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Association Between -1607 1G/2G Polymorphism of *MMP1* and Temporomandibular Joint Anterior Disc Displacement with Reduction

Alberto Sierra Rosales[1], Eduardo Alberto Villaseñor Rodríguez[2], Christian Lorena López González[2], Edy David Rubio Arellano[2], Susan Andrea Gutiérrez Rubio[2], Teresa Arcelia García Cobián[2]

[1]Departamento de Clínicas Odontológicas Integrales, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara
[2]Instituto de Terapéutica Experimental y Clínica, CUCS - Centro Universitario de Ciencias de la Salud; Universidad de Guadalajara

Correspondence: Teresa Arcelia García Cobián, Centro Universitario de Ciencias de la Salud, Edificio P primer Piso, Sierra Mojada 950, Col. Independencia CP 44340 Guadalajara, Jalisco, Mexico. Tel: +33-10-58-52-00. e-mail: tgcobian2002@yahoo.com.mx

Anterior disc displacement with reduction (DDWR) is considered one of the most common disorders within the temporomandibular joint (TMJ), with a prevalence of 41% in adults. Matrix metalloproteinases play an important role in the degradation of the TMJ and the matrix metalloproteinase 1 (MMP1) 1607 1G/2G polymorphism increases the local expression of MMP1 thus leading to accelerated degradation of the extracellular matrix. The objective of this study was to evaluate the association between the 1607 1G/2G polymorphism of *MMP1* gene and DDWR in a group of Mexican individuals from western Mexico. A total of 67 unrelated individuals, between the ages of 18 and 36 years, of both genders, were included in this study. Study participants with DDWR were required to meet the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD), while a second control group of 90 individuals without DDWR were also included. Both groups were required to have paternal and maternal ancestry (grandparents) of the same geographic and ethnic region. Genotypes were determined using the nested PCR technique. The 1G/2G polymorphism was found in 68.7%, followed by 2G/2G in 25.4% and 1G/1G in 6.0% of the cases group. While the prevalence in the control group was 55.5% for the 1G/2G polymorphism, 26.6% for 1G/1G and 17.7% for 2G/2G. An association was found between the 2G allele of the 1607 1G/2G polymorphism of *MMP1* gene and the presence of DDWR in the patients of western Mexico.

Key Words: temporomandibular disorder, anterior disc displacement, single nucleotide polymorphism, matrix metalloproteinase, mmp-1

Introduction

Temporomandibular disorders (TMD) encompass a group of musculoskeletal and neuromuscular conditions that include the temporomandibular joints (TMJ), the masticatory muscles and all associated tissues (1).

These disorders impact approximately 5-12% of the general population in the United States (2). Recent studies report the prevalence among adolescents in other nations between 9.0% and 48.7%, according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (3).

The most common subtypes of TMDs include pain-related disorders, such as myofascial pain, arthralgia, and disorders associated with the TMJ, primarily internal derangements, degenerative joint disease, including associated biopsychosocial conditions (4,5).

Internal derangements are defined as deviations in the position or form of the tissues within TMJ capsule; DDWR occurs when the articular disc is anteriorly displaced relative to the condylar head in the closed mouth position and restores (reduces) its normal physiological relation with the affected condyle during mouth opening, the disc reduction is usually accompanied by articular noises (6). DDWR is the most common internal derangement encountered in adults and corresponds to 41% of TMD clinical diagnoses (7).

Anterior disc displacement without reduction (ADDwoR) is present when the disc is unable to return to its normal position, which leads to a decrease in mouth opening and is usually painful (8). In a study of prevalence and associated factors conducted in Mexico, at the University of Campeche with 506 patients aged 14-25 years, at least 46.1 % of patients had some grade of TMD, while anterior disc displacement with reduction was the most prevalent with 15.6% (9).

The articular disc of the TMJ is formed basically by proteoglycan aggregates and collagen fibers that are composed mainly of type 1 and 2 collagen; its physiologic maintenance is through a balance between degradation of collagen fibers performed by matrix metalloprotease (MMP) and their inhibitors, tissue inhibitors of metalloproteinase (TIMP) (10,11). An association has been reported between accelerated collagen degradation and the increased production and overexpression of MMP1 in the synovial fluid of the TMJ of patients with osteoarthritis (12-14).

