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Estrogen Receptor- α Polymorphisms and Predisposition to TMJ Disorder

Margarete Cristiane Ribeiro-Dasilva^{*}, Sérgio Roberto Peres Line[†], Maria Cristina Leme Godoy dos Santos[†], Mariana Trevisani Arthuri[‡], Wei Hou[§], Roger Benton Fillingim^{*}, and Célia Marisa Rizzatti Barbosa^{||}

^{*}Department of Community Dentistry and Behavioral Science, University of Florida, Gainesville

[†]Department of Morphology, Dental College of Piracicaba, State University of Campinas (Unicamp), São Paulo, Brazil

[‡] Department of Physiology, Dental College of Piracicaba, State University of Campinas (Unicamp), São Paulo, Brazil

[§] Department of Epidemiology and Health Policy Research, University of Florida, Gainesville

^{||} Department of Prosthesis and Periodontology, State University of Campinas (Unicamp), São Paulo, Brazil

Abstract

Temporomandibular joint disorders (TMJD) affect women with greater frequency than men, and sex hormones may contribute to this female predominance. Therefore, this study investigated whether estrogen receptor- α (XbaI/PvuII) single nucleotide polymorphisms (SNPs) are associated with TMJD in women. DNA was obtained from 200 women with TMJD (100 with chronic pain and 100 with signs of TMJD but no pain) diagnosed according to the Research Diagnostic Criteria for Temporomandibular Disorder (RDC/TMD) and 100 control women without TMJD. Restriction fragment length polymorphisms of polymerase chain reaction products were used to analyze XbaI and PvuII SNPs in DNA fragments. A model directly characterizing specific DNA sequence variants based on the risk haplotypic structure implemented with the EM algorithm was used to analyze the data. The [GC] haplotype of the XbaI locus was significantly more prevalent in both TMJD groups when compared with the control group ($P = .0012$). Specifically, the [GC] haplotype was more prevalent within the painful TMJD group versus the control group (OR = 3.203, 95% CI = 1.633, 6.284) and in the TMJD no pain versus the control group (OR = 2.51, 95% CI = 1.267, 4.97). In conclusion, the presence of [GC] haplotype in the XbaI locus may increase the susceptibility of women to develop TMJD.

Perspective: This study suggests that a polymorphism in the estrogen receptor may increase the risk of women developing temporomandibular joint disorder. This finding may elucidate the interindividual differences in the contribution of estrogen to TMJD, the genetic influences on TMJD predisposition, and may serve as the basis for future treatment tailoring, which could enhance outcomes for these patients.

Keywords

Temporomandibular disorder; sex hormones; estrogen receptor; polymorphism

Temporomandibular muscle and joint disorders (TMJD) are the most common cause of chronic pain in the orofacial region.¹¹ The prime manifestations of these disorders are disc displacement with clicking or crepitus sounds produced during mandibular function and persistent, recurring, or chronic pain in the temporomandibular joint (TMJ).³⁷ Epidemiologic data consistently have shown that women are at greater risk for TMD compared with men,^{9, 24} though the reasons for this female predominance have not been determined. Interestingly, women experience more inflammation, facial pain, and tenderness in jaw muscle and temporomandibular joint than men.^{41,53} Some evidence suggests that increased inflammatory response can lead to loss of the articular cartilage.³³ Therefore, sex differences in inflammation may also explain the higher prevalence of TMD among women.⁵³ Moreover, other inflammatory diseases such as osteoarthritis and rheumatoid arthritis are also more prevalent among women than men.^{1,47} However, sex differences in pain processing may also play a role, since abundant evidence demonstrates that women display greater sensitivity to experimental pain than men.^{12,13} These sex differences in both inflammation and pain sensitivity could be driven by common underlying mechanisms, such as the influence of gonadal hormones. That TMD is equally prevalent before puberty and that the higher prevalence of TMD in females emerges in young adulthood²⁵ may implicate sex hormones in the pathophysiology of this condition.⁶ However, the mechanism of action of these hormonal effects is still unknown and is a matter of debate.¹⁴

