

POLYMORPHISMS OF THE NOGGIN GENE AND MANDIBULAR MICROGNATHIA: A FIRST APPROXIMATION

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ABSTRACT

Mandibular micrognathia is a deficiency in mandibular growth that prevents tooth contact during mastication, interferes with phonation and even causes sleep apnea. Studies show that mutant mice for *chd* (chordin) and *nog* (noggin) genes, which are modulators of the Bone Morphogenic Protein (BMP), had mandibular defects ranging from mandibular hypoplasia to micrognathia and agnathia. The human *NOG* gene was the first BMP antagonist identified and it is essential for various late events in mandibular development, which require modulation of the BMP activity. The aim of this work was to determine the presence of *NOG* gene polymorphisms in families with mandibular micrognathia and analyze its phenotype. Four families with mandibular micrognathia were included in this study. Blood samples were taken from the participating individuals through

venipuncture and DNA was extracted. The fragments of interest were amplified using the Polymerase Chain Reaction (PCR) and the Single Nucleotide Polymorphisms (SNPs) of the *NOG* gene reported in the NCBI data base were analyzed through direct sequencing. The SNP rs1348322 was present in homozygote form in the subjects from all the families, where Cytosine is changed to Adenine in position 112 of the exon of the *NOG* gene. The SNP rs 1236187 did not show any clear result. This result suggests that there may be population polymorphism, or markers that are seldom polymorphic for our population. It is therefore necessary to continue with the search for the relationship of the *NOG* gene with mandibular micrognathia.

Key words: micrognathia, mandibular, noggin, polymorphism, gene, SNPs.

POLIMORFISMOS DEL GEN NOGGIN Y EL MICROGNATISMO MANDIBULAR: UNA PRIMERA APROXIMACIÓN

RESUMEN

El micrognatismo mandibular, deficiencia en el crecimiento de la mandíbula, no permite que los dientes entren en contacto durante la masticación, interfiriendo con la fonación y produciendo inclusive apnea del sueño. Estudios con ratones mutantes para el gen *chd* (*chd*) o *noggin* (*nog*) moduladores de las proteínas morfogénicas óseas (BMP) presentaron defectos mandibulares, que van desde hipoplasia mandibular, pasando por micrognatia hasta agnatia. El gen *NOG* humano fue el primer antagonista de BMP identificado y es esencial para varios eventos tardíos del desarrollo mandibular, que requieren modulación de la actividad de las BMP. El objetivo del trabajo fue determinar la presencia de polimorfismos del gen *NOG* en pacientes con micrognatismo mandibular y analizar su fenotipo. Se tomaron 4 familias con micrognatismo mandibular; muestras de sangre fueron tomadas por venopunción a los individuos participantes, el ADN fue extraído, se

realizó la amplificación de los fragmentos correspondientes a los polimorfismos rs 1236187 y rs 1348322 mediante PCR (Reacción en Cadena de la Polimerasa) y se analizaron los SNPs del gen *NOG* reportados en la base de datos NCBI, mediante secuenciación directa. El SNP rs 1348322, se presentó en forma homocigota en los individuos de todas las familias, donde se da el cambio de una Citosina por una Adenina en la posición 112 del exón del gen *NOG*. El SNP rs 1236187, no arrojó ningún resultado en forma clara.

Este resultado sugiere que posiblemente pueden tratarse de polimorfismos poblacionales, o de marcadores poco polimórficos para nuestra población, por lo tanto es necesario continuar en la búsqueda de la relación del gen *NOG* con el micrognatismo mandibular.

Palabras clave: micrognatismo, mandibular, noggin, polimorfismo, gen, SNPs.

INTRODUCTION

Mandibular growth takes place under strict genetic control. Several genes involved in the development process determine the pathological and physiological conditions of this tissue, i.e., one or more alterations

of these genes lead to the development of certain phenotypes¹.

Mandibular micrognathia is characterized by deficiency in mandibular bone growth, which prevents teeth from making contact during mastication, inter-

feres with phonation and even causes sleep apnea, a breathing disorder known as sudden infant death syndrome in infants.

It has been shown that mandible development is controlled by a number of genes and family of genes which include *Msx1*, *Dlx*, *Shh*, *Bmp*, *Fgf* and *Wnt2*; and many studies of the mechanics of its development have focused on the effects of one tissue on another, the effects of the genes that regulate the expression, transcription factors and growth factors, among others³⁻⁵.

Several studies of recombinant protein expression and application in mice and chickens suggest that Bone Morphogenetic Proteins (BMPs) induce bone and cartilage formation; meanwhile their ectopic application conduce to apoptosis then produce lack of mandibular growth, which suggest BMPs have a role in mandibular formation⁶⁻⁸.