Previous functional studies have reported the -1607 1G/2G polymorphism of *MMP1* gene leads to an increase in local MMP1 concentration, which contributes to accelerated degradation of the extracellular matrix (15, 16).

In 2011, Planello found an association between the *MMP1* polymorphism and degenerative disorders of the TMJ in patients who had been diagnosed using magnetic resonance (17). Luo et. al. (18), for their part, found an association between this polymorphism and ADDwoR in patients with and without temporomandibular osteoarthritis. Many proteins constitute the TMJ of which type 1 collagen is one of the main components of the joint and, is mostly degraded by type 1 metalloproteinase. Patients with the MMP1 –1607 2G/2G genotype, included in this study, could manifest increased concentrations of MMP1, leading to a higher degradation of collagen and, potentially have an increased risk for developing DDWR. For this reason, the objective of our study was to evaluate the association between the 1607 1G/2G polymorphism of *MMP1* gene and DDWR in a group of Mexican individuals from western Mexico.

Material and Methods

This study was conducted in a group of 67 unrelated Mexican individuals from the western region of the country, between the ages of 18 and 36 years, of both genders, from 2013-2019. Every participant was required to sign an informed consent form with approval of the University of Guadalajara Committees (Conbioetica14cei01920130619); participants were selected and clinically evaluated by an expert Prosthodontist based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) (19).

A control group of 90 individuals from the same region, without TMD, was also included. Both groups were of Mexican ancestry and without a history of facial trauma, unrelated, without association with any syndrome. The sample size was calculated with the formula for comparison of two independent proportions regard to the anterior disc displacement of the temporomandibular joint prevalence, which is within 18 to 36% (20). It was considered a confidence level of 95%, a statistical power of 80%. According to this, we needed 82 participants nevertheless 157 participants were included.

Extraction and analysis of genomic deoxyribonucleic acid (gDNA)

After informed written consent was obtained from the participants, 6 mL of peripheral blood was drawn from the forearm using tubes with ethylenediaminetetraacetic acid (EDTA), for the extraction of gDNA.

The extraction of gDNA was carried out using the modified Miller technique and the Genomic DNA Purification Kit (Promega, 2014) on total leucocytes (21). The 1607 1G/2G (rs1799750) single nucleotide polymorphism (SNP) of the *MMP1* gene was amplified using nested PCR to determine the insertion-deletion. The first round of amplification was conducted using 5`GGAGTCACTTCAGTGGCA3` (forward) and 5`CAACACTTTCCTCCCCTT3` (reverse) primers in a volume of 10 µL, containing 3 µL of Master Mix (Promega Corporation), 0.4 µL of each oligonucleotide (0.1pm/µL), 0.1 µL of Taq polymerase, 4.6 µL of deionized water and 1.5 µL of gDNA; PCR was carried out, elevating the temperature of the mix for 5 min to 94°C for the initial denaturation, followed by 30 cycles of denaturation at 94°C for 30 s, an alignment at 52 °C for 30 s, an extension at 72°C for 30 s and a final extension at 72°C for 10 min.

Subsequently, an aliquot of 2 µL of the first PCR was amplified using the following primers 5`GGAAATTGTAGTTAAATAATTAGAAAGA3` (forward), 5`GATTGATTTGAGATAAGTCATATCC3` (reverse), under the conditions described above.

Lastly, the amplified fragments were subjected to electrophoresis in a 6% polyacrylamide gel using a buffer solution (0.5x TBE), at 170-200 volts for 30-60 min, and the gel was stained with 0.2% silver nitrate. The amplified products obtained were 138 and 279bp for the 1G/1G homozygote, 279, 138 and 192bp for the 1G/2G heterozygote and 192 and 279bp for the 2G/2G homozygote. The samples were documented with a key and until statistical analysis, they were identified as case or control.

Statistical Analysis

Gene counting was used to determine allele and genotype frequency. The Hardy Weinberg equilibrium estimation and association analysis were calculated by chi-square, Fisher exact test and logistic regression, using a Finetti test program at ihg.gsf.de/cgi-bin/hw/hwa1.pl and IBM-SPSS v20.

Results

The DDWR group was comprised of 47 women and 20 men, who were single and between the ages of 18-36 years (mean = 21.43 ±3.163 years). 58.5% of these patients reported facial pain, 55.4% had nocturnal and daytime bruxism, 72.3% presented jaw locking, 78.5% reported headache or migraine and 47.7% reported tinnitus (Table 1).