Steroid hormones, particularly estrogen, act through their receptors (estrogen receptor- α [ER α] and estrogen receptor- β [ER β])^{23,29,43} in the periphery as well as the central nervous system (CNS),²⁸ producing effects on the inflammatory process^{32,45} as well as on central pain transmission.^{7,44,55} For example, estrogen can directly act on monocytes and macrophages to regulate the production of cytokines (eg, interleukin-1 [IL-1],¹⁷ IL-6,³⁴ and tumor necrosis factor- α [TNF- α]).^{36,42} The cytokines IL-1 β and IL-6 are present in the TMJ synovium during inflammation,²² and IL-1 and TNF- α promote cartilage reabsorption, inhibit synthesis of proteoglycans, and promote inflammation in the majority of TMD structures.^{33,40} Additionally, monocytes/macrophages are the immune cells present within the synovial tissues and are also frequently recruited in synovial inflammation, suggesting that a majority of IL-1 and TNF- α released within the joint may originate from those immune cells.¹⁵ Finally, TMJD, especially when associated with acute trauma, internal derangements, or osteoarthritis, often includes an inflammatory component.^{2,20,21} Therefore, estrogen as well other sex hormones can play an important role in pain severity and TMJD predisposition. As a result, a genetic variation at the ER α could lead to significant modifications in the physiological role of estrogen and consequently in TMJ derangements.

The most studied single nucleotide polymorphisms (SNPs) on the ER α are the PvuII (T-397C) and XbaI (A-351G) sites. These sites were previously associated with higher prevalence of arthritis in women^{3,51} as well as skeletal changes in female symptomatic TMJ osteoarthritis patients (TMJOA)²⁴ and pain severity in TMJOA patients.¹⁹ Moreover, the product of this gene is an important signal transduction pathway mediator,^{38,42} and PvuII and XbaI are expressed in a variety of cell types, including trigeminal neurons³⁵ and chondrocytes and articular cells of bone tissue.^{38,50} These polymorphic sites were also previously linked with numerous conditions including osteoarthritis,^{8,51} Alzheimer's disease,^{4,27} migraine,³² obesity,⁴³ breast cancer,^{5,31} leiomyomas,²⁸ endometriosis,²⁰ bone mineral density,^{11,16,52} and coronary artery disease,³⁹ suggesting that XbaI and PvuII may have functional effects. However, their direct functional effects have yet to be characterized.

Therefore, this study analyzed the relationship of these 2 polymorphisms, PvuII (T-397C) and XbaI (A-351G), in the first intron of the ER α gene, and the diagnosis of TMJD among Brazilian women.

Materials and Methods

Volunteers

A total of 300 women, from 18 to 44 years old, were enrolled from the patient pool from the Integrated Dental Clinic at the Dental College of Piracicaba, Brazil. The participants were interviewed and asked to complete a detailed questionnaire on personal and family medical history, TMD symptoms, and age of onset, frequency, severity, and treatment and its response. Next, the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) questionnaire¹⁰ was completed for each participant. This research was approved by the Brazilian Institutional Review Board, and informed consent was obtained prior to commencement.

Participants were divided into 3 groups with 100 patients in each group. Age and self-reported racial group are described in Table 1. The inclusion criteria for group 1 (signs and symptoms of TMJD) and group 2 (only signs of TMJD without pain) were presence of disc displacement (DD) with reduction (R); without reduction with limited opening (WRLO); without reduction and without limited opening (WRWLO). The exclusion criteria were presence of pain/inflammation elsewhere in the body, or taking contraceptives or any other medication.

The difference between groups 1 and 2 was that group 1 had the added inclusion criterion of arthralgia for more than 6 consecutive months, whereas for group 2 the presence of arthralgia was a criterion for exclusion. For the control group all participants were healthy, without any signs or symptoms of TMJD. The control group was age matched to the TMJD groups.