BMP requirements in mandibular development are not yet clear, but it is known that their signalling leads to apoptosis and that this signal is strongly regulated by several effectors such as twisted gastrulation (*tsg*), *Pax-9* and *FGF*, which reduce or inhibit it, and also by antagonists⁹ such as chordin (*chd*) and noggin (*nog*).

Chd and *nog* are structurally unrelated proteins that bind BMPs in the extracellular space to prevent activation of their receptors. These two antagonists are derived from the first branchial arch and the genes that encode them are expressed in the pharynx during early mandibular growth and in the late mandibular process^{10,11}.

Experiments with mice mutant only for the gene that encodes (*chd*) or only for the gene that encodes (*nog*), showed that they had mandibular defects, with moderately dysmorphic mandibles, without defects in tooth development. This implies that these genes are functionally redundant in the promotion of mandibular growth in heterozygotes (*chd* *-/-*; *nog* *+/-*) but in double mutants (*chd* *-/-* ; *nog* *-/-*) phenotypes range from mandibular hypoplasia to micrognathia to agnathia^{3,4}.

Stottman et al., 2001⁴, also found that in these studies there is incomplete penetrance and variable expressivity related to the micrognathia phenotype, one of the most drastic defects in skeletal development, and that phenotype variability can be related to sensitivity to the levels of BMP antagonists. Thus, results conclude that

chd and *nog* are individually necessary for normal development of the first branchial arch derivatives.

Therefore, these antagonistic genes seem to be essential to mandibular development in mice, maintaining the signalling balance, and their absence produces a reduction in the expression of *FGF8* and increases the apoptotic activity of *BMP*, which shows phenotypically in mandibular asymmetries¹².

The *nog* gene was the first BMP antagonist identified that can bind and inhibit *BMP-4* and takes part in the formation of the head and other dorsal structures. It seems to be essential to several late events in mandibular development, which require modulation of *BMP* activity and in the three main development phases of the first branchial arch¹³.

The human *Noggin* (*NOG*) gene is a gene with only one exon, mapped on chromosome 17 q22. According to Valenzuela et al.¹⁴, the region encoding the *NOG* gene is an open reading frame of 696 nucleotides that encode a polypeptide of 232 amino acids. *NOG* can attenuate the effects of the *BMPs* in osteoblasts and limit their action on skeletal cells.

Five dominant mutations in the *NOG* gene were identified in unrelated Spanish families with proximal phalangism and multiple joint fusion¹⁵. In 2001, Semonin et al.¹⁶ reported three mutations in the *NOG* gene in three Spanish families with progressive ossifying fibrodysplasia. These mutations resulted in the alteration or suppression of a portion of the gene.

To date, there is no report on whether the *NOG* gene participates in lack of mandibular growth. Therefore the aim of this research was to determine the presence of polymorphisms of the *NOG* gene in Colombian families with mandibular micrognathia and to analyze their phenotype.

MATERIALS AND METHODS

Population

This project was approved by the ethics committee at the School of Dentistry at the Javeriana University, Bogotá, Colombia. The population included 4 families from Bogotá with members affected and unaffected by mandibular micrognathia, (each family was made up of two subjects affected by mandibular micrognathia and one unaffected), who

Table 1: Measurements for diagnosing mandibular micrognathia.

1. Size of lower maxilla: <ol style="list-style-type: none"> Distance in millimetres (mm) from Condylion (Co) to Gnathion (Gn) – Bimler Peter. Distance in mm from Gonion (Go) to Pogonion (Pg) – Legan and Burstone. Distance in mm from point Xi to the point of intersection of line (Xi – Pm) and (A – Pg) – Ricketts Robert.
2. Height of the ascending branch of the lower maxilla: <ol style="list-style-type: none"> Distance in mm from Articular point (Ar) to Go point - Bimler Peter. Distance in mm from Go point to the horizontal line (FH) on the plane (C-Go) – Legan and Burstone. Distance in mm from CF to Go – Ricketts Robert.
3. Maxillary ratios: <ol style="list-style-type: none"> EFFECTIVE MEAN LENGTH – EFFECTIVE MANDIBULAR LENGTH (table of compound standards). Ratio between maxillary length and mandibular length ((Co-A) and (Co-Gn)) - McNamara. MAXILLARY LENGTH – MANDIBULAR LENGTH. Ratio between distance in mm (Co to A) and (Co to Gn) – Ecker and Fish. <p>To ensure that the measurements are not affected by alterations in chin size, the following measurement was used to check that it is within normal parameters:</p>
4. Chin size: <ol style="list-style-type: none"> Distance in mm from the projection of point B to the Tangent to Pg perpendicular to plane (Go-M) – Legan and Burstone.

