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Genetic Polymorphism in MMP9 May Be Associated With Anterior Open Bite in Children

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Anterior open bite (AOB) has a multifactorial etiology caused by the interaction of sucking habits and genetic factors. The aim of this study was to evaluate the association between AOB and polymorphisms in genes that encode Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). Four hundred and seventy-two children that presented at least one sucking habit were evaluated. Children were examined clinically for the presence of AOB. Genomic DNA was extracted from saliva. Genotyping of the selected polymorphisms in MMP2, MMP3, MMP9, TIMP1 and TIMP2 was carried out by real-time PCR using the TaqMan method. Allele and genotype frequencies were compared between the groups with and without AOB using the PLINK® software in a free and in a recessive model using a chi-square test. Logistic regression analysis was implemented ($p \leq 0.05$). Two hundred nineteen children had AOB while 253 did not. The polymorphism rs17576 in MMP9 was significantly associated with AOB ($p=0.009$). In a recessive model GG genotype was a protective factor for AOB ($p=0.014$; OR 4.6, 95%CI 1.3-16.2). In the logistic regression analysis, none of the genes was associated with AOB. In conclusion, the polymorphism rs17576 (glutamine for arginine substitution) in MMP9 was a protective factor for AOB.

Key Words: polymorphism, genetics, anterior open bite, dental malocclusion.

Introduction

Anterior Open Bite (AOB) is a dentoalveolar or skeletal malocclusion in the vertical plane (1), with the lack of contact of opposing teeth when posterior teeth are in occlusion (2). AOB malocclusion is multifactorial condition involving a combination of many factors. Sucking habits and facial characteristics such as bone, teeth and soft tissue alterations are involved in AOB etiology (3).

Non-nutritive sucking habits such as finger and pacifier sucking (4,5) and nutritive sucking habit like breast and bottle-feeding (6,7) are reported to be involved in AOB etiology. It is also possible that AOB is influenced by genetic factors (3,8), in which genes that play a role in facial bone growth and development, teeth and soft tissue, may be associated with AOB.

Matrix metalloproteinases (MMPs) are a group of enzymes responsible for the degradation of most extracellular matrix proteins (9,10). The MMPs are inhibited by specific endogenous tissue inhibitors of metalloproteinases (TIMPs). Both, MMPs and TIMPs, are involved in tissue modelling and remodeling (11-13) and play an important role during organogenesis, growth and normal tissue turnover (14).

Although there is no published paper on the association between AOB and genetic factors, it is plausible to hypothesize that genetic factors act in combination with sucking habits establishing the AOB in children. Therefore, the aim of this study was to evaluate the association between polymorphism in MMP2, MMP3, MMP9, TIMP1 and TIMP2 genes with AOB.

Material and Methods

Subject Screening

This study was submitted and approved by the Human Ethics Committee of Antônio Pedro Hospital of Universidade Federal Fluminense (Process #811.473). All parents or caregivers were informed about the study and signed an informed consent.

The sample was collected in 30 public daycare centers from Nova Friburgo, a city in the State of Rio de Janeiro, in the southeast region of Brazil, from May 2012 to October 2013. The parents/caregivers answered a questionnaire about the children's characteristics and habits.

Children with primary dentition whose parents reported at least one sucking habit were selected regardless the type of sucking (finger sucking, pacifier sucking and bottle feeding use).

The selected children were clinically examined by a

pediatric dentist and AOB were considered when occurred with a minimum vertical gap of 0.5 mm between the maxillary and mandibular incisors in centric occlusion. Children with history of previous orthodontic treatment or speech therapy, children with other malocclusion than AOB and children with syndromes or oral cleft were not included.

DNA Extraction and Real-Time PCR Assay

Genomic DNA for molecular analysis was extracted from saliva using a previously reported method (15). Genotyping of the selected polymorphisms in MMP2, MMP3, MMP9, TIMP1 and TIMP2 was carried out by real-time PCR using the Stratagene Mx3005P (Agilent Techologies, SAN Diego, CA, USA) with TaqMan method that expresses each sample as a homozygotic or heterozygotic according to the detected fluorescence.

Statistical Analysis

Student's t test was used to evaluate age difference and chi-square test was used to analyze gender and ethnicity distribution between the group with AOB and the group without AOB, using the Epi Info 7.0 software at a 5% level of significance.

PLINK® software was used to evaluate genotype and allele distribution between the group with AOB and the group without AOB in a free model and in a recessive model. For MMP2, MMP3, MMP9 and TIMP2, that are located in autosomal chromosome, chi-square test was used. For TIMP1, located in a sex chromosome, a multivariate analysis was performed and gender was used as a covariant in the model. The level of significance was set at 5%.

