Myo1H GENE SINGLE NUCLEOTIDE POLYMORPHISM rs10850110 AND THE RISK OF MALOCCLUSION IN THE ROMANIAN POPULATION

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Keywords: *dental anomalies, genetic marker, Matrilin 1*

Abstract: *Background. The Matrilin-1 gene is thought to be involved in the formation of the filamentous network of the cartilage's extracellular matrix and implicitly in the formation of the mandibular condyle cartilage, very important in different malocclusions. Objectives. The purpose of this study was the association of the marker Myo1H (rs10850110) with malocclusions. Methods. The sample comprises 47 patients with different malocclusions. The control group included 11 individuals. Cephalometric measurements were performed. The genotyping was done by sequencing. Results. Significant differences concerning allele and genotype distribution were observed between different malocclusions and the control group. The genotypes were distributed in the population according to Hardy-Weinberg equilibrium. Conclusions. Our findings are in agreement with other studies that emphasize the importance of the Myo1H gene in the determination of Class I, II and especially Class III phenotypes.*

Cuvinte cheie: *anomalii dentare, marker genetic, Matrilin 1*

Rezumat: *Introducere. Se presupune că gena pentru Matrilin-1 este implicată în formarea reţelei de filamente din matricea extracelulară a cartilajului, implicit şi în formarea cartilajului condilului mandibular, foarte important în numeroase malocluzii. Obiective. Scopul acestui studiu a fost asocierea markerului genetic Myo1H (rs10850110) cu malocluziile. Metode. Lotul experimental a fost alcătuit din 47 de pacienţi. Grupul de control a cuprins 11 indivizi. Au fost efectuate măsurători cefalometrice. Genotiparea a fost realizată prin secvenţializare. Rezultate. Diferenţe semnificative au fost observate între diferite clase de malocluzii şi lotul martor. Distribuţia genotipurilor în populaţie a respectat echilibrul Hardy-Weinberg. Concluzii. Rezultatele noastre susţin ipoteza că gena Myo1H are un rol important în dezvoltarea caracteristicilor fenotipice specifice malocluziilor de Clasă I, II şi în special III.*

INTRODUCTION

Dental malocclusion might be the result of a disproportion either between the size and number of the teeth and the size of the jaws, which would produce crowding or spacing, or between the size and shape of the maxilla and/or mandible. Changes in soft tissue morphology or in perioral musculature might also be responsible for an abnormal occlusion.

According to Angle's (1) classification, there are three classes of malocclusion, with appropriate subdivisions depending on the presence of other abnormalities. Patients with class I present crowding (most frequently) or spacing in the anterior region of the dental arch.

Class II anomalies comprise many combinations of skeletal (maxillary prognathism - midface protrusions II/1 - or mandibular retrognathism II/2) and dentoalveolar components (maxillary alveolodental protrusion or mandibular alveolodental retrusion).

Class I and II anomalies are relatively common in the human population, which means that many young people have orthodontic problems.(2-4)

Class III malocclusion can be caused by maxillary skeletal retrusion (even maxillary hypoplasia, also known as false mandibular prognathism or midface retrusion), mandibular skeletal protrusion (mandibular prognathism), maxillary alveolodental retrusion, mandibular alveolodental protrusion or the combination of these. MP is often the acronym used for this disorder (mandibular prognathism).

The prevalence of MP varies with age (5,6), ethnic groups (7,8) and sex, being more frequent in females than males.(9,10) In Asian populations, such as Korean, Chinese and Japanese the incidence of MP is higher than in European ones.(10-12) The etiology of MP has been attributed to various genetic inheritance patterns, influenced also by multiple environmental factors. The studies support an autosomal-dominant mechanism of inheritance (10,13,14), usually polygenic, but monogenic inheritance was also reported.(8)

We also noticed the inheritance of some dental anomalies in first degree relatives: mother-son or mother-daughter, mother-sister-children (unpublished data).

PURPOSE

The purpose of this study was to identify genes that possibly regulate maxillary or mandibular growth in different dental anomalies, using Single Nucleotide Polymorphisms (SNPs) as genetic markers. In this respect, we characterized a fragment of the *Matrilin-1* gene (cartilage matrix protein), a non-collagenous protein, secreted by chondrocytes and expressed dominantly in cartilage. This protein is thought to be involved in the formation of the filamentous network of the cartilage's extracellular matrix (15), and implicitly in the

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Article received on 03.04.2013 and accepted for publication on 01.10.2013
ACTA MEDICA TRANSILVANICA December 2013;2(4):281-284

formation of the mandibular condyle cartilage, very important in many malocclusions.(16)

To our knowledge, until now, similar studies were not done on the Romanian population.