Table 2: Primers used for evaluation of Noggin gene SNPs. Prepared using the PRIMER 3 programme.

SNPs	Primer Forward	Primer Reverse	Fragment size
rs 1236187	CAGTCCACCACCCTCTAGC	TGGGACCGTATATACACACACAT	150 pb
rs 1348322	TGTGTGTATATACGGTCCCAGTTT	CGAAGGGCACTGGAATATAAA	190 pb

visited the School of Dentistry at the Javeriana University for consultation. Informed consent was obtained from all participants after explaining the project to them.

Clinical analysis

A clinical-genetic check was conducted to verify that participants did not have any kind of genetic syndrome. Clinical and radiographic dental examinations were used to determine the presence of micrognathia and analyze its phenotype. Profile radiographs were obtained, on which linear measurements were taken and analyzed (cephalometry) (Table 1).

DNA extraction and amplification by PCR

DNA was extracted from 10ml of peripheral blood from all subjects by salting out¹⁷ and stored in a freezer at -20°C until it was used. The fragments corresponding to polymorphisms SNP rs 1236187 and SNP rs 1348322 were amplified by PCR (Polymerase Chain Reaction), using the primers from the PRIMER

3 program (<http://primer3.sourceforge.net/>) (Table 2), under the following conditions:

For a total volume of 25µl master Mix, 1 µl of taq polymerase (Promega 5 u/µl), 0.25 µM of each primer, 0.25 µM of dNTPs, 2 mM of MgCl₂, 2.5 µl of buffer (Promega) and 1 µl of DNA (50 ng/µl). The PCR conditions were: one initial denaturalization at 95°C for 10 minutes; 30 cycles of denaturalization at 94°C for 1 minute, annealing temperature 56°C for both pairs of primers, for 1 minute and an extension at 72°C for 1 minute (BioRad Thermocycler). The final extension was done at 72°C for 7 minutes. The fragment was viewed in 1.5% agarose gel using ethidium bromide.

Detection of polymorphism

The amplified product was purified using the Wizard DNA Purification System Kit (Promega). The presence of the single nucleotide polymorphism (SNP) of the NOG gene reported in the NCBI SNP Database, and Ensembl, (www.ncbi.nlm.nih.gov)

Table 3: Cephalometric measurements for determining mandibular micrognathia.

The table shows that there is differential growth among the subject's skeletal components, regardless of gender.

Range of measurements for males		
Variable	x_i	$\pm a$
Go - Pg	74,79	3,6
Xi - Pm/ Pg	74,46	3,5
Ar - Go	46,00	7,8
CF - Go	62,72	6,0
Co - Gn	113,71	7,4
Range of measurements for females		
Variable	x_i	$\pm a$
Go - Pg	73,51	4,5
Xi - Pm/ Pg	73,21	4,4
Ar - Go	43,20	9,9
CF - Go	60,58	7,6
Co - Gn	111,05	9,4



Fig. 2: Phenotype characteristics of the subject corresponding to family 1. A) The patient was found to have a convex facial profile, and was diagnosed according to facial morphological index as Leptoprosopic and with facial asymmetry. B) The patient had increased overbite and overjet, and oval upper and lower arch.

and the facial pattern was hypodivergent. Increased overjet, convex profile and class II molar and canine relationship were observed. Anterior upper and lower dental crowding and everted lower lip were also common (Fig. 2).

Detection of polymorphism

The analysis of *NOG* gene polymorphisms through sequencing showed SNP rs 1348322 polymorphism, present in homozygote form in the individuals from all the families. It was found that a Cytosine was substituted by an Adenine in position 112 of the *NOG* gene exon (Fig. 3). Regarding the other SNP rs 1236187 polymorphism, no clear result emerged even after running the test several times.

DISCUSSION

Malocclusions have been considered to be the result of a combination of both dental and skeletal disharmonies. Nevertheless, specialists have pointed out the role of heredity as a cause of malocclusion, because several craniometric and cephalometric studies support the idea that face shape is the product of each individual's genotype¹⁸⁻²³.