With respect to the genotypic frequency of the polymorphism in cases, the most frequent genotype associated with DDWR was 1G/2G (68.7%), followed by 2G/2G (25.4%) and 1G/1G (6.0%). The most frequent genotypes observed in the control group were 1G/2G in 55.5%, 1G/1G in 26.6% and 2G/2G in 17.7%. The 2G allele was most prevalent in the experimental group (Table 2).

The comparison with Fisher's exact test showed a significant difference ($p<0.001$) between the affected group and the control group, as well as between the patients and the 1000 genome project ($p<0.001$). Differences were also

observed between wild type homozygous and heterozygous groups (p = 0.001 with an OR of 5.520) as well as regard to homozygous (p = 0.0022 with an OR of 6.375) and in dominant model comparison (p=0.00081) with an OR of 5.727. When comparing Mexican descendants of the 1000 genome project with the patients in our study, similar results were observed (Table 2).

No association was observed between the polymorphism and the presence of facial pain, jaw locking, bruxism, tinnitus or headache.

Discussion

Internal derangements of the TMJ are defined as deviations in the position and shape of the tissues within the capsule, and anterior disc displacement with reduction is considered one of the most common of these disorders (7). This condition is generally stable, painless and causes little to no discomfort for the patient (22), however, previous studies have shown that individuals with the 2G/2G genotype could potentially develop degenerative disorders (17, 18).

As reported by the literature, the prevalence of TMD varies according to age and the population being studied, hence, the global prevalence of these disorders among adolescents has been reported in 9.0% to 47.7% (3). The presence of signs and symptoms has been observed in 16% – 88% of the general population (23). In Mexico, Casanova-Rosado et. al. (9) conducted a study in 506 Mexican individuals from the southeast region of the country, between the ages of 14 to 25 years, and observed that 46.9% had a TMD. They determined that DDWR was the most common TMD found in 15.6% and that the prevalence was higher among women (52.9%) than men (27.9%) with an average age of 17.6 ± 2.9 years.

A greater influx of women was observed in our study, which was to be expected given that, on average, TMD occurs anywhere from 1.2 to 2.6 times more in women than in men, with women being on average two-fold risk to suffer from this condition than men (24). However, since this study was open to the general population, no frequency can be assumed, nevertheless more than half of our participants (70.1%) were women.

Pain is a frequent symptom in these temporomandibular conditions (5,24,25). Luo et. al. reported that 100% of their patients suffered from facial pain, with 71.6% reporting their pain as mild, while 24.9% reported it as moderate and 3.5% as severe (18). For their part, Planello et. Al (17). observed that the prevalence of pain was 73.9%, which coincides with the current study in which 58.5% of patients (38 cases) reported facial pain.

The frequency of bruxism in existing literature

Table 1. Clinical characteristics within a group of patients with anterior disc displacement with reduction

Clinical Characteristics	Cases	Percent
Facial Pain	38	58.5%
Headache	51	78.5%
Opening Limitation	23	35.4%
Jaw Locking	47	72.3%
Nocturnal Bruxism	36	55.4%
Daytime Bruxism	36	55.4%
Tinnitus	31	47.7%

Table 2. Distribution of genotypes, alleles, observed differences and the relative risk between cases, controls and the 1000 genome project

Polymorphism -1607 1G/2G	Genotypes n (%)				Alleles n (%)		Total
Group	1G/1G	1G/2G	2G/2G	1G/2G+2G/2G	1G	2G	
Cases	4(6.0)	46 (68.7)	17 (25.4)	63	54 (40.3)	80 (59.7)	67
Controls	24 (26.6)	50 (55.5)	16 (17.7)	66	98 (54.4)	82 (45.6)	90
Chi-square (p)	ref	10.19 (0.001)	9.30 (0.002)	11.23 (<0.001)	ref	6.15 (0.013)	7.93 (0.004)
OR (CI 95%)	1	5.520 [1.780-17.117]	6.375 [1.809-22.465]	5.727 [1.881-17.437]	1	1.771 [1.126-2.785]	2.358
1000 genomes	27 (42%)	28 (43.75)	9 (14.06)	37 (57.81)	82 (64.06)	46 (35.93)	64
Chi-square (p)	ref	21.25 (<0.001)	16.74 (<0.001)	23.77 (<0.001)	ref	14.81 (<0.001)	17.05 (<0.001)
OR (CI 95%)	1	11.08 [3.510-35.035]	12.75 [3.389-47.964]	11.49 [3.728-35.430]	1	2.641 [1.603-4.352]	3.430

varies from 22-31% (26), which was very different from observations in the current study in which 55.4% of patients reported nocturnal and daytime bruxism and 72.3% reported jaw locking. The increased frequency of these complications could be due to increased occupational stress, given that the participants of this study were medical and dentistry students, with long hours of studying and who are prone to sleepless nights and are generally less physically active.