METHODS

The sample comprises 47 patients with different types of malocclusions: 10 patients – Class I with an average age of 18.3 years (7 females and 3 males); 19 patients – Class II/1 with an average age of 18.3 years (8 females and 11 males), 3 patients– Class II/2, with an average age of 17.3 years (all females) and 15 patients – Class III with an average age of 21.1 years (8 females and 7 males). The control group included 11 individuals (9 female and 2 male) with an average age of 31 years.

We performed cephalometric measurements in sagittal plane, measuring Steiner's SNA, SNB, ANB, Down's A-B plane and the Wits appraisal (AoBo).

SNA angle indicates whether or not the maxilla is normal, prognathic or retrognathic. The mean value is $82° \pm 2°$; values less than 82° indicate a maxillary retrognathism, while angles larger than 82° would indicate a maxillary prognathism.

SNB is used to assess whether the mandible is positioned anterior or posterior to the cranial base. The mean value is $80° \pm 2°$. Values less than 80° indicate mandibular retrognathism, while larger ones prognathic mandible.

The ANB angle indicates the magnitude of the skeletal jaw discrepancy. Normal values vary between 0° and 2°. An ANB angle greater than 2° indicates a Class II tendency, an angle below 0° suggest a Class III tendency.

The AB-Plane angle measures the relation to the anterior limit of the apical bases related to the facial line (N-Pog). The mean value is −4.6°. Negative values (under −9°) suggest a Class II facial pattern, while positive values indicate a Class III facial pattern. The Wits appraisal (AoBo) is a linear measurement used to assess the antero-posterior jaw disharmony. A linear difference is measured between the projection of points A and B to the occlusal plane. According to the Wits appraisal, the average jaw relationship is 0-2 mm. The smaller values indicate a Class III pattern, the greater ones indicate a Class II skeletal discrepancy.

For the genetic analysis genomic DNA was extracted from oral mucosa cells, using Animal and Fungi DNA Preparation Kit from Jena BioScience.

The gene fragment (803 bp) containing the rs10850110 SNP (12q24.11) was amplified using the primers:

H1For 5'-AATTCTGTCTGCTCCGCATC-3' and

H1Rev 5'-ATTTCCATCCAATGGTGCAG-3'.

The PCR mixture (50 µl) contained 0.5 µM of each primer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 200 ng of gDNA and 1.75 U of Go Taq Flexi DNA polymerase (Promega). Standard amplification conditions were 95ºC for 4 min, 35 cycles: 94ºC for 30 sec, 56ºC for 30 sec and 72ºC for 1 min, final extension 10 min at 72ºC. The PCR products were purified from agarose gel using the Wizard® SV Gel and PCR Clean-Up System (Promega). The DNA sequencing was done by chain-termination method using the H1For primer.

RESULTS

The cephalometric variables are shown in table no. 1. From the 58 analyzed patients, 18.96% had normal occlusion, 17.24% Class I malocclusion, 37.93% Class II malocclusion, and 25.86% Class III malocclusion. Class II malocclusion is the most prevalent pattern in our study, but since the sample size of this study was modest, we cannot conclude that this Class is prevalent in Romanian population.

MP is clinically heterogeneous and may result not only of purely mandibular prognathism, but also from maxillary hypoplasia or a combination of the two.

Table no. 1. Cephalometric measurements and genotypes for rs10850110

Class	Patient	Age (y)	Gender	SNA (°)	SNB (°)	ANB (°)	AoBo (mm)	AB-plane (°)	SNP H1 10850110
I	1	18	M	80	78	2	-1	-3	AG
	2	17	F	82	79	3	2	-9	GG
	3	13	M	83	79	4	2	-7	AA
	4	30	F	81	76	5	1	-9	AG
	5	16	F	80	77	3	1,5	-6	GG
	6	25	F	83	80	3	1	-4	AG
	7	15	M	82	79	3	1	-2	GG
	8	20	F	83	81	2	1,5	-5	GG
	9	17	F	81	80	1	1	-3	AG
	10	12	F	79,8	75,2	4,6	0	-8	GG
II/1	11	18	M	71	68	3	2,5	-3	GG
	12	23	F	-	-	5	3	-9	GG
	13	14	M	74	70	4	2,5	-10	GG
	14	25	M	82	78	4	4	-10	GG
	15	19	F	86	80	6	3	-7	AG
	16	14	M	82	77	5	3	-9	GG
	17	13	F	85	79,5	5,5	3	-10	GG
	18	12	M	82	78	4	4	-11	AG
	19	12	F	83	77	6	4	-9	GG
	20	14	M	84	78	6	3	-7	AG
	21	14	M	82	79	3	3	-5	AG
	22	12	M	83,2	78,7	4,5	3	-9	GG
	23	33	F	84	76	8	4	-12	GG
	24	12	M	82	77	5	4	-10	GG
	25	12	M	83	79	4	3	-8	AG
	26	14	M	79	73	6	6	-11	AG
	27	23	F	86	72	14	12	-23	GG
	28	38	F	85	79	6	3	-12	GG
	29	27	F	74	69	5	6	-13	AG
	30	19	F	79	74	5	7	-8	GG
	31	17	F	79	73	6	6	-10	GG
	32	16	F	81	77	4	5	-10	GG
III	33	20	M	79	77	2	-4	2	GG
	34	24	M	78	82	-4	-2	6	GG
	35	20	F	73	74	-1	-3	0	GG
	36	16	F	76	77	-1	-13	0	GG
	37	21	M	84	90	-6	-10	7	AG
	38	15	M	81	85	-4	-2	0	AG