Sampling

The sampling of buccal epithelial cells was performed as previously described by Trevilatto and Line.⁴⁸ Briefly, individuals rinsed their mouths with mouthwash, containing 5 mL 3% glucose solution for 1 minute. After mouthwash, a sterile wood spatula was used to scrape oral mucosa. The tip of the spatula was then inserted into the retained mouthwash solution. Buccal epithelial cells were pelleted by centrifugation at 5000 g for 10 minutes. The supernatant was discarded and the cell pellet resuspended in 500 μ L of extraction buffer solution [10 mM Tris-HCl (pH 7.8), 5 mM ethylenediamine tetra-acetic acid (EDTA), and 0.5% sodium dodecyl sulphate (SDS)]. Finally, the samples were frozen at -20°C until used for DNA extraction.

DNA Extraction

After thawing, the samples were incubated overnight (ON) with 100 ng/mL proteinase K (Sigma Chemical Co, St Louis, MO) at 37°C with agitation. DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation. DNA was dissolved in 200 μ L TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA]. The concentration was estimated by measurements of OD 260 nm.

Polymerase Chain Reaction

The sequence from T-397G locus in the ER α gene promoter was PCR amplified with primers 5'-GATATCC AGGGTTATGTGGCA-3' (forward) and 5'- AGGTGTTGCC TATTATATTAACCTTGA - 3' (reverse). PCR was carried out in a total volume of 50 μ L, containing 500 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 1 μ M of each primer, 200 μ M of each dATP, dCTP, dGTP, and dTTP, and 2.5 U *Taq* DNA polymerase (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The solution was incubated for 5 minutes, 40 seconds at 95°C , followed by 35 cycles of 1 minutes, 40 seconds at 95°C ; 1 minute, 40 seconds at 61°C ; and 1 minute, 40 seconds at 72°C ; with a final extension of 72°C for 7 minutes, 40 seconds.

A fragment of A-351G of the ER α gene promoter was PCR amplified with primers 5'–GATATCCAGGGTTA TGTGGCA – 3' (forward); 5'–AGGTGTTGCCTATTATATT AACCTTGA – 3' (reverse). PCR was carried out in a total volume of 50 μ L, containing 500 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 1 μ M of each primer, 200 μ M each dATP, dCTP, dGTP, and dTTP, and 4 U Taq DNA polymerase (Amersham Pharmacia Biotech AB). The solution was incubated for 5 minutes, 40 seconds at 95°C, followed by 35 cycles of 1 minute, 40 seconds at 95°C; 1 minute, 40 seconds at 61°C; and 1 minute, 40 seconds at 72°C; with a final extension of 72°C for 7 minutes, 40 seconds.

Restriction Fragment of Length Polymorphism

A 3.5- μ L aliquot of ER α (T-397C) PCR products was digested with 4 units of XbaI (New England Biolabs, Inc, Beverly, MA) in a final volume of 20 μ L. For analysis of ER α (A-351G) polymorphism a 3.5- μ L aliquot of ER α PCR products was digested with 0.6 units of HgaI (New England Biolabs, Inc) in a final volume of 20 μ L. The digestions were carried out at 37°C for 16 hours. The sizes of the fragments generated by the restriction enzymes were previously described.^{3,24,51}

Gel Electrophoresis

The total amount aliquot of the digest was mixed with 3 μ L of loading buffer and electrophoresed on a 10% vertical nondenaturing polyacrylamide gel at 20 mA. The gel was silver stained by DNA Silver Staining Kit (Amersham Pharmacia Biotech AB).

Statistical Methods

All statistical analyses in this article were performed by using SAS v9.1. A *P* value of less than .05 was considered statistical significant.

Single SNP Analysis

The genotype and allele frequencies of single polymorphisms were calculated by direct counting and then dividing by the number of subjects to produce genotype frequency. The significance of the differences in observed frequencies of each polymorphism in test groups was assessed by χ^2 test.

Two SNP Haplotypic Analyses

Our 2 SNPs are genotyped and each SNP has 2 alleles (A, G, and T, C respectively). Therefore there were 4 possible haplotypes: [AT], [AC], [GT], and [GC]. The frequencies of the 4 haplotypes can be denoted by $P_{[AT]}$, $P_{[AC]}$, $P_{[GT]}$ and $P_{[GC]}$. Assuming Hardy-Weinberg equilibrium, 9 genotypes and their frequencies can be constructed in Table 2 based on the frequencies of haplotypes.