Logistic regression analysis was implemented using duration of the sucking habit, breastfeeding, ethnicity, gender and polymorphisms MMP2, MMP3, MMP9, TIMP1 and TIMP2 as covariates in order to evaluate possible interactions.

The standard chi-square test was used to test for deviation from Hardy-Weinberg equilibrium.

Results

Among the 472 analyzed children, 219 had AOB and 253 had normal occlusion. The age ranged between 2 and 6 years old. AOB was more frequent in girls than in boys (p=0.015; OR 1.5, 95%CI 1.0-2.2). Age and ethnicity were not different between groups (p>0.05). In 88% (n=417) of the cases, the mothers reported breastfeeding their children for at least 12 months.

Table 1 presents the results of the genotypes and alleles distribution between the groups.

The polymorphism rs17576 in MMP9 (p=0.009) was significantly associated with AOB. In a recessive model (GG versus AG+AA), GG genotype was a protective factor for AOB (p=0.014; OR 4.6, 95%CI 1.3-16.2). The other analyzed polymorphisms were not statistically different between the groups (p>0.05).

The logistic regression analysis is presented in the Table 2. There was no association between the polymorphisms in MMP2, MMP3, MMP9, TIMP1 and TIMP2 and AOB (p>0.05). Breastfeeding was not associated with AOB in the logistic regression analysis (p=0.09).

Discussion

AOB is commonly classified as skeletal or dental malocclusion. The dental open bite is generally found in

Table 1. Genotype and allele distribution between the groups

Gene	Genotype distribution (%)							Allele distribution (%)					
	Group without AOB (n=219)			Group with AOB (n=253)			p value	Group without AOB (n=219)		Group with AOB (n=253)		p value	OR (IC 95%)
MMP2	CC 29(14.3)	CT 89(43.8)	TT 85(41.9)	CC 21(11.7)	CT 84(46.9)	TT 74(41.3)	0.710	T 147(36.2)	C 259(63.8)	T 126(35.2)	C 232(64.8)	0.770	0.95 (0.71-1.28)
MMP3	GG 25(11.6)	AG 68(31.5)	AA 123(56.9)	GG 23(13.1)	AG 53(30.1)	AA 100(56.8)	0.881	G 118(27.3)	A 314(72.7)	G 99(39.1)	A 253(60.9)	0.800	1.04 (0.76-1.42)
MMP9	GG 16(10.7)	AG 57(38.2)	AA 76(51.0)	GG 3(2.5)	AG 61(51.7)	AA 54(45.8)	0.009	G 89(29.9)	A 209(70.1)	G 67(28.4)	A 169(71.6)	0.709	0.93 (0.63-1.35)
TIMP1	CC 26(14.8)	CT 51(29.0)	TT 99(56.2)	CC 18(11.8)	CT 66(43.4)	TT 68(44.7)	0.359	C 103(29.3)	T 249(70.7)	C 102(33.5)	T 202(66.4)	0.237	1.22 (0.87-1.69)
TIMP2	GG 9(4.7)	GT 48(25.0)	TT 135(70.3)	GG 3(1.8)	GT 34(21.0)	TT 125(77.1)	0.196	G 66(17.2)	T 318(82.8)	G 40(12.3)	T 284(87.7)	0.072	0.67 (0.44-1.03)

the anterior region and is associated with sucking habits. On the other hand, the skeletal open bite is often related to excessive vertical growth of the dento-alveolar complex, especially in the posterior molar region (3) According to Lin et al. (2013) (3) the classification of AOB as either skeletal or dental is difficult, and it is probably a result of both factors in combination with sucking habits. Therefore, the present study selected a sample of children that presented sucking habits, in which AOB phenotype was characterized according to the clinical characteristics.

The effects of sucking habits on the development of the occlusion were under investigation for decades and several studies demonstrated their association with different types of malocclusion (4,16-20). The main question arising from these studies is whether genetic factors are a risk or a protective factor for AOB. For this reason, a genetical analysis was performed to evaluate if the MMP family contributes to the etiology of the AOB.

MMPs are a group of enzymes responsible for the degradation of most extracellular matrix proteins during growth (14). MMPs and TIMPs are expressed by osteoblasts (21-25) and play a role in bone development, modeling and remodeling. The present results demonstrated that MMP9 was associated with AOB. It was observed that GG genotype in MMP9 acted as a protective factor for AOB. MMP9 protein plays an important role in extracellular matrix remodeling by cleaving denatured collagen and type IV collagen in the basement membrane (24). It is interesting to note that the studied polymorphism rs17576 in MMP9 is a non-synonymous substitution characterized by the amino acid substitution of glutamine to arginine (NCBI). Although the impact of this polymorphism on protein function is unknown up to now, a previous study suggested that this polymorphism could lead to partial loss of function in extra-cellular matrix remodeling (25). The obtained results also suggested that this polymorphism presents a functional alteration that plays a role in the establishment of AOB.