	39	21	M	82	90	-8	-13	12	-
	40	42	M	82	84	-2	-2,5	-2	AA
	41	18	F	77	76	1	-9	0	GG
	42	12	F	76	78	-2	-2	0	AA
	43	7	M	78	84	-6	-4	1	GG
	44	29	F	76	89	-13	-14	13	AG
	45	35	F	80	88	-8	-9	8	GG
	46	24	F	80	82	-2	-2	2	GG
	47	13	F	81	82	-1	-5	2	GG
	48	25	F	80	77,5	2,5	1	-5,6	GG
	49	28	F	81	80	1	0	-4	GG
	50	39	M	82	80	2	0	-4,5	GG
	51	31	F	81	79	2	1	-4,6	GG
	52	35	F	80,5	80	0,5	0	-3	GG
Control	53	24	F	83	80,5	2,5	1	-6	GG
	54	24	F	82,5	80	2,5	2	-5	GG
	55	36	F	82	81	1	1,5	-4,5	GG
	56	32	M	83	81,5	1,5	0	-3	GG
	57	30	F	80	79	1	1	-4	GG
	58	37	F	82	81	1	0	-4	GG

The distribution of alleles and genotypes in individuals with Class I, II and III malocclusions and the control group was statistically analyzed using Chi-square test (table no. 2). Significant differences ($p < 0.05$) between the allele frequencies in subjects with Class I, II, III relative to the control were observed. Regarding the genotypes, we noticed significant differences only between Class I, II and the control. The genotypes were distributed in the population according to Hardy-Weinberg equilibrium (*p*-value = 0.3475).

Table no. 2. Genotype and allele distribution of SNP rs10850110

	Genotype			Allele	
Class I	GG	AG	AA	G	A
	5	4	1	14	6
	*p=0.0270**			*p=0.0055***	
Class II	15	7	0	37	7
	*p=0.0350**			*p=0.0478**	
Class III	9	3	2	21	7
	p=0.0858			*p=0.0114**	
Class I, II, III	29	14	3	72	20
	p=0.0552			*p=0.0160**	
Control	11	0	0	22	0

DISCUSSIONS

Although the genetic sequences in human population are remarkably similar, the genomes may differ at about 1,200 bases on average, having other bases as normal. These genetic differences, known as SNPs, serve as genetic markers to associate genes and diseases. The International HapMap Project (haplotype map) tries to identify most of the approximately 10 million SNPs estimated to occur commonly in human genome (snp.cshl.org/hapmappopulations.html.en). Among SNPs, we selected the marker *Myo1H* (rs10850110) locus 12q24.11 responsible for the synthesis of Matrilin 1, an important protein involved in the regulation of maxillary and mandibular growth. For rs10850110, the ancestral allele is G, allele A being the mutant one.

In spite of the small size of the sample, our findings are in agreement with other studies that emphasize the importance of the gene for Matrilin-1 in the determination of Class I, II and especially Class III phenotypes (16, 17). At the 12q24.11 locus, Tassopoulou-Fishell *et al*. (17) pointed out that in an interval of 397,305 bp, another 4 genes flank *Myo1H*. Even if *Myo1H* is the most important gene in mandibular prognathism, we cannot rule out the possibility that other genes are also involved.

CONCLUSIONS

Our findings support the opinion that *Myo1H* gene has an important function in the development of the craniofacial complex. Implicitly, the muscle function might have a more important role in this process than previously thought.

We think that the knowledge of various genes involved in dental anomalies, as well as their mechanism of action will be of great help to orthodontists in order to think out the most efficient strategies for diagnostic, prevention and therapy.

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