연구 목적** : 한국인에서 VEGF상의 단일염기다형성과 일배체형이 잔존치조제 흡수와 갖는 연관성을 밝히기 위해서이다. **재료 및 방법** : 연세대학교 치과대학병원에서 치료받은 부분 또는 완전무치악의 120명의 환자들을 선정하였다. (70.93 ± 9.28 years) 하악 잔존치조제의 높이는 미국 보철학회의 규약에 따라 측정하였다. VEGF 유전자의 세가지 변이, 즉 rs1570360, rs25648, and rs3025039가 tag-SNP으로 선정되었다<sup>10</sup>. 단일염기다형성과 잔존치조제 높이와의 연관성 분석을 위하여 Student's t-test와 ANOVA 를 사용하였다. 5% 이상의 발현빈도를 보이는 일배체형 역시 동일한 방법으로 선정되고 분석되었다. **결과** : 세 개의 tag SNP은 통계학적 연관성을 보이지 않았지만, 두번째로 높은 발현빈도수를 가지는 A-C-C 일배체는 우성 그룹에서 잔존치조제 흡수와 유의한 연관성을 보였다. (P value=0.042). **결론** : 두번째로 높은 발현빈도를 보이는 일배체 A-C-C가 우성

그룹에서 더 적게 흡수된 하악 잔존치조제와 통계적 연관성을 보였다. 다른 개별적 tag SNP과 일배체는 유의한 차이를 보이지 않았다. 이 연구의 결과를 통해 한국인에서 발치 후 잔존치조제의 흡수량에 대해 유전적 연관성 검사를 가능하게 할 수 있을 것으로 기대된다.

**핵심되는 말** : 단일염기다형성; VEGF; 일배체; 잔존치조제 흡수; 하악 무치악