Table 2 lists all 9 genotypes at 2 SNPs, possible diplotypes within each genotype, and their frequencies. Note that all genotypes except the double heterozygote have only 1 known diplotype. For the double heterozygote AG/TC, there are 2 possible unknown diplotypes [GC][AT] and [AC][GT]. Let *p* denote the relative frequency of diplotype [GC][AT] within the genotype GA/CT, therefore *p* can be expressed as in Liu et al.²⁶

$$\pi = \frac{P_{[GC][AT]}}{P_{[GC][AT]} + P_{[GT][AC]}}.$$

The frequencies of subjects in each genotype {10,29,16,34,173,18,10,8,2} are also listed as the observations. The log-likelihood function of the 4 unknown haplotype frequencies ($P_{[GC]}$, $P_{[GT]}$, $P_{[AC]}$, $P_{[AT]}$) given 9 observed genotypes (G) and observation frequencies (O) is then written as previously reported²⁶:

$$\begin{aligned} \text{Log } L(P_{[GC]}, P_{[GT]}, P_{[AC]}, P_{[AT]} | G, O) \\ \propto 2 * 10 * \log(P_{[GC]}) + 29 * \log(P_{[GC]} P_{[GT]}) \\ + 2 * 16 * \log(P_{[GT]}) + 34 * \log(P_{[GC]} P_{[AC]}) \\ + 1 * 173 \log(P_{[GC]} P_{[AT]} + P_{[AC]} P_{[AT]}) \\ + 18 * \log(P_{[GT]} P_{[AT]}) + 2 * 10 * \log(P_{[AC]}) \\ + 8 * \log(P_{[AC]} P_{[AT]}) + 2 * 2 * \log(P_{[AT]}) \end{aligned}$$

An iterative Expectation-Maximization algorithm developed by Liu et al²⁶ can be implemented to estimate the unknown haplotype frequencies. In the E step, the relative frequency of diplotype [GC][AT] within the genotype GA/CT is calculated by function (1) denoted by $\hat{\pi}$. In the M step, the haplotype frequencies can be calculated using:

$$\begin{aligned} \hat{P}_{[GC]} &= (2 * 10 + 29 + 34 + 173 * \hat{\pi}) / 2 * 300, \\ \hat{P}_{[GT]} &= [2 * 16 + 29 + 18 + 173 * (1 - \hat{\pi})] / 2 * 300, \\ \hat{P}_{[AC]} &= [2 * 10 + 34 + 8 + 173 * (1 - \hat{\pi})] / 2 * 300, \\ \hat{P}_{[AT]} &= [2 * 18 + 8 + 173 * (1 - \hat{\pi})] / 2 * 300. \end{aligned}$$

The E step and M step are taken repeatedly until the estimates converge.

After haplotype frequencies were obtained from the EM algorithm, haplotypic effects can be estimated by recategorizing the 10 diplotypes into 3 diplotype groups ([GC] is the risk reference haplotype): [GC][GC] - no risk presence reference haplotype; [GC][$\overline{GC}$] - 1 risk presence reference haplotype; and [$\overline{GC}$][$\overline{GC}$] double presence risk reference haplotype.

The frequencies of haplotypes formed by T-397C and A-351G polymorphisms [AC], [AT], [GC], and [GT] were calculated by using the EM algorithm. The χ^2 was used to test the association between the haplotypes and test groups. Four χ^2 tests were calculated for each haplotype [AC], [AT], [GC], and [GT] by using the 3 recategorized groups: double presence of risk reference haplotypes, 1 risk presence reference haplotype and no risk presence reference haplotype (Table 3). The risk reference haplotype can then be determined from the 4 haplotypes [AC], [AT], [GC], and [GT] by selecting the highest log-likelihood value of the χ^2 tests. Furthermore, the risk associated with haplotypes was calculated as the odds ratio with 95% confidence intervals.