Some evidences reinforce the hypothesis that genetic factors play an important role in the AOB etiology. Although some children presented sucking habits, they did not end up developing AOB. It may be hypothesized that some genes act as a protective factor against AOB development.

This study has some obvious limitations. It is important to emphasize that it was decided to adopt a level of significance of 5% to avoid type I error and to make it possible to identify small genetic effects of the genes on AOB etiology. Therefore, it is possible that the association between MMP9 rs17576 and AOB is a false-positive association since post-hoc analysis was not performed. Additionally, the logistic regression analysis was not able to confirm the results of the univariate analysis.

Another limitation of this study is the sample selection. Children were selected from daycare centers. These children did not present radiograph exams that allowed evaluating their morphological facial pattern. Different cephalometric measures are used to identify open bite tendency (26). Another fact to be highlighted is that an otorhinolaryngologic exam was not performed in order to evaluate nasal breathing and mouth breathing. This topic has been explored by some researchers in the past few decades. It is well known that the characteristic features of the increase in lower facial height and open bite are consistent with those attributed to nasal obstruction (27-29). A study performed in teenagers using respirometric techniques to compare the breathing behavior of normal and long-faced patients demonstrated that the long-faced subjects had significantly smaller components of nasal respiration (29).

Although the present study did not observe an association between breast feeding and AOB, a previous study demonstrated that extending breastfeeding for 12 months was associated with a 3.7 times lower chance of having anterior open bite (30). It is possible that this result did not present a statistical significance due to the fact that 88% of the sample had extended breastfeeding.

To the best of the authors' knowledge, this is the first study to evaluate genetic factors involved in the etiology of the AOB. Further studies should be performed in order to confirm whether MMP9 is a protective factor for AOB, including other possible involved factors.

The polymorphism MMP9 rs17576 (glutamine for arginine substitution) was a protective factor for AOB.

Table 2. Regression analysis in the both cohorts

Gene	Reference	Genotype	p-value	OR (95%CI)
MMP2	CC	CT	0.97	1.01 (0.41-2.49)
		TT	0.83	1.09 (0.45-2.66)
MMP3	CC	CT	0.71	0.84 (0.32-2.15)
		TT	0.33	0.65 (0.27-1.57)
MMP9	AA	AG	0.96	1.01 (0.48-2.14)
		GG	0.14	0.18 (0.01-1.79)
TIMP1	CC	CT	0.38	1.61 (0.55-4.71)
		TT	0.33	0.61 (0.22-1.65)
TIMP2	GG	GT	0.11	0.55 (0.26-1.15)
		TT	0.43	0.47 (0.07-3.07)

Note: OR (95% C.I.)=Odds ratios; 95% confidence intervals. Covariates included duration of the sucking habit, breastfeeding, ethnicity, gender and polymorphisms.

The obtained results support the hypothesis that AOB is a multifactorial condition in which the combination of factors plays an important role.

Resumo

A mordida aberta anterior apresenta uma etiologia multifatorial causada pela interação entre hábitos de sucção e fatores genéticos. O objetivo deste estudo foi avaliar a associação entre mordida aberta anterior e polimorfismo nos genes que codificam as metaloproteinases da matriz (MMPs) e seus inibidores teciduais (TIMPs). Foram avaliadas 472 crianças que apresentvam pelo menos um hábito de sucção. As crianças foram clinicamente examinadas para avaliar a presença de mordida aberta anterior. DNA genômico foi extraído da saliva. A genotipagem dos polimorfismos selecionados em MMP2, MMP3, MMP9, TIMP1 e TIMP2 foi realizada por PCR em tempo real, usando o método de TaqMan. As frequências alélicas e genotípicas foram comparadas entre os grupos com e sem mordida aberta anterior usando o software PLINK®. Duzentas e dezenove crianças apresentavam mordida aberta anterior enquanto 253 não a apresentavam. O polimorfismo rs17576 em MMP9 estava significativamente associado com mordida aberta anterior (p=0,009). No modelo recessivo (GG versus AG+AA) o genótipo GG foi um fator protetor para mordida aberta anterior (p=0,014; OR 4,6; 95%CI 1,3-16,2). Concluindo, o polimorfismo rs17576 (substituição de glutamina por arginina) em MMP9 está associado com mordida aberta anterior. Os resultados obtidos suportam a hipótese de que fatores genéticos estão envolvidos com a etiologia da mordida aberta anterior.

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