There is a high prevalence of otologic signs and symptoms among adult patients with TMD, which could be explained by different theories such as the proximity of the structures to the TMJ, the ear canal and the shared innervation. The results of a meta-analysis showed that the most frequent symptoms were blocked ear sensation (74.8%), otalgia (55.5%), tinnitus (52.2%), vertigo (40.8%) and hearing loss (38.9%), which is similar to those found in our study in which 47.7% of patients reported hearing noises or buzzing in their ears (27).

The -1607 1G/2G SNP has also been shown to increase the expression, transcription, and activity of collagenase, which could potentially be associated with the severity of the condition from its onset (15). Two previous studies carried out in Brazilian and Chinese populations have observed that the polymorphism could possibly be linked to increased degradation of the TMJ. Luo et. al. conducted a study in 141 Chinese patients in which they observed that individuals with the 2G genotype were 1.91 times more likely to develop ADDwoR in patients with and without temporomandibular osteoarthritis. For this reason, special attention and care must be given to patients with this genotype who also suffer from ADDwoR or osteoarthritis, given the association between this polymorphism and increased MMP1 activity, which leads to increased catabolism of articular fibrocartilaginous tissue and degradation (12). Planello et. al. (11), also conducted a study in Brazilian individuals, in which, patients with a homozygous 2G/2G genotype were 2.47 times more likely to develop degradation of the TMJ. In Accordance with this study, where carriers of the 2G allele had almost 2-fold risk, further, individuals with the homozygous 2G/2G genotype could be 6.3 times more likely to develop DDWR, and 5.7 times greater in the dominant model compared to the control group. Lastly, since the 2G allele was twice as prevalent in the study population compared to the controls, its presence may be a risk factor for the development of DDWR in this population from western Mexico.

No association was observed between the studied symptoms and the polymorphism, perhaps due to the sample size of subgroups when separated by clinical characteristics.

The limitations of this study were that the serum levels of MMP1 were not measured and the damage was not confirmed by radiological examination.

This polymorphism is associated with the development of DDWR in the group of patients from western Mexico. The presence of the 2G allele could be considered as a risk factor for the development of DDWR in this western Mexican population.

Resumo

O deslocamento anterior do disco com redução (DADR) é considerado um dos distúrbios mais comuns na articulação temporomandibular (ATM), com prevalência de 41% em adultos. As metaloproteinases da matriz desempenham um papel importante na degradação da ATM e o polimorfismo 1607 1G/2G da metaloproteinase da matriz 1 (MMP1) aumenta a expressão local da MMP1, levando à degradação acelerada da matriz extracelular. O objetivo deste estudo foi avaliar a associação entre o polimorfismo 1607 1G/2G do gene *MMP1* e a DADR em um grupo de indivíduos mexicanos do oeste do México. Um total de 67 indivíduos não relacionados, com idades entre 18 e 36 anos, de ambos os sexos, foram incluídos neste estudo. Os participantes do estudo com DADR foram obrigados a cumprir os Critérios de Diagnóstico de Pesquisa para Disfunções Temporomandibulares (CDP/DTM), enquanto um segundo grupo controle de 90 indivíduos sem DADR também foi incluído. Ambos os grupos tinham ascendência paterna e materna (avós) da mesma região geográfica e étnica. Os genótipos foram determinados pela técnica de nested PCR. o polimorfismo 1G/2G foi encontrado em 68,7%, seguido por 2G/2G em 25,4% e 1G/1G em 6,0% do grupo de casos. Enquanto a prevalência no grupo controle foi de 55,5% para o polimorfismo 1G/2G, 26,6% para 1G/1G e 17,7% para 2G/2G. Foi encontrada uma associação entre o alelo 2G do polimorfismo 1607 1G/2G do gene *MMP1* e a presença de DADR nos pacientes do oeste do México.

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