Results

The haplotype with the highest log-likelihood was the [GC]. The overall model showed that the presence of [GC] was significantly higher in the TMJD with pain compared with control group ($P = .0028$) and when the TMJD groups were put together (TMJD total = TMJD no pain + TMJD with pain) the P value was .0012 (Table 3). The odds ratio analysis showed that patients carrying the haplotype [GC] increased significantly the risk to develop signs of TMJD by 2.51 times when the TMJD no pain group was compared with control group (OR = 2.51, 95% CI = 1.267 to 4.97). The same haplotype [GC] increased significantly in 3.203 times the risk to develop TMJD with pain when TMJD with pain group was compared with control group (OR = 3.203, 95% CI = 1.633 to 6.284). However, the risk type [CG] was not significantly associated

with higher risk to develop symptoms of TMJD when the TMJD no pain group was compared with TMJD pain group (OR = 1.276, 95% CI = 0.712 to 2.288).

Discussion

The PvuII and XbaI polymorphic sites are located on intron 1, and whether these sites have functional consequences is unknown. However, polymorphisms on introns could affect mRNA production, as these sites may contain transcriptional regulatory sequences.⁵² For example, the Sp1 polymorphism, located in the first intron of the collagen-type I α -1 gene, is known to change the mRNA transcription, leading to decreased bone mineral density and increased fracture risk.⁵⁰ Similarly, the PvuII–XbaI polymorphic sites on the first intron of the ER α gene could influence gene expression. Additionally, a recent report showed that the PvuII polymorphism is located within a potential bMyb binding site, which regulates transcription efficacy of a reporter gene.²⁵ Alternatively, the location of a variable length of the (TA)_n VNTR in the promoter of the ER α gene could also affect gene transcription. Furthermore, previous studies have shown that a VNTR in proximity to a promoter can have a significant influence on transcriptional regulation.¹⁸ However, other polymorphic sites in the estrogen receptor gene might similarly influence TMJD predisposition.

Our results show that the presence of genotype [GC] haplotype in the ER α gene may indicate that TMJD is related to receptor dysfunction. Although the exact mechanism is unclear, 3 hypotheses can be proposed about the association between the ER α gene and TMJD predisposition. First, the haplotype [GC] may directly affect the level of ER α through transcriptional regulation. Alternatively, the haplotype [GC] may be in linkage disequilibrium with an exonic polymorphism that has a direct influence on ER α protein function. Finally, the polymorphism may be linked with another unidentified mutation adjacent to the ER α gene affecting the estrogen action on chondrocytes⁴⁴ and/or the action of estrogen on monocytes and macrophages altering the release of inflammatory mediators such as cytokines, IL1,¹⁵ IL6,³⁴ tumor necrosis factor- α (TNF- α),^{36,42} and nitric oxide.^{30,46}

Also, our findings showed no differences in haplotype frequencies between the TMJD no pain group when compared with the TMJD pain group, but differences emerged when comparing both TMJD groups to the control group. One possible interpretation of these results is that this rare haplotype is associated with the mechanism of TMJ pathology but is not associated with pain related to TMJD. The presence of 1 risk haplotype [GC] was twofold higher in the combined compared with control group, and the presence of 2 risk haplotypes was only observed in the TMJD group and not in the control group (Table 3). Obviously, additional studies are warranted in order to replicate these findings.

The clinical implications of these findings, while speculative, suggest that estrogen action may be of importance after a trauma in the temporomandibular joint. For example, this polymorphism could influence inflammatory responses by altering estrogen's effects on chondrocytes, which could lead to an increased risk of developing TMJD.

Also, we cannot rule out that this polymorphism may also influence the level of estrogen in brain areas responsible for pain processing and also could alter functioning of the opioid system, consequently increasing the pain perception.^{49,55} As reported for Zubieta et al,⁵⁵ when gonadal steroid levels are low, women are less capable of suppressing deep pain by the activation of the μ -opioid system. Moreover, administration of exogenous estrogen increased pain-related μ -opioid receptor binding and reduced pain sensitivity.⁴⁴

Recently, Kang et al (2007),¹⁹ studying the association of Pvu II and Xba I polymorphisms and pain susceptibility in female temporomandibular joint osteoarthritis (OA), showed that TMJ OA patients carrying the [GC] haplotype were found to have a significantly higher risk

of moderate or severe pain compared to those without the GC haplotype, suggesting that ER- α genotype may be associated with pain susceptibility in female TMJ OA patients.