Thus, it is clear that genotype contributes to phenotype variation, and this has been shown in several studies that examined the differences and similarities among the members of some families and the dis-

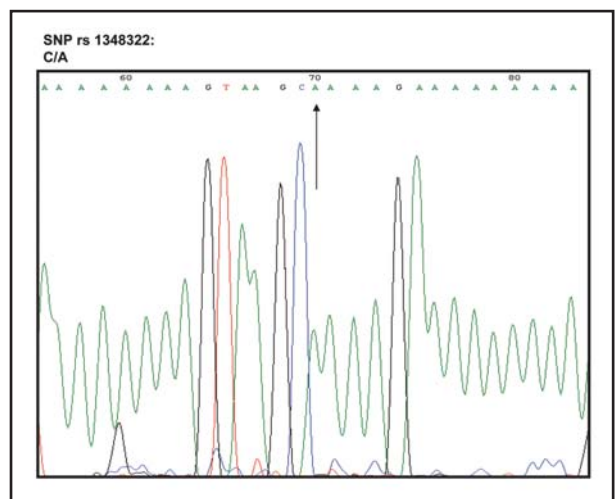


Fig. 3: Sequencing in the subject. The panel shows SNP rs 1348322 polymorphism where a C is substituted by an A. This alteration was present in all the subjects studied (affected and unaffected).

inction between phenotype and genotype, which is essential for understanding heredity and development of organisms¹⁹⁻²⁷. Mandibular micrognathia defined as an anteroposterior skeletal dysplasia of

the lower maxilla is the primary anatomical cause of class II malocclusion, since the mandible serves as the base for dental arches, and alterations of its morphology and growth alter the occlusal relationships and function. This study assessed 16 affected and unaffected subjects from 4 unrelated families in whom the analysis of micrognathia was based on the observation of 25 different clinical parameters defined as phenotypical prevalences. In the data obtained from the analysis of prevalence of these measurements between fathers and their offspring, and mothers and their offspring, several similarities were found in the different measurements. Nevertheless, the most relevant and significant results were between mothers and their offspring, where it was found that in particular the measurements Xi – Pm/Pg, CF – Go, Co – Gn, Nasolabial (Cm-Sn-Ls), have a significance level lower than 7%, suggesting that these measurements related to mandibular size tend to be passed on from mothers to daughters and not fathers to sons, showing a possible relationship to gender. There have been similar findings in previous studies regarding overall craniometric dimensions among members of the same family^{22,25,28}. In 1991, Harris and Johnson²⁹ showed that measurements of tooth dimensions seem to be closer between members when deciduous dentition is fully erupted, but begin to diverge substantially during adolescence and young adulthood. However, the opposite is true for craniofacial dimensions, where there is increasing similarity of measurements in adulthood. This study of subjects from families with mandibular micrognathia shows major similarities in craniofacial measurements. However, it is noteworthy that although mandibular micrognathia was diagnosed in all the families, the differences among the measurements of each of the families are not statistically significant, making them similar to each other. This might imply that the environment definitely plays an important part as an additional component, influencing the phenotypical characteristics of the individuals. According to Garn et al.³⁰, this “cohabitation effect” makes the members of a family resemble each other more than they would even

if they shared the genes of common ancestors. Although there are studies describing the genes that act on the development of mandibular tissue, there are no reports proving the relationship between phenotypes and the genes responsible for mandibular shape and size, due to the large number of genes involved in facial development, including mandibular growth. Nevertheless, QTL (quantitative trait loci) analysis in mice has identified chromosomal regions involved in the anteroposterior regulation of the mandible, suggesting more than 10 candidate genes for the development of the mandible, which may be related to mandible size³¹⁻³³. In this study, we analyzed *NOG* gene polymorphisms in families with mandibular micrognathia because it is an antagonist gene that plays a part in modulating a BMP activity, which seems to control much of the growth and development of the mandibular processes³⁴. Furthermore, recent research has revealed serious anomalies in the skeletal development of mice with homozygotic mutations in the *nog* gene such as holoprosencephaly, which is often associated to micrognathia and agnathia¹³. This is a new study, as there are no reports in Colombia or in the literature describing the possible relationship between polymorphisms (reported in the NCBI database) of the *NOG* gene, and individuals with mandibular growth deficiency. Sequencing clearly showed the presence of SNPs 1348322 polymorphism of the *NOG* gene in all the individuals studied. These results do not show a relationship between the polymorphisms described in *NOG* and mandibular growth deficiency, possibly because they may be population polymorphisms, or because these markers are not sufficiently polymorphic for our population. Therefore further molecular studies are needed to enable other populations and regions of this gene to be analyzed as regulating regions, thus continuing with the search for how *NOG* is related to mandibular micrognathia. In addition, these studies will contribute in the future as a base to find the genetic cause of sleep apnea, which is closely related to this entity.

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