The present study is limited by a small sample size and by the admixed Brazilian population, which limits the generalizability of the data. Additionally, the present genetic study includes only data from articular pain but not muscle pain, and whether similar findings would emerge among patients with myalgia is not known. Considering that estrogen receptors are also present in the muscles,⁵⁴ additional research including individuals with muscle pain is warranted.

Similar to Kang et al (2007),¹⁹ within the limitations of this pilot study, our results suggest that women carrying the [GC] haplotype at the locus A-351G in their ER α gene have 3.2 times greater susceptibility to TMJD than those who do not carry this genotype. Therefore, this paper supports previous studies regarding the influence of estrogen in TMJD. Further studies are needed to replicate these results and to determine whether ER α is associated with individual differences in pain perception among women with TMJD.

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Table 1

Distribution of Age and Self-Report Racial Groups Among Control and TMD/DD Groups

	Control	TMJD Without Pain	TMJD With Pain
Age (y)	31.01	31.34	30.29
Mean ($\pm$ SD)	8.36	8.36	7.99
Ethnic group (%)			
White	76	74	82
Afro-American	16	18	18
Asiatic	08	08	0

Table 2
Nine Genotypes, Possible Diplotypes Within Each Genotype, and Their Frequencies at Two SNPs

Genotype		PVUII	Possible Diplotype	Diplotype frequency Expected	Relative Frequency within each genotype	Observed Frequency	Risk Type
XbaI							
GG	CC		[GC][GC]	$P_{[GC][GC]} = P^2_{[GC]}$	1	10	[GC][GC] [*]
GG	CT		[GC][GT]	$P_{[GC][GT]} = 2P_{[GT]}P_{[GC]}$	1	29	[GC][GC] [†]
GG	TT		[GT][GT]	$P_{[GT][GT]} = P^2_{[GT]}$	1	16	[GC][GC] [‡]
GA	CC		[GC][AC]	$P_{[GC][AC]} = 2P_{[GC]}P_{[AC]}$	1	34	[GC][GC] [†]
GA	CT		[GC][AT]	$P_{[GC][AT]} = 2P_{[GC]}P_{[AT]}$	π		[GC][GC] [†]
			[GT][AC]	$P_{[GT][AC]} = 2P_{[GT]}P_{[AC]}$	$1-\pi$	173	[GC][GC] [‡]
GA	TT		[AT][GT]	$P_{[GT][AT]} = 2P_{[GT]}P_{[AT]}$	1	18	[GC][GC] [‡]
AA	CC		[AC][AC]	$P_{[AC][AC]} = P^2_{[AC]}$	1	10	[GC][GC] [‡]
AA	CT		[AC][AT]	$P_{[AC][AT]} = 2P_{[AC]}P_{[AT]}$	1	8	[GC][GC] [‡]
AA	TT		[AT][AT]	$P_{[AT][AT]} = P^2_{[AT]}$	1	2	[GC][GC] [‡]

^{*} [GC][GC], no presence of risk type
[†] [GC][GC], presence of only 1 risk type
[‡] [GC][GC], double presence of risk type.

Table 3

Distribution of Risk Type [GC] Throughout the Groups

Frequency	Control	TMJD Without pain	TMJD With pain	TMJD Total ^{**}
[GC][GC] [*]	85 (85%)	68 (68%)	63 (63%)	130 (65%)
[GC][GC] [†]	15 (15%)	29 (29%)	30 (30%)	59 (29.5%)
[GC][GC] [‡]	0 (0%)	3 (3%)	7 (7%)	10 (5%)
Total (n)	100	100	100	200
χ^2			$P = .0028^1$	$P = .0012^2$

^{**} TMJD total = TMJD without pain + TMJD pain.

¹ χ^2 test when compared TMJD with pain and control groups.

² χ^2 test when compared TMJD total and control groups.

^{*} [GC][GC], no presence of risk type.

[†] [GC][GC], presence of only 1 risk type.

[‡] [GC][GC], double presence of risk type.